PATHOGENESIS OF INTESTINAL INFECTIONS

MICROBIOLOGICAL AND PATHOLOGICAL PRINCIPLES

Edited by
M. V. Voino-Yasenetsky
and
T. Bakács

AKADÉMIAI KIADÓ, BUDAPEST

PATHOGENESIS OF INTESTINAL INFECTIONS

Edited by
M. V. VOINO-YASENETSKY
and T. BAKÁCS

This book is the result of long-standing Hungarian-Soviet collaboration in studies on basic problems of the pathogenesis of dysentery, salmonelloses, and enteric infections caused by *Escherichia coli*. The contributors, pathologists and microbiologists, describe the human pathology of the diseases as well as the infectious processes induced by enteropathogens in animals. The chief aim of the authors has been to find the most essential link in the pathogenesis, i.e. to pinpoint the interaction between the organisms and the host. Special attention has been paid to the correct assessment of pathological events and further to advantages and disadvantages of various animal models. All available information on human pathology and animal experiments from early works up to the most recent ones has been considered critically in the light of the authors' own experience. General questions of the pathogenesis of enteral infections are considered in the introductory chapters where also different approaches to the study of various infectious processes, including nonintestinal ones, are discussed.
The book is primarily intended for the specialist (clinicians, microbiologists, pathologists and others) engaged in the above problems, but may also be of interest to workers in other fields of infectious pathology.

AKADÉMIAI KIADÓ
Publishing House of the Hungarian Academy of Sciences
BUDAPEST

ISBN 963 05 1131 2

PATHOGENESIS OF INTESTINAL INFECTIONS

MICROBIOLOGICAL AND PATHOLOGICAL PRINCIPLES

Edited by
M. V. Voino-Yasenetsky
and
T. Bakács

AKADÉMIAI KIADÓ, BUDAPEST

PATHOGENESIS OF INTESTINAL INFECTIONS

Edited by
M. V. VOINO-YASENETSKY
and T. BAKÁCS

This book is the result of long-standing Hungarian-Soviet collaboration in studies on basic problems of the pathogenesis of dysentery, salmonelloses, and enteric infections caused by *Escherichia coli*. The contributors, pathologists and microbiologists, describe the human pathology of the diseases as well as the infectious processes induced by enteropathogens in animals. The chief aim of the authors has been to find the most essential link in the pathogenesis, i.e. to pinpoint the interaction between the organisms and the host. Special attention has been paid to the correct assessment of pathological events and further to advantages and disadvantages of various animal models. All available information on human pathology and animal experiments from early works up to the most recent ones has been considered critically in the light of the authors' own experience. General questions of the pathogenesis of enteral infections are considered in the introductory chapters where also different approaches to the study of various infectious processes, including nonintestinal ones, are discussed.
The book is primarily intended for the specialist (clinicians, microbiologists, pathologists and others) engaged in the above problems, but may also be of interest to workers in other fields of infectious pathology.

AKADÉMIAI KIADÓ
Publishing House of the Hungarian Academy of Sciences
BUDAPEST

ISBN 963 05 1131 2

PATHOGENESIS OF INTESTINAL INFECTIONS

MICROBIOLOGICAL AND PATHOLOGICAL PRINCIPLES

PATHOGENESIS OF INTESTINAL INFECTIONS

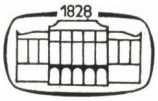
MICROBIOLOGICAL AND PATHOLOGICAL PRINCIPLES

Edited by

M. V. VOINO-YASENETSKY

and

T. BAKÁCS



AKADÉMIAI KIADÓ · BUDAPEST 1977

Chapters 2, 6, 7, 8, 16 translated by
A. RÉDEI

English text edited by
B. LÁNYI

ISBN 963 05 1131 2

Printed in Hungary

CONTENTS

PREFACE

In spite of the tremendous progress that has been made in the prevention and treatment of infectious diseases the problem of enteric infections can by no means be regarded as outdated. We still hear about outbreaks of cholera, and recently a dysentery epidemic with more than 8000 fatal cases was caused by *Shigella dysenteriae* 1, a microorganism which was thought to have entirely disappeared. Dysentery due to *Sh. sonnei* and *Sh. flexneri* continues to appear all over the world. The cases are less dangerous but not infrequently they respond to therapy poorly and are liable to have a protracted course. The importance of typhoid fever and salmonelloses must not be underestimated either. Some members of the *Escherichia coli* group play a more important part in human and animal pathology than it has previously been supposed.

It would appear that, especially in the highly industrialized countries with a dramatic development in public installations like piped water systems, drainage, sewage treatment and with an adequate control of "noxious" foodstuffs, such infections can be strongly restricted and perhaps completely eliminated.

Expectations, however, have only been partially fulfilled. By the first half of our century morbidity due to these diseases had decreased, and the endemic areas had in many countries been totally or, at least, partly eliminated. But no further improvement has occurred and there has even been a deterioration in the last 30 years.

The development of sanitary installations has been slower than would have been necessary even in the richest countries, especially as regards the quantity and quality of drinking water, drainage and the neutralization of sewage. This is typical not only of industrialized areas, but also of smaller towns and even of rural districts. As a result of this paradoxial situation, water- and food-borne epidemics again occurred in the most developed countries, e.g. the recent epidemic of typhoid fever conveyed by potato salad in Baden-Württemberg, F.R.G. Mechanized animal-husbandry and the meat processing industry have helped to spread certain enteric bacteria.

The situation is even more alarming in countries of the third world. According to the data of WHO, every year about 500,000,000 people are affected by infection with enteric bacteria. Water and food-borne infections regularly occur in these countries, where piped water, drainage and

sewage treatment are almost unknown. In addition, undernourishment of the population renders them more susceptible to these pathogenic bacteria.

Escherichia coli and *Shigella* can mostly be found in the background of enteric infections of uncertain origin. This has made it necessary to introduce further sanitary measures as aspecific protection, since specific measures like vaccination proved insufficient.

Accordingly, investigations had to be extended to intracellular parasitism and the problem had to be approached from the aspect of tissue immunity. The aim of these studies has been to elucidate the pathomechanism of enteric infections and probably also a more efficient preventive activity.

In recent years interest has turned towards agents of enteric infections. Previously unknown biological properties of these organisms playing a part in the pathogenesis of enteric diseases have been described. The capacity of shigellae to parasitize the epithelial cells and the ability of cholera vibrios to produce peculiar toxic substances responsible for the typical clinical symptoms may be listed among these properties.

Data obtained on the pathogenesis of cholera have been dealt with in a number of extensive reviews. There is an obvious need for reviewing recent data on bacterial infections of the intestine such as shigelloses, salmonelloses as well as escherichioses. The contributors of the present volume undertook this work all the more willingly because their own research in this field helped them to reconsider the available literary information in the light of their own observations.

The advances reported on have been partly due to recent development in the fields of immunohistochemistry, electron microscopy, etc. but also to the joint effort of representatives of different fields, e.g. the teams organized by S. B. Formal in the U.S.A. The results of French (S. Szturm-Rubinsten, D. and M. Piéchaud) and Japanese (H. Ogawa and R. Sakazaki, N. Tanaka and others) authors are also well known.

The keratoconjunctivitis model devised by the Hungarian microbiologist, B. Serény, in the early 1950's is undoubtedly the prime source of modern concepts as regards the pathogenic properties of dysentery agents. Morphological investigations of experimental shigella keratoconjunctivitis undertaken by P. Rácz on the initiative and under the guidance of Professor M. V. Voino-Yasenetsky in 1958, served as the starting point for a lasting scientific collaboration of Hungarian and Soviet investigators in the study of enteric infections. The present monograph sums up the results of this collaboration.

The results presented have been obtained in animal experiments. Time and again doubts are raised as to the usefulness in human pathology of information obtained in this way. Another problem may also arise namely the one relating the applicability of the findings of these experiments in clinical practice, i.e. to naturally occurring infectious diseases. The answer is to obtain as much information as possible on the pathological processes in the human body as well as on the effect of challenging experimental animals with pathogenic organisms by this or that route. These problems are extensively discussed in our book. We also wished to submit compara-

tive analysis of the various experimental models widely (though not always justifiably) used in experimental work on enteric infections.

Though enteric infections are distinguished by certain peculiarites, their pathogenesis cannot be understood without taking into consideration the dynamics of development of other infectious processes. This made the discussion of some general problems necessary, particularly in the introductory chapters of the book. Therefore, we believe that it might prove useful not only to researchers specially concerned with enteric diseases but to all those interested in the problems of infectious pathology.

We are pleased to acknowledge the assistance of many colleagues who participated in the research this book has been based on, especially L. S. Bibinova, T. N. Khavkin, Yu. E. Polotsky, V. L. Belyanin, E. M. Dragunskaya and B. M. Ariel of the Department of Pathology of the Institute of Experimental Medicine, Academy of Medical Sciences, USSR, B. Serény, P. Rácz, K. Tenner, J. Oláh and B. Rédey of the Hungarian National Institute of Public Health, E. M. Novgorodskaya, T. A. Avdeeva, M. K. Voino-Yasenetskaya, V. A. Arbuzova, N. R., Vasser and L. A. Smirnova of the Pasteur Research Institute of Epidemiology and Microbiology (Leningrad) as well as E. S. Snigirevskaya of the Institute of Cytology, Academy of Sciences of the USSR. The authors are grateful to the laboratory and technical assistants for their help in carrying out the complex and time-consuming investigations.

The Editors

PART I

CHAPTER 1

GENERAL PRINCIPLES FOR THE STUDY OF THE PATHOGENESIS OF INTESTINAL INFECTIONS

by

M. V. VOINO-YASENETSKY

BIOLOGICAL GROUNDS OF THE INTERRELATION BETWEEN HOST AND MICROORGANISM

Though the aetiology of most infectious diseases has become sufficiently clear, the essence and causes of the whole complex of intricate pathophysiological, morphological and immunological changes occurring in the body during infection are still being studied. In the chain of events underlying some complex phenomenon, the main and the most essential link—the lack of which makes the phenomenon impossible—should be found first. In the case of infectious diseases this very link is the direct interaction between the host and pathogenic organisms, in other words, the infectious process.

Without sufficient knowledge of the infectious process underlying a certain disease, it is impossible to understand its pathogenesis and to estimate correctly the importance of the symptoms.

Infection is nowadays regarded as a kind of parasitism and, accordingly, the general biological laws that govern the evolution and interrelations between all living beings are also valid as regards the development of infectious diseases. The pathogenic properties of organisms are often considered as their ability to invade a susceptible host and cause disease. However, it is not the disease that is essential for the microorganisms (its numerous manifestations reflecting the defensive responses of the host are, in fact, even dangerous for them), but they need a medium suitable for their growth and multiplication. It is equally difficult to assume that a certain host-susceptibility to harmful agents has developed in the course of evolution. More possible is the formation of a resistance or defence against them. Metchnikoff (1905), who was the first to adopt a biological approach to infectious diseases, was accused of attributing to microorganisms the capacity to act rationally in the "struggle" between the host and organisms (Dubos, 1954). The most primitive forms of life frequently demonstrate a striking expedience in their behaviour. Having originated during evolution and not governed by rationality, this expedience, however, is rather relative: with some change in the environment, the properties underlying it may become harmful for the microorganism itself. Moreover, the absence of any rationality is also inherent in defence reactions of a host exposed to microbial invasion. Even in man leukocyte emigration and macrophage activity are independent of the host's will and consciousness. Such spontaneous responses may occasionally prove to be useful not to the host but to the parasite that contrived to get adapted to them.

Considering the host's defence reactions, it is usually assumed that they are directed against the agents of infectious diseases. An encounter with pathogenic organisms, except for epidemic outbreaks, is not so frequent. On the other hand, there are always vast numbers of other microorganisms in our environment which appear to be harmless but are capable of growing in the tissues and fluids of our body. It is from these microbes that the body must mainly be defended. This defence is sufficiently reliable and such agents gain free access to the tissues only after the death of the host.

Under certain conditions many of the physiological functions of the body may acquire a protective character. However, the principal role in preventing infection is played by the barriers separating the internal medium from the environment, as well as by the special mechanisms acting against the microorganisms that have somehow penetrated the tissues or the blood.

External barriers formed by the epithelial tissue of various structures do not only serve as mechanical obstacles. The secretion of certain cells and glands and the activity of cilial epithelium, peristalsis etc. (Anitschkow, 1960) may all contribute to the elimination of microorganisms from mucosal surfaces. Macrophages of lung alveoli, engulfing both dust particles and organisms reaching the lungs also have a barrier function. Still, external barriers cannot be considered perfect, and frequently they may be damaged. Therefore, the second, internal, line of defence (phagocytes and non-specific antibodies) must always be ready for action.

Lysozyme and other humoral factors are known to be most efficient regarding "banal" organisms. Likewise, the cells capable of phagocytosis (leukocytes, elements of the reticulo-endothelial system) are most successful in coping with facultative pathogenic organisms always present on the surface and in the open cavities of the body. But in most organs the number of phagocytic cells is rather low. Therefore, as a response to microbial penetration through the tissues, an inflammatory reaction arises which, according to Metchnikoff, is essentially an aggregation of leukocytes in the affected area. Such a response to the parasite may pass unnoticed for the host and, apparently, occurs much more frequently than assumed.

Consequently, the development of a progressive, pronounced infection is only possible if one of the following two conditions is present: (*i*) impairment of the defensive powers of the body or (*ii*) the presence of special agents capable of overcoming the mechanism of natural immunity.

The impairment of defensive functions creates a ground for non-contagious diseases developing as autoinfections by organisms inhabiting the skin, intestine and respiratory tract. In animal experiments it is possible to reproduce purulent pneumonias, cholangitis, appendicitis etc. by merely damaging various external barriers (Anitschkow, 1960). Similarly, attenuation of phagocyte response caused, e.g. by cortisone or X-rays may result in fatal infection due to the body's "own" (indigenous) microorganisms.

Infections caused by staphylococci, pneumococci and other potentially pathogenic agents are termed banal, though some biological characteristics of the agents penetrating the tissue do influence their development. The specificity of the pathogenesis is more evident in contagious diseases.

In the life cycle of agents causing infectious diseases there are three main stages, namely, (*i*) penetration into the internal medium of another, (usually defined) living organism, (*ii*) presence and multiplication of the parasite in the host, (*iii*) excretion of the parasite to the environment. The first and the last stages, which are extremely important from the epidemiologic standpoint have been studied rather thoroughly (Zhdanov, 1964). The fate and behaviour of pathogenic organisms which settle in the host are less well known.

The properties of bacteria grown on artificial media have been studied in detail, and a vast amount of information has been obtained on the physiology, biochemistry and genetics of various organisms including the agents of infectious diseases. At the same time, it has long been suspected and has by now become a firm belief that pathogenic organisms residing in the body may display some peculiar properties unobservable *in vitro*.

Great attention is devoted to the problem of vital activity of the organisms within the host (Howie and O'Hea, 1955; Felton, 1957; H. Smith and Taylor, 1964). Such studies at the cellular level have been made in tissue cultures and isolated cells obtained from exudates. Nevertheless, in the body parasites may encounter, in addition to tissues suitable for their growth, a number of obstacles created by natural defence mechanisms. Little is known at present of these complex effects in the host, especially as regards certain infectious diseases. Still, the data of the literature and the results of our own studies made in collaboration with microbiologists make it possible to draw some general conclusions mainly as to bacterial and protozoal infections.

The first and rather important conclusion is that the initial response of the body to the penetration of organisms into its tissues runs the same course irrespective of the properties of the parasite. This early non-specific response efficiently protects the body against common (facultatively pathogenic) organisms. The specific response to unknown antigenic stimuli, on the other hand, takes some time to develop corresponding to the time required to bring the specific antibodies into action. The main events determining the development of infectious disease occur during this period. The mechanisms by which certain pathogens resist the bactericidal effect of blood serum and lysozyme are not fully known. Some are known to protect themselves by exotoxin secretion or by the production of substances named aggresins, leukocidins etc. interfering with the emigration of leukocytes and with phagocytosis. But the majority of pathogenic agents are unable to counteract the leukocytic reaction and engulfment by phagocytes.

The fate of phagocytosed bacteria may be different. Saprophytes and slightly virulent bacteria perish rather quickly and are digested by polymorphonuclear leukocytes and macrophages. Some species of pathogenic organisms captured by these cells may not only remain alive but may multiply rapidly, thus destroying the phagocyte itself. The causative agents of tuberculosis, leishmaniasis, scleroma and a number of other human and animal infections have become adapted to a prolonged existence inside macrophage-type cells. They grow and feed at the expense of these cells without causing them any great damage, occasionally they do not even

interfere with their division, and survive in daughter cells. The host cell may become hypertrophic acquiring a particular shape ("epithelioid", "typhoid", "leprotic" cells), and is destroyed only when densely packed with parasites. Microorganisms inhabiting the cells may be devoid of certain digestive enzymes which are not necessary for such an existence, though they usually also preserve their ability of growing freely in the tissues (facultative intracellular parasites). Other kinds of bacteria (e.g. agents of leprosy) and some protozoa (malarial plasmodia, in particular) are obligatory intracellular parasites. The highest degree of parasitism is characteristic of viruses: when infecting the cell, they only transmit the information encoded in the RNA which induces the production of new viral particles.

The above well-known facts show the diversity of the behaviour of pathogenic organisms in the host. In addition, the agents of different infectious diseases exhibit a peculiar tropism to certain tissues and organs,whereupon some organisms (viruses, particularly) parasitize not only phagocytes but also epithelial, muscular and nerve cells as well as erythrocytes.

SIGNIFICANCE OF INTRACELLULAR PARASITISM OF MICROORGANISMS

The settling of various organisms in certain host cells is frequently accounted for by their need for a particular medium and adequate conditions for nutrition. Moulder (1962) has pointed out that all parasites, except the obligatory ones, are able to multiply outside the cells as well indicating that food demand is not the sole determinant of parasitic habit. However, he still discerns other factors only in view of the metabolic peculiarities of the parasite. Under the conditions of intracellular existence this ability to take up large, complex and metabolically active molecules is clearly of great advantage. In fact, without this ability, little is gained by living inside a cell in preference to outside (Moulder, 1962).

Having adapted themselves to the existence in some cells the microorganisms obtain nutrition and a safe shelter against the defence reactions of the body. This is the main advantage of intracellular parasitism, obviously determining the course of evolution of many pathogenic agents. Parasites not susceptible to the digestive enzymes of macrophages or settling in some other cells are far from being always resistant to polymorphonuclears, the most active phagocytes. Polymorphonuclears are not dangerous for the microorganism residing inside a living cell, as they cannot penetrate the cell covered with an intact membrane. However, leukocytes immediately attack the parasites released after the death of the host cell protecting them. This is evident in malaria, rickettsiosis and psittacosis (ornithosis), the biochemistry of which was studied by Moulder.

Burnet's rickettsia, even when overfilling the macrophage, fail to attract leukocytes until the former is destroyed (Fig. 1-1*a*). Polymorphonuclears accumulate and phagocytize parasites only after the destruction of the host cell (Fig. 1-1*b*). Quite similar phenomena were also observed in our

laboratory in experiments with ornithosis (Tolybekov and Krasnik, 1966). To prove the role of polymorphonuclears in malaria was more difficult. Malarial plasmodia inhabiting erythrocytes and circulating with them in the blood stream are supposed to be phagocytized only by reticulo-endothelial cells of the spleen, liver and bone marrow. Studying the pathology of tropical malaria we had the opportunity to examine the placentae of women in labour at different stages of the cyclic course of the infection. In the intervals between the attacks, the intravillar spaces of the placenta were packed with a great number of erythrocytes infected with small, maturing schizonts. Leukocytes were almost absent; the presence of large macrophages overloaded with pigment particles was characteristic (Fig. 1-2*a*, *b*). During labour, at the peak of the attack when matured parasites divided into daughter cells are released from the erythrocytes destroyed by them, the placenta is literally flooded with polymorphonuclears devouring the plasmodia that have not yet penetrated fresh erythrocytes (Fig. 1-2*d*). In other infectious diseases the importance of leukocytes in the defence of the body is not quite realized. Up to the present there are different views regarding the presence of polymorphonuclears in some specific granulomas. Firstly, in histological preparations stained commonly without using the oxidase reaction, granulocytes are not always discernible.* In foci of inflammation, especially in post-mortem material, the nuclei of polymorphonuclears are frequently swollen and spherical, similar to the undefined "round cells" so frequently described in granulomas (Florey, 1962; Voino-Yasenetsky and Zhabotinsky, 1970). Secondly, not all investigators consider the periodicity and focal character of polymorphonuclear emigration connected with the release of the pathogenic agent from the cells infected by them. This is rather obvious in scleroma (Fig. 1-3*a*, *b*).

Organisms existing as parasites in macrophages or some other cells and acquiring at the same time a certain resistance to polymorphonuclears have apparently the greatest chance of survival. As it will be shown later, such a resistance is indeed possessed by the most virulent strains of bacteria. Far less frequently, organisms acquire the ability to parasitize polymorphonuclears themselves. This is supposed to be characteristic of gonococci and meningococci.

It is highly probable that the properties of parasites settled in any cell may change to some extent. There is a noticable difference in morphology between salmonellae growing freely and those living inside macrophages (see Chapter 15). The peculiar life cycle of some pathogenic protozoa inside the epithelial cells of animal carriers is known. In the course of infection the behaviour of the cells towards parasites may change. Phagocytes of immunized animals successfully digest the organisms against which they were quite helpless before (Metchnikoff, 1905; Pokrovskaya and Kaganova,

* For the elective demonstration of granulocytes we prefer Sudan-α-naphthol staining suggested by Goldmann (1929,*a b*, 1933) for lipids bound to proteins. As regards leukocytes, this method provides the same results as the oxidase reaction. Neutrophilic and eosinophilic granules are stained brown while lipid inclusions are stained yellow or orange. Safranine may be substituted for Sudan III (Margolin, 1948).

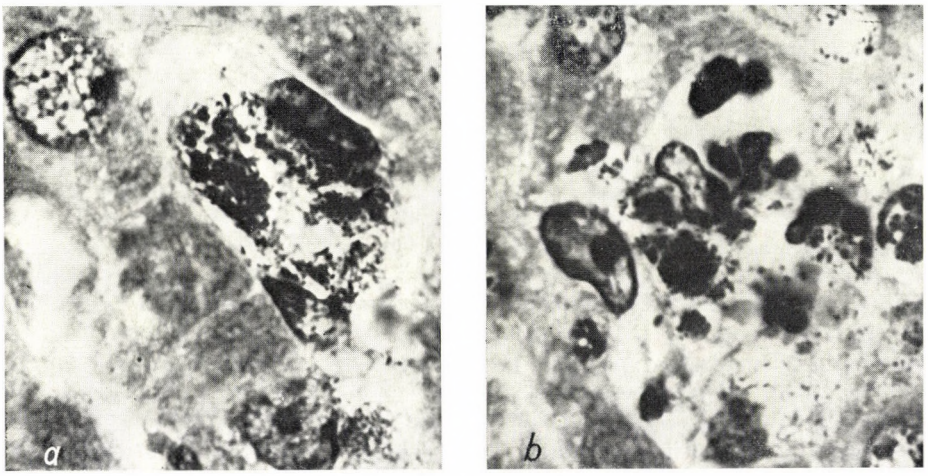


Fig. 1-1. *Coxiella burneti* in the guinea pig liver. *a* Abundant growth in Kupffer cell. *b* Phagocytosis of rickettsia by leukocytes after cell destruction. Leishmann stain, ×1750 (courtesy of T. H. Khavkin)

1947; Suter and Ramseier, 1964). This is not connected with the effect of humoral factors of defence: the infected cells are not penetrated by specific antibodies (Rous and Jones, 1916); in addition, such stimulation is observed even in the absence of antibodies (Mackaness and Blanden, 1967).

Neither antibodies nor bacteriophages (Cefalu et al., 1963; Manolov and Kosturkov, 1965; Bakhutashvili, 1968, 1969) are dangerous for the organism growing in the cells. Some drugs including certain antibiotics are also ineffective against such organisms.

The effect of antibiotics on intracellular parasites has been discussed extensively in the literature (Suter, 1954; Smadel, 1963; Planelyes and Kharitonova, 1965; Smirnov, 1966; V. N. Solovyev, 1968; Thorpe and Marcus, 1967; Bonventre et al., 1967). Most authors note that antibiotics have only a slight effect, or none at all, on the organisms present in the cells. It should be noted that the results of investigations carried out mainly in tissue cultures are controversial. Apparently, some antibiotics can pass the cellular membrane (e.g. rifampicin) (Osada et al., 1972*a*, *b*; Lobo and Mandell, 1973). Yet, radical cure of infections caused by intracellular parasites is still a difficult problem.

While intracellular parasitism is undoubtedly advantageous for the microorganism, the advantage of growth of some of them on the surface of epithelial cells is less clear. Such a localization of pathogenic agents was revealed by Lindner (1921) in many infectious diseases of the conjunctiva. Some of his descriptions and figures are quite convincing, indeed, while others only suggest the adsorption of organisms to dying epithelial cells, as pointed out by Hoffman and Frank (1966). These authors found various organisms on the surface of (and in their opinion, even inside) the epithelium in

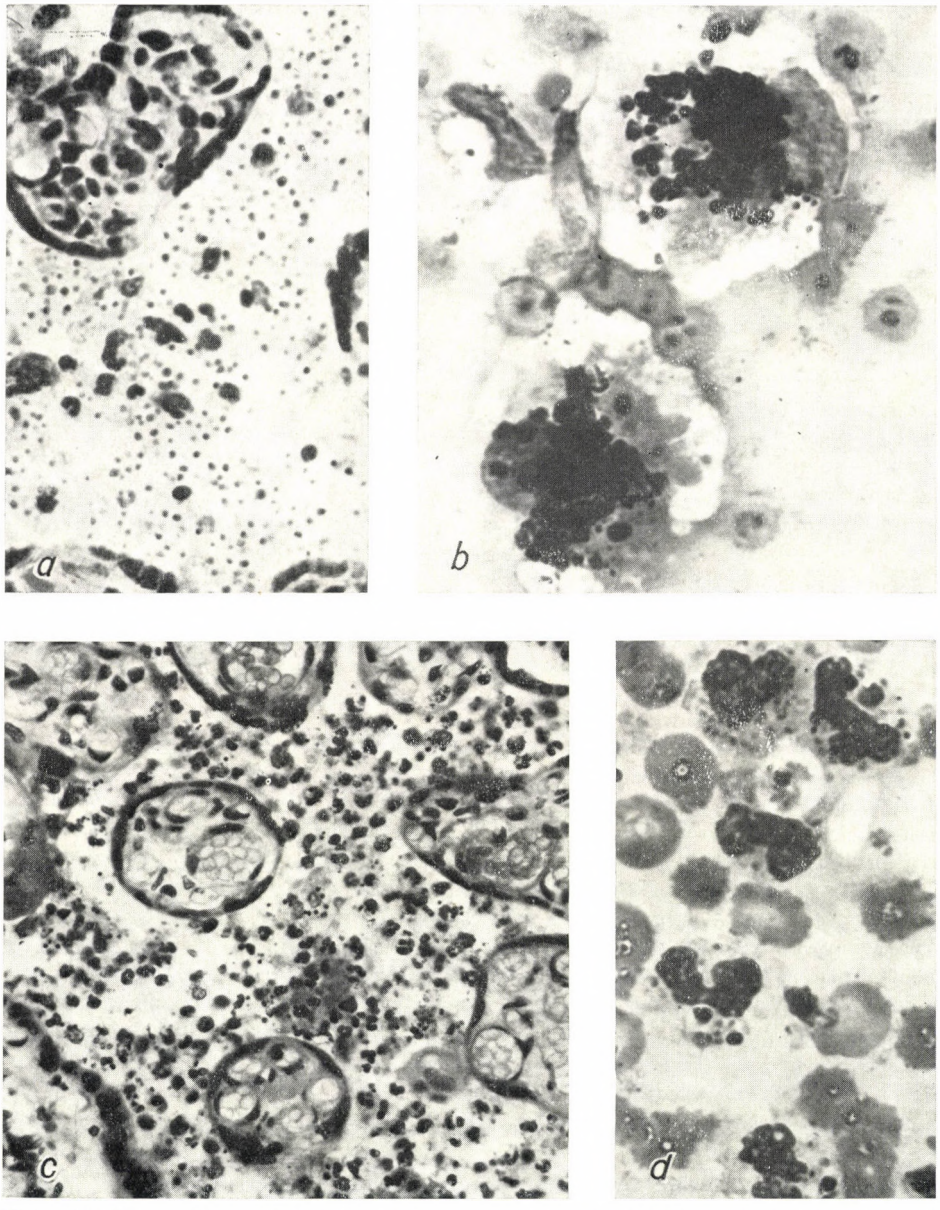


Fig. 1-2. *Plasmodium falciparum* and phagocytes in intervillar spaces of placenta. *a* and *b* Maturation of parasites present inside the erythrocytes (in *b* erythrocytes are not stained). *c* Abundant accumulation of polymorphonuclears at the division stage of parasites. *d* Polymorphonuclears phagocytizing dividing forms of the parasite and young parasites released after the destruction of erythrocytes. Placental blood smears, Giemsa stain, ×900 (*a* and *b*); histological preparations, thionine (*c*) and haematoxylin-eosin (*d*), ×380 (Voino-Yasenetsky, 1950)

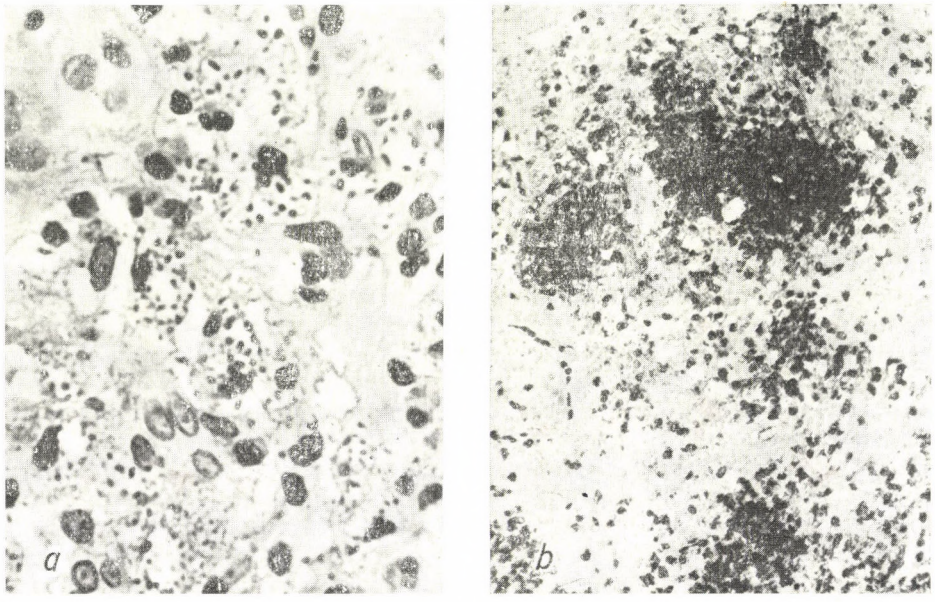


Fig. 1-3. *a* Lack of reaction to scleroma agents growing inside Mikulicz's cells. Haematoxylin-eosin, ×800. *b* Focal aggregates of granulocytes near disintegrated infected cells. Goldmann's Sudan-α-naphthol, ×120 (Voino-Yasenetsky and Yampolskaya, 1956)

scrapings from the oral cavity and the vagina of healthy humans, rabbits, and hamsters. It would be unreasonable to deny the possibility of microbial growth on the epithelium, especially in view of the fact that the same localization of pathogens was observed in the respiratory tract in whooping-cough (Brown and Brenn, 1931; Voino-Yasenetsky and Khay, 1957; Tarasova, 1958; Robbins, 1967), in the urethra in gonorrhoea (Ward and Watt, 1972) and also in the intestine in some of its diseases as will be described below. In the opinion of Ward and Watt, the adherence of gonococci to the surface of the urethral epithelium of man prevents the washing off of parasites by urine at the very onset of gonorrhoea (later they penetrate deep into the mucosa). Such an adaptation is likely to be useful also for the organisms inducing diarrhoea.

INFECTIOUS PROCESS AND INFECTIOUS DISEASE

The concept of the infectious process is more restricted than that of the disease, and it does not comprise all the typical (though not necessarily obligatory) manifestations. On the other hand, it refers to all forms of infections including latent ones, not manifested as a disease. It is rather rare that the clinical-anatomical complex characteristic of an infectious disease may be completely attributed to the vital activity of a parasite. Thus, the grave condition in tetanus is due to the action of the toxin secreted

by *Clostridium tetani*, and the injurious effect of toxins play a part in the pathogenesis of a number of other diseases. A great number of manifestations of the disease reflect the active and—in most cases—useful response of the host.

The defensive role of cough in clearing the respiratory organs or the role of increased peristalsis of the intestine relieving it of harmful contents does not need any justification. Veselkin (1963) proved the usefulness of pyrexia, a symptom typical of many infectious diseases. Fever, although evoking fatigue, contributes to immunological and some physicochemical processes which help to overcome the infection.

All such reactions are expedient only to a certain extent. Infrequently, they may become pathologic and may persist after the cure of the infectious process (cough after whooping-cough, intestinal disorders after dysentery). Pathogenic microorganisms are undoubtedly capable of using and even evoking some host reactions beneficial to them. Cough and diarrhoea may contribute to the elimination of infection, but at the same time, the spread of the causative agent may initiate new infections. The aggressivity of animals suffering from rabies ensures the chain of the transmission of the virus. Thus, the above statement that microbial parasites do not need the illness of the host, should not be stipulated.

In the present book it is not possible to discuss the general problems of infectious pathology in detail. Toxic substances and allergic phenomena as well as other, still incompletely known factors play a part in the development of infectious diseases. Nevertheless, infection should not be looked upon as intoxication, and the misuse of the concept of allergy must likewise be avoided. The pathogenesis of infectious diseases cannot be studied without the thorough analysis of a complex of causes and sequences, lesions and reactions. Thus, e.g. the phagocytes themselves, which digest the captured organisms also perish rather frequently. Formerly, endotoxins released by the destroyed organisms were believed to kill the phagocytes; recently, disintegration by their own lysosomes and enzymes is supposed to play the main role. The antigen-antibody complex resulting from a seemingly useful immunological response may prove to be toxic. These considerations are complicated by the fact that the results of entirely different pathologic processes may be similar.

The problems of the pathogenesis of intestinal diseases have not yet been solved. The points we succeeded in elucidating will be discussed in the following chapters. It appears that research on intestinal barrier systems and on the location of bacteria will contribute to the solution of these problems.

EPITHELIAL BARRIER AND COMMON MICROBIAL INTESTINAL FLORA

The epithelial layers of the skin, respiratory tract and urinary tract have a mechanical defensive function. In the gastrointestinal tract the barrier role of the mucosa is combined with complex processes of digestion and absorption of nutrients and also, as it has recently become known, with

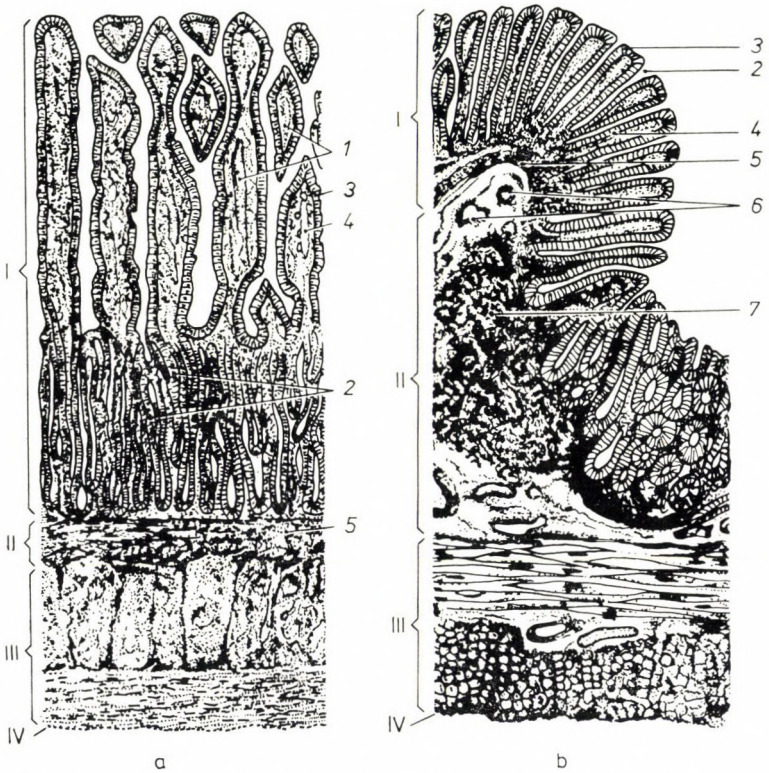


Fig. 1-4. Structure of the wall of small (*a*) and large (*b*) intestine. *I*, mucous membrane; *II*, submucosa; *III*, muscularis externa; *IV*, serosa. *1*, Villi; *2*, crypts; *3*, epithelium; *4*, lamina propria; *5*, muscularis mucosae; *6*, blood vessels, *7*, lymph follicle (from Eliseev et al., 1972)

the production of secretory immunoglobulins and bacteriolytic enzymes (Kraft and Kirsner, 1971; Erlandsen et al., 1974; Poger and Lamm, 1974; Rowley, 1974; Peeters and Vantrappen, 1975). In various parts of the tract, the mucosal structure has similar features (Fig. 1-4).

The monolayer epithelium of the intestinal mucosa consists of prismatic cells (also termed absorptive or main cells) and goblet cells producing mucus; the latter are most abundant in the colon. Paneth's cells and enterochromaffin cells with specific granules in the cytoplasm are situated deep in the ileal crypts. The epithelial lining is separated from the so-called tunica or lamina propria by a thin basal membrane. The lamina propria is composed of loose connective tissue resembling lymphoid tissue. Its base is formed of reticular fibres containing plasma cells and a number of cells resembling reticulo-endothelial cells with lymphocytes among them. Granulocytes are less frequent.* In the terminal ileum the lamina propria contains typical

* Patzelt (1936) described the cellular composition of the lamina propria of various animals in great detail. Results of electron-microscopic studies in mice have been reported by Deane (1964).

lymphoid aggregates, the Peyer's patches. The border of the lamina propria is formed by a thin muscular layer, called muscularis mucosae, separating the lamina propria from the tunica submucosa. Next to this, the muscular layer is found.

Some important details of the structure of the intestinal wall have recently been revealed by modern histological and cytological techniques (Shestopalova et al., 1960; Trier and Rubin, 1965; Rifaat et al., 1965; Aruin, 1967; Trier, 1967; Crane, 1968; Toner, 1968; Ito, 1969; Lojda et al., 1970; Rubin, 1971; Kaye et al., 1973; Owen and Jones, 1974).

The cytoplasm of intestinal epithelium has the same ultrastructural components as other cells. There are many mitochondria and a moderate amount of free ribosomes; the endoplasmic reticulum and the Golgi apparatus are pronounced. In the apical portion lysosome derivatives, shapeless, electron-dense inclusions, bordered by a membrane, can be observed. Near the apical surface the cell organelles are absent, but the cytoplasm is denser here and has a fine fibrillar structure (terminal web).

Much attention has been devoted to the outer epithelial surface directed to the intestinal lumen. Previously, it was assumed to be covered with a cuticulum resembling a brush with its hairs glued. Electron-microscopic observations showed the "brush" to be formed of narrow (0.08–0.1 μ in width), finger-like prominences of cytoplasm and to be covered, like the whole cell, with a three-layer plasma membrane (see Fig. 22-11, p. 316). Reaching 0.5–1.5 μ in height, they are densely packed; their number on each cell, according to different authors, amounts to 1000–3000. These structures, the microvilli, increase the absorptive surface of the gut manifold.

There is a so-called fuzzy (extraneous) coat, up to 1.5 μ in thickness, overlying the microvilli. It consists of a felt-like network of extremely fine fibrils originating from the plasma membrane of microvilli. This coat probably acquires such an appearance after fixation, and has *in vivo* a homogeneous gel-like structure (Ito, 1969). Ito believes it to be a particularly developed glycocalyx, obviously present in all cells. At any rate, all investigators consider mucopolysaccharides of the fuzzy coat not to be related to the mucus produced by goblet cells.

On lateral surfaces as well as on microvillar apexes occasional small rounded bodies covered with a membrane are found (Shnitka, 1964; Rifaat et al., 1965; Donellan, 1965; Takeuchi et al., 1968; R. C. Brown et al. 1969). Their nature is not clear. Rifaat et al., Donellan and Brown et al. believe them to be merely cross-sections of protrusions originating from microvilli. Rifaat et al. consider them possible fixation artifacts. It is noteworthy that the parasitic nature of such extrusions seems to be ruled out. As pointed out by Shnitka, the absence of a central nucleoid distinguishes them from viruses.

The brush border is PAS positive, its microvilli are intensely stained with thionine. It is not always possible to reveal them in this way using common formalin fixation (especially in autopsied material).

The interest devoted to the fine structure and biochemical peculiarities of the outer surface of the intestinal epithelium is fully justifiable. This is the site where not only absorption but also partial digestion of nutrients —

called "membrane or contact digestion" by Ugolev (1965, 1967)—occurs. Furthermore, Ito (1964), Trier (1967), Komissarchik and Ugolev (1970) consider the mucopolysaccharide coating as a barrier defending the epithelial cells.

The mucus produced by goblet cells plays an important defensive role in the colon having rough contents extremely rich in microorganisms. The structure and function of these cells were described in detail by Florey (1960, 1962).

Considering the barrier role of the intestinal epithelium, the connection of its single cells is of interest. This connection is most stable between the apical portions of the cells where the so-called junctional complex is seen. One of its components, the zonula occludens, is situated just below the microvilli and is characterized by a dense confluence of the plasma membranes of adjacent cells. In the intermediate zone that follows, the contiguous membranes are not fused but connected by means of an unknown substance. Peculiar structures (desmosomes), located deeper, link certain areas of lateral surfaces of adjacent cells, without surrounding them completely. In the lower (basal) parts of the epithelium the connection is far less stable; between the cells there are spaces the width of which depends upon the functional state of the intestine. These spaces have an irregular shape as the lateral surface of the cells is quite tortuous. The epithelial cells seem to have no definite links with the basal membrane; presumably they merely adjoin it. Thus, the epithelial layer is, obviously, able to alter its position, which is necessary in view of the fact that cell division occurs deep in the crypts. Gradually maturing, the young epithelium is pushed out of the crypts until the old cells are shed at the villar tips. This process takes a rather short time: 3 days in experimental animals and 3–8 days in man (Lipkin, 1965; Timashkevich, 1966; Lipkin and Bell, 1968). In case of a physiological detachment of the cells no defects (not even transient ones) appear in the intestinal epithelial lining. At the same time, the absence of a stable connection between its cells and the basal membrane accounts for the fact that after human or animal death the epithelium may fall off the mucosal surface very rapidly and sometimes almost completely.

As has already been mentioned, the lamina propria underlying the epithelium exhibits a variable structure. The variability of its cellular elements has been confirmed by biopsy in healthy subjects (Rubin and Dobbins, 1965). The lamina propria is less developed in the colon than in the ileum; its macrophages lying nearer to the outer surface contain a certain number of granular inclusions (Donellan, 1965). The lamina propria may be the site of infectious processes as will be seen below. In addition, because of its resemblance in structure to lymphoreticular tissue, the lamina propria may participate in immune processes (Kraft and Kirsner, 1971).

Maturation and differentation of the lamina propria (like all lymphoid and reticulo-endothelial tissues) are distinctly connected with antigenic stimuli. In germ-free animals reared under sterile conditions the intestinal mucosa, and especially its lamina propria, are underdeveloped; after colonization of the gastrointestinal tract by some organisms, however,

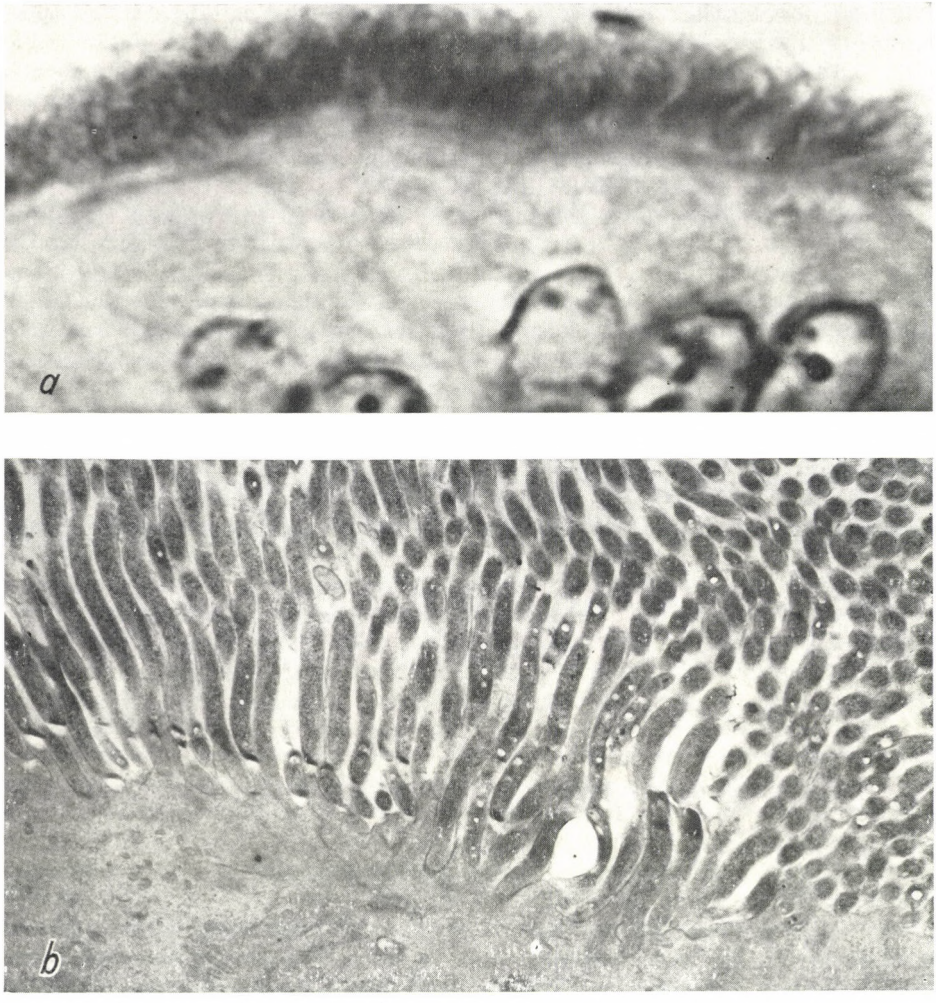


Fig. 1-5. Numerous spirochetes on the surface of epithelial cells of the monkey's large intestine. *a* Leishman stain, ×2600. *b* Prepared with osmium tetraoxide fixation and embedded in Epon, ×23 000. (Voino-Yasenetsky et al., 1975)

the usual appearance of the mucosa might soon be observed (H: Gordon, 1960; Sprinz, 1962; Abrams et al., 1963; Donaldson, 1968; Bauer, 1968; Khlystova and Zaitsev, 1971; Miniats and Valli, 1973; Wostmann et al., 1973). This state of the mucosa in healthy humans and animals has been called "physiological inflammation" by Sprinz (1962). Similarly, Dubos et al. (1967) consider the normal microbial intestinal flora to correspond to agents of chronic infectious processes, but having a mild course. Instead of infection, however, it would probably be more correct to speak of a

constant stimulation by enteric organisms of the cells having defensive functions (Miyakawa et al., 1965).

It has been pointed out by Metchnikoff that some of the constant inhabitans of the bowel produce toxic substances and are harmful to the host. However, in general, the intestinal flora is useful and even necessary (Perets, 1955, 1962; Donaldson, 1968; Hentges, 1970; Gorbach and Levitan, 1970; Gorbach, 1971; Lebedeva et al., 1974).

As stated by Hill and Drasar (1975), interest in the human intestinal bacterial flora has greatly increased in recent years. This is, to a large extent, due to new techniques which permit the study of the dominant members of the intestinal flora, the non-sporing strictly anaerobic bacteria. These authors also mention a number of unsolved questions in connection with the functional significance and even the composition of the human intestinal flora. This study was based chiefly on the examination of faeces, i.e. the contents of the distal colonic lumen, whereas the mucosal flora (bacteria growing in close association with the mucosal surface) may differ markedly from this.

Almost all investigators dealing with this subject share the view that common intestinal bacteria interfere, to a certain extent, with the invasion of the intestine by pathogenic organisms. Microorganisms capable of growing in the mucus secreted by goblet cells on the surface of the epithelial lining are considered by Dubos and Savage to play a specific role in host defence (Dubos et al., 1965; Savage et al., 1968, 1971; Savage, 1969, 1970, 1972; Gordon and Dubos, 1971). In mice there are fusiform rods and spiral-like microorganisms appearing in the caecum and in the colon in the 2nd–3rd week of life, and multiplying abundantly in mucoid films. They do not penetrate deep into the epithelium or even into the brush border, as if they formed a second epithelial defensive barrier. Fusiform organisms residing in the mucus were also demonstrated in rats together with some Gram-negative rods and cocci resting on ileal villi (Savage, 1970). Plaut et al. (1967) and Nelson and Mata (1970) reported on similar findings in man.

It is difficult to decide whether or not the microorganisms growing in mucoid layers on the intestinal surface are really useful for the host. However, there is some evidence that these (or similar) organisms are capable of coming into direct contact with the epithelium. Hampton and Rosario (1965) found some rather long, rod-shaped bacteria (*Streptobacillus moniliformis* in their opinion) protruding at various angles over distal portions of the ileal mucosa. Electron microscopy showed these organisms to thrust into the brush border slightly indenting the plasma membrane. The epithelial cell displays certain moderate changes only at the site of bacterial attachment.* Savage and Blumershine (1974), Davis and Savage (1974), Erlandsen and Chase (1974), reporting on similar findings in mice and rats,

* Describing identical findings in the rat intestine, Reimann (1965) states that bacteria are engulfed by epithelial cells. However, he illustrates this with an obviously wrong picture of intracellular localization of an organism which has been obtained by oblique section through the epithelium.

reject the idea that these microorganisms could belong to *Streptobacilli* and call them simply "segmented filamentous microbes".

Quite frequent embedding of spirochetes has been described in the brush border of the colonic and rectal epithelium in man and monkeys (Harland and Lee, 1967; Gear and Dobbins, 1968; Takeuchi and Sprinz, 1969, 1970; Lee et al., 1971; Takeuchi et al., 1971, 1974; Takeuchi and Zeller, 1972; Voino-Yasenetsky et al., 1975). Over large portions of the gut this border is occasionally wholly substituted by densely arranged spirochetes (Fig. 1-5). It is surprising that there are no alterations in the epithelial cells at sites where spirochetes are attached; also the leukocyte response is absent and no intestinal disorders occur.

LOCALIZATION OF ENTERIC PATHOGENS IN MAN

Bacteriological studies of patients' stools prove that the agents of enteric infections are present in the contents of the digestive tract. Therefore, a purely toxic effect was for a long time attributed to these organisms when trying to explain the pathogenesis of such diseases. This is, hovewer, a simplification not suitable for accounting for the essence of all enteric infections.

The intestinal epithelial barrier does not only prevent the passing of organisms abundant in the digestive tract (especially in its distal portions), but also that of several noxious substances. When undamaged, it is almost impermeable to endotoxins of Gram-negative organisms (see Chapter 4), like the agents of typhoid fever, dysentery and *E. coli*-enteritis. Hence, for the development of infectious processes in the intestine a disturbance of this defence, or the presence of parasites capable of overcoming it, is necessary.

Intestinal diseases caused by protozoa are a good example. *Entamoeba histolytica* is quite harmless while it is found in the intestinal lumen, but when, for some still unknown reason, it penetrates the intestinal wall, amoebic dysentery will develop. These parasites invade the colonic submucosa and attack the tissues by help of their enzymes, while leukocytic response is almost absent. The pathogenesis of colitis produced by *Balantidium coli* is almost identical (Voino-Yasenetsky, 1964*a*).

The pathogenic properties of *Giardia lamblia* are not quite clear. Shakhnazarova (1962), Zamsheck et al. (1963), Rubin and Dobbins (1965), Takano and Yardley (1965), Hoskins et al. (1967), M. M. Solovyev (1968), Barbieri et al. (1970), Brooks et al. (1970), Erlandsen and Chase (1974) observed that these organisms inhabited not only the intestinal lumen of humans but were also attached to the mucosal surface. Infrequently they even penetrated the epithelial cells (Morecki and Parker, 1967; Brandborg et al., 1967; Brandborg, 1971; Mueller et al., 1973). They are pathogenic because of damaging the absorbing epithelial surface of the small intestine which may lead to impairment of host feeding (Alp and Hislop, 1969; Ament and Rubin, 1972; Erlandsen and Chase, 1974). Mueller et al. (1973) believe that, by

attaching themselves to the mucosa, *Giardia* seem to ingest superficial enterocyte structures (the fuzzy coat, microvilli).

Some coccidiae, extremely widespread among animals, are intracellular parasites. Almost all of their life cycle takes place in the intestinal epithelial cells. The particulars of this cycle were described in detail by Kheisin (1967). It should be pointed out that the cell invaded by a large parasite remains viable while it grows and multiplies (Scholtysek, 1968). The same occurs in human coccidiosis, a rare, but occasionally lethal disease (Brandborg et al., 1970).

Epithelial cells of the insect digestive tract provide a site for the development of causative agents of certain human diseases, such as malaria and rickettsiosis. The host's vital functions do not appear to be impaired: the mosquito keeps feeding on human blood and infects its victim with plasmodiae (sporozoites) that invade its salivary glands. Still more striking is the harmless and obviously mutually beneficial symbiosis of the host and certain bacteria settling in the intestinal epithelial cells of insects (Steinhaus, 1947, 1949; A. Koch, 1960).

The capability of enteric bacteria for intracellular parasitism was first established in typhoid. Granulomatous alterations in lymphatic formations of the intestine and mesentery in this disease had long been known. Using modified Romanovsky-Giemsa staining, Goodpasture (1937) and Adams (1939) were the first to detect very small bacteria in the cellular cytoplasm of such granulomas. The cells harbouring the organisms—characterized by moderate basophilia—were named plasma cells. Planelyes and Forshter (1946, 1947) suggested that these cells were, in fact, not plasma cells, but young reticular cells or histiocytes. Still they were not right in stating that the organisms invaded lymphocytes during experimental typhoid infection in mice. Recent data presented in Chapters 13–18 permit to assume that salmonellae parasitize macrophage-type cells.

The ability of shigellae to exhibit intracellular parasitism was a more unexpected finding though, almost since the very moment of their discovery, these organisms were suspected not only to inhabit the intestinal lumen but also to invade its walls (see Chapter 4). They were assumed to grow extracellularly, forming aggregates in areas of erosion and ulcerative defects of the affected mucosa. Studying the morphology of experimental keratoconjunctivitis produced by shigellae, Piéchaud et al. (1958) observed these bacteria rather frequently in the cytoplasm of corneal epithelial cells. They put forward the idea that the epithelial cells might serve as "a last shelter for parasites", saving them from phagocytosis by leukocytes. Later, after studying experimental shigella cystitis Szturm-Rubinsten and Piéchaud (1963) assumed that the epithelial cells themselves had defensive functions by phagocytizing microorganisms.

LaBrec and Formal (1961) came to a different conclusion. Studying the intestine of guinea pigs infected with shigellae after a special pretreatment, they concluded that dysentery organisms only pass through the epithelium and settle deeper in the loose tissue of the lamina propria underlying the epithelium. Formal, LaBrec and their co-workers made a series of investigations into the localization of shigellae; the results were reported

in their papers published in the years 1963–67. Meanwhile, data had accumulated proving the capacity of shigellae to inhabit the epithelial cells and to grow in them e.g. in the cornea (Rácz and Serény, 1962; Levenbuk and Andreeva, 1962; Rácz, 1963), in the urinary bladder (Levenbuk and Andreeva, 1962, 1965*a*) and in the respiratory tract (Voino-Yasenetsky and Voino-Yasenetskaya, 1962*a*, *b*) of experimental animals. Certainly, these results did not allow the conclusion that intraepithelial parasitizing by shigellae occurs in the intestine when dysentery runs its normal course. Evidence of this was, however, obtained in spontaneous and artificially induced dysentery in monkeys (Voino-Yasenetsky, 1963; Voino-Yasenetsky and Khavkin, 1964; Ogawa et al., 1966*a*). Then also American investigators (Takeuchi et al., 1968) agreed with our conclusions on shigellae being localized precisely in the intestinal epithelial cells. Information on the localization of shigellae in the host and on the role of these organisms in the pathogenesis of dysentery infection is discussed in greater detail in Chapters 4–12.

The properties of pathogenic *E. coli* described in Chapters 19–22 proved more diverse. Some peculiarities of growth *in vitro* as well as certain antigenic features of these organisms resemble those of salmonellae and shigellae, suggesting some genetic connections between them. The behaviour of *E. coli in vivo* confirms this point. Experiments with animals revealed that like salmonellae, all *Escherichia* (pathogenic or non-pathogenic) are able to multiply in macrophage-type cells (Polotsky and Arbuzova, 1967; Polotsky et al., 1968). However, such intracellular parasitizing of *E. coli* is of rather short duration and is usually restricted to one cycle of bacterial multiplication. When the cell overfilled with the organisms perishes, they are released and readily destroyed by polymorphonuclears. Varieties of *E. coli* causing dysentery-like diseases in man inhabit, similarly to shigellae, the epithelial cells (Polotsky and Arbuzova, 1967; Ogawa et al., 1968*b*; Polotsky et al., 1968, 1971; Formal et al., 1971*a*). *E. coli* organisms associated with infantile enteritis grow on the surface of the intestinal epithelial lining (Ilgner, 1956). Finally, in recent years some particular strains of *E. coli* causing cholera-like diseases in humans and producing enterotoxins have been found (Formal et al., 1971*a*; Sack et al., 1971; Avdeeva et al., 1973*a*). These organisms grow extracellularly (see Chapter 22).

The localization of the microorganisms causing cholera is not fully understood. They multiply abundantly in the liquid filling the intestinal lumen during the course of the disease and seem to possess the ability to come into direct contact with the mucosa.

Koch (1883) detected great amounts of vibrios in the intestinal contents of patients who had died of cholera. Histological studies demonstrated that at autopsies made soon after death or some hours later small "rod-shaped organisms" did not only densely colonize the epithelial surface of ileal villi, but they also penetrated it and even invaded deep layers of the intestinal wall. Koch, however, warned that his findings might be taken for post-mortem growth of putrefactive bacteria observed in "not quite fresh cadavers". This compels us to be cautious in dealing with similar reports by Babeș (1885), Tizzoni and Cattani (1888) and other authors, who had

not paid attention to the period elapsing between the death of the patients and autopsy.* Stoerk (1916), working with a large material, absolutely rejected the possibility of a prevital penetration of tissues by vibrios. At early autopsies vibrios were found only in the intestinal contents and partly on the epithelial surface "as if stuck". This is in agreement with the observations of Dutt (1967) as well as with the results of experimentally induced infection of animals and experiments on isolated intestinal segments (Goodpasture, 1933; LaBrec et al., 1965; Cruickshank et al., 1966; Patnaik and Ghosh, 1966; Dutt, 1967; Freter, 1969; Elliot et al., 1970). Penetration of vibrios into the epithelial cells or deeper mucosal layers has not been observed in these experiments. However, Avtsyn et al. (1971) have recently described that *V. cholerae* administered into the ileal lumen of 10–12-day old suckling rabbits resided not only on the mucosal surface but also penetrated the cytoplasm of single epithelial cells.

With the use of electron microscopy there appeared a considerable interest in "Whipple's disease", in spite of its rare occurrence. This disease is characterized by the accumulation of large macrophages containing lipids and PAS-positive granular inclusions in the intestinal mucosa and submucosa (as well as in mesenteric lymph nodes and in some organs). Whipple himself (1907) who was the first to describe such lesions, found some small organisms detectable with Levaditi's silver impregnation method in mesenteric lymph nodes. Yet, for a long time this disease was considered to be the sequel of metabolic disturbances. At present, its infectious nature is almost certain. Most investigators recognize the small "bacteria-like bodies" found in the tissues at intestinal biopsy as genuine organisms. The forms of their division have been described and antibiotic therapy has proved successful.** These organisms lodge in the lamina propria. They are predominantly arranged between the cells but many are captured by macrophages (histiocytes). In these cells the organisms are commonly enclosed in phagolysosomes and undergo destructions.

Trier et al. (1965) and Dobbins and Ruffin (1967) established that the agents of Whipple's disease were also phagocytized by polymorphonuclears appearing in the affected tissues. Other authors, studying a few cases, failed to note the active role of polymorphonuclears. Dobbins and Ruffin revealed characteristic small organisms in the epithelium of the small intestine as well. They pointed out that in the cytoplasm of basal parts of the epithelial cells only single, well preserved organisms were seen whereas in the apical parts, their degenerative forms were embedded in large phagolysosomes in the form of aggregates. According to these authors, the pathogenic agents of Whipple's disease penetrate the epithelium from beneath, from the lamina propria, but do not survive there. Similar findings were described by Watson and Haubrich (1969), Roberts et al. (1970), and Pages (1971).

* Post-mortem changes that had not been taken into account led to the erroneous concept about the almost complete shedding of epithelial lining of ileal mucosa in cholera (for details see Voino-Yasenetsky and Zhabotinsky, 1970).

** For relevant literature see the reviews of David (1967), Otto and Begemann (1970), Greenberger et al. (1971), Aruin et al. (1971).

Watson and Haubrich, however, were very cautious in discussing the ways of the agent's penetration through the epithelial cells. They showed great amounts of such bacteria to be present between the epithelial cells, the lateral surfaces of which were exceedingly convoluted in their lower portions. Hence, the sections may have given a false impression of intra-epithelial localization of the organisms. Yet, the possibility of finding bacterial clumps in the upper (apical) parts of epithelial cells is doubtless. There, they are surrounded by a membrane and appear to have been destroyed. It follows that the epithelial cells are able to restrict as well as to digest the parasites that have entered them.

In addition to pathological processes developing mainly in the intestine, the mucosa of the latter may serve as the portal of entry for agents of other infectious diseases. Such diseases are, for example, brucellosis, listeriosis and pseudotuberculosis, which we do not discuss here, dealing with "enteric" pathogens only.

CHAPTER 2

BIOLOGICAL FEATURES OF ENTERIC BACTERIA CAPABLE OF INTRACELLULAR PARASITISM

by

B. SERÉNY

The family Enterobacteriaceae consists of peritrichous or non-motile Gram-negative rod-shaped bacteria. They ferment glucose rapidly with or without gas, reduce nitrates to nitrites and grow on simple culture media (Report of the Enterobacteriaceae Subcommittee, 1958). The family, by means of biochemical methods, is divided into groups and subgroups, which are subdivided into serogroups and serotypes. The serotypes can be classified into biotypes and into phage-types (Kauffmann, 1956*a*).

The family Enterobacteriaceae is made up of a number of related organisms, many of them having common antigenic factors. The arbitrarily established groups are not sharply delineated within the family; they form dense centres consisting of serologically and biochemically more or less homogeneous bacteria, many of which are serologically related (Ewing, 1953; Report, 1958).

The modern classification system of the Enterobacteriaceae has passed the test of practice. By means of the available biochemical and serological procedures the overwhelming majority of the isolated strains of enterobacteria may be relatively easily identified. Certain strains, however, present difficulties even with the most careful examination. A few of the numerous publications on such intermediate forms are as follows. The strains of Piéchaud et al. (1965) corresponded to Shigella, but developed strong saccharolytical activity, and were, therefore, classified into a new group, "Parashigella". Trifonova (1965) concluded that strains identified as *E. coli* O25, O28, O102 belonged to shigellae (*Sh. sofia*, *Sh. scholtensii*, and *Sh. niemi*, respectively). Kerekes (1965) isolated an intermediate *Shigella-E. coli* strain which corresponded to the sorbitol positive variant *Sh. flexneri* 2a, but fermented rhamnose and salicin. With this he induced experimental shigella keratoconjunctivitis in guinea pigs.

The criteria of our present system of classification have not included the pathogenicity of Enterobacteriaceae. It has been found that only certain groups of enteric bacteria are able to penetrate the cells of the intestinal mucosa (i.e. of intracellular parasitism), and there is a close connection between intracellular parasitism and the pathogenicity of these microorganisms. Only those strains are able to induce intestinal infections which enter the cells of the intestinal mucosa and grow there.

CLASSIFICATION OF INTRACELLULAR PARASITIC BACTERIA

Enterobacteriaceae may be classified into two main groups, namely intracellular parasites, and bacteria not growing inside cells. Part of the intracellular parasites multiply almost without exception in the epithelial cells of the mucosa (intraepithelial parasites), while other intracellular parasites attack macrophage-type cells.

INTRAEPITHELIAL PARASITES

All bacteria belonging to the *Shigella* group (except phase II of *Sh. sonnei* and a few others) are virulent when freshly isolated. In human dysentery (Bakács et al., 1970) and in monkey dysentery (Voino-Yasenetsky, 1963; Formal et al., 1971*a*) they penetrate the epithelial cells of the intestinal mucosa, where they grow and destroy the epithelial cells producing local inflammation. They penetrate the epithelial cells of the conjunctiva and of the cornea in the guinea pig's eye (Piéchaud et al., 1958; Rácz et al., 1960; Levenbuk and Andreeva, 1962). Experimental nasal infection of albino mice (Voino-Yasenetskaya's model) affects the epithelial cells of the bronchi (Voino-Yasenetsky and Voino-Yasenetskaya, 1962*a*, *b*). Experimental oral infection of starved guinea pigs (Formal's model) results in penetration of the organisms into the epithelial cells of the intestine (Formal et al., 1965*d*; Bibinova et al., 1968*a*, *b*). After experimental vesical infection of guinea pigs (Bingel's model) the bacteria enter the epithelial cells of the bladder (Szturm-Rubinsten and Piéchaud, 1963; Tenner et al., 1971*b*); when given into the isolated intestinal loop of the rabbit (De's model) they penetrate the epithelial cells and multiply there (see Chapter 22). In guinea pigs pretreated with streptomycin and in albino mice after oral infection (Freter's model) a prolonged excretion of the pathogenic agent was demonstrated (Freter, 1955, 1956; Rauss et al., 1966*a*, *b*). In experimental vaginal infection of mice (Kashiba's model) a purulent secretion was observed (Kashiba et al., 1967). In orally infected cats intraepithelial parasites induced a clinical picture similar to dysentery (Sergeevich, 1954). Shigellae are able to infect cell cultures and elicit a cytopathogenic effect (see Chapter 5). Avirulent shigella cultures are either unable to enter, or penetrate only a very small part of the epithelial cells and fail to multiply there. Virulent forms of different serotypes of shigellae are epithelial parasites. In this case intraepithelial parasitism is a group property.

There is a genetic homology between the Shigella and *E. coli* groups (Luria and Burrous, 1957; Falkow et al., 1963; Schneider and Falkow, 1964; Stenzel, 1965). Because of the close relationship, these two groups were ranged in the same main divison (Ewing and Edwards, 1960). The numerous serological connections of the *Shigella* and *E. coli* groups are well known (Ewing, 1953), and many intermediate forms between these two genera were described (Manolov, 1958, Stenzel, 1962*c*; Piéchaud et al., 1965; Trifonova, 1965).

The *Escherichia* group occupies a central place in the family Enterobacteriaceae, having numerous relationships with the other groups. The greatest amount of common antigens, shared with other groups, is found in the genus *Escherichia*. Owing to this fact some authors suggest that in the course of evolution, this genus might have been the progenitor of all other Enterobacteriaceae groups (Polotsky, 1967, 1970). As regards intracellular parasitism, a similar connection may be established between *E. coli* and other enterobacteria. Certain *E. coli* serotypes are intraepithelial parasites, others are more or less capable of multiplying in macrophages while the rest exhibit no intracellular parasitism (see Chapters 20–22).

Novgorodskaya (1968, 1970) (see Chapter 19) divides enteropathogenic *E. coli* (EEC) into two categories. The first category includes organisms causing epidemic infantile gastroenteritis (EEC-I or "*E. coli* dyspepsiae"), the second category comprises serotypes inducing dysentery-like diseases in adults and children [EEC-II or "shigelloid coli" (Rédey, 1964*a*, *b*), or "dysentery-coli type" (Stenzel, 1965)]. Serotypes known as common inhabitants in the bowel or causative agents of extraintestinal diseases are termed as non-enteropathogenic (NEEC) organisms, and cannot be included into these categories. According to Rédey (1970) the following *E. coli* serotypes may be classified as intraepithelial parasites: O28a28c:K73(B18), O29, O32, O112a112c:K66, O115, O124:K72(B17), O135, O136: K78(B22), O143 and O144, i.e. the so-called "shigelloid coli" serotypes. These and some other *E. coli* serotypes are called by Novgorodskaya (1966*b*, *c*, 1968, 1970), Novgorodskaya et al. (1966*a*, 1968, 1970), Novgorodskaya and Polotsky (1972) Shigella-like group of EEC-II (see Chapter 19).

This category is intermediate between *Shigella* and *E. coli* as regards its biochemical and serological properties. The O antigens of several serotypes are identical with *Shigella* antigens (see Chapter 19). Novgorodskaya and Polotsky (1972) noted that *E. coli* strains classified into category II had fermentation properties closer of those of *Shigella* than other *Escherichia*. Earlier a similar view was published by Sakazaki et al. (1967). *E. coli* O115 strains, as examined with keratoconjunctival test and by oral administration to human volunteers, produced typical dysentery. These strains fermented lactose late or not at all, did not decarboxylate lysine, were non-motile and produced no gas from glucose and other sugars (Trabulsi and Fernandes, 1969). *E. coli* O124, O143 and O144 failed to produce lysine decarboxylase (Serény, 1963; Costin and Olinici, 1965; Stenzel, 1965; Aldová and Lázničková, 1967). Although O124 strains show a relatively narrow fermentation spectrum (Stenzel, 1965), there are cultures which ferment lactose rapidly (Kolta, 1967). Using the data of twelve authors, Vasser (1968) summarized the biochemical activities of serogroup O124 and mentioned that some O124 cultures ferment lactose promptly while others failed to attack this sugar. As regards their biochemical activity, keratoconjunctivitis-inducing members of *E. coli* O136 might be looked upon as intermediates between *Shigella* and *Escherichia*: they produce gas from glucose, do not ferment lactose, do not decarboxylate lysine, are non-motile and part of them fail to utilize citrate (Aldová and Lázničková,

1967). In biochemical activity, except for lysine decarboxylase, serogroup O144 is similar to other *Escherichia* strains (Aldová and Lázničková, 1967).

The disease induced by serotypes of the second category resembles shigellosis (Serény, 1963; Rédey, 1964*a*, *b*; Novgorodskaya, 1968). In animal experiments (Voino-Yasenetskaya's, Bingel's, Formal's, De's models and cell cultures) they behave like intraepithelial parasites (Rédey and Csizmazia, 1960; Stenzel, 1962*a*, *b*, Serény, 1963; Novgorodskaya et al., 1964*a*, 1966*b*; Ogawa et al., 1966*b*, 1968*b*; Polotsky and Arbuzova, 1966, 1967; Polotsky et al., 1968, 1971; Novgorodskaya, 1968, 1970; Polotsky and Vasser, 1970*a*, *b*; Formal et al., 1971*a*). Shigella-like *E. coli* strains may induce a reaction in animals different from that caused by shigellae. Polotsky and co-workers (Polotsky and Arbuzova, 1966, 1967; Polotsky et al., 1968; Ariel et al., 1968; Novgorodskaya et al., 1968*a*) found that *E. coli* O28a28c, O124 and O143, examined with Voino-Yasenetskaya's model, similarly to other *E. coli* types, multiplied in the alveolar macrophages within the first 24 h, while salmonellae multiplied in cycles (Voino-Yasenetsky, 1964*b*).

Cross-immunity furnishes further data on the homology of intraepithelial enteric parasitic bacteria. Cross-immunity was unambiguously demonstrated not only between different *Shigella* serotypes, but also between *Shigella* and shigella-like *E. coli* serotypes (Stenzel, 1962*b*, *c*; Serény, 1963; Istrati et al., 1963*a*; Istrati and Istrati, 1964*b*; Ogawa et al., 1966*b*). Cross-immunity has never been shown between shigellae and salmonellae.

PARASITES OF MACROPHAGES

The intraepithelial parasites form a well-defined group. This is not true for the parasites of macrophages, which mainly multiply in the cytoplasm of the macrophage-type cells. Histological changes induced by them are at first similar to the picture of infections caused by shigellae and shigella-like *E. coli*. Macrophage-type cells, as a reaction to intraepithelial parasites, may also appear among the polymorphonuclear leukocytes infiltrating the subepithelial area and later some macrophages containing bacteria in their cytoplasm may be discernible in the subepithelial tissue (Tenner et al., 1971*b*). These organisms, however, generally do not damage the epithelial cells, but enter the lymphoreticular tissue, where they are phagocytized by the macrophages (Rácz et al., 1970; Tenner et al., 1971*a*).

Some *Salmonella* serotypes *(S. typhimurium*, *S. enteritidis* and possibly also *S. typhi)*, which may induce experimental salmonella keratoconjunctivitis when freshly isolated, seem to occupy an intermediate position between intraepithelial and macrophage parasites (Stenzel, 1962*b*, *c*; Istrati et al., 1963*a*; Gleiberman et al., 1964). Six hours after infection with *S. typhimurium*, many bacteria may be seen in the epithelial cells of the conjunctiva. These salmonellae multiply mainly in the area above the lymphoreticular tissue. Later the number of these cells decreases, while the number of macrophage-type cells of the lymphoreticular tissue increases (Bakács et al., 1970; Tenner et al., 1971*a*). It may be established in general that the majority

of *Salmonella* serotypes possess the ability of multiplying in the macrophage-type cells and of destroying them (Voino-Yasenetsky, 1964).

A greater part of freshly isolated *S. paratyphi-A* and *S. paratyphi-B*, *S. typhi*, *S. rostock*, *S. meleagridis*, *S. muenchen* strains, though inducing no macroscopical change in the guinea pig's eye, gain access to the blood-stream and the viscera (Serény, 1955*a*, 1957; Moore, 1957; Trabulsi, 1965). Using Voino-Yasenetskaya's model, freshly isolated and old strains of *S. typhimurium* and *S. heidelberg* were found to begin multiplying 4–5 days after infection in the lung of the mouse. *S. paratyphi-B* multiplies persistently but has no fatal effect; *S. typhi* does not multiply at all (Arbuzova, 1964*b*). Occasionally salmonellae are discernible in the bronchial epithelium (Ariel and Bernovskaya, 1970). Similar findings were reported in the "lung model" with EEC-I (*E. coli* dyspepsiae), which first showed multiplication in macrophages but were killed after 24 h (Polotsky et al., 1968) (see Chapter 21). In Bingel's model fresh strains of *S. enteritidis* and *S. java* were highly virulent, while *S. typhimurium*, *S. bareilly* and *S. reading* were moderately virulent. On the whole, bladder salmonellosis results in less significant changes than cystitis shigellosis (Stenzel, 1960). *S. typhimurium* and *S. enteritidis* penetrate epithelial cells of the bladder and multiply there (Bakács et al., 1970). In Formal's model *S. typhimurium* enters the epithelial cells of the small intestine (Takeuchi, 1967). In mice, *S. typhi* and *S. paratyphi-B* easily penetrate the intestinal mucosa after oral infection; however, they usually remain in the intestinal tract, where they persist rather long (Ørskov and Kauffmann, 1936). *S. typhimurium* on the other hand, penetrates the intestinal mucosa, multiplies in the peripheral lymph nodes and enters blood circulation causing fatal infection in albino mice after 5–10 days (Ørskov and Kauffmann, 1936; Belyanin, 1968). *S. typhimurium* multiplies well in guinea pig peritoneal macrophage culture and destroys macrophages. *S. paratyphi-B*, on the other hand, is killed in the macrophages (Arbuzova, 1964*a*). After salmonella keratoconjunctivitis a cross-protection develops against other *Salmonella* serotypes (Gleibermann et al., 1964) but not against *Shigella* (Rauss, 1968). Between *Listeria monocytogenes* and *S. typhimurium* a certain form of cross-immunity (mutual bactericidal effect of the macrophages) may be found in albino mice (Blanden et al., 1966).

Accordingly *Salmonella* serotypes may show different reactions in various animal models. This seems to be obvious from their different aetiological role in natural human and animal enteric infections.

The uncertain limits of this grouping are demonstrated by the observation that the keratoconjunctivitis-negative *Arizona* strains multiply in pulmonary tissue (Voino-Yasenetskaya's model) and kill a part of the animals. In cell cultures and oral infection of albino mice they elicit an effect similar to that of *S. typhimurium* strains (Arbuzova, 1970*b*).

In Table 2-I we summarized the grouping of intracellular parasitic Enterobacteriaceae. Biological properties are presented as described by Moller (1954), Report (1958), Kauffmann (1956*a*), Rédey (1964*a*, *b*), Aldová and Lázničková (1967), Aldová et al. (1968), Ewing (1968), Novgorodskaya (1968), Trabulsi and Fernandes (1969).

TABLE 2-I

Subdivision of intracellular parasitic Enterobacteriaceae

	Motility	Gas (in glucose)	Lactose	Ammonium citrate	Indole	H_2S	Lysine decarboxylase (Carlquist)	Glutamic acid decarboxylase
Intraepithelial parasites								
Shigella	−	−	−	−	*d*	−	−	+
Shigella-like EEC-II ("shigelloid coli")	− *d*	+	+ or ×	−	+	−	− *d*	+
Macrophage parasites								
EEC-I (*E. coli* dyspepsiae) and NEEC	+ *d*	+	+ or ×	−	−	−	+	+
Salmonella	+	+	−	+	−	+	+	−

Note: + positive within 1 – 2 days,
× delayed or irregularly positive
d different biochemical types

Part of the pathogens emanating from the disintegrating macrophages are phagocytized by leukocytes (Bakács et al., 1970). Intranasal experimental infection with enteropathogenic *E. coli* strains belonging to the first category (EEC-I) and with non-enteropathogenic *E. coli* (NEEC) has no fatal outcome; the microorganisms do not penetrate the epithelial cells of the bronchi, but multiply in alveolar macrophages the first day following challenge (Polotsky et al., 1968) (see Chapter 21). Filotti (1965) examined 22 *E. coli* dyspepsiae cultures with De's model and found 12 positive strains among them. *E. coli* strains originating from urinary tract infections were avirulent with the test. EEC-I induced inflammation in the isolated intestinal loop of the rabbit (see Chapter 22). In albino mice, after oral ingestion of these bacteria, no fatal infection developed; the organisms were not recovered from the blood and viscera of mice (Arbuzova et al., 1970). These organisms may also multiply and elicit a cytopathogenic effect in cell cultures originating from human amnion (see Chapter 20).

The categories of intracellular parasitism overlap the taxonomic groups, i.e. organisms differing in intracellular parasitism may belong to the same biochemical group. This difference between taxonomic groups and grouping on the basis of parasitism is still more striking if one considers the virulent and avirulent variants of a single serotype, or even of the same strain. Hence a closer connection between biochemical activity and intracellular parasitism cannot be expected.

As a further conclusion it should be emphasized that association between intracellular parasitism and pathogenicity has so far only been verified for intraepithelial parasites.

INTERRELATION OF PATHOGENICITY AND BIOLOGICAL PROPERTIES

A close correlation has been found between intraepithelial parasitism and pathogenicity (LaBrec et al., 1964; Formal et al., 1971*a*; Voino-Yasenetsky, 1963, 1966*a*, *b*, 1970). Conjunctival infection, a simple experimental model for the examination of pathogenicity, is the oldest method used in such studies (Serény, 1955*a*, *b*) and the most suitable for mass investigations (Formal et al., 1971*b*). Rédey (1957, 1960, 1964*a*, *b*) demonstrated that the virulence of *Shigella* and shigella-like *E. coli* strains, as tested by the keratoconjunctival model, is valid for man. Experiments by other authors (Istrati, 1961; Istrati et al., 1964*a*; Stenzel, 1962*b*; Trabulsi and Fernandes, 1969) confirmed the results of Rédey.

The available incomplete data are not sufficient to recognize virulent bacterial cells on the basis of their morphological and staining properties. Roelke (1943) showed that the "*Klatsch-Praeparatum*" (imprints) made from S-colonies of *Sh. sonnei* consisted uniformly of short, thick rods. The cells of the R-colonies resembled filamentous fungi. Oblique illumination shows virulent *Shigella* colonies to be fluorescent (see below). From the examination of Akatova (1964, 1965), virulent cells seem to be less thick than avirulent ones. Florey (1933) has stated that bacteria from a dense *S. typhimurium* suspension, when put on the surface of the small intestine invade the epithelial cells, and may be seen occasionally in the form of coccoid, faintly painted rods. Morphological examinations in shigella keratoconjunctivitis (see Chapter 7) have shown shigellae penetrating epithelial cells after conjunctival infection to have grown in size compared with extracellular bacteria. In smears prepared from the conjunctival discharge salmonellae form foci in the cytoplasm of epithelial cells called salmonella-stars (Tenner et al., 1971*a*). Intracellular shigellae are shorter and have less distinct outlines right after conjunctival infection than in the late phase of the disease (Lin et al., 1964). Voino-Yasenetsky (1964*b*) has observed in salmonella pneumonia of mice that the pathogenic agent at first becomes longer, then shorter; stains poorly and regains its usual shape only in the necrotic tissues after having been released from the cells. In the opinion of Manolov (1968) all virulent *E. coli* O124 cultures consist of capsulated bacteria.

Virulent cultures of *E. coli* O124 may be actively motile by help of peritrichous flagella (Manolov, 1968). Flagella, however, do not promote penetration into epithelial cells, as non-motile shigellae and *E. coli* may also enter the epithelial cells of the mucosa.

It has been supposed that adhesion of shigellae to the intestinal mucosa happens by means of fimbriae. Fimbriated *Shigella* and *E. coli* cultures become rapidly attached to the colonic mucosa in model experiments, while non-fimbriated bacteria fail to do so. However, certain newly isolated *Sh. dysenteriae*, *Sh. flexneri* and *Sh. sonnei* serotypes have no fimbriae (Duguid and Gillies, 1957; Duguid, 1968). As in keratoconjunctival test non-fimbriated shigellae may be virulent, there is no direct connection between this property and pathogenicity. Falkow et al. (1963) showed that fimbriated hybrids had a lower growth rate than the parent strains

and other hybrids. Fimbriated *S. typhi-E. coli* hybrids were avirulent in mouse experiments (Baron et al., 1960).

Only virulent shigellae are able to attach to the surface of the cells and stimulate the ruffling movement of the cell membrane, resulting in the incorporation of the bacteria. A very vivid movement of intracellular shigellae may be observed in the cell. Therefore, shigellae entering the cells seem to have an autonomous moving capacity (Ogawa et al., 1968*a*).

According to Takeuchi et al. (1965) non-flagellated shigellae enter the lamina propria of the intestinal mucosa within 12 h in significant numbers. There is obviously an interaction between the bacteria and cytoplasmic organelles (especially endoplasmic reticulum and Golgi apparatus). Rácz et al. (1970) and Tenner et al. (1970) have observed electron-microscopically the direct transfer of the pathogenic agent from one corneal epithelial cell to another.

Stenzel (1961*c*, 1962*c*) found no parallelism between virulence and growth rate; therefore, a reduction in the multiplication rate *in vitro* cannot be responsible for decreased virulence. Falkow et al. (1963) examined the multiplication of virulent and conjugated strains *(in vivo)* using Formal's model. The virulent parent strain and a virulent *E. coli-Shigella* hybrid multiplied well in the intestine of the guinea pig. Other hybrids multiplied weakly or not at all. There is ample evidence of a direct correlation between multiplication *(in vivo)* and virulence (Serény, 1955*a*, 1957; Voino-Yasenetsky and Voino-Yasenetskaya, 1962*a*, *b*).

According to Bogdanova (1965), the change in virulence is reflected in the electrokinetic potential of the culture which is higher in avirulent *Sh. flexneri* variants than in the original virulent strains.

The morphological properties of enterobacterial colonies may be associated with their pathogenicity. Virulent strains form S-colonies, while the R-form is avirulent. *Sh. sonnei* cultures, shown to be avirulent by the keratoconjunctival test, correspond morphologically to phase II, as demonstrated by Rauss (1955) and confirmed by others (Serény, 1955*a*, 1957; Gekker et al., 1957*a*, *b*; Manolov, 1957*a*, *b*; Siroko, 1957, 1958; Szturm-Rubinsten et al., 1957; Gorea, 1959; Noskov, 1959; Mackel et al., 1961; Stenzel, 1962*b*, *c*). The pathogenicity may, however, be lacking in S-form *Sh. sonnei* cultures (Gekker et al., 1957*a*, *b*; Serény, 1957, 1960*a*). Kotelko et al. (1960) have differentiated four morphological variants of *Sh. sonnei* colonies, two of which were virulent.

Formal et al. (1965*b*) have observed less transparent avirulent colonies of *Sh. flexneri* 5 among the characteristic transparent virulent colonies in agar cultures. The avirulent variants have been used as genetic recipients in the course of hybridization with *E. coli* K-12 donor strain. Most of the recombinants, gained in this way, were avirulent and formed colonies characteristic of avirulent strains. Some of the hybrids, however, seemed to be virulent; most of them regained the colony morphology of the original strain. Accordingly, the connection between colony morphology and virulence was not always close.

Colony structure can be more exactly studied by oblique illumination (Walters et al., 1954; Cooper et al., 1957; Belaya, 1970*a*). This technique

is suitable for differentiation between virulent and avirulent colonies of *Sh. flexneri* (Falkow et al., 1963). The method has not been unanimously accepted (Watkins, 1960).

We have recommended the use of oblique light with varying angles of incidence. Using our method, virulent parts of a *Sh. sonnei* colony may be recognized by virtue of their characteristic fluorescence. Kerekes (1962) was able to differentiate between virulent and avirulent colony variants in *Sh. flexneri* cultures. We examined (Serény, 1963) *E. coli* O124:K72(B17) cultures and found that colony structure reflected virulence in this organism as well. Rauss (1963*a*, *b*, 1968) applied our method with success, and demonstrated a close correlation between virulence measured with the keratoconjunctival test and certain properties of shigella colonies. Refractive colonies contained virulent clones. On the basis of colony structure, Andreeva et al. (1965) derived variants different in virulence from the parent shigella culture. Akatova (1965) demonstrated that intensity and colour of fluorescence of bacterial colonies were related to the phenomenon of light diffraction, determined by two factors: the thickness of microbial cells and their relative position to the direction of light.

The source of wild *Shigella* strains has no influence on their pathogenicity; strains isolated freshly from the faeces of carriers and from that of patients were equally virulent (Nakumara, 1967; Kerekes, 1968).

There is a not too close connection between biochemical activity and intracellular parasitism. Shigella-like *E. coli*, belonging to the intraepithelial parasites, are intermediates between *Shigella* and other *E. coli* serotypes as regards their biochemical properties (Serény, 1963; Sakazaki et al., 1967; Trabulsi et al., 1967; Aldová et al., 1968; Novgorodskaya, 1968; Rédey, 1970). Gleiberman et al. (1964) showed that mainly anaerogenic *S. typhimurium* strains are able to elicit salmonella keratoconjunctivitis in the eye of the guinea pig. Though certain differences have been noted between the sugar fermenting capacity of virulent and avirulent shigella cultures by Shantarenko and Tesla (1960), no correlation is accepted between the two properties (Serény, 1955*a*, 1957).

The factors of the virulence of shigellae have been studied only for a very short time. Accordingly, it is evident that further investigations are necessary.*

* This chapter was completed by B. Serény in 1971. Some additional data are presented in the following chapters. Biological properties and genetics of shigellae have been discussed comprehensively by Timakov and Petrovskaya (1972).

CHAPTER 3

METHODS APPLIED IN THE STUDY OF THE PATHOGENESIS OF INTESTINAL INFECTIONS

by

M. V. VOINO-YASENETSKY

As it has been shown in Chapter 1, agents of infectious diseases often display certain important characteristics when observed only in the host. This may be revealed by means of bacteriological methods combined with morphological investigations. However, bacteriologists apparently prefer to use biochemical methods though, as they themselves admit, these investigations have not as yet been very successful (Dubos, 1954, 1957; Garber, 1960; Moulder, 1962; Woods and Foster, 1964; A. L. Smith, 1964; Petrovskaya, 1967; H. Smith, 1968; Rowley, 1971). It seems quite possible that the behaviour of microorganisms depends on chemical or physical factors. However, first it is necessary to know how the parasite in question behaves in the host and to observe its interrelation with various tissue elements, first of all with phagocytes.

In common histological preparations pathogenic Gram-negative bacteria cannot be distinguished from rods normally inhabiting the intestinal tract. With the fluorescent antibody technique (Coons, 1958) it is possible to identify not only certain microorganisms but also their breakdown products, as well as specific antibodies produced by host cells. This method has bridged the gap separating bacteriology from pathology and has become a useful tool in many fields of research, especially in immunology. It also contributes significantly to the investigation of enteric infections, though there are still considerable difficulties in this sphere.

Intestinal lesions occurring in dysentery or typhoid fever can only be studied at autopsy. However, alterations in the intestine set in very early, within hours or even minutes after death, thus preventing a correct evaluation of the findings. Moreover, death rates in enteric infections have been reduced considerably, and today one can hardly expect to obtain fresh autopsy specimens for studying the most interesting acute stages of the disease. For reasons to be dealt with later, relatively little information has been gained from intestinal biopsy specimens in dysentery and typhoid fever. Therefore, the main sources of information on the pathogenesis of these infections are still the animal experiments.

Investigations of the pathology of human infectious diseases are based on so-called model experiments. Inoculation of rabbits or guinea pigs with pathogenic agents fails to produce a true nosological model (Voino-Yasenetsky, 1966*c*, 1969). Even if the best approximation is attained (e.g. salmonellosis in rabbits, influenza or pertussis infection in mice), in animals

the disease runs a somewhat different course; some characteristic symptoms fail to appear, the outcome is different, etc. The principles of modelling, however, do not presuppose a complete identity between model and its original. It is merely assumed that the main and essential features coincide.

It is generally considered that the closer the experimental animal is to man in its anatomical and physiological properties the more valuable is the model experiment. The greatest importance is usually attached to experiments performed on monkeys.* This is undoubtedly correct, but studies in experimental pathology cannot entirely depend on this expensive animal, which is not very convenient to handle. Besides, monkeys are known to be resistant to certain organisms pathogenic for man. At the same time, for some reason, these organisms may cause severe disease in mice or guinea pigs. It should be noted that for this purpose such unnatural procedures are sometimes used as to make the reliability of the results questionable.

Indeed, it is doubtful whether some of these models may be termed "experimental dysentery" if the mice only succumb to enormous doses of *Sh. flexneri* injected intraperitoneally. According to some sceptical authors, these experiments can be termed "injectional" rather than infectious. Nevertheless, certain questions of pathology or therapy may occasionally be answered by these experiments. In fact, inoculation of bacteria into the conjunctival sac or respiratory tract of mice, a procedure which might seem utterly inadequate, has led to the discovery and investigation of a highly important property of these organisms, namely their capacity to grow within epithelial cells.

In general, experiments on animals are only justified when their purpose is clearly realized and the limitations and relativity of the results are recognized. These conditions can only be obtained with a careful study of the model representing no more than a certain aspect of the natural pathologic process under investigation.

Different procedures for reproducing human enteric diseases in animals are described in detail in handbooks on experimental techniques (Sarkisov and Remezov, 1960; Wundt, 1966). These descriptions, however, only deal with the technical aspects. Even Frenkel's paper (1969) containing some interesting general statements, only enumerates techniques and animals that may be used in studies on infectious pathology. Some information on the nature of pathological processes occurring in animals inoculated in different ways with shigellae, salmonellae and escherichiae is available from other reports presenting the results of model experiments with these organisms. These data will be considered critically in the following chapters. At the same time, it seems appropriate to consider some of the general aspects of animal experiments.

* DuPont et al. (1969) infected humans with *Shigella*, terming the disease a dysentery model. But is this really a model? It is genuine dysentery that has been deliberately induced (which is hardly permissible).

ENTERAL INOCULATION OF ANIMALS WITH PATHOGENIC ENTERIC BACTERIA

The most natural way of inoculating with enteric bacteria is to feed them to animals with their diet. A similar procedure is the administration of bacilli through a stomach tube; this method increases the likelihood of infection and provides a possibility of determining more exactly the amount of bacteria necessary for the development of infection. However, this procedure, without any pretreatment, is only successful in monkeys inoculated with shigellae or in mice infected with mouse-pathogenic salmonellae. Attempts to infect other animals in a similar manner proved to be unsuccessful. Occasionally, cats and rabbits could be infected with agents of dysentery (see Chapter 10), but the results were only rarely reproducible. The authors of these reports apparently dealt with particularly virulent strains, losing virulence gradually, which is a feature characteristic of dysentery and other enteropathogenic bacteria.

Animals failing to contract the disease after inoculation with a pathogenic organism are often termed non-susceptible or insensitive to a certain infection. It was, however, shown in Chapter 1 that many organisms, including saprophytes that would be able to inhabit organs and tissues of animals are prevented by host defence from penetrating these tissues and from multiplying in an environment suitable for their nutrition. Pathogenic organisms are able to overcome it, but only in some particular animal species. Other animals should not be termed non-susceptible. They should rather be regarded as resistant. For a correct evaluation of procedures used in experimental infection of animals which normally resist usual methods of inoculation, it is important to keep this distinction of terms in mind.

As early as 1885, Koch, who was then studying cholera, found that vibrios given orally in great amounts induced no disease in guinea pigs, because they were killed in the animals' stomach. Fatal infection occurred, however, if the same vibrio was introduced into the duodenum bypassing the stomach. In a series of elegant experiments Koch studied the digestion of guinea pigs and found that, in contrast to man, food was not churned in their stomach, but was deposited in layers as ingested and stayed there for a considerable time during which the highly acid gastric juice destroyed the vibrios. The small intestine, where alkalinity proved favourable for the agent of cholera, was traversed rapidly by particles discharged from the stomach. Koch found that oral infection of guinea pigs was successful after preliminary neutralization of the gastric contents with sodium bicarbonate solution and intraperitoneal administration of 1 ml of opiate to suppress intestinal peristalsis.

In similar experiments with *S. typhi* Koch, as well as W. Sirotinin (1886), obtained inconclusive results. Later, by introducing the agent into the duodenum, Valdman (1931) succeeded in infecting rabbits with *S. typhimurium*, to which they were "non-susceptible" on oral administration. As shown by Kucheryavy (1956), rabbits fed with milk can be infected with salmonellae. The organisms probably survive within flakes of casein,

formed owing to the curdling of the milk in the stomach. Besides, as observed by Smirnov (1966), with this mode of administration salmonellae appear in the duodenum within as little as 3–5 minutes.

In the case of shigellae a safe passage through the gastric barrier was insufficient to ensure their habitation in the intestine. Attempts were made to introduce shigellae directly into the intestinal lumen of laboratory animals (per rectum or through the caecum) — but without success. Assuming that injury of the intestinal mucosa promotes the development of infection, the mucosa was first irritated with ammonia solution (Grigoryev, 1891) or croton oil (Shiga, 1898), then dysentery bacilli were introduced. This method proved occasionally successful in experiments on cats but the irritating or injuring procedures used by Avetikyan in experiments on mice, provoked no infection.

Shigella infection was more successful when artificial impairment of the digestive function was associated with certain procedures affecting the general condition of the animal. Thus, Kazarinov (1903, 1904) used Koch's method successfully in experiments on rabbits (intragastric administration of sodium bicarbonate and intraperitoneal opiate prior to infection) after a three-day fast. A similar procedure was adopted by Formal et al. (1958) who produced dysentery infection in guinea pigs; they modified their method subsequently (Formal et al., 1959) using carbon tetrachloride instead of starvation.

It seems more or less obvious how neutralization of gastric juice and impairment of intestinal peristalsis affect enteric infection in "non-susceptible" animals. It is much more difficult to explain the part played by starvation in the method of Koch as modified by Kazarinov, or that played by carbon tetrachloride poisoning in Formal's modification of Koch's method. Formal noticed fatty dystrophy of the liver in starved animals. That is why he replaced starvation with carbon tetrachloride injection, causing even more severe liver lesions. These lesions have been studied in great detail (Myren, 1956; Hübner, 1965; Krishnan and Stenger, 1966; David, 1967; Thom and Schlicht, 1970; et al.), but it is still far from clear how they contribute to the onset of enteric infection.

The mechanism of local effects on the intestine, contributing, as reported by a number of investigators, to the development of infectious processes after enteral inoculation of animals is even less clear. In addition to the above-mentioned administration of irritating agents (croton oil, etc.) which appear to promote shigella infection, attempts have been made to produce similar effects by preliminary sensitization of the intestine with killed bacilli (Rubinshtein and Skavronskaya, 1950). As this procedure yielded inconsistent or contradictory results, no significant role could be attributed to allergic phenomena in early dysentery.

Numerous experiments and observations demonstrate that the development of enteric infections depends to a considerable extent on the general condition of the experimental animal, or rather, on mechanisms underlying so-called natural immunity. It has been proved by a number of investigators that the injection of corticosteroids, ionizing radiation as well as other factors depressing phagocytic activity, increase the severity of experimen-

tal salmonella infection. In experiments by Troitsky et al. (1958) ionizing radiation was found to activate latent (subclinical) chronic dysentery in monkeys and to promote *Sh. flexneri* infection in rabbits. It is not clear in what manner enteric infections are influenced by heat or cold (Kucheryavy, 1957; Miraglia and Berry, 1962), ultra-high frequency radiation of different intensities (Sviridov, 1970) and other agents used by investigators for various purposes.

The experiments may be influenced by factors sometimes ignored, such as the diet and its vitamin content (Newberne et al., 1968; Haltalin and Nelson, 1970; and others). It is worth noting that evidence has been obtained on seasonal variations in the resistance of animals (Belaya, 1958; Formal et al., 1961*a*). The possibility that the virulence of bacteria used for animal inoculation may not have been uniform, cannot be excluded either.

It seems difficult to explain the individual differences in the resistance of laboratory animals. Indeed, inoculation of a group of mice, rats, guinea pigs or rabbits in the same manner never results in infection taking the same course in each animal. In view of the unavoidable irregularity of the results of these experiments, microbiologists have adopted the index LD_{50} representing the amount of organisms causing death in half of the animals. It usually remains unknown why the other half survives.

Lines (or strains) of mice have been selected that display particularly high or low resistance to salmonellae. Even in these animals individual variations cannot be excluded. Experimental results may be influenced by various occult diseases or latent infections, currently occurring in laboratory animals. In the special colony of mice (NCS), which were free of pathogenic organisms, experiments of Schaedler and Dubos (1964) produced more uniform results than is usual.

The normal inhabitants of the intestine interfere with the development of experimental enteric infection. The suppression with antibiotics of the vitality of these organisms may decrease the LD_{50} considerably (by several tens of thousands) as tested in enteral salmonella infection (Bonhoff et al., 1954). The same measure has been found to enhance cholera infection in guinea pigs (Freter, 1955). In this way shigella-resistant animals (mice and guinea pigs) can be infected with shigellae (Freter, 1956; Cooper, 1959), or the intestine of mice can be colonized with alien strains of *E. coli* (Rauss and Kétyi, 1960; Ashburner and Mushin, 1962). Successful experiments have also been made on germ-free animals (Formal et al., 1961*a*; Mushin and Dubos, 1965), as well as on newborn animals, a few days after birth, when enteric organisms have not yet been established (Kétyi, 1964). In these cases enteric infection with shigellae or pathogenic *Escherichia* strains usually results only in a more or less prolonged symptomless excretion of bacteria with the faeces or in the death of the animals from a generalized infection (see Chapter 22).

The fact that normal intestinal bacteria exert a protective effect by interfering with the establishment of pathogens is of considerable practical importance and has long been discussed by clinicians and microbiologists (Perets, 1955, 1962), but still lacks a reliable explanation. The probability of a direct antagonism was suggested and attributed to colicin production

by saprophitic *E. coli* (Halbert, 1948; Levine, 1963; Kudlay and Likhoded, 1966; Filichkin, 1969). On the other hand, Freter (1956), Hentges (1969) and other investigators noted that the growth of shigellae was inhibited by non-colicin-producing strains of *Escherichia*. According to the hypothesis of Kétyi (1965), common colon bacilli establish close contact with the intestinal epithelium and prevent other bacteria from doing the same. This hypothesis, however, does not agree with the observations of pathologists proving that such intimate contacts with the intestinal epithelium are established only by pathogenic strains of *E. coli* which grow on its surface or within the cytoplasm of its cells (see Chapters 19–22). Freter (1962) discussed a possible competition for nutrient substances among organisms, while Bonhoff et al. (1964) considered shigellae and salmonellae to be unadapted to the usual pH of colonic contents, depending on the production of acetic and butyric acids by bacteroids occurring here. But in Hentges' (1969) opinion none of these factors *per se* are dangerous for shigellae.

These explanations, however, provide no clue as to why escherichiae and bacteroids inhabiting mainly the colon interfere with the vitality of salmonellae, cholera vibrios and enteropathogenic *E. coli*, multiplying in the small intestine. In discussing their experiments in germ-free guinea pigs, Formal et al. (1961*b*) suggested that the protective role of escherichiae against shigella infection depended on the stimulation of host defence mechanisms by saprophites, rather than on direct antagonism between microorganisms.

Donaldson (1967*a*, *b*) reviewed the results of investigations in germ-free animals and concluded that a normal microbial population of the intestine influenced the microscopic structure of the intestinal wall as well as the development of the reticulo-endothelial system and the aptitude for defence. Still, it remains to be explained why a single dose of streptomycin, 24 hours before inoculation with shigellae, makes naturally resistant mice susceptible to shigella infection. The remarkable investigations of Abrams and Bishop (1966) should be mentioned in this connection. They studied the multiplication of *S. typhimurium* in the intestine of germ-free and conventional mice; their first impression was that a normal microbial population was favourable for the development of intestinal mucosa, thereby making it less permeable for bacilli. After more careful experimentation, however, they had to deny their theory, concluding that the normal flora does not influence mucosal resistance directly, but may alter infection by affecting intestinal emptying. Indeed, the significance of intestinal peristalsis had already been regarded by Koch as a factor in resistance when he used opiates for its suppression in the experiments described above.

The inhibition of peristalsis is the main feature of another model of enteric infection, introduction of the infective agents into an isolated segment of the intestine. Technical details and the results with *Shigella*, *Salmonella* and *Escherichia* are dealt with in Chapter 22.

Assuming that "enterotropism" is an inherent property of enteropathogens, attempts have been made to provoke intestinal lesions typical of typhoid fever, by injecting the agent intravenously or subcutaneously

(Besredka, 1925; Sanarelli, 1926; et al.). Valdman (1955, 1964) proved these experiments to be inconclusive. This also applies to more recent experiments by Morgunov et al. (1962, 1963), who attempted to provoke typhoid fever or dysentery by subconjunctival administration of the agent. The evidence (particularly the pathomorphological findings) presented in these papers is not convincing, and the method has not been used to any further advantage.

It is obvious why investigators endeavour to produce enteric infections with intestinal lesions. It seems doubtful, however, whether these attempts are always justified. While in monkeys dysentery runs a course practically identical to that in man, the same infection in guinea pigs involves the intestine only after preliminary injury, and the symptoms are not typical of the disease. Certain features inherent in these experimental diseases complicate the evaluation of the results of experiments based on methods elaborated by Koch and Kazarinov or Koch and Formal. Besides, they are complicated technically. No wonder, therefore, that these models have not become widely used.

Enteral inoculation of salmonellae has been applied more widely. *S. typhimurium* or *S. enteritidis* produce intestinal lesions in rats and mice which are very similar to those of human typhoid fever. As will be shown in Chapter 17, however, this apparent similarity conceals some very significant differences, so that comparison between typhoid fever and experimental salmonellosis can only be made with many restrictions.

Thus, even if enteral inoculation of animals with agents of enteric infections is successful, the investigator has to proceed with care when evaluating the results. Unless merely the death rates of infected animals are recorded, time-consuming studies are necessary to establish the fate of the agent in the intestine by cultural, microscopic and other methods. That is why methods of parenteral infection are commonly preferred, particularly when large numbers of animals are used. It is, therefore, relatively simpler to observe the process of infection induced by enteric bacilli outside the alimentary tract.

PARENTERAL INOCULATION OF ANIMALS WITH *SHIGELLA*, *SALMONELLA* AND *ESCHERICHIA*

Subcutaneous, intravenous, intraperitoneal and even intracerebral administration of various bacteria and viruses is widely used by bacteriologists and immunologists. This also concerns agents not known either to elicit peritonitis or encephalitis, nor to spread by the haematogenous route. In addition to these currently used methods, enteric infection might also be studied with the keratoconjunctival tests (Zoeller and Manoussakis, 1924*a*; Serény, 1955*b*), with injection of bacteria into the urinary bladder of guinea pigs (Bingel, 1943*a*), or into the respiratory tract of mice (Voino-Yasenetskaya, 1957). These procedures are regarded as extremely artificial by some investigators, who, however, do not hesitate to inject shigellae

into the blood stream or peritoneal cavity. There are also authors who do not believe in using experimental models involving unnatural situations.

Model experiments are not only used because the original situation is poorly accessible. Modelling as a method of research involves the breaking down of complex phenomena into single components which can thus be more expediently analysed. As stated above, a model is supposed to reflect only the main features of the phenomenon under consideration, while aspects of secondary importance may not necessarily resemble the original. In the case of infectious disease, consequently, it must be clear in the first place to what extent one or another model reflects features characteristic of this particular infectious process. Considering this, the experiment, even if performed outside the digestive tract, may prove to be highly instructive in the investigation of infections which under normal conditions affect mainly the intestine.

Thus, model experiments must be based on preliminary information on the nature of the phenomenon under study. It would be impossible otherwise to decide which is the main factor and which are only secondary phenomena. From the nature of the lesions, insight into their causative agent and pathogenesis might be gained. Nevertheless, information available on the mechanism of action of these agents as well as on the immediate causes of the pathologic processes they evoke is still far from being complete. Accordingly, blanks in our knowledge on the pathogenesis of infection are filled by various hypotheses. Trends in experimental research and the choice of models depend on these hypotheses.

At first sight the most important features of enteric diseases appear to be their clinical manifestations and the lesions occurring in the alimentary tract. If dysentery was still to be regarded as a clinico-anatomical complex of symptoms, animal experiments should be expected in the first place to reproduce the characteristic intestinal dysfunction and to provoke colitis. It is hardly worth mentioning, however, that diarrhoea (as well as cough or fever) are, in fact only consequences of some more important pathological process. Inflammation of the intestinal mucosa (colitis), too, is the sequel of some primary cause.

In admitting dysentery to be a specific infectious disease nothing has as yet been said of the immediate causes of the functional and morphological alterations inherent in it. For a long time the principal role in the development of the disease was attributed to toxic products of shigellae, rather than to the organisms themselves. It was assumed that toxins (or endotoxins), being absorbed first, did not affect the intestines directly but injured the intestinal mucosa while being eliminated from the blood stream. This hypothesis justified experiments involving intravenous, intraperitoneal or subcutaneous administration of shigellae or their disintegration products. These experiments were in current use and investigators were convinced that they reproduced genuine dysentery in animals by this unusual route of inoculation.

It may be assumed, finally, that the process underlying dysentery is not simple intoxication but rather the peculiar interaction between parasite and host, as discussed in Chapter 1. The approach applied to experimental

investigation should, accordingly, be different. This hypothesis can only be verified in experiments which make it possible to observe the complete pathological process including the parasite's vitality and the host's responses. These observations should not necessarily be made on the intestinal mucosa. Shigellae are known to cause in some animals (and occasionally in man) lesions of the conjunctiva or cornea, and the mucosa of the urinary bladder or respiratory tract. If the specific infectious process proves to develop similarly on different mucous membranes, then shigella keratoconjunctivitis, cystitis, or pneumonia may serve as convenient models.

The situation is similar to other enteric infections with unclarified pathogenesis. However, it would be wrong to choose the experimental methods in order to favour a single hypothesis, or to use certain experimental models and to reject others. Only if the following conditions are fulfilled can a sound opinion be formed: (*i*) current hypotheses must be reconsidered critically; (*ii*) pathological processes induced in animals by inoculation of agents of enteric infections by different routes must be studied comprehensively; (*iii*) results of experiments must be correlated with reliable data on the natural course of the infection in humans. Therefore, we shall have to reconsider the informative or practical value of model experiments in the investigation of enteric infections after having presented all relevant experimental findings.

PART II

CHAPTER 4

HUMAN DYSENTERY AND HYPOTHESES ON ITS PATHOGENESIS

by

M. V. VOINO-YASENETSKY

Striking clinical manifestations—diarrhoea with exceedingly frequent bloody mucous stools, tenesmus and abdominal pain—were so characteristic of dysentery that it has been acknowledged as a clinical entity since ancient times. Its distinction was also justified by the peculiar morbid anatomical features involving the most distal parts of the alimentary tract. In acute cases the mucous membrane of the large intestine is thickened and turns to a dark pink, almost red colour. It is coated with haemorrhagic mucous and purulent or greenish-white layers of exudate. When these films are absent or insignificant, the case is classified as catarrhal colitis; if the coat is thick, as fibrinous colitis. This group may include so-called croupous forms (with readily detachable layers of exudate) or diphtheritic forms (with dirty greenish-gray or brownish coats adhering firmly to the intestinal wall). Usually dysenteric lesions are particularly severe in distal parts of the colon, although they may be equally marked in other portions, occasionally involving even the terminal ileum. These lesions are not necessarily continuous, being frequently interspersed with areas of almost healthy mucosa. As a rule, the crests of folds are affected more severely.

Even in early stages of dysentery, defects of the intestinal mucosa appear as erosions or ulcerations. At later stages the morphology is characterized by a number of defects of different shape and size (ulcerative colitis). Association of ulcer with remnants (or recurrence) of active inflammation complicates morphological classification in colitis, and, therefore, intermediate forms have also been distinguished, such as catarrhal-ulcerative and fibrinous-ulcerative colitis. At the same time, there may be cases of positive intravital or post-mortem bacteriological findings in which the mucous membrane shows an almost normal appearance at autopsy.

HISTOLOGY OF DYSENTERY COLITIS

Gross intestinal lesions that occur in dysentery are described almost uniformly by different pathologists, only disagreeing as regards the classification of various forms of colitis. Considerable disagreement may, however, be found concerning the microscopic changes.

Thus, according to Fischer (1929), MacCallum (1945), Skvortsov (1946), Abrikosov (1957), Davydovsky (1956), Dvizhkov (1964) and a number of

other authors, the catarrhal form (or stage) of colitis in dysentery is mainly due to congestion and oedema of the mucosa, as well as to enhanced production of mucus. The leukocyte response may be slight, or even absent, while profuse desquamation of the epithelium frequently occurs, and surface necroses of the mucosa can often be observed.

According to other investigators, the initial stage of dysenteric colitis is associated with inflammatory processes with the active participation of leukocytes. As early as 1866, Heubner described seropurulent exudation with focal liquefaction (rather than necrosis) of the mucosa. Similar observations were made by Bibinova (1954, 1967) who noted that this liquefaction often, though not invariably, tends to occur at sites of the colonic mucosa where lymphatic follicles are embedded. In the opinion of Aschoff (1928) dysentery is initiated by leukocytic infiltration of the mucosa and small defects of the epithelium coated with fibrino-purulent layers (resembling mushrooms and displayed more clearly by the oxidase reaction). At the same time, Aschoff also mentioned the necrotizing form of colitis, associated with a more expressed destruction of surface layers of the mucosa. An important point in these cases was that the necrotized tissue was usually densely infiltrated with leukocytes, attributed by Aschoff to a previous inflammatory response. In very severe lesions necrosis involved also the submucosa and was accompanied by a massive deposition of fibrin.

Aschoff's description of severe forms of colitis was contradicted by Siegmund (1929), Skvortsov (1946) and Abrikosov (1957) who maintained that "fibrinous" colitis, defined in terms of the appearance of the mucosa, was in fact not accompanied by profuse deposition of fibrin. However, Löhlein (1921), Lorentzen (1923) and other investigators found a more or less dense fibrin network in some of their cases. But sometimes leukocytes and debris prevailed in the film-like layer of exudate. According to Strukov (1971) a distinction between croupous colitis (with easily detachable films) and diphtheritic colitis can be made according to the depth of fibrin deposition.

An old argument still remains unsettled on dysenteric lesions involving the solitary follicles (lymphoid nodules) to be found scattered deep in the colonic mucosa. Felsen (1936, 1945) claimed that dysenteric colitis was initiated by toxic damage of these follicles. However, it appears from his descriptions and photomicrographs, that he mistook perfectly normal structures for pathologic ones: a certain degree of hyperplasia of these follicles occurs frequently, especially in children, and dimple-like depressions over their apex are also normal formations. According to Löhlein (1921) and Maksimovich (1960), these depressions allow a closer and more prolonged contact of dysentery toxin with the mucosa, while the follicles themselves display some resistance to the action of the toxin. If they are damaged, this occurs at a later period, and, evidently, the injury is due to secondary infection.

It seems possible that secondary infection plays a decisive part in the development of ulcerous lesions of the mucosa in protracted dysentery. The immediate cause, however, of the occurrence of long-standing, often multiple ulcers which may even extend beyond the mucosa, remains to be elucidated.

Ill-defined and conflicting views on the microscopic morphology of intestinal lesions in dysentery have, up to recent times, led to a number of unsound hypotheses on the pathogenesis of the disease. Current hypotheses will be discussed below.

DIFFERENT FORMS OF DYSENTERIC INTESTINAL LESIONS

The different grades of severity of colitis may be assumed to represent various stages of a pathological process, starting as a mild catarrh, then growing gradually in severity to culminate in fibrinous (diphtheritic) inflammation. Accordingly, in most handbooks on morbid anatomy catarrhal, fibrinous and ulcerative colites are regarded as stages, rather than forms of dysentery. It is usually noted, however, that transition from one stage to another may be lacking. Indeed, even if the disease had run a protracted course, autopsy may reveal no more than catarrhal inflammation of the colonic mucosa. On the other hand, colitis may be of the fibrinous type even in patients dying on the 1st to 3rd day of the disease. It appears, therefore, more correct to distinguish different forms of dysenteric colitis, the peculiarities of which depend on several factors, rather than exclusively on the duration of the disease.

It seems reasonable to suppose that the form of intestinal lesion occurring in the individual patient might depend on the host's response to the infection. Mild catarrhal colitis has mostly been observed in young children. Thus, extensive post-mortem findings, including a total of 1434 cases (Vishnevet-

TABLE 4-I

Intestinal lesions revealed post-mortem in patients of different ages who died of dysentery

(Voino-Yasenetskaya et al., 1942)

Age group (years)	Forms of colitis (per cent)					
	catarrhal	fibrinous	gangrenous	ulcerative	chronic catarrhal	No. of cases
Under 1	68.4	20.0	—	5.6	6.0	424
1–2	56.0	27.5	—	10.1	6.4	502
2–3	43.2	31.0	0.7	12.2	12.9	139
3–5	41.0	31.3	1.2	19.3	7.2	83
5–10	24.1	32.1	1.5	37.2	5.1	137
10–20	20.6	23.8	2.4	40.5	12.7	126
20–30	12.1	9.1	1.5	62.5	14.8	264
30–40	4.9	5.9	1.5	65.9	21.8	202
40–50	8.4	6.7	1.1	55.3	28.5	179
50–60	11.7	4.2	1.7	48.3	34.1	120
Over 60	14.1	3.8	2.0	52.8	27.3	106

skaya, 1941), revealed that almost half of the patients aged under 3 years had been affected with catarrhal colitis (48.7 per cent), while between 9 and 12 years of age the incidence of this form was only 25.6 per cent. Detailed information on our own post-mortem findings in 2382 children and adults is presented in Table 4-I.

It is apparent from these data that the distribution of different forms of colonic lesions among persons succumbing to dysentery at various ages was far from being uniform. The incidence of catarrhal colitis decreased down to a minimum level between 30 and 40; then it increased slightly. Fibrinous colitis occurred most frequently in children aged 2–10, though it never formed more than one-third of all intestinal lesions in these age groups. A striking feature was the gradual growth of incidence of ulcerative colitis, found to prevail in the age group of 20–40. Later, the incidence of ulcerative colitis decreased slightly again, while that of chronic catarrhal intestinal lesions rose. Lesions characteristic of acute dysentery prevailed in children, while those of chronic dysentery occurred mainly in adults.

Despite the striking dependence of post-mortem findings on age, the intestinal wall cannot be assumed to respond to infection in a different manner at different periods in human life. Cases of diphtheritic or croupous colitis were not infrequent in infants, while in adults the local lesion was occasionally restricted to catarrhal inflammation. It is thus evident that the prevalence of catarrhal dysentery in young children, revealed at autopsy, was only relative.

It is well known that shigella infection in adults may cause but a relatively slight diarrhoea with a simple catarrhal inflammation of the colonic mucosa detected by sigmoidoscopy. These cases are not included in autopsy statistics. But a diarrhoea easily tolerated by an adult, who sometimes even fails to consult a doctor, may prove fatal for a child. A similar conclusion was drawn by Vishnevetskaya (1941), but Skvortsov (1946) considered that death of young children from an essentially mild dysentery is actually due to vital functions being upset, which may be fatal for the young organism. It remains to be determined whether dysenteric infection itself can either be severe or relatively mild.

COMPARISON OF PATHOLOGICAL AND BACTERIOLOGICAL FINDINGS

Clinical evidence has shown that *Sh. dysenteriae* 1 is associated with the severest forms of dysentery, whereas *Sh. sonnei* is usually dangerous to young children. However, neither Lorentzen (1923), nor Huebschman (1925), nor Ter-Grigorova (1938) could find any significant difference in the morbid anatomy of dysentery caused by different *Shigella* types. The two former investigators based their conclusions on the results of 10 and 6 post-mortem investigations, respectively. Ter-Grigorova had an extensive material but she, too, used an incomplete classification of dysentery bacilli into "Shiga" and "Flexner" bacilli (the latter including "Y-bacilli" and "Strong's bacilli", as formerly distinguished).

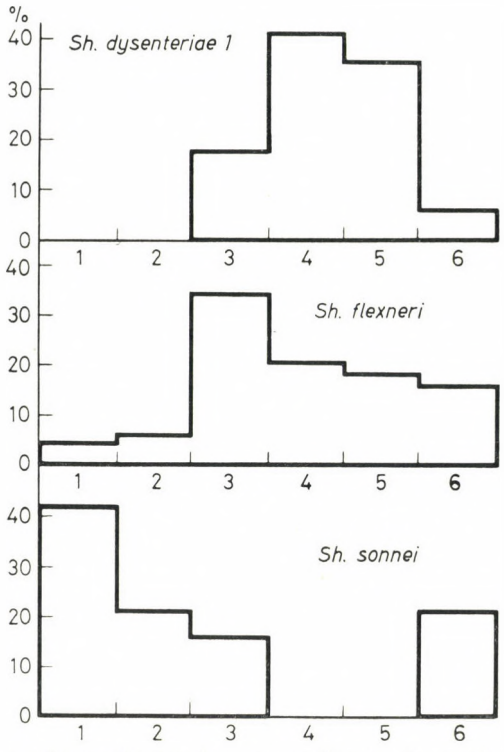


Fig. 4-1. Distribution of intestinal lesions according to the pathogens *Sh. dysenteriae* 1 (121 cases), *Sh. flexneri* (558 cases), and *Sh. sonnei*. *1* Bowel at autopsy without any apparent alterations; *2* slight catarrhal colitis; *3* pronounced catarrhal and catarrhal-ulcerative colitis; *4* fibrinous and fibrinous-ulcerative colitis; *5* chronic ulcerative colitis; *6* chronic catarrhal colitis (Voino-Yasenetskaya et al., 1942)

Figure 4-1 shows the distribution of the forms of colitis referable to the principal types of shigella (Voino-Yasenetskaya et al., 1942). *Sh. dysenteriae* 1 was mostly isolated from cases of fibrinous or ulcerative colitis, and less frequently from severe cases of catarrhal colitis. *Sh. flexneri* occurred both in fibrinous colitis and in the absence of any apparent lesion of the intestinal mucosa.* The mildest lesions were produced by *Sh. sonnei:* in 8 out of 19 cases the intestinal wall was almost normal in appearance at autopsy in young children with so-called toxic dyspepsia.

One cannot claim, of course, that the nature of lesions in dysentery depends entirely on the type of the causative agent and that the response of the host is of no importance. It would equally be erroneous to attribute too great an importance to host reactivity, ignoring differences in pathogenicity among members of the *Shigella* group.

* There is no evidence as yet that differences in the severity of intestinal lesions vary with types within the *Sh. flexneri* group.

It may be seen among the cases presented in Fig. 4-1 that *Sh. sonnei* dysentery occurred much less frequently than *Sh. dysenteriae* 1 or *Sh. flexneri* dysentery. At the time when these observations were made *Sh. sonnei* was not frequent; today *Sh. sonnei* and *Sh. flexneri* have become the leading types among agents of dysentery, while *Sh. dysenteriae* 1 have disappeared almost everywhere. Accordingly, post-mortem examination in rare cases of death from dysentery reveals a prevalence of mild catarrhal or catarrhal-ulcerative colitis. It is usually considered that this decreased severity of intestinal lesions is mainly due to the use of antibiotics. This might be so, but severe cases of colitis are no longer seen post mortem even in cases without adequate treatment. Clinical or sigmoidoscopic examinations yield similar results. This is why we shall have to refer repeatedly to observations made in the past, in speculations on the pathogenesis of dysentery.

HYPOTHESES ON PATHOGENESIS

The clinical resemblance of dysenteric colitis to colitis observed in uraemia or sublimate poisoning has led to the assumption that in each of these cases the intestinal mucosa is injured by toxic products eliminated through it. Studies on the causative agent of dysentery have confirmed, rather than contradicted, this assumption. Attempts to inoculate animals with shigellae by the natural route produced inconsistent, or, more often, negative results (see Chapters 3 and 10). No apparent damage was produced by large quantities of toxic products of these bacteria introduced orally or directly into the intestinal lumen. On the other hand, parenteral administration of live or killed shigellae, or their disintegration products (endotoxin), as well as of *Sh. dysenteriae* 1 exotoxin, sometimes resulted in dysenteriform lesions in the intestine of rabbits and dogs (Conradi, 1903; Flexner and Sweet, 1906; Doerr, 1907; etc.).

Variations of the hypothesis that intestinal lesions in dysentery are due to release of toxin by the bacteria are still popular. Direct damage of the mucosa by toxic products released into the intestinal lumen, appears unlikely at present. Circulating dysenteric toxins are usually said to affect blood vessels of the intestinal wall selectively, either directly or through the mediation of the nervous system (Bibinova, 1932*a*, *b*; Penner and Bernheim, 1942, 1960; Letterer, 1944; Dergachev, 1954). This is an important correction since the possibility of toxins entering the intestinal lumen from the circulating blood has not been proved (Troitsky, 1952; Ado, 1963). Also, agents of dysentery or toxic products arising at their lysis have not been proved to find their way into circulation at the onset of the disease, before the intestine has been damaged.

Bacteraemia is not a characteristic feature of dysentery. Shigellae may but rarely be cultured from the blood, and if so, usually shortly before the patients' death or at post mortem (Fischer, 1929; Lentz and Prigge, 1931). Bacteraemia was not shown in cats, dogs or rabbits after oral inoculation in carefully conducted experiments by Sirotinin and Brodskaya (1959).

Only in young mice inoculated intragastrically with a blunt needle with large amounts of shigellae (up to 10^9) were the agents recovered occasionally from the blood and internal organs 5–15 minutes after inoculation (Cooper and Keller, 1948; Sinay et al., 1951; Obukhova, 1956). Although these investigators attributed their findings to the rapid penetration of bacteria through the gastric or intestinal wall, another route of penetration (discussed in Chapter 15) appears to be more likely. At any rate, these experiments are hardly comparable to the natural course of dysentery.

Since intoxication could not be explained by a direct entry of shigellae into the blood stream, colitis was supposed to depend on the action of endotoxin released in the intestine on disintegration of the bacteria and absorbed through the mucosa. This assumption is not confirmed by observations showing the undamaged intestine to be impervious or almost so to macromolecules similar in size to shigella endotoxin (Clark, 1959; Berczi, 1968; Berczi et al., 1968/1969; Walker and Isselbacher, 1974).

In animals fed with shigella endotoxin (Troitsky and Tumanyan, 1955; Li, 1959) or *E. coli* endotoxin (Fine et al., 1959; Ravin et al., 1960; Ravin and Fine, 1962) labelled with ^{32}P, radioactivity was found in the blood, which might be an evidence of the entry of antigen into circulation. However, radioactive isotopes which are insufficiently bound to endotoxin, may be split in the digestive tract and absorbed independently (Grechko and Sedova, 1960; Ondráček et al., 1966). Thus ^{51}Cr forming a firmer bond with antigens,* failed to get into the blood from the intestine in similar experiments (Sanford and Noyes, 1958).

Certain data indicate that, under abnormal conditions, like starvation or impaired intestinal peristalsis, certain amounts of products of bacterial lysis given in large doses orally may enter the blood circulation. In experiments by Bibinova et al. (1971) (see Chapter 10) part of the guinea pigs that had been starved and given opiates, even died after oral administration of shigella endotoxin. This finding, of course, cannot be accepted as a proof of massive intestinal absorption of toxic products. Formal et al. (1960) showed that LD_{50} of shigella endotoxin was 1700 mg in normal guinea pigs, while only 53 mg in starved animals and 4 mg in those poisoned with carbon tetrachloride. They attributed the results to an impairment of the detoxifying activity of the liver. In the experiments mentioned above, in which toxin was injected into the blood stream subcutaneously or intraperitoneally, extremely large, almost lethal doses were needed. Even these, as noted by Flexner and Sweet (1906), gave rise only to vascular disorders and desquamation of the small bowel epithelium. Lesions resembling certain forms of dysenteric colitis were only found in a few dogs and in about one-fourth to one-third of the rabbits that had died 1–3 days after injection (Conradi, 1903; Flexner and Sweet, 1906; Doerr, 1906; Olitsky and Kligler, 1920; Bibinova, 1932*a*, *b*). As a rule, these lesions (ulcers and fibrinous colitis) were located in the caecum without involving the sigmoid and rectum, which are the parts most severely affected by dysentery.

* Gupta and Reed (1969) have shown that different isotopes are bound to different structures of bacteria; e.g. ^{51}Cr labels capsular polysaccharides.

The lesions which are sometimes found in the caecum after parenteral administration of shigella toxin are no proof of any particular "tropism" of the toxin for this section of the intestine. It was demonstrated by Fell et al. (1961) that changes induced by impaired circulation healed rapidly in the small intestine, while in the large intestine, inhabited by numerous organisms, injured areas of the mucosa tended to become infected and ulcerated. A similar role of intercurrent bacterial infection in inflammation of the caecum in rabbits was claimed by some investigators, who studied the results of parenteral administration of shigella toxin (Doerr, 1907; Bibinova, 1932*a*, *b*; Letterer, 1944). These authors, however, realized that their results might have suggested that toxic products of shigellae exerted a selective haematogenic action on this particular section of the intestine. Indeed, Letterer (1944), who believed that primary toxic vascular injury was the decisive event in the development of dysenteric colitis, claimed that all subsequent phenomena were no longer specific for dysentery.

The question arises whether toxic products of shigellae do, in fact, exert a specific action. Since *Sh. dysenteriae* 1 is the only exotoxin producer, intestinal lesions are usually attributed to endotoxin present in all members of the *Shigella* group. Moreover, the action on the host of endotoxins produced by a variety of bacteria is essentially the same, in contrast to the selective activity of exotoxin (Burrows, 1951; Gilbert, 1960).

Later it became evident that under uniform conditions, i.e. in lethal parenteral intoxication, similar vascular disorders and intestinal lesions are brought about by endotoxins of *E. coli*, *Salmonella*, *Brucella* and *Serratia marcescens* (Franke, 1944–1945; Sandritter and Heymann, 1953; Golubeva, 1954; Heymann, 1955; MacLean and Well 1956; Weil and Spink, 1957; Lillehei and MacLean, 1958; Mullens et al., 1964). Filtrates of *Proteus* cultures (Güldner, 1936), exotoxins of *Sh. dysenteriae* 1, of *Corynebacterium diphtheriae* (Penner and Bernheim, 1942; Penner and Klein, 1952; Sandritter and Heymann, 1953; Heymann, 1953, 1955) and of staphylococci (Rigdon and Leff, 1936) had similar effects. Moreover, injections of parasympathomimetic agents (Moeller and Kirsner, 1954) or histamine (Brasher et al., 1955; Fell et al., 1961) acted in the same manner. Similar findings may be seen in different forms of shock (Noble and Collip, 1942; Penner and Bernheim, 1942; Kroneberg and Sandritter, 1953; Ross, 1957; Weil and Spink, 1957; Lillehei and Washington, 1957; Sanford and Noyes, 1958; Fine et al., 1959; Reinert et al., 1964; Sandritter, 1965; Wray and Thomlinson, 1969). It has been suggested that these pathological processes may be the result of homeostatic responses (Penner and Bernheim, 1942) apparently related to the Selye syndrome (Sandritter and Heymann, 1953; Kroneberg and Sandritter, 1953; Heymann, 1953, 1955).

Thus, all the basic assumptions underlying the hypotheses of a resorption-elimination mechanism alleged to be responsible for the intestinal lesions in dysentery, prove to be unfounded. Since it has been demonstrated that the mere presence of shigellae in the intestinal lumen fails to produce colitis either directly or indirectly, it remains to be supposed that the agent of dysentery tends to colonize within the intestinal wall.

EARLIER DATA AND CONJECTURES ON LOCALIZATION OF SHIGELLAE IN THE INTESTINAL MUCOSA

It is well known that, when the diagnosis of dysentery is supported by clinical and epidemiological evidence, isolation of shigellae from the faeces is not always possible, even if cultures are made during the first days of the disease, and if stools have the typical appearance containing mucus and blood. Bacteriological diagnosis can, however, be made in no more than half of the clinical cases of dysentery. It was due to this fact that the symptom complex of dysentery was suggested to be of poly-aetiological origin. Doubts were even raised as to the pathogenic role of the *Shigella* group (Davydovsky, 1956; etc.). At the same time bacteriologists disclosed the important fact, which still has not been adequately explained, that insistent, repeated culturing of faeces may often result in isolation of the agent of the disease. Shneerson et al. (1941) noted fluctuations in the faecal excretion of shigellae even in the course of a single day. This may only be possible if shigellae grow at a hidden site and enter the intestinal lumen at irregular intervals.

When Shiga (1898) obtained post-mortem cultures from three patients who died of dysentery, he found that shigellae could be isolated both from the surface of the damaged mucosa and from inside; those recovered from deeper layers tended to grow in purer cultures. Gross (1919) made post-mortem examinations early (1–5 hours after death) and cultured Shiga bacilli in 6 cases and Flexner bacilli in 5, taking the specimens from the tunica submucosa (after removing the intestinal mucosa). Šikl (1920)

TABLE 4-II

Connection between ante- and post-mortem culturing of shigellae and the condition of the colon

(Voino-Yasenetskaya et al., 1942)

Autopsy diagnosis	Ante-mortem cultures		Post-mortem cultures	
	No. of cases	Percentage of positive cultures	No. of cases	Percentage of positive cultures
Absence of intestinal lesions				
Control	14	0	94	0
Dyspepsia	264	6.4	292	8.9
Colitis				
Slightly catarrhal	58	20.7	130	31.5
Pronounced catarrhal	136	39.7	110	86.3
Fibrinous and fibrinous-catarrhal	189	52.4	85	83.5
Ulcerative	292	39.7	108	65.7
Catarrhal-ulcerative	117	47.0	61	67.2
Chronic catarrhal	205	21.5	127	44.1
Total	1275		1007	

obtained positive results in 59 out of 96 autopsy cases, culturing films of exudate or mucus from the surface of the gut and the contents of small cysts or abscesses. Both Shiga and Šikl noted that the bacteria were cultured mainly from the more severely affected parts of the intestine.

Shigellae were isolated from cultures taken from the surface of or inside the mucosa in dysentery by Solovyeva et al. (1938), Ter-Grigorova (1938), Izrailimsky (1939) and other investigators. Our observations, shown in Table 4-II, included a comparison, in a large number of patients, of faecal and post-mortem cultures (suspensions from specimens of the intestinal wall). As seen from the table, post-mortem cultures taken from intestinal wall tissue were, in all forms of colitis, 1.5 times or twice as frequently positive as faecal cultures, although these were repeated several times.

The purpose of these studies, carried out in Central Asia, was to elucidate the causative agents of intestinal infections which were then prevalent. Cultures of specimens from the colon wall proved beyond any doubt that the majority of severe cases of colitis (catarrhal and fibrinous) were of a dysenteric nature. At later stages of the disease—in cases of ulcerative or chronic catarrhal colitis—the percentage of positive post-mortem cultures was lower, but still somewhat higher than ante-mortem. In these cases negative cultures were not accepted to exclude the diagnosis of dysentery or as an admission of any other reason for the intestinal lesions.* When similar studies were undertaken some years later in another locality in western Ukraine—strikingly similar, in fact, almost identical results were obtained (Voino-Yasenetskaya and Voino-Yasenetsky, 1948). Thus, one may state that bacteriological confirmation of dysentery depends on some common factors, such as the technique of examination** and the peculiarities of pathogenesis of the infection.

In experimental animals Sirotinin and Brodskaya (1959) found the greatest amount of shigellae in mucus and in the mucosa of the large bowel, whereas they demonstrated very few bacteria in the muscular coat of the intestinal wall, similarly to the findings of Shiga (1898). Shigellae were sometimes detected outside the intestinal wall, namely in regional lymph nodes. These observations are of no particular importance, since lymph nodes are known to arrest any organism spreading beyond the local focus of infection. Gindes (1940) reported that at autopsy shigellae were more frequently detected in mesenteric lymph nodes than in the intestine. Grinfeld et al. (1959) cultured *Sh. sonnei* or *Sh. flexneri* from lymph nodes in several cases where the intestine was unaffected and the patients had died of non-dysenteric diseases. It was assumed that shigellae may survive

* In the area surveyed, the incidence of protozoal diseases—amoebiasis and balantidiases—was low; the belief, current among practitioners, that intestinal diseases were related to malaria, was not confirmed (Voino-Yasenetsky, 1943). As to the cases of mild colitis, their agents might have been enteropathogenic *Escherichia coli* not known at that time.

** Specimens for post-mortem cultures were taken from a small area of the intestine, usually from the rectum, before its lumen had been opened and the mucosa examined. In some cases negative cultures were due to the masking effect of abundant growth of *Proteus* in the nutrient media.

for a long time within lymph nodes. The fact that lymph nodes and follicles may act as reservoirs of shigellae was supported by Morgunov et al. (1965). These views are not new. Westenhöfer (1918) suggested that the pathogenesis of dysentery was related to that of typhoid fever or intestinal tuberculosis, where primary lymph node involvement is a characteristic feature. Although this alleged analogy was criticized severely (Löhlein, 1921; etc.), later it was claimed again by Felsen (1936, 1945) that the primary event in the development of dysenteric colitis affected lymph follicles of the colonic mucosa. The problem will be considered later, it may be stated here, however, that Felsen's claims are denied by the majority of pathologists today.

Important information on the location of shigellae could be expected from microscopic investigations. Up to recently, however, bacteria were hardly ever mentioned in reports on histology of dysenteric colitis. Ingrowth of various bacteria including Gram-positive ones through the intestinal wall (Grigoryev, 1891; Maksimovich, 1960) should evidently be attributed to post-mortem changes. With routine bacterioscopic methods, shigellae cannot be distinguished from other bacilli, e.g. *E. coli*. Lorentzen (1923) studied autopsy specimens from 16 cases of dysentery, obtained from Gross, who performed post-mortem investigations, as mentioned above, soon after death. Lorentzen applied various methods for staining the sections taken from the intestine and revealed a wide range of different organisms in surface layers of inflammatory scab-like deposits, while deeper, at the boundary with remnants of mucosa, there were only accumulations of short, coarse rods. He considered the latter to be dysentery bacilli. In two of these cases, which had run a very brief course, clusters of the same bacilli were seen in the surface layers of the mucosa that was not so severely damaged, and also "*in den oberflächlichen Epithelien und in den Epithelzellen einzelner in die Noduli versenkter Drüsenschläuche*". Lorentzen restricted his report to this brief description (without giving any illustrations), and apparently did not attach particular significance to his finding. At any rate, he failed to mention intraepithelial location of shigellae in the summary, merely saying that they penetrated the intestinal mucosa from the lumen. It should be noted that in areas where the epithelial layer had been destroyed Lorentzen sometimes found bacilli resembling shigellae even in the connective tissue of the lamina propria. Here they were arranged singly or in clusters and some of them were engulfed by histiocytes.

Lorentzen's investigations were not continued. Pathologists made various assumptions in their descriptions of lesions affecting the intestine in dysentery, attempting to explain their origin, but seldom tried to search for organisms in the intestinal wall. Letterer (1944) who used only Gram's method of staining (which is apt to reveal only superinfecting organisms, rather than the agent of dysentery) still ventured to claim: "*Ich glaube nicht, dass man aus dem färberisch oder kulturell geführten Nachweis von Ruhrerregern im Darmlumen oder in der Darmwand, auf oder in der Schleimhaut, Endgültiges schliessen darf*" (p. 692). It was found, however, that the explanation of the perplexing pathogenesis of dysentery was to be sought in the peculiar behaviour of its agent in the host.

Some of the particular biological features of shigellae have become known recently from morphologic studies of cells and tissues infected with these organisms. These studies were prompted by successful attempts to reproduce dysenteric infection outside the digestive tract, namely in the urinary bladder, conjunctiva, cornea and lungs of laboratory animals.

Thus, information on the essence of intestinal lesions in dysentery is rather controversial and is in itself insufficient for understanding the pathogenesis of this infectious disease. The possibility of the damaging effect of shigella endotoxins has not been proved and now it seems rather improbable. However, data stating that dysentery agents invade not only the intestinal lumen but also its walls, have long been available, and they are very important for evaluating the results of the latest experimental investigations dealt with in the following chapters.

CHAPTER 5

INTRAPERITONEAL CHALLENGE, CELL CULTURE AND CHICK EMBRYO EXPERIMENTS WITH SHIGELLAE

by

T. N. KHAVKIN and V. L. BELYANIN

INTRAPERITONEAL CHALLENGE WITH SHIGELLAE

Experiments with intraperitoneal inoculation of shigellae have been carried out since the beginning of this century. At first dogs, cats, rabbits and guinea pigs (Rosenthal, 1903; Raczynsky, 1904) were used; today the white mouse is preferred. Sufficiently high doses (varying widely in different cultures) induce symptoms of intoxication in mice: flaccidity, hyperthermia, convulsions, paralysis accompanied occasionally by diarrhoea and haemorrhagic colonic lesions. Shigellae usually multiply abundantly in the blood and internal organs. After receiving sublethal doses, some of the animals recover. Addition of mucin to cultures of shigellae increases their pathogenicity several hundred thousand or even million-fold (Sidbjerg and Hansen, 1943, cit. by Wundt, 1966).

The ability of shigellae to produce the above syndrome is usually attributed to toxic properties of the organisms (Selter, 1910; Wundt, 1966). For a long time these properties were regarded as signs of virulence and pathogenicity of shigellae. Intraperitoneal inoculation of animals was even regarded as a "peritoneal model" of dysentery and was used both for evaluating the virulence of different cultures and for studying the pathogenesis of dysentery. Time and again doubts were raised as to the correctness of this method. One of the arguments had been that oral inoculation of volunteers or monkeys with shigellae, found highly pathogenic in the peritoneal test, failed to cause dysentery (Shaughnessy et al., 1946*a*, *b*; Branham et al., 1949). The results of vaccination of human subjects and those of intraperitoneal tests with the vaccines on mice were likewise discordant (Troitsky, 1958*a*).

The most convincing arguments against experimental intraperitoneal inoculation of shigellae were advanced in the last decade, when their ability to multiply in epithelial cells, rather than their toxicity was shown to be the main factor of their specific pathogenicity. No parallelism was found between tests demonstrating the parasitic capacity of shigellae in the epithelium and experiments with intraperitoneal challenge of white mice. Cultures giving positive keratoconjunctival tests may be of low pathogenicity for mice when inoculated intraperitoneally, and, on the contrary, cultures failing to penetrate the epithelium and incapable of producing specific intestinal lesions, may be fatal to mice in the intraperitoneal test. If the bacteria of a culture lose their capacity to multiply in epithelial cells, there need not be a parallel significant decrease in the dose

necessary to cause the death of intraperitoneally inoculated mice. This prompted LaBrec et al. (1964), Stenzel (1962*b*, *c*, 1966), Nakamura (1967), Ogawa et al. (1967*a*), Petrovskaya and Blinova (1971) to consider intraperitoneal inoculation unsuitable for evaluating the pathogenic properties of shigellae.

Nevertheless, intraperitoneal inoculation is still in use in laboratory practice. It is applied in tests to detect virulence of shigellae, to determine the toxicity of their products and to analyse the effects of various external agents (drugs, radiation, etc.) on the organisms, as well as in experiments on active and passive immunity. It has also been attempted to correlate, with some restrictions, the syndrome provoked in mice with certain aspects of human dysentery (McGuire and Floyd, 1958).

The results of intraperitoneal inoculation are usually evaluated in terms of mortality (or survival) of the inoculated animals. The culture is administered to groups of mice in different dilutions, sometimes with the addition of 5 per cent mucin. Some of the animals die within a few hours after inoculation, and the deaths are recorded for three days. The results of the experiment are statistically analysed and expressed in terms of lethal or infective doses. Within each serotype of *Shigella*, lethal doses of different cultures may vary considerably but, as mentioned above, these differences are not correlated with the ability of any individual culture to grow within epithelial cells. In addition to the establishment of lethal doses, some investigators also prepared cultures from the peritoneal fluid, blood and internal organs, recording usually the bacterial counts in the fluid or tissue suspension.

With this kind of experiment, relationships between the bacteria and the cells in inflammatory exudates remain unknown. Unless it becomes clear what actually happens in the peritoneal cavity, it is impossible to form an adequate opinion of the nature of the pathological process.

Evidence derived from experimental studies on peritoneal exudate *in vitro* is inconclusive because it merely indicates that peritoneal cavity cells (polymorphonuclear leukocytes and macrophages) are capable of phagocytizing shigellae. It is, therefore, not possible to draw reliable conclusions from the data obtained with a number of different experimental techniques, as to the factors affecting phagocytosis. The difference in the properties of *Shigella* strains should not be neglected either.

Freidlin (1961, 1962) observed a pronounced phagocytosis of *Sh. flexneri* (previously not tested for epitheliotropism) by peritoneal cavity polymorphonuclear leukocytes of guinea pigs and detected reactive changes in the latter. The phagocytic ability of leukocytes taken from vaccinated animals increased tremendously. On the other hand, according to the observations of Calabi (1970), shigellae showed no distinctive liability to being phagocytized by polymorphonuclear leukocytes and bacterial resistance to phagocytosis was independent of the result of the keratoconjunctivitis test in guinea pigs. Up to now no evidence has been obtained on the relation between shigellae and peritoneal macrophages or on reactive processes occurring in the peritoneal tissue.

Experiments were performed in collaboration with Arbuzova (Pasteur Institute of Epidemiology and Microbiology, Leningrad), who was respon-

sible for bacteriological investigations aimed to elucidate the fate of bacilli introduced into the peritoneal cavity, and the reactive processes induced in the peritoneum of white mice inoculated with *Sh. sonnei*.

Two strains were studied: a pathogenic strain (giving a positive keratoconjunctival test in guinea pigs) and a non-pathogenic strain (negative with the same test). Different doses of 18-hour cultures of these strains were administered to the animals. The peritoneal fluid and the gastrocolic omentum were investigated. The omentum was studied since it is known to possess a phagocytic mechanism and to contain large numbers of histiocytes and macrophages (Maximow, 1927). The omentum is more apt to rapid and active response to microorganisms or foreign bodies than other peritoneal structures (Wilson and Miles, 1964). Moreover, spread preparations of the omentum are convenient for cytological examination.

The animals were sacrificed, 8 at a time, every 3–5 minutes, 3, 6, 9, 12, 24 and 48 hours after inoculation. Specimens were taken from 4 animals for bacteriological study, and from 4 animals for morphological investigation from each group of 8 animals. Separate cultures were made from the omental tissue and peritoneal fluid. Before grinding the omentum in saline, it was thoroughly rinsed.* Preliminary experiments showed that in this manner bacteria adsorbed onto the surface of the omentum could be removed. For cultures of peritoneal fluid, the peritoneal cavity was washed with 0.5 ml of saline, which was then collected and poured on a nutrient medium.

Both strains, irrespective of their capacity for intraepithelial multiplication, produced an infectious process. Immediately after inoculation a considerable part of the bacteria penetrated the blood; the rest remained distributed between the omentum and peritoneal fluid. Both organisms multiplied in the omentum, but mainly in the peritoneal fluid. Nine to 12 hours after inoculation the amount of bacteria cultured from the fluid was manifold compared to the initial values.

By this time the inflammatory response of the peritoneum was quite apparent. It was of an exudative nature, similar to that obtained after intraperitoneal administration of indifferent particles or other organisms. Accumulation of polymorphonuclears, as well as of macrophages (monocyte-type cells and omental histiocytes) was observed.

Independently of the strain and dose of shigellae, within the first 12 hours after inoculation, polymorphonuclears prevailed in the inflammatory exudate; 15–30 per cent of the cells of the exudate were macrophages. The intensity of inflammation, however, was different. With absolutely lethal doses of shigellae, inflammation was slight and infiltration of the omentum was scarce, occurring in minute foci. With sublethal doses, there was a stronger response.

As mentioned above, the omentum adsorbed only a small proportion of the bacteria introduced. Not less than 3 million organisms were needed for

* The method for the bacteriological investigation of the omentum is described in Chapter 14.

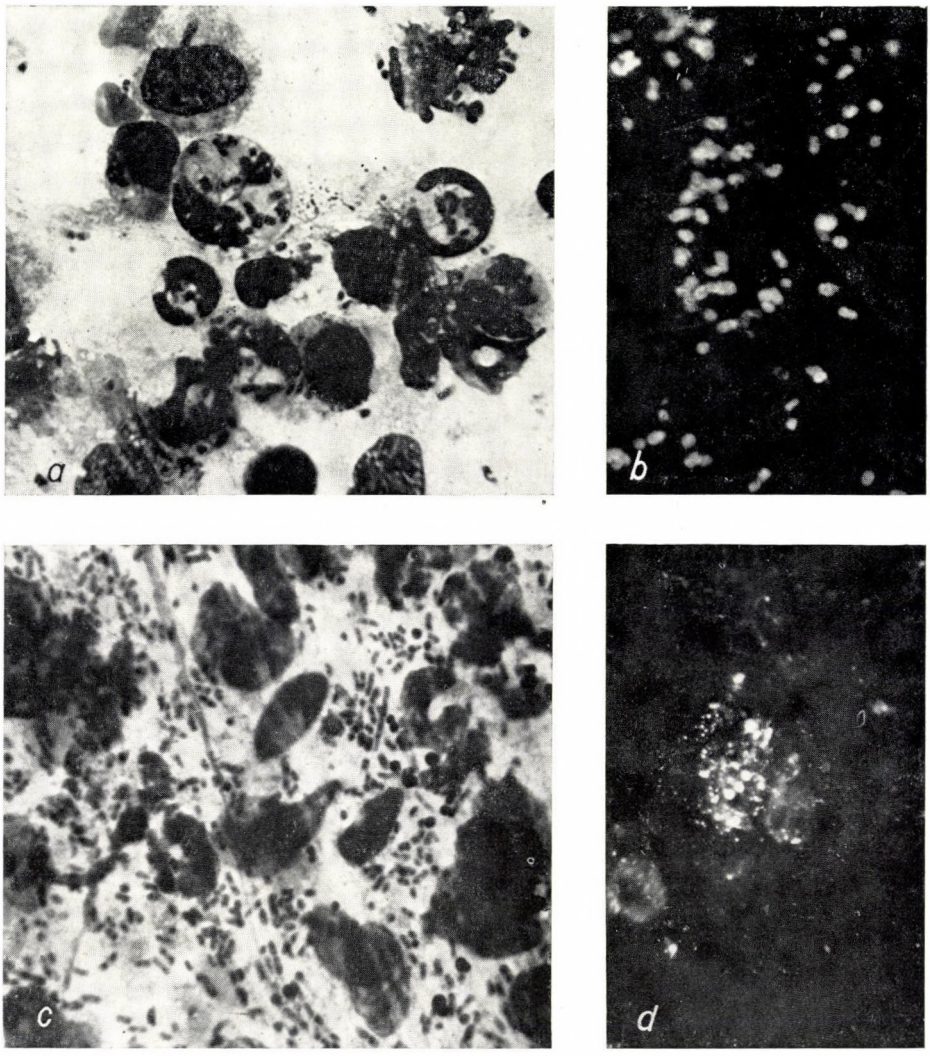


Fig. 5-1. Omental spread preparations of white mouse after intraperitoneal inoculation with *Sh. sonnei*. *a* 9 h after inoculation with 25×10^6 pathogenic organisms, Romanovsky stain. *b* 24 h after inoculation with 7×10^6 pathogenic organisms, immunofluorescent stain. *c* Similar picture, Romanovsky stain. *d* 24 h after inoculation with 3×10^6 non-pathogenic organisms; the picture shows a few bacteria and debris; immunofluorescent stain, $\times 960$

their successful recovery from the omental spread preparations and from the peritoneal smears. When the dose was high enough, the bacteria were revealed both extracellularly and in the cytoplasm of leukocytes, macrophages and histiocytes of the omentum. Even after inoculation with lethal doses of shigellae, the infiltrating leukocytes and macrophages contained engulfed organisms (Fig. 5-1*a*).

The state of phagocytized bacteria and of the cells that ingested them, showed that both an accomplished and an unaccomplished phagocytosis occurred in the peritoneum. Part of the shigellae were digested: they no longer had sharp outlines, were swollen and stained poorly. Others retained their usual morphological features and were even dividing. Similarly, among the leukocytes and macrophages that had ingested bacteria, there were cells showing no signs of injury, while others were definitely damaged (pyknotic nuclei, caryorhexis and considerably vacuolized or intensely basophilic cytoplasm). Finally, on the surface of the omentum microcolonies of shigellae were seen among the cell debris. Evidently, these colonies started to grow from surviving bacteria, released from destroyed phagocytes.

The lesions observed 9–12 hours after inoculation depended on the amount of organisms introduced into the peritoneal cavity. Microscopic findings closely correlated with the results of cultivation. Inoculation with lethal doses led to abundant multiplication of shigellae, while the inflammatory response in the peritoneum was suppressed. In the mice sacrificed after 24 hours, or succumbing at earlier periods, shigellae penetrated the entire omentum (Fig. 5-1*b*) and invaded the peritoneal fluid. Phagocytosis was infrequently seen and signs of digestion of bacteria were absent (Fig. 5-1*c*).

Inoculation with sublethal doses led to two types of infection: (*i*) either to an unrestrained growth of the agents in the peritoneal cavity and a suppression of the inflammatory response resulting in the death of the animals; or (*ii*) to a decrease in the number of bacteria resulting, by the end of the second day, in a complete clearance of shigellae of the peritoneal cavity and the subsequent recovery of the animals. When the outcome of infection was favourable, the number of bacteria revealed in histological preparations also decreased sharply, although the inflammatory infiltration of the peritoneum, mainly with macrophages, became more pronounced. At this period shigellae were only detected in the omentum with the fluorescent antibody technique. The bacteria formed small groups, or were arranged singly. Some of them maintained their characteristic outline with a brightly fluorescent rim, while of others, only the remnants were seen as amorphous lumps (Fig. 5-1*d*). In some of the mice sacrificed 48 hours after inoculation, no trace of antigenic material was found in the peritoneum.

Thus, parallel cultural and microscopic investigations of the peritoneum revealed a regular pattern of the infectious process following intraperitoneal inoculation with bacteria. This process was independent of the epithelium-invasive properties of shigellae. Since the peritoneum is not covered by epithelium, only the interaction between the infective agent and leukocytes, as well as macrophages of local and haematogenous origin, might be observed in the peritoneal cavity. The results of this interaction determine the outcome of the process: either a complete clearance of the peritoneum

of bacteria by phagocytosis, or a suppression of the inflammatory response and the death of host from septicaemia and intoxication.

Our observations fail to support the assumption that shigellae multiply within peritoneal macrophages (see below). There was no evidence of intracellular multiplication similar to that observed in salmonellosis (see Chapter 14). Although dividing shigellae were occasionally seen within leukocytes or macrophages, there was no way to decide whether they were dividing within the cell, or had been engulfed at the moment of division.

The results of these investigations strengthen the doubts raised in connection with the use of intraperitoneal inoculation for testing the pathogenic properties of shigellae. At the same time, intraperitoneal inoculation may be useful for studying the interaction between shigellae and phagocytic cells or for solving certain other problems related to shigellosis.

CELL CULTURE STUDIES

Since the 1960's, cell cultures have been widely used in the study of *Shigella*. The ability of these bacteria to multiply in growing cells was first described by Watkins (1960), and Gerber and Watkins (1961) and later also by other investigators (LaBrec et al., 1964; Bakhutashvili, 1967, 1968, 1969; Formal et al., 1967*a*; Nakamura, 1967; Ogawa et al., 1967*a*, *b*; 1968*a*; Yamada and Ogawa, 1967; Andreeva and Bakhutashvili, 1968; Bakhutashvili and Gulevich, 1968; Ogawa, 1970; Calabi, 1970; Belaya, 1970*a*; Nosova et al., 1970, 1972).

An important fact established in relation to the pathogenicity of these bacteria was, that only those strains multiplied in cell cultures which were epitheliotropic in animal experiments, i.e. which caused keratoconjunctivitis in guinea pigs and were pathogenic for orally infected monkeys and starved guinea pigs.

Cell culture methods and infectious procedures as well as the evaluation of the results vary somewhat according to different authors; the technique in general is as follows:

Suspensions of 24 h bacterial cultures are pipetted into tubes or dishes containing coverglasses with growing cells. One or two hours later these coverglass cultures are washed and transferred into a fresh medium. Antibiotics are added to prevent bacteria from multiplication in the medium, although Gerber and Watkins (1961), and Cefalu et al. (1963), showed that certain antibiotics inhibited shigella multiplication in the medium as well as within the cells. Cefalu et al. (1963) successfully replaced antibiotics with specific immune serum. Inhibition of the extracellular growth after the addition of antidysentery phage was observed by Bakhutashvili (1967). If the medium is free of bacterial growth inhibitors, the coverglasses with the infected cells are washed every 2–3 h with subsequent transfer into a fresh medium.

Wilder and Elberg (1973) believed that extensive washings failed to eliminate all the bacteria from the medium. In experiments with *Listeria* they showed that continuous phagocytosis of bacteria which grew in the

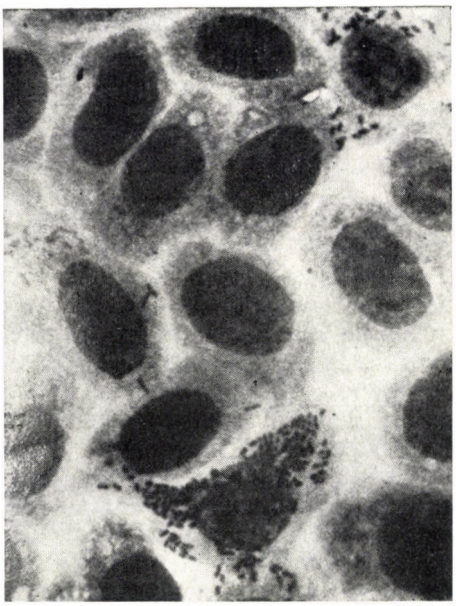


Fig. 5-2. HeLa cell monolayer 7 h after inoculation with pathogenic *Sh. flexneri*, Giemsa stain, ×420 (Formal et al., 1965*a*)

maintenance media appeared to account for the changes observed in the number of intracellular organisms.

As a rule, the results of cell and tissue culture experiments are read not later than 24–28 h periods, some experiments being limited to 2–8 h. The results are evaluated either in terms of infected cell counts in stained preparations or by estimating the number of organisms in the cultured cells. Sometimes the number of destroyed cells and the damage to cell layers are also taken into account.

Differences in the methods of infected cell cultivation can influence the ultimate results (Calabi, 1970) and hamper their comparison. It is also difficult to compare the results of bacteriological and microscopic examinations. The bacteriological techniques only reveal living bacteria in the cultures, while microscopic examination detects destroyed organisms as well.

It should be noted that avirulent shigellae can sometimes cause damage and death of these cells without penetrating them (Nakamura, 1967). Moreover, virulent organisms destroy infected cells at various rates. The ability to inhabit the growing cells correlates only with the epitheliotropism of shigellae; the cytotoxic properties of the organism play no significant role (Bondarenko et al., 1973*a*, *b*). Therefore, the intracellular bacterial multiplication remains, up to now, the most reliable indication of positive results. Besides, it is difficult sometimes to distinguish microscopically intracellular bacteria from bacteria adsorbed onto the cell surface.

Differences in experimental procedure and evaluation of the results of cell cultures infected with shigellae hamper the quantitative comparison of the data of different authors. However these differences do not diminish the significance of the discovery made with the aid of cell cultures, i.e. the selective ability of only epitheliotropic shigellae to penetrate the cells. It should be noted that the experimental results vary not only because of the differences in technique but also because of the divergent cell resistance to shigellae and the varying degree of intracellular multiplication among the representatives of the genus *Shigella*.

Special comparative investigations carried out by Bakhutashvili (1967) and Ogawa et al. (1967*a*, *b*) showed that shigellae possessed no species or organ-specific tropism to animal cells. Moreover, they were able to grow both in primary cultured, and in established cell lines. However, the multiplication rate and the amount of infected cells varied from culture to culture. Ogawa et al. demonstrated that 24 hours after infection the bacteria were found within no more than 5 per cent of cells in established cell lines (HeLa, L, human embryo intestinal epithelium) while in some primary cultured lines (rabbit cornea, mouse lung) 70–90 per cent of cells were affected as early as 6 hours after infection. Certain cell lines appeared to be completely resistant to pathogenic shigellae (Bakhutashvili, 1967; Nosova et al., 1970).

There are few data on the alteration of cells infected with microorganisms. Usually, the bacteria in such cells accumulate around the nucleus and sometimes fill the cell processes (Fig. 5-2). Frequently the cells retain their usual appearance for several hours. Bakhutashvili and Gulevich (1968) described mitosis of infected cells. The signs of injury as well as the destruction of the cells can be seen shortly after infection. The cell damage is preceded by the activation of cell metabolism and, in particular, by an increase in acid phosphatase activity (Jervis and LaBrec, 1966), which is a non-specific response to infection and is observed in cell cultures infected by a number of bacteria and viruses (Khesin, 1967). In the course of bacterial multiplication there is a decrease in the cell acid phosphatase activity, indicative of lysosomal damage; karyopyknosis and destructyon of the cytoplasm have also been observed (Jervis and LaBrec, 1966).

Experiments have also been made with cultures of peritoneal macrophages, which retain their strong phagocytic activity *in vitro* and are able to destroy the antigens taken up by them (Jacoby, 1965; Bennett, 1965, 1967*a*, *b*; Furth and Cohn, 1968). In contrast to other cells, the macrophages can engulf both pathogenic and non-pathogenic shigellae. However, pathogenic strains usually multiply actively and kill the phagocyte, while non-pathogenic ones grow slowly and are ultimately digested by the cell (Cefalu and Puglisi, 1967*a*; Belaya et al., 1968; Bondarenko et al., 1973*a*). The most striking differences between pathogenic and non-pathogenic strains are observed when the macrophages are infected with small doses (Cefalu et al., 1963); large doses of bacteria can destroy the cell layer independently of the organisms' virulence (Yee and Buffenmyer, 1970).

Ogawa et al. (1967*b*) showed that *Sh. flexneri* multiplied more actively than *Sh. sonnei*. They attributed this difference to the virulence of the for-

mer. However, the possible correlation between the multiplication rate of different shigellae in cell cultures and their virulence for animals has not yet been thoroughly studied.

The correlation between epitheliotropism of shigellae and their ability to multiply within culture cells allowed us to consider tissue cultures as an additional model which might be used for the determination of the specific pathogenicity of different shigellae, in addition to the keratoconjunctival and pulmonary tests and oral infection of starved guinea pigs. However, it is evident that standard and optimum experimental procedures must be elaborated for use in routine practice. Standardization should evidently involve the choice of cell lines, media, number of cells and bacteria, the method of prevention of extracellular bacterial multiplication as well as time schedule and analysis of results.

Infected cell cultures can also be used for theoretical studies. However, dedifferentiation (dependent on cell origin and preparation method) and the lack of the integrative influence of the host organism (Khlopin, 1946; Alov et al., 1969) may interfere with the interpretation of the results obtained. It was demonstrated that *in vitro* shigellae multiplied in cells in which they never occurred *in vivo*. Sometimes their propagation in such cells was more intensive than in those susceptible to infection *in vivo*.

As an example let us quote the experimental results with infection of guinea pig corneal cells in a primarily trypsinized culture consisting of two cell types: epithelial cells and fibroblasts (Ogawa et al., 1967*b*). Shigellae were shown to multiply more intensively in connective tissue cells than in the epithelium, susceptible to experimental keratoconjunctivitis in guinea pigs. These authors found that cell cultures from human intestinal epithelium, which is the predilectional site of shigella multiplication *in vivo*, were resistant to these bacteria.

Some investigators used tissue cultures in studies on the general phenomena of interaction between shigellae and the host cell. In this respect, the cinemicrographic studies of Ogawa et al. (1968*a*) and of Bakhutashvili (1969) are of great interest. They showed that, in contrast to avirulent strains of *Shigella*, the virulent ones could attach themselves to the cell surface. According to Ogawa et al. (1968*a*), at the attachment sites the cytoplasm showed a vigorous ruffling movement, invaginations and pinocytotic vesicles were formed and incorporation of the bacteria could be observed. The killed bacteria, even those of highly virulent strains, lost their capacity to become attached to cultured cells and failed to penetrate them.

Using a cinemicrographic method, Ogawa et al. (1968*a*) described the autonomous movements of shigellae and their polarity within the cells; one end of bacterium, "the head" always pointed in the direction of bacterial movement. They did not draw a definite conclusion concerning the mechanism of bacterial incorporation into the tissue culture cells which, as they noted, could be rather difficult to apply to the host. It remains to be elucidated whether this process is active or passive. If the process is passive, than it should be established whether it is connected with the normal cytoplasmic movement or whether it is the result of changes in the

colloidal state of the cytoplasm produced by the bacteria. Nevertheless, the data pointing to the role of the viability and autonomous movement of shigellae during the interaction with the cells deserve further consideration.

CHICK EMBRYO EXPERIMENTS

The ability of shigellae to multiply in chick embryos was described by Weil and Volentine in 1940. They made an attempt to use the infected embryo as a model for their investigations on the process of shigella infection. Further studies have shown that chick embryos are of little use for this purpose, but they can be employed for testing pathogenicity, virulence and toxicity of different strains.

Usually the embryos are infected on the 9th to 11th day; later they become resistant to shigellae. The bacterial culture is inoculated either into the extraembryonic area (i.e. yolk sac, surface or cavity of chorio-allantois) or into the yolk vein. The technique of inoculation into the extraembryonic area is the same as described by Schmidt and Lennete (1965) and Sokolov (1965) for viruses and rickettsiae. Kétyi (1966*b*) described the methods of intravenous shigella infection in detail. The results of infection are evaluated on the basis of the number of embryos dying and of cultural data.

The bacteria inoculated into the yolk sac reach the embryo more rapidly than those applied onto the chorio-allantoic surface. Death of the embryos sets in within 24–72 hours after infection depending upon the zone of inoculation, on the dose and on the properties of the strain used (Kabanova, 1952; Erlandson et al., 1958).

When inoculated intravenously the bacteria reach the embryo directly and may cause its death within the first 12 hours before they multiply significantly. Kétyi (1966*b*) suggested that rapid fatality was due to the effect of shigella toxin. Bacterial cultures unable to kill the embryo when inoculated into the extraembryonic zone were lethal when injected intravenously (Rauss et al., 1967).

Injected in relatively large doses (tens of thousands or millions of organisms), shigellae multiplied intensively and caused the embryo's death, independently of the pathogenicity of the culture for man and experimental animals. This fact aroused scepticism in a number of authors as regards the adequacy of chick embryo for shigella pathogenicity tests. According to Stenzel (1962*b*, *c*) both the chick embryo and the intraperitoneal method are of little value for this purpose. However, other authors do not share his doubts.

Belaya (1959, 1962*b*) inoculated embryos with different cultures and found significant differences between their effect in correlation with their pathogenicity in the keratoconjunctival test in guinea pigs. Fresh cultures with a positive keratoconjunctival test were able to cause 100 per cent embryonic death even when a small number of bacteria (2–200 cells) were inoculated into the extraembryonic zone. Bacteriological examination showed that these bacteria grew rapidly and abundantly in the allantoic fluid, blood, liver and brain. On the other hand, the minimum lethal dose

for the embryo was much larger ($2 \times 10^3 - 1 \times 10^{10}$ cells) if the culture was non-pathogenic in the keratoconjunctival test. The multiplication curve for these bacteria reflected a slow and gradual increase in the amount of viable bacteria which, even at the end of the experiment, did not reach the level which causes embryonic death. Shigellae were found in small amounts in the embryo's blood and organs but never in the brain.

Belaya found that in shigellae maintained for a long time in artificial medium, the loss of the ability to penetrate the corneal epithelium was accompanied by a decrease in pathogenicity for the chick embryo. The minimum lethal dose of such strains increased more than 100 000-fold, as compared with the parent organism. These observations were confirmed by Petrovskaya and Blinova (1971).

The changes occurring in the infected chick embryo, and the interrelations between the bacterium and chick embryo are almost unknown. There are only few data available on haemorrhage, purulent exudates and inflammatory infiltrations developing in envelopes of the embryo (Weil and Volentine, 1940; Erlandson et al., 1958; Elchinova, 1960). Levenbuk and Andreeva (1965*b*) observed shigellae in the cells of yolk and chorio-allantoic ectoderm. Nevertheless, the pathological processes occurring in the embryo have not yet been clearly defined, and the usefulness of the chick embryo test applying extraembryonic inoculation has not been established. The intravenous embryo infection, as Kétyi (1966*b*) assumed, can mainly be used for the evaluation of toxicity but parallel tests, using other experimental models, should be performed.

As shown by many researchers, when shigellae are administered intraperitoneally, an exudative inflammation of the peritoneum, accompanied by an abundant inflow of leukocytes, develops. Large bacterial doses rapidly suppress the defensive response and the animals die of sepsis. When relatively small doses are used, the organisms disappear from the peritoneum and the animals recover. The development of the infectious process in the peritoneal cavity does not depend on the capacity of shigellae to penetrate the epithelium and does not demonstrate pathogenic properties of the strains studied.

The ability of shigellae to multiply inside cells of cultures *in vitro* is correlated with their pathogenicity in enteral challenge and keratoconjunctival tests.

In intravenous challenge chick embryos die due to the effect of toxic products of shigellae. With other modes of challenge, generalized infection of the embryo arises regardless of the pathogenic properties of the strain.

CHAPTER 6

EXPERIMENTAL SHIGELLA KERATOCONJUNCTIVITIS

by

B. SERÉNY

Two decades have passed since the appearance of the first reports on the experimental conjunctival infection of guinea pigs and rabbit with *Shigella* and the description of the pathological processes produced thereby (Serény, 1954, 1955*a*, *b*). Within a short time, the method became widely used and thus the relevant literature can hardly be surveyed. The applied technique undoubtedly plays a role in its popularity, since the method is very simple, and the reading of the results is also simple and convenient because the provoked pathological process appears in the cornea and conjunctiva. Merely these advantages, however, would not have inspired so many researchers to apply this method repeatedly; its popularity can mainly be attributed to its reliable reproducibility. A further advantage of the method is the opportunity to study the interaction between the organisms and the host from different aspects. With this method of experimental conjunctival infection, shigellae and some other enteropathogenic microorganisms can be identified and their virulence determined; moreover, the model can be applied advantageously in serological, immunological and genetical experiments.

THE KERATOCONJUNCTIVAL TEST

History. Artificial conjunctival infection has been introduced in animal experiments by Calmette (1928), who pointed out that this kind of experimental infection is an equivalent of the infection ensuing within natural conditions.

First *Shigella dysenteriae*, then *Salmonella typhi*, *S. paratyphi-A* and *S. paratyphi-B* were used by Zoeller and co-workers (Zoeller and Bastouil, 1924; Zoeller and Manoussakis, 1924*a*). At that time sensitization with bile (Besredka, 1919) was of great importance in the induction of intestinal infections. The severely damaging effect of sterile cattle bile on the eyes of guinea pigs was established in preliminary experiments (Zoeller and Bastouil, 1924); a 75 per cent solution of the bile caused visible corneal turbidity lasting for 3–4 days. The eyes of the experimental animals were treated with this concentrated bile solution before the conjunctival infection, then the *Shigella* culture was introduced into the bottom of the conjunctival fornix. In this way keratoconjunctivitis developed, in 50 per cent

of the infected guinea pigs, followed by spontaneous healing. Reinfection caused keratoconjunctivitis again. With the same technique Zoeller and Bastouil (1924) could induce experimental keratoconjunctivitis with *S. paratyphi-A* and *S. paratyphi-B* cultures; recent observations failed to confirm this finding (Serény, 1955*a*, 1957; Gekker et al., 1957*a*, *b*; Yakhnina and Kuznetsova, 1959; Gekker, 1964). The explanation of the contradictory results was that the French authors administered the bacteria into tissues damaged artificially.

Bacterial flora of the guinea pig's conjunctival sac. Bacteria present in the normal guinea pig's eye are shown in Table 6-I (Serény, 1955*a*).

TABLE 6-I

Bacteria cultured from conjunctival samples of healthy guinea pigs

Organism	Incidence*	Average number of colonies**	
		on agar	on blood-agar
Corynebacterium pseudodiphtheriticum	100	29	28
Staphylococcus albus	100	7	18
Spore-forming aerobic bacilli	46	0.5	1.2
Sarcina	20	0.15	0.20
Escherichia coli	14	0.55	0.76
Staphylococcus aureus	6	0.10	0.06
Streptococcus	5	—	0.05
Neisseria catarrhalis	2	0.08	0.07
Proteus	2	0.03	0.01

* In per cent of samples.
** No. of colonies per plate, average of 100 plates.

Experimental animals and technique of bacterial cultures. Usually guinea pigs, rarely rabbits are used for the conjunctival test. For the identification of a freshly isolated suspected *Shigella* strain, sex, age, weight and colour of the animals may be neglected. For experiments to be evaluated statistically, a strictly homogeneous group of animals should be chosen.

Before the infection, each experimental animal should be examined, their weight and their temperature should be measured, and the condition of their eyes should be checked. Under special conditions, culturing of the conjunctival sample is desirable, in order to exclude the presence of enterobacteria (Serény, 1955*a*; Mackel et al., 1961).

The strain to be examined may be cultured in liquid (broth, peptone water, synthetic) or on solid (blood-agar, agar, etc.) medium. Optimum culturing time is 16–24 h. Depending on the weight of the animal, 0.01–0.02 ml should be inoculated into the conjunctival sac of the guinea pig.

With strains used for continuous examinations care should be taken to delay a decrease in virulence during storage (lyophilized or on Dorset egg culture-medium in a refrigerator). Subcultures should be made from colo-

nies appearing most suitable at oblique illumination (Serény, 1961*b*; Kerekes, 1962). Loss of virulence may be prevented by passage of the strain on the guinea pig's eye, or by the use of shigellae obtained from the exudate of chronic shigella keratoconjunctivitis (Belaya, 1961*a*). Selection of the colonies on the basis of their virulence, judged by their colony structure, provide a further means for the preservation of virulence. Serial inoculations without checking the colony structure will result in a fast decrease, or total loss, of virulence (Serény, 1955*a*, *b*; Gekker et al., 1957*a*, *b*; Noskov, 1959; Shantarenko and Tesla, 1960; Stenzel, 1963*a*; Ciufeco, 1970).

Technique of conjunctival infection. The bacterial culture should be inoculated into the space between the tarsal and the bulbal conjunctiva (the bottom of the conjunctival sac). Then the palpebrae should be kept closed for about one minute. Infection may be carried out with a platinum loop or glass rod (Serény, 1955*a*). Bacterial suspensions may be inoculated with a Pasteur pipette (Serény, 1958*a*) or with a thin injection needle fitted to a tuberculin syringe (Istrati and Istrati, 1964*a*, *b*). Virulent shigellae inoculated to the interpalpebral zone stick very quickly, therefore there is no need for the technical manipulations applied by some authors to ensure a successful conjunctival infection. Also, the simple technique resembles natural infection more closely.

The exact infective dose remains a problem in conjunctival infection. The bacterial count of the inoculum may be estimated by the usual methods within a reasonable limit of accuracy. The number of bacteria taking part in the infection is, however, far from being identical with the number introduced. As the eyeball is not situated in a closed cavity and is also in connection with the nasal cavity *via* the nasal duct, the inoculated bacteria may leave the site of entry within a short time. Yet, a greater part of the bacteria seem to remain in the conjunctival sac and participate in the induction of the infection. Washing of the conjunctival sac one minute after conjunctival infection does not prevent the development of shigella keratoconjunctivitis (Serény, 1955*a*, *b*). Conjunctival infection with graded doses is performed in biological experiments for mathematical-statistical analysis (Serény, 1958*b*, 1960*b*; Stypulkowska, 1962; Cross and Nakamura, 1970).

The degree of virulence may be estimated by use of a strain cultivated in liquid culture medium (Stypulkowska, 1962) or by use of a desiccated strain (Serény, 1960*b*). These methods allow the estimation of the minimum infective dose (MID) or the medium infective dose (ID_{50}), the values of which may vary considerably according to the strain used.

Infections conveyed by a contact route depend on the conditions under which the animals are kept. In overcrowded cages shigella keratoconjunctivitis will most probably spread to healthy animals. As excretion of the bacteria decreases with the progress of the disease, the possibility of spontaneous infections is reduced later on.

Infection may spread from one eye to the other in the same animal (Szturm-Rubinsten et al., 1957; Noskov, 1959).

Symptoms and course of shigella keratoconjunctivitis. The symptoms and course of shigella keratoconjunctivitis may be influenced, on the one hand,

TABLE 6-II

Incubation period of shigella keratoconjunctivitis after conjunctival infection with different numbers of bacteria

Technique	No. of bacteria	No. of animals developing signs/ after days				Total
		1	2	3	4	
Inoculation with loop	10^9	228	11	3	—	242
Inoculation with glass rod	10^9	15	—	—	—	15
Instillation	10^8	40	10	—	—	50
Instillation	10^7	34	40	5	—	79
Instillation	10^6	3	10	—	1	14
Total		320	71	8	1	400

by the general status of the host and, on the other, by the quantitative and qualitative relations of the pathogenic microorganism. The pathological process may change considerably due to the development of immunity (reactions of the immune host will be discussed later in this Chapter).

Incubation period. Immediately after conjunctival infection cytological alterations can be observed (Serény, 1955*a*; Rácz, 1963) and fluctuations in the number and resistance of the microorganisms are also evident.

The cellular reaction developing after conjunctival infection consists mainly in an appreciable increase in cellular elements (chiefly polymorphonuclear leukocytes) as early as 1–2 h after the infection; the cytoplasm of large mononuclear cells contains a conspicuously high number of bacteria (see Chapter 7). At first, pathogenic microorganisms inoculated into the conjunctival sac show signs of decay, and the resistance of the surviving ones is also reduced. Then surviving bacteria begin to multiply and their resistance increases (Serény, 1955*a*).

Estimation of the incubation period is arbitrary to a certain degree; the time lapse between the conjunctival infection and the first observable alteration seems to be the best approximation.

In case of a conjunctival infection with a great number of virulent shigellae, the first macroscopically observable alterations usually occur on the conjunctiva; the conjunctival sac and the plica semilunaris are hyperaemic and swollen, then chemosis and a mucopurulent exudate appear. The first signs of conjunctivitis might be observed within 5 h after conjunctival infection in 10 per cent, within 6 h in 6 per cent, within 7 h in 9 per cent, within 8 hours in 23 per cent, and within 12 h in 32 per cent of the animals. In the remaining 20 per cent, the first signs develop later.

Reduction of the infective dose results in a longer incubation period (Table 6-II).

Following conjunctival infection with minimum doses of virulent *Shigella* cultures or with cultures of low virulence, the first lesions on the cornea appeared as late as after 5–7 days in some of the cases (Gekker et al.,

1957*a*, *b*; Manolov, 1957*a*, *b*; Szturm-Rubinsten et al., 1957; Siroko, 1958; Gorea, 1959; Yakhnina and Kuznetsova, 1959; Shantarenko and Tesla, 1960; Dunareanu and Brindus, 1961; Mackel et al., 1961.; Gleiberman et al., 1964; Lin et al., 1964; Ciufeco, 1970; Kandyurina, 1970*a*, *b*).

Conjunctivitis. Shigella keratoconjunctivitis usually starts with fever and with acute conjunctivitis accompanied by abundant exudation. Acute conjunctivitis in itself is not characteristic of shigella infections, because it may be produced by other bacteria (mainly salmonellae). Some minor alterations (hyperaemia, oedema) may be frequent on the conjunctiva of normal guinea pigs (mainly albinos) and cannot, therefore, be regarded as signs of infection. The criteria of experimental conjunctivitis include significant changes appearing on a completely normal conjunctiva. Conjunctival lesions usually not observed under physiological conditions are: strong erythema and swelling of the conjunctival sac, resulting in the appearance of a circular, red, thread-like formation between the tarsal and the bulbar conjunctiva. Hyperaemia and oedema extend to the plica semilunaris of the bulbar conjunctiva, and are observable on the following days, when chemosis spreads without swelling of the conjunctival sac.

Chemosis is a reliable sign of the development of conjunctivitis and also of its healing. The bulbar conjunctiva and the underlying connective tissue become highly oedematous, so that the conjunctiva overlaps the sclera and covers it almost totally: as long as chemosis lasts, the sclera cannot be seen. This symptom is observable as early as the beginning of conjunctivitis, and persists even when other symptoms (exudation, hyperaemia) have subsided. The onset of chemosis depends on the virulence of the culture and on the infective dose; the higher the virulence and the greater the number of the infecting organisms, the earlier this sign will develop. Chemosis is usually evident on the day following conjunctival infection; with less virulent strains and smaller number of bacteria it appears after 2 or 3 days or may be absent. It usually lasts for 2–3 weeks, but its duration varies with the strain used; it may cease after 4 days, or may persist for a longer time, occasionally for more than a month (Serény, 1955*a*).

Shigella keratoconjunctivitis is usually accompanied by the production of abundant exudate. Microscopically, the exudation starts immediately after conjunctival infection; the mucopurulent exudate, however, becomes visible only about 6–24 h later. Its volume gradually increases and reaches the maximum about 2–3 days after the conjunctival infection, when a turbid, white, tough, mucopurulent exudate fills the interpalpebral zone almost totally; occasionally it covers the whole bulb. The abundant exudate may run over the confines of the interpalpebral zone, and, while trickling down the fur of the animal, may propagate the infection. Tough exudate may make the palpebrae stick together. The infected eye is often held closed for a few days. The volume of the exudate usually decreases after one week. Microscopic examination shows the presence of numerous polymorphonuclear leukocytes for several days following macroscopic recovery.

Inflammatory oedema rapidly spreads to the palpebrae, which become strongly swollen. Not only a marked narrowing of the interpalpebral zone but also ectropium may occur. The severely swollen conjunctiva causes

a bulging of the palpebra, which first assumes an oblique position, then it may be turned out almost entirely. Thus the tarsal conjunctiva becomes detached from the cornea.

The above-mentioned symptoms appear quickly and an acute, intense, purulent conjunctivitis develops in most of the animals as early as one day after the conjunctival infection.

The tarsal conjunctiva may be pale because of severe oedema and increased tissue pressure; no vascularization can be observed. Hyperaemia of the bulbar conjunctiva appears in the form of a red, violet-iridescent ring around the cornea. During the second week isolated pin-head-sized infiltrations appear on the conjunctiva, the confluence of which results in the development of larger spots with a thick, mucopurulent content. Smaller ulcerations may also be observed.

After a few days the inflammatory oedema decreases, the ectropium ceases and the palpebrae regain their original thickness and position. The exudation also lessens, finally the sclera becomes visible. Some symptoms, particularly the moderate thickening of the palpebrae and chemosis may persist for a longer period (Serény, 1955*a*; Gekker et al., 1957*a*, *b*; Manolov, 1957*a*, *b*; Siroko, 1957, 1958; Szturm-Rubinsten et al., 1957; Gorea, 1959; Piéchaud and Szturm-Rubinsten, 1959; Yakhnina and Kuznetsova, 1959; Dunareanu and Brindus, 1961; Mackel et al., 1961; Rácz and Serény, 1962; Stenzel, 1962*b*, *c*; Rácz, 1963; Ciufeco, 1970).

Keratitis. Inflammation of the conjunctiva is always accompanied by keratitis when the conjunctival infection has been carried out with an adequate number of virulent shigellae. Conjunctivitis appears earlier and is usually well established by the time the first signs of keratitis are observed on the cornea. Sometimes the corneal lesion is only associated with a mild conjunctivitis, or observable signs of conjunctivitis may even be absent.

A typical shigella keratoconjunctivitis is characterized by a diffuse, rough turbidity extending to the whole corneal surface. Occasionally the alterations start as pin-point-sized small infiltrations. The strong turbidity of the cornea is associated with an increased infiltration and with oedematous changes (Rácz, 1963).

A few days after the conjunctival infection the cornea loses its brilliance, becomes faint, a mild opacity appears in the region of the limbus, and small epithelial erosions, stainable with fluorescein, develop. On the following day the cornea becomes totally opaque and its turbidity increases progressively. On the 1st to 4th day the cornea becomes unclean, white coloured and turbid as a result of rough, diffuse infiltration and oedema masking the contours of the pupil so that the iris and pupil cannot be observed by focal illumination. The entire corneal surface stains with fluorescein.

The margin of the bulbar conjunctiva appears at the border of the turbid cornea as a narrow, hazy, unclean, white, hardly prominent frame. The alterations observable in the course of subsequent days seemingly start from this region. The circular detachment of the epithelium, which is formed on the cornea and consists in reality of debris and leukocytes (Rácz, 1963), starts from the limbus and results in a progressive denudation of the loosened, swollen, red cornea. In the "red ring" phase the cornea is

ulcerated (Rácz, 1963), becomes severly infiltrated with leukocytes and thickens four to five-fold in consequence of intense oedema. On the 3rd to 5th day the swollen bulbar conjunctiva extends over to the cornea without a definite borderline. It may give the impression that the conjunctiva has grown onto the cornea. The area of the rough coat decreases gradually within the red ring and disappears on the 7th to 14th day of the disease. The gradually broadening ring is covered with epithelium and is unstainable with fluorescein.

As soon as the coat has detached itself from the cornea, but frequently earlier, swelling and loosening decrease, the red colour fades, and the cornea regains its normal flat shape. Histologically the thickness of the cornea decreases and the oedema disappears (Rácz, 1963). The process starts from the periphery and progresses towards the centre in the form of a gradually broadening ring. When it reaches the centre, the eyeball is covered by a dark, highly turbid, uneven, rough surfaced formation. The flattening of the cornea occurs usually between the 10th and 20th day of the disease (Serény, 1955*a*).

Simultaneously, with the gradual flattening of the loosened cornea, a vascularization may be observed, both superficially and deeper in the cornea. The superficial vessels resemble the branches of a tree, while the deep ones look like brushes or brooms, and do not extend beyond the margin of the cornea. The higher number of superficial vessels suggests that the process takes place mainly in the superficial layers of the cornea.

The formation of the new epithelial layer of the cornea does not mean an accomplished reparation; in this state the cornea has not yet regained its original state. Its surface is rough and granulated, and it has not regained its normal transparency. In animals with light-coloured fur complete gross recovery ensues within a few days. In dark-furred animals, however, a longer period (from the 16th–21st days to the 18th–23rd days) may be necessary for complete recovery or the cornea may even remain in a permanently turbid state. Occasionally a few smaller or larger pigmented spots may persist (Serény, 1955*a*, *b*; Gekker et al., 1957*a*, *b*; Manolov, 1957*a*, *b*; Szturm-Rubinstein et al., 1957; Siroko, 1957, 1958; Yakhnina and Kuznetsova, 1959; Dunareanu and Brindus, 1961; Mackel et al., 1961; Stenzel, 1962*b*, *c*; Rácz, 1963; Gleiberman et al., 1964; Lin et al., 1964; Ciufeco, 1970).

Body temperature. In shigella keratoconjunctivitis the body temperature of guinea pigs raises over the normal level, but is usually not more than 40.5 °C, and never exceeds 41 °C. A rise in temperature on the day of the infection occurs only exceptionally (6.6 per cent). It is most frequent on the first (76 per cent), and second (17.4 per cent) day after the infection. In about half of the cases the fever lasts 24–72 h; in about 25 per cent, 2–6 hours; and in about 25 per cent, 6–24 h. Later the temperature decreases below 40 °C and remains at this level until the end of the disease (Serény, 1955*a*; Ciufeco, 1970).

Body weight. A loss of body weight occurs in some of the animals 2–3 days after the conjunctival infection. A few days later the weight begins to increase (Serény, 1955*a*; Ciufeco, 1970).

Bacteraemia. Bacteraemia lasting for a short time can be relatively frequently revealed in shigella keratoconjunctivitis. The number of bacteria reaching the blood is small. The bacteraemia has no significance in the pathogenesis of keratoconjunctivitis; shigellae usually enter the circulation via the lesions of the eye. Exceptionally a shigella-sepsis may develop (Serény, 1955*a*; Andreeva, 1962; Levenbuk and Andreeva, 1962; Lin et al., 1964; Ciufeco, 1970).

Recovery of shigellae from infected eyes. Virulent shigellae inoculated into the conjunctival sac can be cultured from ocular discharge from the very beginning of the infection until healing.

The course of shigella keratoconjunctivitis can be adequately followed by culturing. The number of shigellae in the exudate increases after a transient decrease following conjunctival infection. The increase of the bacterial count precedes the development of local lesions. As long as the acute state of shigella keratoconjunctivitis lasts, the number of shigellae remains at a maximum level. The local lesions usually persist even after the specimens become negative for shigellae. The presence of bacteria influences the process of healing; after their disappearance, recovery is not hindered. In the majority of animals with shigella keratoconjunctivitis the conjunctival exudate becomes negative in 2–3 weeks.

In the acute stage shigellae may be cultivated from different parts of the eye (conjunctiva, cornea, capsula of Tenon), but the bacteriological examinations of the internal organs and the faeces yield usually negative results (Serény, 1955*a*, *b*; Gekker et al., 1957*a*, *b*; Manolov, 1957*a*, *b*; Szturm-Rubinsten et al., 1957; Siroko, 1957, 1958; Gorea, 1959; Yakhnina and Kuznetsova, 1959; Mackel et al., 1961; Strongovskaya, 1963; Gleiberman et al., 1964; Lin et al., 1964; Ciufeco, 1970; Cross and Nakamura, 1970).

Serological examination. Agglutinins for shigellae cannot be detected in the blood serum of healthy animals. Application of living avirulent or killed *Shigella* cultures not inducing a reaction in the eye, may give rise to specific antibody production due to the absorption of endotoxins. In keratoconjunctivitis type-specific agglutinins appear on the second week, and rapidly reach a maximum titre (1:80–1:640). From the third week on the agglutinating capacity of the blood serum decreases and drops to zero in 2–3 months. Antibody production may be entirely absent (Serény, 1955*a*; Gekker et al., 1957*a*, *b*; Gekker and Belaya, 1968; Gorea, 1959; Noskov, 1959; Shantarenko, 1959; Yakhnina and Kuznetsova, 1959; Belaya, 1964; Gleiberman et al., 1964; Lin et al., 1964; Aldova et al., 1968; Ciufeco, 1970; Cross and Nakamura, 1970). The haemagglutinin titre of the blood serum runs parallel with agglutination (Belaya, 1964; Aldova et al., 1968). The increase in the number of protective antibodies during shigella keratoconjunctivitis is similar to that in human dysentery. Development and specificity of these antibodies are shown in Tables 6-III and 6-IV. There is no correlation between the protective effect and the agglutinin titre of the serum (Serény, 1958*b*; Ogawa et al., 1966*b*; Aldova et al., 1968). Specific haemagglutinins may be found in the conjunctival exudate, as well as in the cornea, sclera and iris of infected guinea pigs (Lin et al., 1964; Aldova et al., 1968).

TABLE 6-III

*Protective capacity of guinea pig serum against the homologous strain after recovery from shigella keratoconjunctivitis**

Ist series of experiments

(*a*) Number of survivals in groups of 25 mice

Serum dose, ml	*Sh. flexneri 3* rabbit hyperimmune serum	Guinea pig serum	
		after recovery from keratoconjunctivitis	untreated animals
0.1	—	—	13
0.025	—	17	10
0.00625	24	10	6
0.0015625	19	9	—
0.000390625	12	—	—

(*b*) Percentage protective capacity

Serum	Relative potency	Fiducial limits
Rabbit hyperimmune serum	100	—
Guinea pig sera:		
after recovery from keratoconjunctivitis	4.2	0.61–12.6
untreated animals	0.61	0.14–1.50

IInd series

(*a*) Number of survivals in groups of 25 mice

Serum dose, ml	First experiment (11th January)		Second experiment (1st February)	
	Sh. flexneri rabbit hyperimmune serum	Guinea pig serum after recovery from keratoconjunctivitis	Guinea pig serum after recovery from keratoconjunctivitis	Untreated animals
0.1	—	—	—	12
0.025	—	21	15	11
0.00625	24	20	11	4
0.0015625	23	16	6	—
0.000390625	16	—	—	—

* Conjunctival infection: *Sh. flexneri* 3 and *Sh. sonnei* "S" (in the IIIrd series).
Method (mouse protection test): subcutaneous immunization followed, one hour later, by intraabdominal challenge with a 0.5 ml suspension containing 0.08 μg dried *Sh. flexneri* 3 *(Sh. sonnei)* in 5 per cent mucin.
Observation period: 3 days.

TABLE 6-III *(cont'd)*

(*b*) Percentage protective capacity

Serum	Relative potency	Fiducial limits
Rabbit hyperimmune serum	100	—
Guinea pig serum after recovery from keratoconjunctivitis	10.9	1.3–41.2
Guinea pig sera:		
after recovery from keratoconjunctivitis	100	—
untreated animals	14.5	2.2–62.9

IIIrd series

(*a*) Number of survivals in groups of 25 mice

Serum dose, ml	*Sh. sonnei* "S" rabbit hyperimmune serum	Guinea pig serum	
		after recovery from keratoconjunctivitis	untreated animals
0.1	—	—	3
0.025	—	9	2
0.00625	21	5	1
0.0015625	9	1	—
0.000390625	7	—	—

(*b*) Percentage protective capacity

Serum	Relative potency	Fiducial limits
Rabbit hyperimmune serum	100	—
Guinea pig sera:		
after recovery from keratoconjunctivitis	3.8	0.9–9.5
untreated animals	0.2	0.01–0.8

Chronic shigella keratoconjunctivitis. There are great differences in the course of keratoconjunctivitis infections. In the majority of the cases (about 90 per cent) there is a spontaneous healing within 2–3 weeks, while 5 per cent recover within a month. In about 3 per cent recovery takes 2–3 months. Finally in about 2 per cent of the cases chronic keratoconjunctivitis may develop (Serény, 1955*a*), which may, however, be as high as 13 per cent (Gorea, 1959).

The development of chronic keratoconjunctivitis is determined by the condition of the host and not by the properties of the invading organisms. This is suggested by the fact that only part of the animals infected simultaneously with the same microorganism develop chronic disease, moreover, a

TABLE 6-IV

*Protective capacity of guinea pig serum against the homologous strain after recovery from shigella keratoconjunctivitis**

Ist series

(*a*) Number of survivals in groups of 25 mice

Serum dose, ml	Guinea pig serum		
	after recovery from keratoconjunctivitis induced by		untreated animals
	Sh. sonnei	*Sh. flexneri*	
0.1	—	4	3
0.025	10	2	2
0.00625	6	1	1
0.0015625	4	—	—

(*b*) Percentage protective capacity

Serum	Relative potency	Fiducial limits
Homologous guinea pig serum	100	—
Heterologous guinea pig serum	1.7	$8.9 \cdot 10^{-6}$–11.2
Untreated guinea pig serum	0.95	$3.3 \cdot 10^{-15}$–8.5

IInd series

(*a*) Number of survivals in groups of 25 mice

Serum dose, ml	*Sh. sonnei* "S" rabbit hyperimmune serum	Guinea pig serum	
		after recovery from keratoconjunctivitis by *Sh. sonnei*	untreated animals
0.1	10	10	11
0.025	9	9	9
0.00625	4	7	2

(*b*) Percentage protective capacity

Serum	Relative potency	Fiducial limits
Heterologous rabbit hyperimmune serum	1	—
Guinea pig sera:		
after recovery from keratoconjunctivitis	1.85	0.17–109.4
untreated animals	1.12	0.23–5.98

* *Serum:* pooled guinea pig serum obtained during convalescence following shigella keratoconjunctivitis induced with *Sh. flexneri* 3.
Control serum: Pooled guinea pig serum obtained during convalescence following shigella keratoconjunctivitis induced with *Sh. sonnei* (Ist series) and *Sh. sonnei* "S" rabbit hyperimmune serum (IInd series).
Method (mouse protection test): gradual subcutaneous immunization followed, one hour later, by intraabdominal challenge with 0.5 ml suspension containing 0.08 μg dried *Sh. sonnei* in 5 per cent mucin.
Observation period: 3 days.

simultaneous infection of both eyes of the same animal may result in a chronic process in only one of the eyes. In the majority of the cases chronic shigella keratoconjunctivitis is a primary disease running its course before sufficient immunity could develop.

Chronic shigella keratoconjunctivitis responds well to aimed treatment with antibiotics. A spontaneous recovery is also possible, the first sign of which is the absence of bacteria from the conjunctival exudate. Afterwards the loosened cornea flattens and more or less regains its usual appearence. Chemosis also ceases, but the eye heals with permanent lesions (Serény, 1955*a*, *b*, 1957; Gekker et al., 1957*a*, *b*; Manolov, 1957*a*, *b*; Szturm-Rubinsten et al., 1957; Gekker and Belaya, 1958; Siroko, 1957, 1958; Gorea, 1959; Yakhnina and Kuznetsova, 1959; Shantarenko and Tesla, 1960; Dunareanu and Brindus, 1961; Mackel et al., 1961; Stenzel, 1962*b*, *c*; Belaya, 1964; Istrati and Istrati, 1964*a*, *b*; Lin et al., 1964; Ciufeco, 1970).

Sepsis. A shigella sepsis may occur exceptionally after conjunctival infection (0.7 per cent). At a later stage of the disease (3–4 weeks) the local process may flare up, the bacterial count, showing previously a decreasing tendency, may rise suddenly and the body temperature may also increase. The general condition of the animal worsens rapidly and it soon dies. At autopsy the weight of the spleen is found increased and in the liver there are irregularly shaped, compact, yellow foci, 4–5 mm in diameter. Shigellae may be cultivated from different organs (liver, spleen, omentum, suprarenal glands, myocardium, ileum, colon) as well as from all parts of the eye (Serény, 1955*a*).

The prognosis of shigella keratoconjunctivitis is good. The disease is liable to spontaneous healing and only exceptionally does it end in death.

FACTORS DETERMINING THE DEVELOPMENT OF SHIGELLA KERATOCONJUNCTIVITIS

The primary infection of a guinea pig's eye with a large dose of a virulent *Shigella* culture results in shigella keratoconjunctivitis in every case. The consequent occurrence of experimental shigellosis would suggest that a successful infection depends only on the virulence and the quantity of the bacterium. However, serial conjunctival infections carried out with lower numbers of bacteria have proved that the development of the disease depends not only on the microorganism but also on the host. The interaction between the microorganisms and the host may be observed immediately after the conjunctival infection (leukocyte emigration to the conjunctival exudate, or total destruction of bacteria).

THE PATHOGENIC AGENT

Species and serotypes of Shigella. The most obvious difference between shigella keratoconjunctivitis induced by *Sh. flexneri* and by *Sh. sonnei* is in the intensity and duration of the disease. The number of *Sh. sonnei*

cells usually decreases rapidly in the conjunctival exudate at the end of the first week. In *Sh. flexneri* infection the decrease occurs, as a rule, later. Both disease and recovery have a more rapid course with *Sh. sonnei* as compared with *Sh. flexneri*. In *Sh. flexneri* infections, the exudation is more intensive and lasts longer in most of the cases. Strains belonging to subgroups B, C and D elicit more purulent lesions than bacteria of the A subgroup (Serény, 1955*a*, *b*; Szturm-Rubinsten et al., 1957; Gekker and Belaya, 1958; Yakhnina and Kuznetsova, 1959; Shantarenko and Tesla, 1960; Istrati and Istrati, 1964*a*; Lin et al., 1964; Ciufeco, 1970).

Infecting dose. A certain correlation can be demonstrated between the dose of shigellae and the incubation period. A reduction in dose results in an increase of the incubation period (see Table 6-II); if smaller doses are applied, the bacteria at first multiply on the conjunctiva, until they reach an appropriate number to elicit an inflammatory response. Less resistant (less virulent) microorganisms are destroyed by the defence mechanisms of the host. Larger infecting doses contain a greater number of resistant bacterial cells capable of an early intracellular multiplication.

The bacterial count of the inoculum influences the course of the disease to a certain degree. With very small doses the symptoms of conjunctivitis are usually very moderate, only isolated infiltrations appear on the cornea during the first few days and the whole course of the disease may be shortened. A massive infection, however, is characterized by a rapidly ensuing acute inflammation (Serény, 1955*a*; Lin et al., 1964). Virulent shigellae given in lower numbers than the minimum infecting dose are, as a rule, destroyed. Sometimes they may be cultured from the conjunctival exudate for several hours.

Virulence. In this chapter virulence is interpreted as the disease-inducing capacity of shigellae in the guinea pig's eye. In relation to shigella keratoconjunctivitis, any *Shigella* strain is qualified as virulent if it is able to elicit keratoconjunctivitis on the eye of a healthy guinea pig in doses of less than 10^7 organisms. Reproducible results are obtained only if at least 10 guinea pigs are used for each of the graded doses and both eyes of the animals are infected. The results should be statistically analysed. The degree of virulence varies greatly from strain to strain. The ID_{50} may range between 10^2 and 10^7 cells (Serény, 1958*b*, 1960*b*, 1962*c*; Stenzel, 1961*c*; Rauss et al., 1967).

Shigella strains isolated freshly are virulent (with the exception of *Sh. sonnei* phase II), but they lose their pathogenic capacity on storage, e.g. after 3–12 months on Dorset culture medium in the refrigerator (Serény, 1955*a*, *b*; Gekker et al., 1957*a*, *b*; Siroko, 1957, 1958; Piéchaud et al., 1958; Belaya, 1959; Gorea, 1959; Yakhnina et al., 1960*a*; Mackel et al., 1961; Stenzel, 1962*b*, *c*; Gleiberman et al., 1964; Mirzoev et al., 1966; Cefalu and Puglisi, 1967*b*; Nakamura, 1967; Kerekes, 1968; Ciufeco, 1970; Kandyurina, 1970*a*, *b*). The rate of the decrease of virulence depends on environmental factors (composition of the culture medium, pH, temperature and duration of storage). Sometimes the organism retains its virulence for a surprisingly long time in water (Siroko and Verkholomov, 1965). Virulence is lost more rapidly, if the culture is stored under disadvantageous condi-

tions (e.g. at high temperature). Infrequently, avirulent strains may occur among freshly isolated cultures (Manolov, 1957*a*, *b*; Szturm-Rubinsten et al., 1957; Noskov 1959; Shantarenko and Tesla, 1960; Stenzel, 1962*b*, *c*).

Serial passage of virulent *Shigella* strains on artificial culture media results in the decrease of virulence (Gekker et al., 1957*a*, *b*; Belaya, 1959, 1968; Noskov, 1959; Shantarenko and Tesla, 1960; Stenzel, 1963*a*; Bogdanova, 1965; Serény, 1966; Ciufeco, 1970). The loss of virulence usually ensues suddenly and seems to be the result of a mutation (Stenzel, 1971*b*). Avirulent mutants are easy to obtain from virulent strains by culturing on media containing antibiotics or certain dyes (Stenzel, 1961*b*; Tenner et al., 1969).

The virulence of the strain can be preserved in different ways. One of these methods is the induction of a conjunctival infection with the same strain at intervals of 1–2 months and the maintenance of the culture isolated from the conjunctival exudate (Serény, 1960*a*). *Sh. sonnei* cultures preserve their pathogenicity for a long time if subcultures are made from colonies with star-like formation from time to time (Serény, 1961*b*). Chronic keratoconjunctivitis may also be used for maintaining virulence; in this condition virulent shigellae are released (Belaya, 1962*a*). Lyophilization is not suitable for preserving virulence for a longer period (Belaya, 1962*a*). Reversion of *Shigella* strains to virulence has not been reported (Shantarenko and Tesla, 1960; Mackel et al., 1961; Bogdanova, 1965) unless by genetical manipulation (Formal et al., 1965*b*).

THE HOST

The development, or the absence, of shigella keratoconjunctivitis may be highly influenced by general and local conditions in the host. The susceptibility or the resistance of the host depends on specific and non-specific factors.

The importance of the host's general condition in the development of shigella keratoconjunctivitis is indirectly shown by the fact that there is no obvious difference between the disease-inducing capacity of different *Shigella* strains isolated from various forms of human dysentery, acute and chronic patients, symptomless carriers (Yakhnina et al., 1960; Nakamura, 1967; Kerekes, 1968). The severity and the course of shigella keratoconjunctivitis are associated with the resistance of the animals (Gorea, 1959). Seasonal variations, due to hypovitaminosis, in the course of shigella keratoconjunctivitis, suggest the significance of the general condition of the host (Belaya, 1964). X-ray irradiation of the test animals, however, did not modify the disease (Goryunova, 1963). Sex and body weight of the guinea pigs do not influence the development of the disease or the intensity of the symptoms (Serény, 1955*a*); the colour of the fur, however, is of greater importance. Recovery is more rapid and more perfect in albinos (Serény, 1955*a*; Shantarenko and Tesla, 1960; Stypulkowska, 1962).

Starvation for a short time (3 days) does not reduce the resistance to conjunctival infection (Serény, 1955*a*). Ascorbic acid accelerates the recovery, while B_1, B_{12} and D vitamin administration results in a prolonged excretion of bacteria (Shantarenko and Tesla, 1960). The animal's previous sensitization with a killed *Shigella* culture has no effect on the development of shigella keratoconjunctivitis (Shantarenko, 1959), but after recovery a hypersensitivity may occur against different agents (*E. coli* cultures, killed shigellae), manifesting as mild, quickly healing lesions (Serény, 1958*b*, 1960*b*).

The significance of local factors is proved by the influence of the intactness of the conjunctival epithelium on resistance (Shantarenko and Tesla, 1960). Inoculation into both eyes of the same animal makes it possible to study the role of local factors. Small doses of virulent *Shigella* cultures or medium doses of less virulent strains may affect one of the eyes while the other remains uninjured at simultaneous infection. Differences in the clearance from bacteria and in the time of the clinical recovery may also be noted (Serény, 1955*a*).

Congenital immunity of different animals. Susceptibility of experimental animals to conjunctival infection with *Shigella* cultures is highly different. Guinea pigs and rabbits are most susceptible (Serény, 1955*a*, *b*, 1957, 1960*a*, *b*; Manolov, 1957*a*, *b*; Szturm-Rubinsten et al., 1957; Ciufeco, 1970; Cross and Nakamura, 1970). In these animals about 10^8–10^9 bacteria of a virulent *Shigella* culture without exception induce a typical keratoconjunctivitis. Goats and horses are also susceptible (Rédey, 1960). Inoculation of cats with large doses results in keratoconjunctivitis only in a small percentage. Successful infections are preceded by a long incubation period and local lesions are limited to isolated foci (Serény, 1955*a*, 1957). In hamsters the conjunctival infection is not always followed by lesions of the eye; keratoconjunctivitis developing occasionally is very mild and heals in a few days (Serény, 1955*a*, 1957; Manolov, 1957*a*, *b*). Polecats, white mice, rats, chickens, dogs and monkeys are resistant (Serény, 1955*a*, *b*, 1957, 1960*a*, *b*; Manolov, 1957*a*, *b*; Szturm-Rubinsten et al., 1957; Shantarenko and Tesla, 1960; Ciufeco, 1970). Rédey (1960) was able to produce shigella keratoconjunctivitis in white mice and rats. It is interesting that shigella keratoconjunctivitis may be induced with shigellae on the eye of the monkey after artificial poliomyelitis virus infection (Rédey, 1960).

Acquired immunity. The development of immunity in shigella keratoconjunctivitis can be followed by the use of a suitable technique. Blokhov (1964) demonstrated the establishment of resistance in the course of the disease.

Immunity of the infected eye may be reliably detected by a second conjunctival infection of the eye with graded doses of bacteria (Table 6-V) (Serény, 1958*b*). Such experiments allow one to draw conclusions as to cross-immunity against other *Shigella* types (Serény, 1955*a*, 1957, 1960*a*, *b*; Gekker et al., 1957*a*, *b*; Gekker and Belaya, 1958, Szturm-Rubinsten and Piéchaud, 1958; Gorea, 1959; Kotelko et al., 1960; Gleiberman et al., 1964; Istrati and Istrati, 1964*a*; Lin et al., 1964; Cross and Nakamura, 1970).

TABLE 6-V

Immunity of the guinea pig's eye after recovery from shigella keratoconjunctivitis

Effect of conjunctival infection*	First infection		Second infection	
	No. of eyes	per cent	No. of eyes	per cent
Eye remained intact	474	50.1	95	32.1
Mild conjunctivitis	—	—	13	4.5
Abortive keratoconjunctivitis	—	—	147	50.3
Typical or chronic keratoconjunctivitis	472	49.9	28	9.2
Atypical keratoconjunctivitis	—	—	10	3.5
Shigella sepsis	—	—	1	0.4
Total	946	100.0	294	100.0

* Infecting doses: 10^7 to 10^9 cells.

Local resistance seems to have no serotype specificity. A decrease in the frequency of the pathological reaction after the second conjunctival infection indicates immunity developing following shigella keratoconjunctivitis. Further evidence includes: (*i*) mild abortive diseases often occurring after reinfection; (*ii*) shigellae introduced into the conjunctival cavity after recovery are usually destroyed; (*iii*) reinfection is followed by short-term bacteraemia and hyperpyrexia only rarely (Serény, 1955*a*, 1957). *E. coli* (B or K-12) given simultaneously with the homologous culture increases resistance (Cross and Nakamura, 1970).

Immunity developing in the course of shigella keratoconjunctivitis is only relative and may be overcome by small infective doses. The second keratoconjunctivitis may be even more severe than the primary disease. The increased relative resistance of the healed eye is associated with tissue immunity, due to an altered reactivity of the epithelial cells formed newly in the course of regeneration. Ultrastructural alterations in the epithelial cells of the cornea during the development of cellular immunity are evident from the increased number of mitochondria and from the marked development of the endoplasmic reticulum (Bakács et al., 1970). According to Cross and Nakamura (1970) immunity is the result of the appearance of specific shigella antibodies in the tissues of the eye.

Hypersensitivity analogous to the postdysenteric state of man may also occur and result in inflammatory reaction of the tissues in response to otherwise harmless injuries. It is interesting from an epidemiological point of view, that there is no change in the morbidity rate; the only effect of immunity is that abortive processes occur instead of severe typical symptoms (Serény, 1958*b*) (see Table 6-V).

The resistance of the eye usually increases in the course of repeated reinfections. It also increases when different types or species of *Shigella* cultures are used for consecutive conjunctival infections. In spite of the progressive increase of the resistance, serial reinfections cannot induce a 100 per cent immunity against a massive infection (Serény, 1955*a*, 1958*b*;

Manolov, 1957*a*, *b*; Szturm-Rubinsten and Piéchaud, 1958; Gorea, 1959; Yakhnina and Kuznetsova, 1959; Noskov, 1959; Shantarenko, 1959; Shantarenko and Tesla, 1960; Stenzel, 1962*b*, *c*; Gleiberman et al., 1964; Istrati and Istrati, 1964*a*).

If one eye of the experimental animal is infected earlier and the other later, it may be demonstrated that there are qualitative and quantitative differences between the local resistance of the healed eye and the general immunity of the other eye. The local resistance is more intensive and non-specific, the general immunity is weaker and species-specific (Serény, 1955*a*, 1957, 1958; Gekker et al., 1957*a*, *b*; Manolov, 1957*a*, *b*; Gorea, 1959; Noskov, 1959; Shantarenko and Tesla, 1960; Stenzel, 1962*b*, *c*; Gleiberman et al., 1964; Istrati and Istrati, 1964*a*; Lin et al., 1964; Belaya, 1964).

Recovery from shigella keratoconjunctivitis results in a relative resistance lasting about nine months (Serény, 1955*a*, *b*; Manolov, 1957*a*, *b*; Gekker and Belaya, 1958; Yakhnina and Kuznetsova, 1959).

The immune host may differ from the susceptible one, not only in respect of its unresponsiveness to an otherwise infectious dose of bacteria, but also in the lesions developing after a successful reinfection. Of the numerous varieties, the following main types of reaction deserve mention: (*i*) the eye remains intact; (*ii*) a mild conjunctivitis develops without a keratitis; (*iii*) mild keratoconjunctivitis; (*iv*) typical keratoconjunctivitis; (*v*) atypical keratoconjunctivitis; (*vi*) shigella sepsis (Serény, 1955*a*).

After the second or subsequent infections the growth of the infecting agent is less intensive than after the first infection (Serény, 1955*a*; Cross and Nakamura, 1970). Reinfection is characterized by a less frequent and minor rise in temperature and by an insignificant loss of body weight.

There may be significant differences between the titres of the agglutination reaction during the first shigella keratoconjunctivitis and during the reinfection period. The titre often increases, but it may also decrease. There is no regular correlation between the degree of local reaction after reinfection and the agglutinin titre of the serum, or a close connection between the agglutinating antibodies of the serum and resistance (Serény, 1955*a*; Gekker and Belaya, 1958; Gorea, 1959; Yakhnina und Kuznetsova, 1959; Noskov, 1959; Shantarenko and Tesla, 1960; Mackel et al., 1961; Lin et al., 1964; Cross and Nakamura, 1970). Mouse protective antibodies and the healing of the disease in the course of the first shigella keratoconjunctivitis are likewise not associated (Serény, 1955*a*).

Haemagglutinins in the blood serum and in corneal extracts are not specific either; by haemagglutination inhibition they can be demonstrated two weeks after the healing of the second shigella keratoconjunctivitis (Lin et al., 1964).

Cross-immunity between shigellae and other bacteria. From eye lesions in guinea pigs induced with bacteria other than shigellae, some interesting conclusions may be drawn. Keratoconjunctivitis produced by *Corynebacterium diphtheriae* is not followed by the development of cross-resistance to shigellae (Gekker et al., 1957*a*, *b*; Shantarenko, 1959; Istrati and Istrati, 1964*a*). Neither *Listeria monocytogenes* and *Shigella* (Szturm-Rubinsten and Piéchaud, 1958) nor *Sh. flexneri* 2 and *S. enteritidis* (Stenzel, 1962*c*)

are related in this respect. In contrast, cross-immunity was demonstrated between shigellae and *E. coli* strains pathogenic to the guinea pig's eye (Stenzel, 1962*c*; 1965; Istrati et al., 1963*a*, *b*; Serény, 1963; Istrati and Istrati, 1964*a*).

ANALOGY BETWEEN SHIGELLA KERATOCONJUNCTIVITIS AND IMMUNITY IN HUMAN DYSENTERY

It would be wrong to insist on seeking analogies between experimental and human shigellosis, but the relationship between certain reactions is striking. Clinical, epidemiological, bacteriological and immunological examinations have equally proved the development of a small degree of immunity following human dysentery (Hardy et al., 1942; Sinay, 1950; Polyansky, 1953; Rauss, 1955, 1968). The first infection with shigellae in infancy produces a more or less severe disease; the following infections become more and more localized. The short-term immunity after dysentery can be preserved with frequent stimuli (Rauss, 1963*a*, *b*, 1968). All these statements are also valid for shigella keratoconjunctivitis. Serological similarities in the production of agglutinins and mouse protecting antibodies are also evident (Cooper and Keller, 1947; Rauss and Kétyi, 1952*a*, *b*, 1955; Rauss, 1955).

In spite of the above findings there appears to be a pronounced difference between shigella keratoconjunctivitis and dysentery in respect of the specificity of clinical protection, since immunity developing after human dysentery is usually considered to be type-specific. However, recent observations do not confirm the above opinion. According to Rauss (1955) a cross-immunity develops between *Sh. flexneri* types sharing dominant group antigens (Rauss, 1955). In mice, a resistance is established to *Sh. sonnei* phase I after immunization with *Sh. flexneri* (Istrati et al., 1958*a*). Clinical and epidemiological observations have shown that the infection of man with a single *Shigella* type usually results in an immunity against other types (Dosser, 1953; Rubashkina, 1953; Kamenskaya, 1956; Meshalova, 1955). As immunity in human dysentery and in shigella keratoconjunctivitis displays several important common characteristics, the model seems to be suitable for the investigation of different immunological problems.

Cell-mediated immunity has a decisive role in resistance to shigella keratoconjunctivitis. In our opinion this finding should be considered in human dysentery. The failure of specific prophylaxis against dysentery might be caused by striving only for humoral immunity produced by vaccines. In this manner the number of circulating antibodies could be significantly increased without, however, obtaining clinical protection. It may be supposed that induction of cell-mediated immunity with living, avirulent shigellae would produce better results.

PRACTICAL APPLICATIONS OF THE GUINEA PIG EYE REACTION

It took a relatively short time for the method to become popular and there are several practical as well as theoretical uses of the test. Because of its high selectivity, it is suitable for the isolation of shigellae from faeces and sewage. The faeces should be seeded on agar or on blood-agar medium and the culture should be inoculated into the eyes of guinea pigs (Rédey and Csizmazia, 1960; Bals and Leonesco, 1961; Istrati and Ciufeco, 1962; Avdeeva and Turuleva, 1968).

When testing water, it should be passed through a membrane filter and the conjunctival infection is to be carried out with the culture grown on the membrane (Istrati et al., 1962*a*). By conjunctival infection shigellae that cannot be demonstrated with the usual methods and other keratoconjunctivitis-producing bacteria, may be isolated (Rédey and Csizmazia, 1960; Bals and Leonesco, 1961; Bayramova, 1962, 1963; Istrati et al., 1962*a*, *b*; Blinkin, 1963; Gleiberman et al., 1964; Vörös et al., 1964; Avdeeva and Turuleva, 1968).

The consistent capacity to produce keratoconjunctivitis seemed to be used for the taxonomic definition of *Shigella* organisms (Manolov, 1958, 1959; Manolov et al., 1962; Stenzel, 1962*a*; Trifonova, 1963, 1965; Piéchaud et al., 1965). Stenzel (1964) made a proposal to the Enterobacteriaceae Subcommittee of the International Microbiological Society to include the keratoconjunctivitis inducing capacity in the definition of the *Shigella* genus. However, the Subcommittee has refused the proposal (Carpenter, 1963*a*, *b*).

The shigella keratoconjunctivitis model has been successfully used in other examinations, e.g. in phage multiplication studies (Strongovskaya, 1963) and for the selection of *Sh. sonnei* phase I colonies, etc. (Serény, 1959*a*, 1960*c*, *d*, 1961*b*; Kotelko et al., 1960). The virulence of *Shigella* strains may be preserved for a long time by means of serial conjunctival infections at intervals of one or two months (Serény, 1960*a*; Belaya, 1962*a*). Gekker et al. (1957*a*, *b*) have suggested to use the keratoconjunctivitis inducing capacity of *Shigella* cultures for the selection of vaccine strains. Formal et al. (1967*a*, *b*) used conjunctival infection for checking the virulence of vaccines.

Typical shigella keratoconjunctivitis may be spontaneously conveyed from infected to normal guinea pigs (Serény, 1955*a*; Gorea, 1959; Rédey and Csizmazia, 1960; Istrati and Meitert, 1963). Application of this observation as an epidemiological model has revealed that the antigenic structure of *Shigella* strains shows no essential change during the dissemination of the infection and in the course of the disease (Serény, 1962*a*, *b*) and the phage-type also remains stable (Istrati and Meitert, 1963). In the course of mixed shigella infections an interference can be demonstrated: the suppressed agent disappears from the eye exudate within a few days (Manolov, 1963).

The conjunctival infection may be used for examining the human enteropathogenicity of *Shigella* and *E. coli* strains, as there is a strict correlation

TABLE 6-VI

Infective doses of some enteropathogens as tested by conjunctival infection and by oral infection of human volunteers
(Rédey, 1957, 1960, 1964*a*, *b*)

Organism	Effective dose	Number of bacteria administered orally to man	Reactions in man	Remarks
E. coli O143	10^5	10^9	Fever, bloody stool	—
E. coli O124	3×10^4	10^4	?	Mild symptoms
Sh. flexneri 2a	5×10^2	2×10^3	?	Mild symptoms
Sh. flexneri 2a	5×10^2	2×10^5	+++	8–10 bloody stools
Sh. flexneri 2a, heated	—*	10^{13}	+	Endotoxin intoxication
Sh. flexneri 1b	—**	4×10^{13}	+	Endotoxin intoxication
Sh. flexneri 1b, heated	—**	2×10^{13}	?	Very mild endotoxin intoxication
Sh. sonnei I	—**	2×10^{13}	—	With mucin 1–2 cells caused fatal infection in mice
Sh. sonnei II	—**	2×10^{13}	—	Five different strains

* For conjunctival infection a large inoculum was needed.
** Avirulent with conjunctival infection.

between their pathogenicity to the guinea pig's eye and to humans (Rédey, 1957, 1960, 1964) (Table 6-VI).

Istrati and co-workers (Istrati 1961; Istrati et al., 1963*a*, 1964*a*) confirmed these findings on a number of volunteers. Stenzel (1962*b*) confirmed these results in a self-experiment. Accordingly, the pathogenicity stated by conjunctival infection may be regarded as an indicator of human enteropathogenicity, i.e. strains displaying an affinity towards the conjunctiva of the guinea pig, independently of their biochemical properties, may be regarded as pathogenic for the colonic mucosa of man (Rauss, 1968).

Several workers estimated the active and passive protective effect of dysentery vaccines by parenteral immunization of white mice. However, dysentery vaccines, protective in mice experiments, were not suitable for the prophylaxis of human dysentery (Hardy et al., 1948; Shaugnessy et al., 1964*a*, *b*; Medzhinov, 1953; Higgins et al., 1955*a*; Troitsky, 1958*a*; Istrati et al., 1963*a*; Rauss, 1968). Different kinds of *Shigella* vaccines failed to protect guinea pigs against shigella keratoconjunctivitis (Manolov, 1957*a*, *b*; Noskov, 1959; Stenzel, 1961*b*; Serény, 1962*c*; Stypulkowska, 1962; Arons, 1966; Ogawa et al., 1966*b*; Aldova et al., 1968).

Istrati and co-workers (Istrati et al., 1963*a*, 1964*a*, 1965, 1967, 1968; Istrati and Istrati, 1964*a*, *b*, 1966; Ciufeco et al., 1965) were the first to

report on successful local application of living avirulent *Shigella* variants. They demonstrated that a cross-immunity existed between *Shigella* subgroups. Their variant was not injurious for man at oral administration and remained stably avirulent after serial passage in the intestine of man. The living vaccine was successfully applied in children with chronic shigella excretion. After the experiments of Istrati's team several workers have attempted immunization with avirulent *Shigella* cultures. Ogawa et al. (1966*b*) have found that the local application of an attenuated *Shigella* variant results in a moderate resistance. Inoculating repeatedly with streptomycin-dependent *Sh. sonnei* and *Sh. flexneri* mutants, Sergeev et al. (1967) induced local immunity in the eye of guinea pigs. The vaccine was harmless for monkeys (Sergeev et al., 1968). According to Belaya (1968), avirulent shigellae selected by serial passages on culture media are attenuated variants which retain their capacity to enter the epithelial cells and induce an inflammatory response in the mucosa, which can be demonstrated only with histological methods (Levenbuk et al., 1968). Repeated conjunctival application of attenuated shigella cultures produces a definite protective effect. Monkeys can be successfully immunized with such vaccines administered orally (Belaya et al., 1970); oral administration was harmless in 147 humans (Rubtsov et al., 1970). A great number of avirulent variants were selected with a simple procedure by Tenner et al. (1969). The protective effect of the avirulent variants was established on the guinea pig's eye and strains displaying the most pronounced immunogenic capacity were used in subsequent experiments. In this manner a 80 per cent protection could be reached against shigella keratoconjunctivitis.

It has been demonstrated (Godun, 1962; Kovalev, 1965*a*, *b*) that the dysentery phage exerts no beneficial effect on local administration. In contrast, shigella keratoconjunctivitis reacts well to specific antibacterial drugs and is, accordingly, suitable for estimating the *in vivo* effect of various preparations (Manolov, 1957*a*, *b*; Siroko, 1957, 1958; Manolov et al., 1958; Serény, 1958*a*; Shantarenko and Tesla, 1960). Chloramphenicol alone or in combination with riboflavin is highly effective (Kovalev, 1965*a*, *b*). Of furacillin, syntomycin and streptomycin, the last one shows the most pronounced therapeutic effect at local administration (Strongovskaya, 1963).

The success of specific drug therapy depends on (Serény, 1958*a*):

1. drug sensitivity of the agent,
2. dose and period of administration,
3. defence mechanism of the host.

The conjunctival infection may also be applied for genetic investigations; the virulence of the parent strains may be estimated and virulent and avirulent mutants may be selected. Stenzel (1961*c*, *d*, 1966) found no correlation between the degree of streptomycin resistance and the virulence of hybrids of streptomycin-sensitive and streptomycin-resistant *Sh. flexneri* and streptomycin-resistant *E. coli* K-12. Formal and co-workers (1965*b*) used spontaneous avirulent mutants of *Sh. flexneri* 5 as genetical recipients. Their mating with *E. coli* K-12 cultures resulted in avirulent as well as virulent hybrids. According to the investigations of Petrovskaya and

Lycheva (1970), there is a genetic locus on the chromosome of shigellae adjacent to the streptomycin zone responsible for shigella keratoconjunctivitis inducing capacity.

COMPARATIVE INVESTIGATIONS ON SHIGELLA KERATOCONJUNCTIVITIS AND OTHER EXPERIMENTAL SHIGELLOSES

Piéchaud and Szturm-Rubinsten (1959) carried out conjunctival, subcutaneous, intraabdominal, vaginal, nasal and vesical infections in guinea pigs. It may be concluded from their results that conjunctival infection is the most advantageous experimental technique. The more laborious vesical infection also yielded excellent results. In general, *Shigella* strains pathogenic to the eye also induce cystitis in guinea pigs. A disadvantage of the latter method is that the allergic components may hide the anti-infectious protection. Belaya (1959) compared the results obtained by the conjunctival infection, mouse test and chicken embryo technique. There was a good agreement between the virulence established in guinea pigs and chicken embryos; the virulence found for mice and chicken embryos, however, showed no correlation. Stenzel (1962*b*, *c*) stated that strains displaying pathogenicity for the mucous membranes can induce a disease in the eye of the guinea pig as well as in its bladder; the sensitivity of the latter model is usually superior to that of the former. Vesical infection is less specific than shigella keratoconjunctivitis and its symptoms are also less differentiated. Rauss et al. (1966*a*, *b*, 1967) observed a complete agreement between the mouse intestinal test and the reaction in the guinea pig's eye. According to Formal and co-workers (1965*d*, 1971*a*) the keratoconjunctivitis test and the penetrating ability into HeLa cells agree with the capacity of invading intestinal epithelial cells. Blinova (1970) carried out parallel experiments with nasal (Voino-Yasenetskaya, 1957, 1958; Voino-Yasenetsky and Voino-Yasenetskaya, 1962) and conjunctival infection using *E. coli-Shigella* recombinants. She demonstrated definite differences as to the *in vivo* multiplying capacity of virulent, attenuated and avirulent hybrids by means of nasal infection but not by conjunctival infection. She considered the lung model suitable for the differentation of *Shigella* hybrids which could not be differentiated by shigella keratoconjunctivitis.

DISADVANTAGES OF THE KERATOCONJUNCTIVITIS TEST

1. The abundant eye discharge, containing shigellae in great numbers, may be the source of human laboratory infection and the infection of the experimental animals may be conveyed to healthy animals.

2. Shigella keratoconjunctivitis and human dysentery differ in several respects, namely in the (*i*) infected host, (*ii*) conditions of infection, (*iii*) site of entry of the pathogenic agent, (*iv*) target organ, (*v*) clinical symptoms. Accordingly, the results of conjunctival infection can be applied to human dysentery with numerous restrictions only.

CHAPTER 7

HISTOPATHOLOGY OF EXPERIMENTAL SHIGELLA KERATOCONJUNCTIVITIS

by

M. V. VOINO-YASENETSKY and T. BAKÁCS

In the preceding chapter macroscopic manifestations of shigella keratoconjunctivitis in guinea pigs have been described. In one of his early works Serény (1957) mentioned histologic investigations made by Kerényi, who showed that the inflammatory process began at the site of the conjunctival fold. Then a superficial ulceration of the cornea, accompanied by its oedema and leukocytic infiltration, occurred. With the decrease of inflammation, the epithelial lining was gradually repaired. A similar description of experimental shigella keratoconjunctivitis was given by Siroko (1958). Neither of these works provided any information on the presence or absence of bacteria in the impaired tissues. However, still earlier Serény (1955*a*, *b*) found single rods in the polymorphonuclear leukocytes in smears of inflammatory exudate from the palpebral fissure of infected animals. The bacteria were more numerous in the cytoplasm of sloughed epithelial cells. The same was later reported by Manolov (1959) who believed that shigellae multiplied in the corneal epithelium; in reality, only impaired or destroyed cells appeared in the purulent exudate accumulating in the conjunctival sac. It was, therefore, necessary to find out whether shigellae could colonize viable cellular elements of the conjunctiva and cornea.

Piéchaud et al. (1958) investigated the eyes of 17 guinea pigs sacrificed at different intervals (from 3 hours up to 40 days) after conjunctival infection with shigellae. In addition to haematoxylin and eosin they also used methylene blue for staining histological preparations. The superficial damage of the cornea appeared as early as after 9 h; at 36 h the epithelial lining showed only small islets, but after 2–4 days it became entirely destroyed. By this time the inflammatory response (accompanied by vascularization of the corneal stroma) had subsided but epithelial reparation proceeded very slowly, taking sometimes more than 30–40 days. In the acute stages of infection, rod-like bacteria were seen in the corneal epithelium not only in its superficial layers but also in the well-preserved cells of the basal layer. As mentioned in Chapter 1, the above authors suggested that the epithelium may serve as the "last resort" for shigellae, seeking refuge from phagocytes.

Levenbuk and Andreeva (1962) also noted the penetration of shigellae into the epithelial cells of the guinea pig cornea. The bacteria were larger in the superficial epithelial layer than in the deeper layers. This finding, as well as the increase in the number of bacilli in single cells after

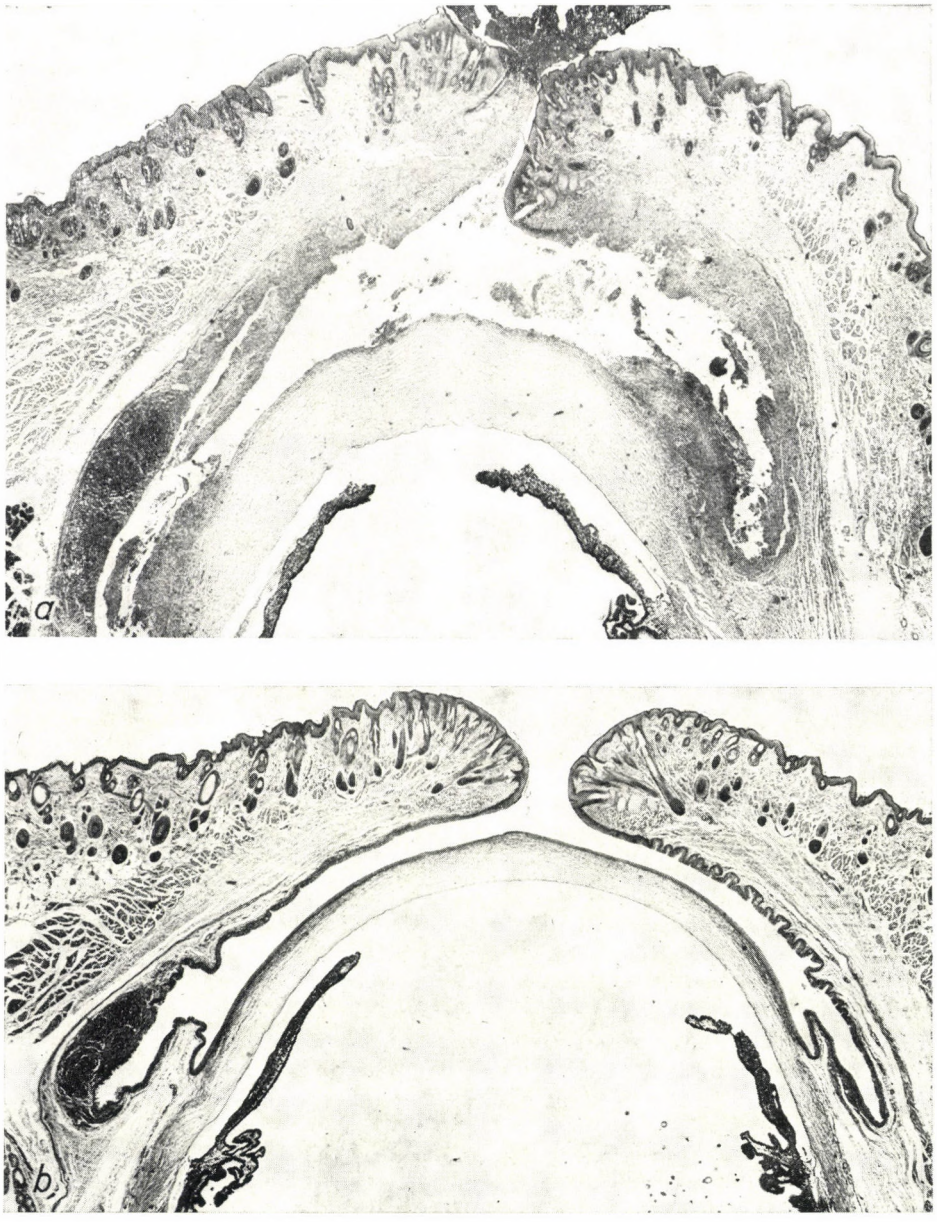


Fig. 7-1. Experimental shigella keratoconjunctivitis. *a* Sagittal section (not passing directly across the centre of the cornea) through the eyelids and the anterior portion of the guinea pig eyeball 48 h after inoculation. Thionine, lens. *b* The same after 16 days. The eyelids have regained their normal appearance but the cornea is still somewhat thicker (especially towards the centre) and denser than normally. Dominici's stain, lens. Note the lymph node in the region of the corneal fornix

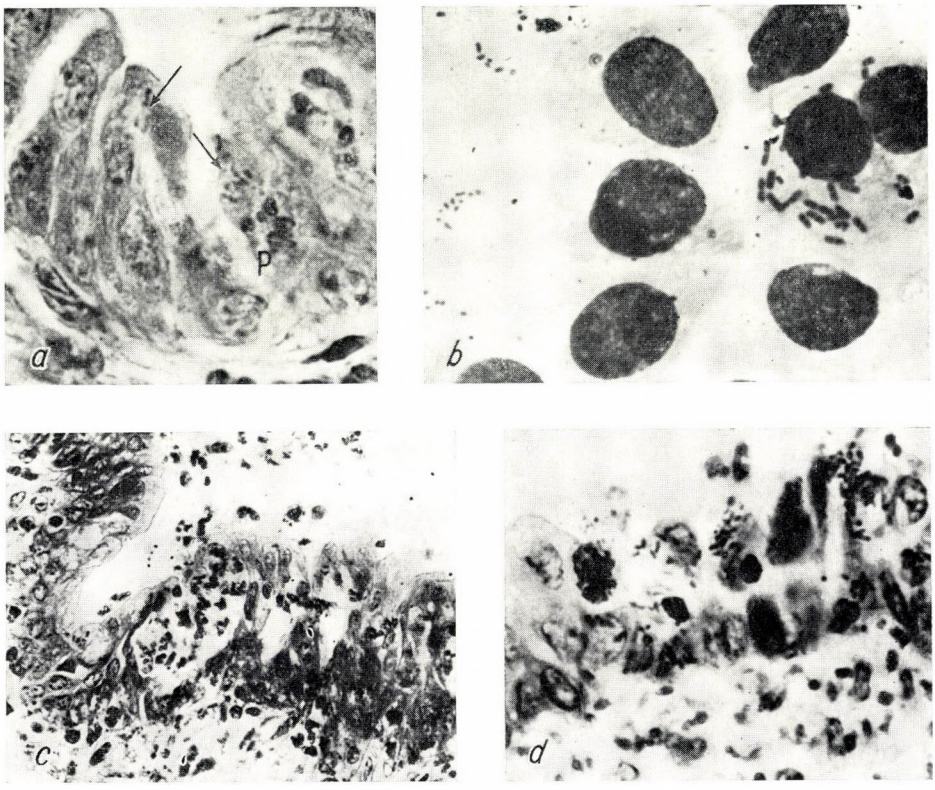


Fig. 7-2. Conjunctival lesions caused by shigellae. *a* Bacteria in the apical part of the epithelial cells (arrows) and two polymorphonuclears (P) passing between the cells 1 h after inoculation. Dominici's stain, ×1000. *b* Organisms of different size in the exudate and inside the desquamated binuclear epithelial cell. Exudate smear 3 h after inoculation. Eosin-azure, ×1180. *c* Formation of a microabscess in the conjunctival epithelium 3 h after inoculation. Thionine, ×475. *d* Erosion with organisms in the cytoplasm of the epithelial cells present along its edges 6 h after inoculation. Thionine, ×1000

the 2nd day, suggested a multiplication inside the epithelial cells. Laboratory *Shigella* strains which have evidently lost their pathogenicity caused no morphological alterations in the eyes even if introduced together with endotoxin.

Attempting to clarify the pathogenic properties of shigellae, Piéchaud et al. (1958) used six different strains of the agent (*Sh. dysenteriae* 1, *Sh. flexneri* 1 and 2, *Sh. sonnei*, *Sh. boydii* 2 and 4) to infect the eyes of 17 guinea pigs. Since each strain was only tested on 1–3 guinea pigs sacrificed at various intervals, their results are not suitable for drawing far reaching conclusions. In the brief description by Levenbruk and Andreeva (1962) of histological alterations of the conjunctiva and cornea, some controversial, apparently artificial, structures were mentioned (bulging

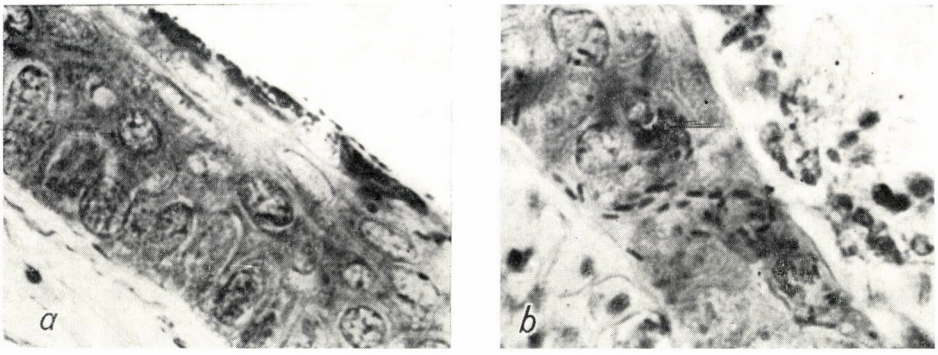


Fig. 7-3. Initial corneal lesions. *a* Shigellae in the superficial epithelial cells 9 h after inoculation. *b* Sloughing of upper layers of the epithelium and bacterial invasion of the deeper ones 18 h after inoculation. Thionine, ×1000

of the nuclei and formation of hollow spaces in the epithelial cells, showing, in fact, the immigration of leukocytes).

In a more detailed histopathological study made at our institutes, about 400 guinea pigs were used and bacteriological and clinical observations were made (Rácz et al., 1960; Rácz and Serény 1962; Rácz, 1963). In accordance with the technique described in Chapter 6, the culture containing shigellae was either dripped into the conjunctival sac (10^8 bacteria) or introduced there by means of a platinum loop (about 10^9 bacteria). With the first method the process developed more slowly, the conjunctival damage was less pronounced and recovery more rapid. The animals were sacrificed at different intervals, from 1 minute to 1 year (not less than 3 animals at a time).

The first experiment was not entirely successful. The corneal epithelium not covered with eyelids dried up very rapidly after the animal had been sacrificed, thus becoming hardly suitable for study. Therefore, in subsequent series the eyeball was extracted together with the eyelids (carefully sutured at first) and the orbital connective tissue for histological study. The best results were obtained by a 2 h fixation in Müller's solution with subsequent transfer of the specimens to Zenker-formol, and embedding in paraffin. Sections were stained with haematoxylin-eosin according to Dominici's method and with carbolic thionine.*

It soon became evident that shigellae were not only able to penetrate living epithelial cells of the conjunctiva and cornea but also to multiply in their cytoplasm (Rácz et al., 1960; Rácz and Serény, 1962), and that development of experimental shigella keratoconjunctivitis (Fig. 7-1) obviously depends on these biological properties of its agents (Rácz, 1963).

* Thionine is recommended for staining Gram-negative organisms in histologic preparations; being more stable than methylene blue it usually gives much better results. It should be noted that not all commercial brands of thionine are suitable for this purpose.

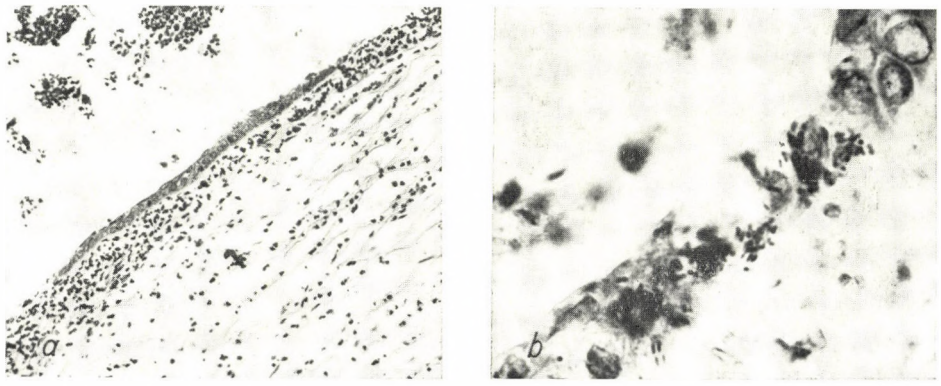


Fig. 7-4. Corneal lesions 24 h after the beginning of the experiment. *a* A small islet of epithelial cells on the surface, oedema and leukocyte infiltration of the proper tissue in the central area of the cornea. Thionine, ×140. *b* Shigellae in the rest of epithelial cells. Thionine, ×1000

Within the first hours after inoculation, the organisms were seen to appear inside the epithelial cells in the region of the conjunctival fornix. At first only a few (Fig. 7-2*a*), then more and more rods could be demonstrated. At the early stage intracellular shigellae were especially large (Fig. 7-2*b*). The infected cells appeared quite normal at the beginning but after the agents had multiplied in them they were destroyed or desquamated (Fig. 7-2*a–d*).

The inflammatory response arose almost simultaneously with the invasion of the epithelium by shigellae (they were never found to be penetrated deeper). As early as one hour after the beginning of the experiment polymorphonuclears emigrated from the small blood vessels into the subepithelial tissue and, in rapidly increasing numbers, they passed between the epithelial cells and partly accumulated there (Fig. 7-2*a* and *c*); then they emigrated into the conjunctival sac forming a purulent exudate. Their ability to engulf and destroy shigellae was evident though in this case phagocytosis was not very pronounced.

The inflammation first appeared in the region of the fornix, later involving the palpebral conjunctiva, and extending onto the cornea after 6–9 hours. Three to 6 hours after inoculation only occasional corneal epithelial cells contained organisms but such cells could readily be observed later. Shigellae appeared first in superficial cells (Fig. 7-3). The affected cells died and were shed in the same way as in the conjunctiva. But here the process was more pronounced, progressing until almost the whole corneal epithelial lining was destroyed (Fig.7-4). The injury remained superficial; in underlying tissues only oedema, haemorrhages and leukocyte infiltration, extending rather far into the loose connective tissue of the orbit, were present. Blood vessels gradually grew into the corneal stroma. It is worth mentioning that no appreciable alterations could be noted in the lymph nodes of the conjunctival fornix.

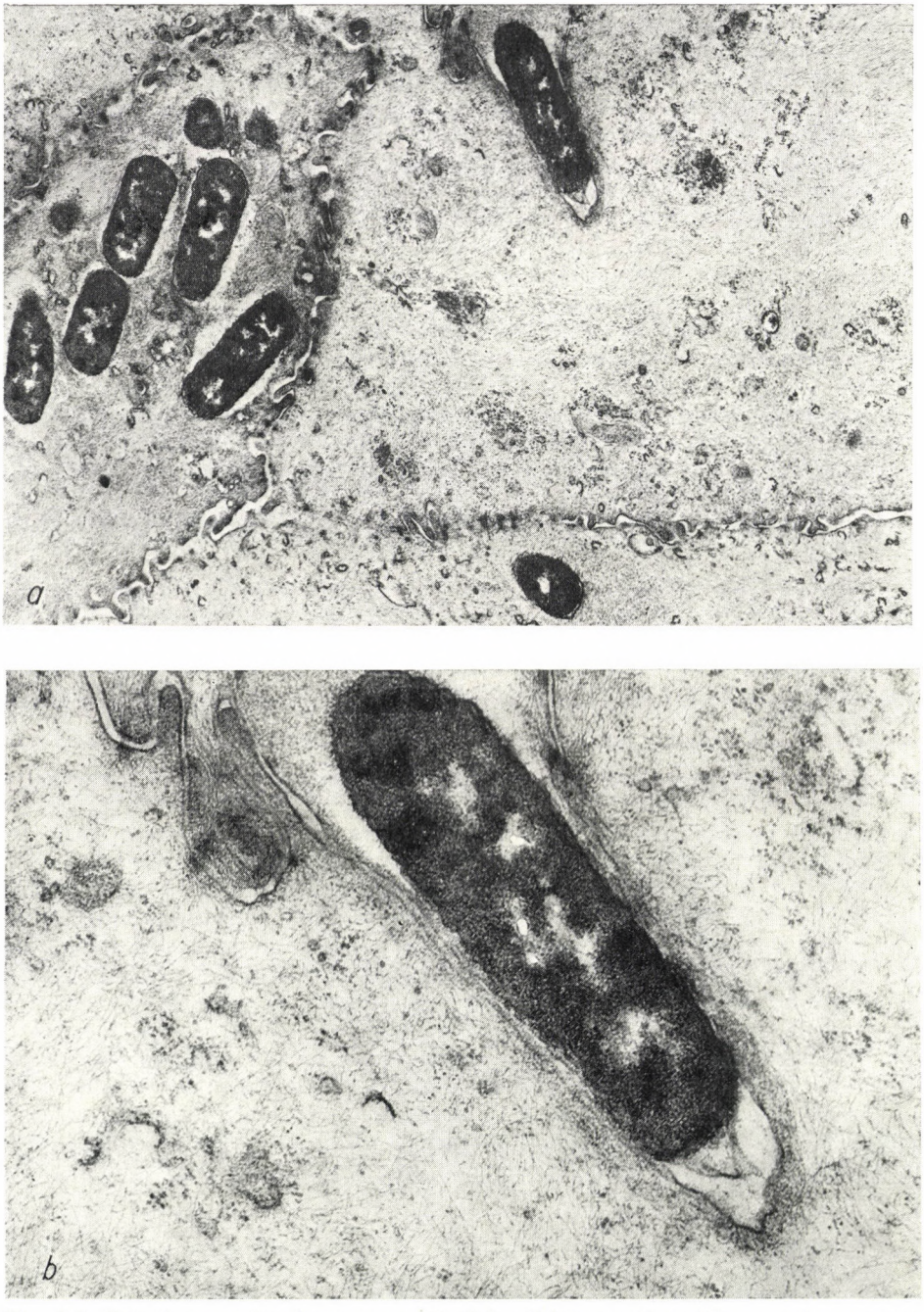


Fig. 7-5. Shigellae passing from one epithelial cell into another. The organism causes indentation of the outer membranes of both cells. 9 h after inoculation. *a* ×9400, *b* ×28,000

When using large doses of *Sh. flexneri*, the pathological signs reached a peak by the end of the first to the third day. From the fourth day onwards the inflammation subsided first on the conjunctiva. As the cellular infiltration and the oedema decreased and the organisms gradually disappeared, the conjunctival epithelial lining was fairly rapidly restored, acquiring its usual appearance by the tenth day. Corneal lesions healed much slower. A rather intense growth of the epithelium, starting from the periphery, i.e. from the bulbar conjunctiva, could be observed from the sixth day of the experiment. The inflammatory response in the cornea usually lasted as long as a fortnight (sometimes even longer). The newly formed epithelium was partly affected by shigellae again.

The conjunctival-type epithelium spreading over the raw surface of the cornea gradually acquired the stratified structure characteristic of the cornea, though it frequently exhibited some initial features suggesting its conjunctival origin. Thus, in certain areas mucus-producing cells were still present. After recovery, pigmentation of the corneal epithelial lining, a normal feature of the conjunctival epithelium in dark-coloured animals, was preserved for a long time.

In additional series of experiments the results of challenge with 10^8–10^9 shigellae of an avirulent strain were studied. These organisms caused only a short-term and slight conjunctival inflammation, not involving the cornea. Shigellae were occasionally seen between the epithelial cells; they did not penetrate the cells and were rapidly phagocytized.* The killed shigellae as well as their endotoxins had hardly any effect, except for a slight leukocyte infiltration of the conjunctiva and an increased mucus secretion by the epithelial cells.

Thus, the development of shigella keratoconjunctivitis in the experiments on guinea pigs was evidently related to the ability of these bacteria to penetrate the epithelial cells and to multiply there. The damaged cells, seemingly unaltered and viable at first, were finally destroyed. This was confirmed during further electron-microscopic studies of the cornea.

The first electron-microscopic investigations (Wessel and Rácz, 1967) cannot be considered successful. Due to the somewhat inappropriate processing of the material, a marked shrivelling of the organisms inside the epithelial cells occurred. As a result, they became surrounded by empty spaces simulating phagocyte vacuoles. In subsequent experiments performed with increased care (Tenner et al., 1970; Bakács et al., 1970) valuable information was obtained not only on shigellae lodging in the epithelial corneal cells but also on the manner of bacterial penetration into these cells.

Figure 7-5 shows the passing of shigellae from one epithelial cell into another. The organism appears to indent the cell and push along with it the membranes of both cells. Figure 7-6*a* also shows a bacterial passage

* According to observations of Levenbuk et al. (1968), *Shigella* mutants possessing reduced virulence preserved the ability to penetrate into the epithelial cells of the guinea pig conjunctiva but did not multiply there and disappeared 12 hours after inoculation. By that time leukocyte infiltration of the conjunctiva, detectable only histologically, also subsided.

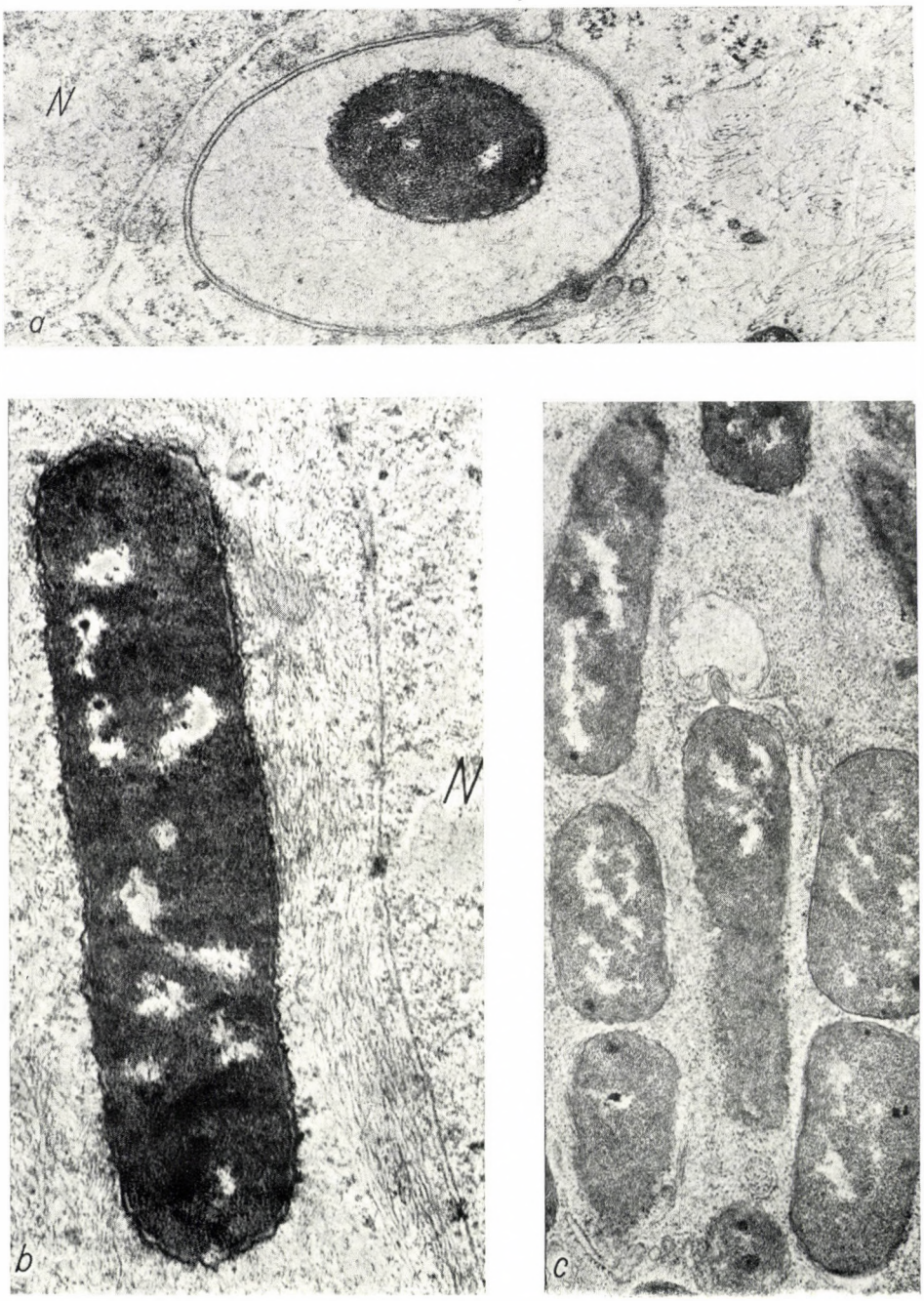


Fig. 7-6. Shigellae in corneal epithelial cells. *a* The organism near the nucleus (N) is surrounded by a double membrane. The ultrastructure of the cell is preserved 12 h after inoculation, ×36,000. *b* Numerous microfibres near a bacterium lying freely in the cytoplasm 48 h after inoculation, ×25,000. *c* A great number of shigellae (some of them dividing partly) in the cytoplasm. There are no membranes around the bacteria 12 h after inoculation, ×17,500

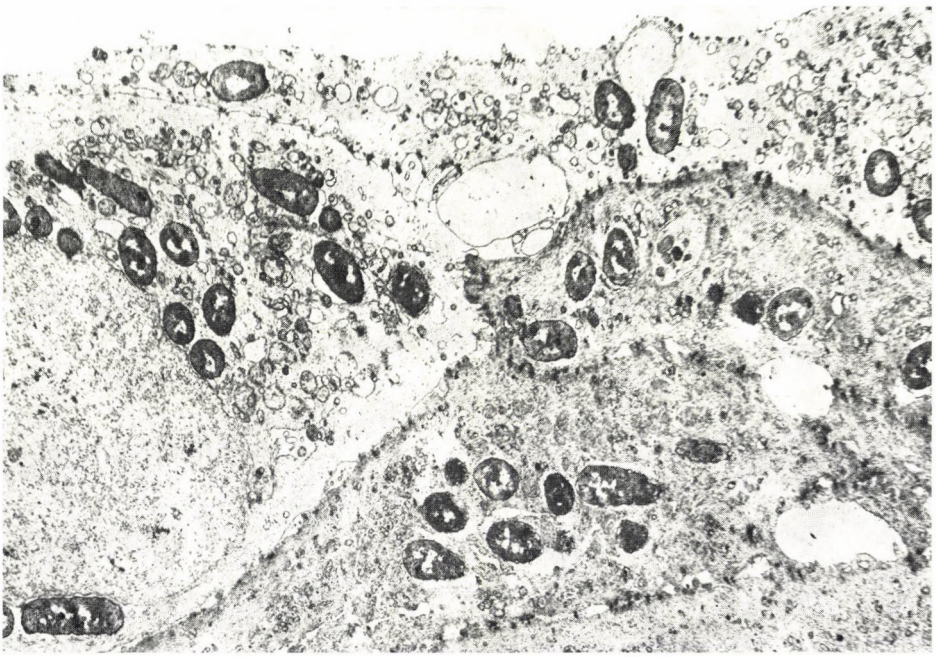


Fig. 7-7. Shigellae in the epithelial cells 12 h after inoculation. Pronounced damage of cellular ultrastructure and destruction of cellular membranes on the surface, × 5500

from one cell into another. The organism is surrounded by a double membrane. It seems probable that the inner membrane and the substance adjoining the microorganism had been brought along from a primarily infected cell, while the outer membrane belonged to the neighbouring cell. Later these membranes obviously disappear since shigellae, as a rule, lie freely in the cytoplasm of corneal epithelial cells (Fig. 7-6*b*, *c*). The cytoplasm directly adjoining the organisms may become denser (see Fig. 7-5). But, usually, as long as the organisms inside the cell are not too numerous, its ultrastructure is well preserved. In the cells of deeper corneal layers signs of lesion are infrequently revealed even if there are many shigellae in the cytoplasm. In such cells (as well as in non-infected ones) the cytoplasm is densely packed with organelles, i.e. an active response to injury can be observed. However, in the outer epithelial layers, as early as 3–12 hours after the beginning of the experiment, gradually increasing areas are seen where dystrophic changes in both infected and non-infected cells are evident. The plasma membrane becomes obviously more fragile than normally and it can easily be destroyed in the process of making preparations (Fig. 7-7).

The lesion of the epithelial cells in outer layers of the cornea are probably due to toxic substances of the purulent exudate accumulating in the conjunctival sac which are the toxic products of shigellae digested by polymorphonuclears. Phagocytosis may be observed in smears from the con-

junctival exudate containing free organisms growing here or released when damaged epithelial cells disintegrate. Shigellae are rarely seen in polymorphonuclears inside the cornea. Leukocytes may be disposed quite near the epithelial cells filled with organisms but they do not penetrate them. Shigellae (obviously free ones) engulfed by polymorphonuclears are found commonly in phagosomes and, being digested, are frequently deformed.

Lin et al. (1964) have also established the fact of shigella multiplication inside the epithelial cells. According to observations of Ogawa et al. (1967*a*), who used the immunofluorescent technique, there is a direct association between the number of infected epithelial cells and the intensity of inflammation in the conjunctiva and cornea.

Eye lesions caused by shigellae similar to those of experimental guinea pig keratoconjunctivitis may occasionally occur in man (Lentz and Prigge, 1931). In this respect the observation of Drozdova and Petrova (1929) is of interest, who described the development of a serious keratoconjunctivitis in a mother two days after the faeces of her child suffering from dysentery got into her eyes.

Thus, the development of experimental shigella keratoconjunctivitis is due to parasitism of the agent in the epithelial cells of the cornea and the conjunctiva. Laboratory *Shigella* strains, which preserve other properties characteristic of these bacteria, but are devoid of this capacity, merely cause a short-term leukocyte response detectable only histologically.

It should be emphasized that shigellae invade undamaged epithelial cells and exert no toxic effect while lodging there. It is the multiplication of the organisms in the cytoplasm that results finally in the destruction of the host cell. Toxic substances appearing in the process of shigella digestion by phagocytes seem to participate in the development of local lesions.

The infectious process in the guinea pig's conjunctiva sets in and is finished more rapidly, while in the cornea it lasts longer and results in the destruction of the epithelial lining.

CHAPTER 8

EXPERIMENTAL SHIGELLA CYSTITIS

by

M. V. VOINO-YASENETSKY and T. BAKÁCS

Shigella cystitis occasionally arises as a complication in dysenteric patients; the number of cases so far described is about 50 (Bingel, 1943*a*). Since these complications were reported mainly in little girls the infection was obviously of ascending nature. Bingel (1943*a*, *b*, 1944*a*) himself successfully reproduced shigella cystitis in guinea pigs, recommending his method as a model for the experimental study of dysentery.

According to Bingel's technique the bladder is at first emptied by means of a thin, glass catheter, then 1 ml of 24 hour shigella broth culture is introduced into it. Female animals are applied as their ureter is shorter and is not curved as it is in males. After the challenge, a rather pronounced oedema of the vulva arises on the first day. Moisture in the genito-anal region and some other general symptoms (anorexia, adynamia) may be present and some of the animals die. Recovery in survivors generally sets in by the 15th day. Bacteriuria is found in the course of the disease. At autopsy of sacrificed guinea pigs shigellae may be cultured from the bladder. Bingel also isolated the agents from other organs but only in animals that have died.

Bingel was the first to demonstrate that only comparatively freshly isolated shigellae were pathogenic, while older strains maintained on artificial nutrient media did not induce cystitis though they retained their ability to produce toxins. Subsequently, Bingel (1944*b*) used his model for a number of experiments aimed at studying the pathogenesis of dysentery. He came to the conclusion that the development of an inflammatory process in the urinary bladder cannot be attributed simply to the effect of shigella toxins on the mucosa or blood vessels. He assumed that the activity of the microorganisms themselves was of importance. He demonstrated histological damage (even necrosis and ulcer formation) in the vesical mucosa. As to the results of bacterioscopy, Bingel (1944*b*) reported that organisms were predominantly present in superficial debris, less frequently they were seen among epithelial cells which were still preserved or in superficial layers of inflammatory infiltrated mucosa. According to his hypothesis, dysentery bacteria, using a certain enzyme (which they possess only for a limited time), penetrate the epithelium, die off and release their endotoxins. Under the influence of endotoxins some part of the tissue becomes necrotized and microorganisms grow in the debris formed.

Letterer and Seybold (1949) criticized Bingel's hypothesis. Based on a number of experiments on guinea pigs they attributed significance to the

effect of shigella endotoxins on mucosal blood vessels in the pathogenesis of dysentery. They described vascular disorders (oedema, haemorrhages) which they thought to arise before epithelial damage; 8–10 h after challenge they observed vacuolar epithelial degeneration (hydropic swelling) and later a destruction and desquamation of the epithelium, resulting in total denudation of certain mucosal areas. The leukocyte response (with leukocyte emigration into the bladder lumen) only appeared in the 14th h of the experiments. They (not stating the method of staining) failed to show organisms in the epithelium and explained all pathological processes observed in the mucosa only as primary toxic damage of blood vessels.

Levenbuk and Andreeva (1962) studying experimental keratoconjunctivitis and cystitis in guinea pigs (see Chapter 7) were the first to report briefly on the presence of shigellae inside the epithelial cell of the bladder mucosa. Later (Levenbuk and Andreeva, 1965*a*) they mentioned that in experimental cystitis the organisms were situated not deeper than the epithelial layer and even when introducing shigellae into the bladder wall, the bacteria proliferated only in epithelial cells.

Szturm-Rubinsten and Piéchaud (1963) studied shigella cystitis in guinea pigs as well, but apparently (it is not quite clear from their paper) they mainly investigated smears from the urinary sediment of infected animals. In the smears they observed the same picture as in the exudate obtained from the palpebral fissure in experimental keratoconjunctivitis. The bladder content displayed many sloughed epithelial cells filled with organisms as well as leukocytes also containing shigellae but in a smaller number. Some infected epithelial cells were destroyed; others, densely filled with organisms appeared well-preserved. Discussing their observations, they raised the problem of the possible defensive role of epithelial cells phagocytizing the causative agents. This question, however, remained unanswered.

Tenner et al. (1971*b*) studied experimental shigella cystitis using Bingel's technique. The same course of the infection was also reported by these authors, but in more detail. Some of the guinea pigs died, others were sacrificed at various intervals after challenge (from 1 up to 144 h).

Gross post-mortem changes were observable as early as 6 h after challenge. Oedema of the bladder wall was slight at first but it gradually increased and extended over the connective tissue of the small pelvis and along the ureters by the 9–24th h. Haemorrhages on the congested vesicular mucosa were discernible; later, at the beginning of the second day, ulcers appeared. Occasionally, single areas of mucosa were covered with yellowish grey films.

As known, the bladder is lined with the so-called transitional epithelium composed of several layers, the upper one being composed of rather large rounded cells. According to Letterer and Seybold (1949) in normal guinea pigs the epithelial lining consists of 3 cellular layers. In a distended bladder the epithelium becomes flattened.

Tenner et al. (1971*b*) found that as early as 1 h after challenge the number of polymorphonuclears in the submucosal blood vessels increased in all animals. Leukocytes were also present outside the vessels, in a small number, i.e. in the connective tissue beneath the epithelium throughout the bladder

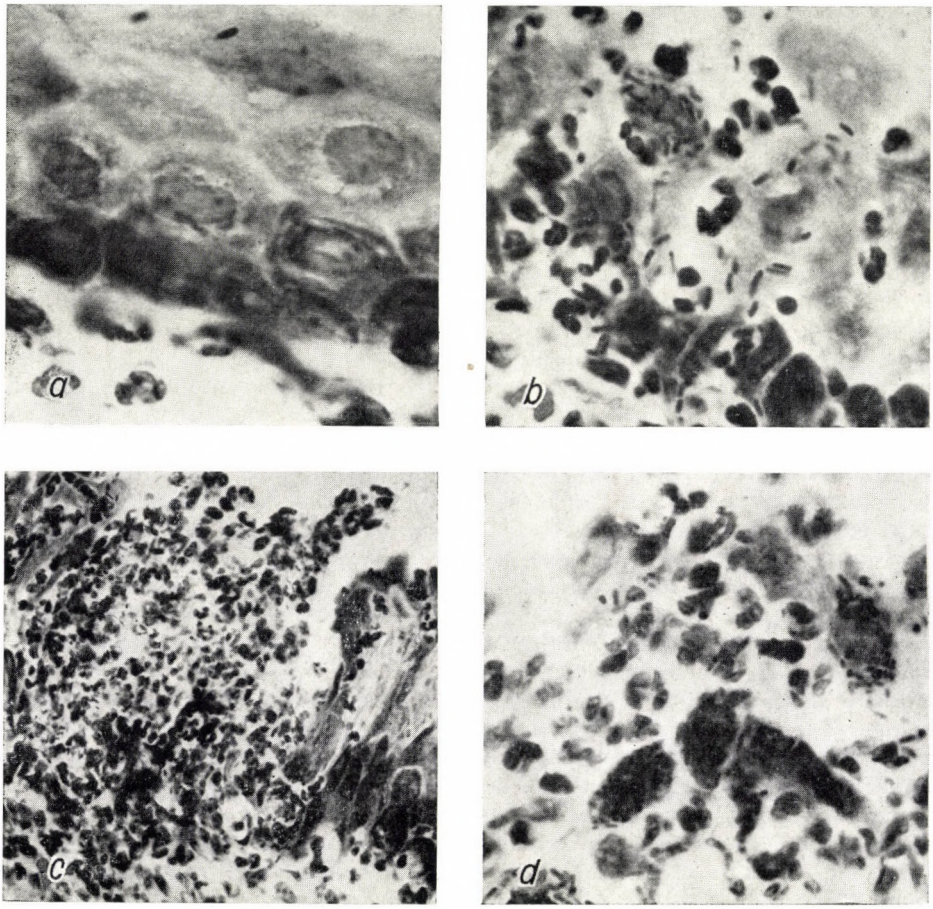


Fig. 8-1. Focal damage and organisms in epithelial cells of guinea pig bladder after introducing *Sh. flexneri* suspension. *a* 1 h after challenge, ×1200; *b* after 6 h, ×1200; *c* after 24 h, ×450; *d* after 24 h, ×1200. Thionine

wall. Slight diffuse oedema of the connective tissue was observed. In the cytoplasm of single epithelial cells having an ordinary structure 1–2 well-stained rods were found (Fig. 8-1*a*). In the course of the first two days the signs of inflammation became more prominent. Three to six hours after challenge a focal leukocyte infiltration appeared in the epithelium (Fig. 8-2). In such foci polymorphonuclears were found between the epithelial cells damaged by shigellae (Fig. 8-1*b*, *c*). The number of the organisms inside the epithelial cells as well as that of the damaged cells increased. This was regarded as evidence of the bacterial multiplication in the cellular cytoplasm and of the possibility of shigella transfer from infected into intact cells.

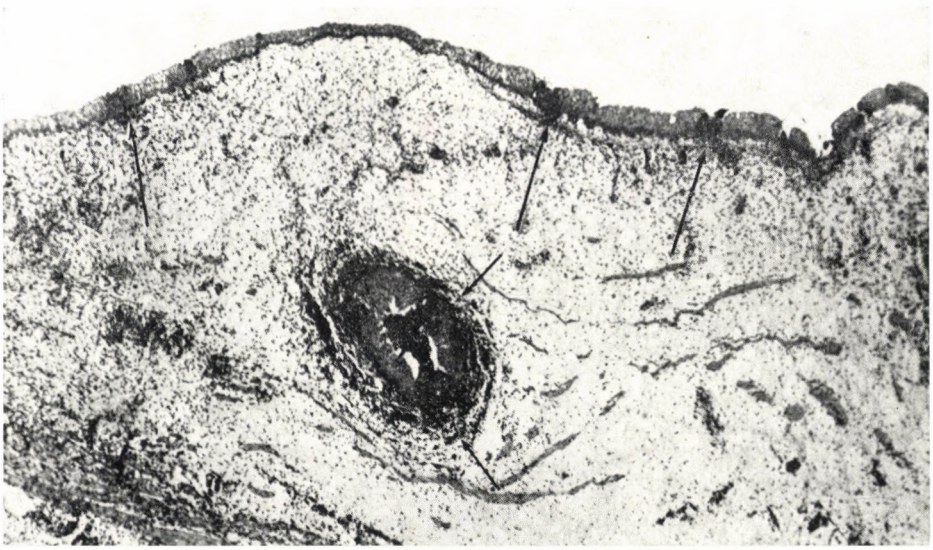


Fig. 8-2. Focal leukocyte infiltration in vesicular mucosa and (below in centre) ureteral mucosa 6 h after challenge; oedema of submucosa. Thionine, × 70

As in shigella keratoconjunctivitis, the nuclei and the cytoplasm of vesicular epithelial cells containing bacteria usually remained well preserved. Signs of degeneration were observed only in cells filled with a great number of organisms. As shown in a yet unpublished electron-microscopic study, shigellae that were situated in the cytoplasm of epithelial cells were dividing and did not display any signs of damage. In contrast, the organisms engulfed by phagocytes exhibited signs of injury and were enclosed in vacuoles which, in addition, contained an electron dense substance.

Infected epithelial cells were finally destroyed or sloughed in increasing numbers (Fig. 8-1*d*). Most of them, evidently, were excreted with the urine. At sites superficial defects of various extension appeared (Fig. 8-3*a*). It is important to note that these defects were confined to the epithelial lining and did not spread to the submucosa, where only a moderate local leukocyte response was observed. In accordance with observations of Bingel (1944*b*), shigellae did not penetrate beyond the epithelial layer and were exceptionally detected in macrophage-type cells in the areas of defects devoid of epithelium. In some places leukocytes accumulated between the epithelial cells forming "microabscesses" as seen in the conjunctival epithelium (see Chapter 7). At early stages, epithelial cells infected by shigellae and undergoing destruction, as well as leukocytes phagocytizing the agents were found in the microabscesses. Later, vacuoles containing fragments of disintegrated cells remained occasionally at these sites (Fig. 8-3*b*). Beginning with the second day, formation of non-specific granulation tissue was noted in the seriously damaged areas, though a simultaneous production of fresh infectious foci was also observed. In many cases similar

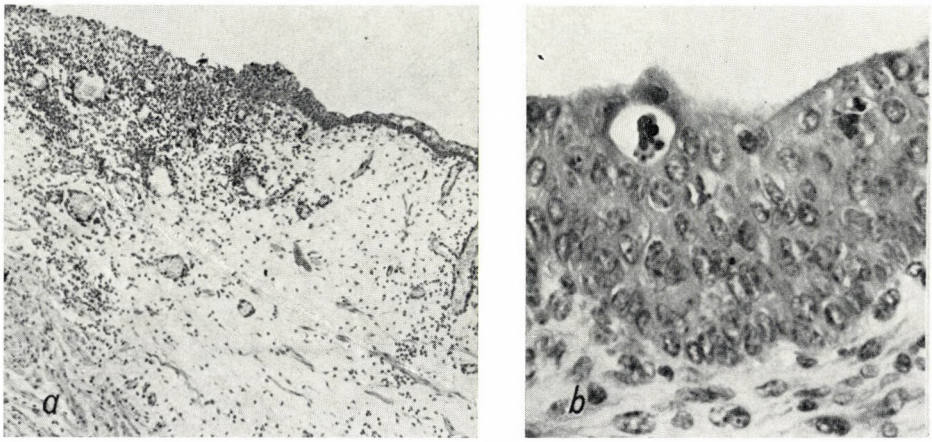


Fig. 8-3. *a* Partial destruction of the epithelial lining 48 h after challenge, ×40. Haematoxylin eosin. *b* Vacuole with leukocyte fragments on its superficial layer after 72 h, ×500. Haematoxylin eosin

pathologic processes were observed in the mucosa of the ureters (see Fig. 8-2) and also in the renal pelvis.

In subsequent experiments, avirulent or killed shigellae and their endotoxins were introduced into the bladder of guinea pigs. Confirming the results of Bingel, no cystitis developed, though in histological sections there was a slight transient accumulation of polymorphonuclears (lasting for 24 h) under the epithelium and a slight oedema. The same phenomena were evoked by introducing sterile broth into the bladder.

Thus experimental shigella cystitis and keratoconjunctivitis have much in common. In both models parasitism in epithelial cells of the mucosa is responsible for the damage observed. In experiments on the bladder focal injuries conceivably connected with shigellae lodging at isolated areas of the epithelial lining are conspicuous . In contrast to descriptions by Letterer and Seybold (1949) such epithelial injuries appear quite soon, as early as a few hours after challenge. At the same time a leukocyte response begins. It is at first diffuse, because of the general irritation of the mucosa by the bacterial suspension introduced in broth then it is strictly limited to sites where shigellae have settled in the epithelium. Vesicular mucosal lesions may be rather extensive but, as a rule, they are confined to the outer surface. Under the epithelial lining only vascular disturbances (oedema, haemorrhages) are seen, which develop simultaneously with the epithelial damage.

Finally, it should be mentioned that shigellae occasionally induce vulvovaginitis in humans (Lemann, 1920; Teveli, 1934; Temme et al., 1969). In guinea pigs the susceptibility of the vagina was observed by Piéchaud and Szturm-Rubinsten (1959) as well as by Levenbuk and Andreeva (1965*a*). Kashiba et al. (1967) even suggested the use of vaginal challenge of young mice as a model for studying shigella infection.

Summarizing, shigellae introduced into the bladder of guinea pigs, penetrate the mucosa and gain entrance into epithelial cells. As in the cornea, shigellae usually settle directly in the cytoplasm and do not produce any perceptible cell damage until having reached great numbers by multiplication. In the bladder the focal nature of inflammatory changes of the mucosa related to its immediate damage by the organisms are clearly visible. The introduction of apathogenic or killed shigellae and their endotoxin into the bladder evokes but a slight leukocyte response similar to that induced by broth.

CHAPTER 9

INTRANASAL CHALLENGE OF LABORATORY ANIMALS WITH SHIGELLAE (THE SHIGELLA LUNG MODEL)

by

M. K. VOINO-YASENETSKAYA

and

M. V. VOINO-YASENETSKY

Air-borne infection of animals as well as intranasal and intratracheal administration of microbial suspension into the respiratory tract has long been employed in laboratory practice. The method is mainly used for studying the development of pneumonia induced by pneumococci, tuberculosis and certain other pulmonary infectious diseases. At autopsy of dysentery patients, focal or lobar pneumonia (Fischer, 1929), occasionally with areas of abscess which may even develop into gangrene, are frequently observed (Polonsky and Voino-Yasenetsky, 1940). However, no direct relation has been proved between these complications and the primary disease and in most cases they are thought to be a secondary infection (Tarasova, 1958). Felsen (1945) described a peculiar "pneumonic type" of dysentery but his work had been based on clinical observations and had not been confirmed by bacteriological examinations.

Thus, there seemed to be no sense in administration of shigellae into animal's respiratory tract. However, having failed to reproduce dysentery in young rabbits by means of oral administration (using a blunt needle), we found that many of the animals developed focal pneumonia. In these cases shigellae were recovered in pure culture from the pulmonary tissue (Bibinova and Voino-Yasenetskaya, 1954). These observations have shown that (*i*) bacteria administered orally may reach not only the oesophagus but the trachea as well; (*ii*) they do not perish in the lungs but are capable of inducing a local infectious process. This observation gave the idea to use the lung infection as a model for studying certain properties of shigellae. A special study (Voino-Yasenetskaya, 1957) demonstrated that the experiments should be made on white mice with a relatively small number (millions) of shigellae as inoculum. Thus their fate in the body can be followed by means of bacteriological and histological examinations performed at different stages of the infection.*

* At about the same time intranasal challenge with shigellae was also used by Vedmina et al. (1956) but they administered high numbers of organisms (up to 2×10^9) and expressed the results in terms of the death rate of the animals. Avtsyn and Berezina (1958), who used the same method for studying the effect of antibiotics, did not publish the results of bacteriologic investigations.

INTRANASAL INFECTION OF MICE WITH SHIGELLAE

Eighteen-hour broth culture (0.05 ml) was introduced under light ether anaesthesia intranasally to white mice weighing 12 to 14 g.

The above volume contained about 10^7 bacteria of which approximately 70 per cent reached the lungs. The actual infecting dose was determined by means of pulmonary tissue cultures taken immediately after inoculation. The lungs of mice killed by ether were finely ground with 1 ml of saline, measured portions of its various dilutions were seeded on agar plates, and the colonies grown were counted. The quantitative assay was repeated in the course of the experiment at certain intervals. Taking into consideration

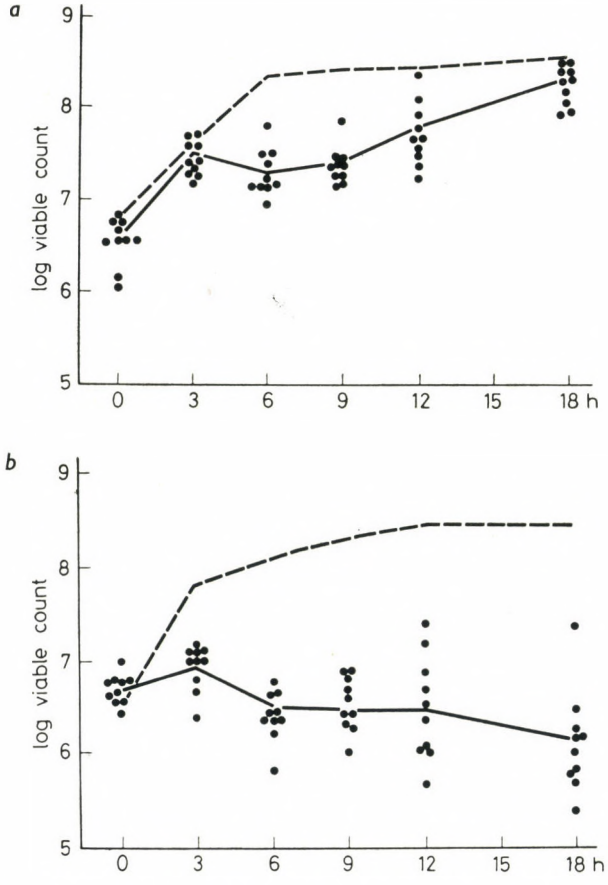


Fig. 9-1. *Sh. sonnei* (*a*) and *Sh. dysenteriae* 2 (*b*) in the lungs of mice at different periods after challenge. Dots denote the number of organisms in the lungs of single mice; continuous line, mean logarithmic values characteristic of periods of the experiment; dotted line, dynamics of multiplication of the same organisms in liquid medium (broth)

the inevitability of individual fluctuations in the development of infection, cultures were made from not less than 5 killed mice each time. Animals dying spontaneously were examined separately.

Figure 9-1 represents the results of two experiments on a lung model using a highly virulent *Sh. sonnei* strain and an almost non-pathogenic strain of *Sh. dysenteriae* 2. In the first experiment almost all the animals died within 24 hours, while in the second one only some mice died of chance complications.

The characteristic features of the curves displaying the growth (Fig. 9-1*a*) and death (Fig. 9-1*b*) of shigellae in the lungs of mice should be noted. In both cases an increased number of organisms were recovered from the lung tissue 3 h after inoculation. With *Sh. dysenteriae* 2 this increase was not considerable but recurred regularly in all similar experiments. A later decrease in the number of organisms, usually by the 6th h after the beginning of the experiment, was regularly observed, independently of the virulence of the organism. The difference in the fate of the two organisms became evident only later. Histological study in these experiments (Voino-Yasenestky and Voino-Yasenetskaya, 1962*a*, *b*) explained the reasons of the above-mentioned phenomena, and presented a certain amount of additional evidence on the peculiarities of the course of experimental shigella infection.

Shigellae could be revealed in eosin-azure or thionine-stained sections of pulmonary tissue immediately after inoculation. At first they were few in number appearing as single rods mostly in the apical portion of the lung as well as about the hili. Within 3 h the number of organisms markedly increased and most of them rested on alveolar walls. Even with the slightly

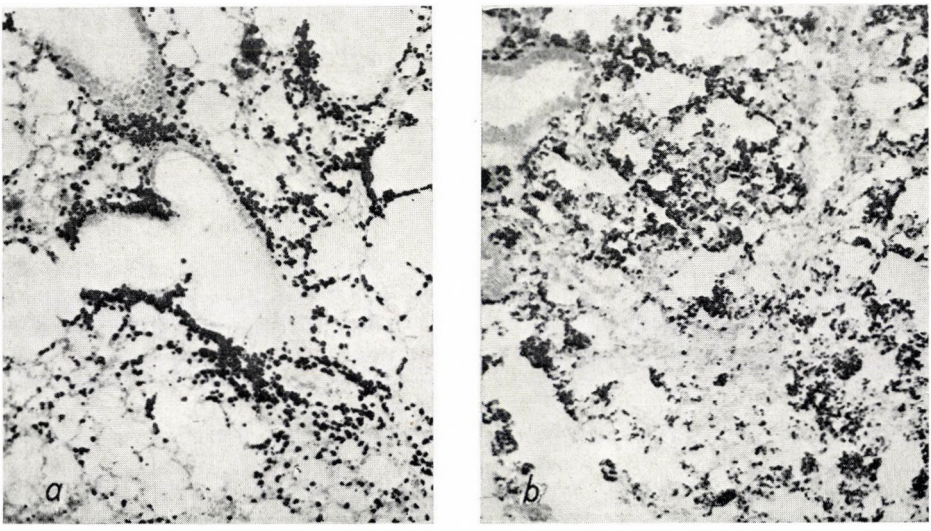


Fig. 9-2. Accumulation of granulocytes (staining dark) in the lungs 2 h (*a*) and 3 h (*b*) after challenge with shigellae. Goldmann's Sudan alpha-naphthol stain, ×100

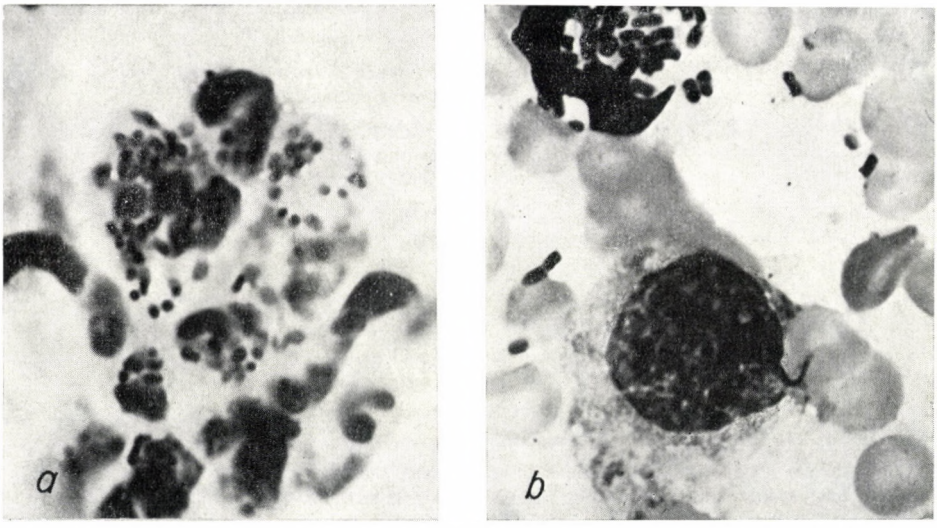


Fig. 9-3. Phagocytosis of *Sh. flexneri* (*a*) and *Sh. sonnei* (*b*) by leukocytes in pulmonary alveoli 6 h after challenge. Absence of phagocytosis by macrophage (*b*, right bottom). *a* Thionine, ×2000; *b* imprint, eosin azure, ×1700

virulent strains of *Sh. dysenteriae* 2 only a small part of the organisms (10–30 per cent) were phagocytized by macrophages which could always be found ready in pulmonary alveoli. This somewhat reduced but did not suppress bacterial multiplication. More virulent organisms of *Sh. sonnei* were scarcely engulfed by these cells and showed the same growth rate as on artificial medium during the first hours (see Fig. 9-1*b*).

The course was different when circulating granulocytes invaded the alveoli. Leukocytes first accumulated in the blood vessels around the small bronchi (Fig. 9-2*a*) but later they appeared mainly in the capillaries of the pulmonary tissue (Fig. 9-2*b*). Approximately from the 3rd h of the experiment, the leukocytes gradually emigrated from the blood stream into the alveoli of apical and perihilar portions of the lung where the bacteria were situated. Having reached the alveoli, the polymorphonuclears immediately started to ingest the organisms. In lung sections and imprints a few polymorphonuclears could be found as early as 2–3 h following the challenge; the phagocytosis reached its climax in the 6th–9th h, when the amount of shigellae in the lungs, determined by cultivation, ceased to rise and even diminished (see Fig. 9-1).

In the pulmonary alveoli leukocytes engulfed all shigellae including those more or less pathogenic to mice (Fig. 9-3*a*, *b*). However, the outcome of the phagocytosis was different.

In our experiment an almost non-pathogenic strain of *Sh. dysenteriae* 2 ingested by leukocytes appeared first as long or short, well-staining rods. After 9 h some of them stained very faintly. Moreover, in the cytoplasm

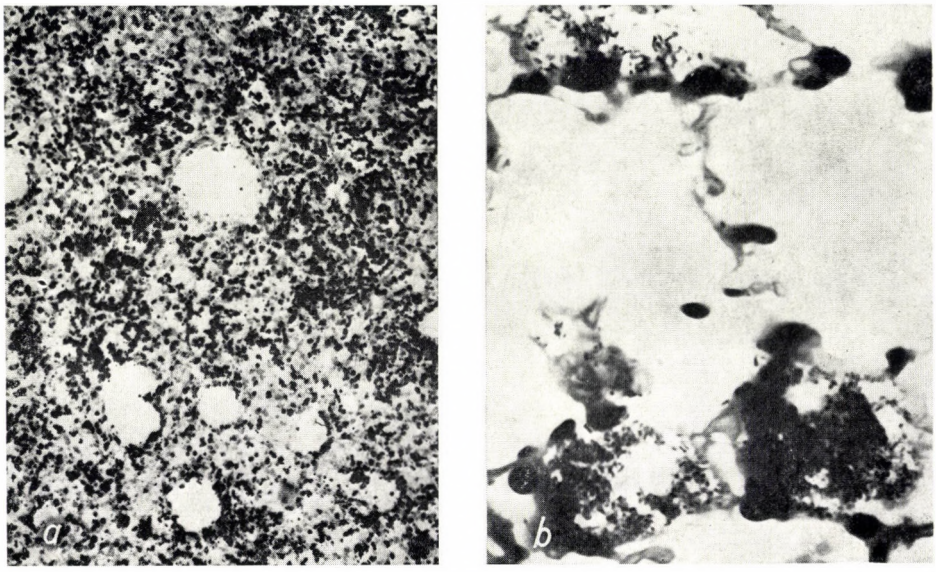


Fig. 9-4. Leukocyte response in the lungs (*a*) and unimpeded bacterial growth in single alveoli (*b*) 9 h after challenge with *Sh. sonnei*. *a* Sudan alpha-naphthol, ×100; *b* eosin azure, ×900

of leukocytes small microbial fragments with uneven ends were frequently observed. Simultaneously with the signs of intracellular digestion of bacteria, the nuclei of leukocytes became considerably deformed.

It should be noted that along with vigorous and successfully completed phagocytosis, single organisms remained free and even formed microcolonies composed of scores of short coccoid rods. By the end of the first 24 h bacterial counts in the lungs had decreased significantly, so that lung sections and imprints rarely contained microbial fragments after another 24 h. However, the presence of viable shigellae ranging from 4×10^3 to 1×10^5 were still detected in the cultures. In the epithelial cells of the bronchi such organisms were present very rarely.

The destruction of *Sh. dysenteriae* 2 organisms was fatal for the leukocytes themselves; later they were engulfed by macrophages or eliminated through the bronchi. Perivascular oedema and haemorrhage were noted frequently, but were less common in certain groups of alveoli. In most mice killed on the 5th–7th day of the experiment the pulmonary tissue presented a practically normal picture. Only small groups of macrophages about the alveolar ducts and accumulation of round mononuclear cells about some arteries and veins showed the pathologic processes that had taken place.

Apparently, bacteria of virulent shigellae engulfed by leukocytes were most frequently destroyed as well: they were observed to become smaller, as if shrinking and were sometimes enclosed into vacuoles. In some other leukocytes fairly well preserved organisms filled the cytoplasm and these

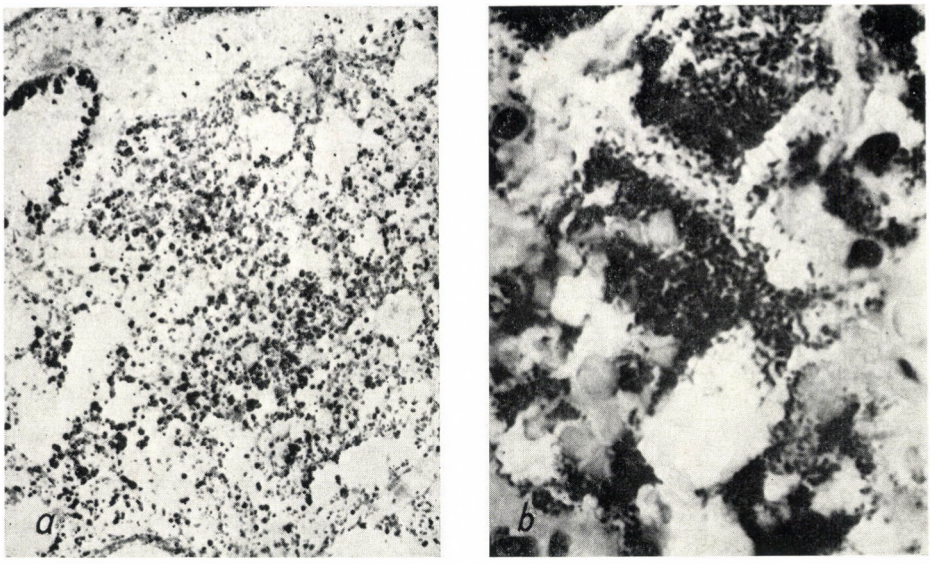


Fig. 9-5. Small number of disintegrating leukocytes in the alveoli (*a*) which are filled with organisms (*b*) 24 h after challenge. *a* Sudan alpha-naphthol, ×100; *b* eosin azure, ×950

cells were characterized by markedly deformed nuclei (see Fig. 9-3*b*). Following the destruction of such a cell, the released organisms were presumably engulfed by other leukocytes. In many cases, however, the alveoli did not contain an adequate number of leukocytes, so that most of the organisms remained free.

The insufficiency of leukocyte response seemed to be particularly noticeable with a highly pathogenic strain of *Sh. sonnei*. At the very beginning of these experiments the polymorphonuclears slowly emigrated from the blood vessels. After 6–9 h, part of the alveoli still contained a considerable amount of granular leukocytes (Fig. 9-4*a*). In these alveoli most bacteria were phagocytized, while other alveoli (frequently adjacent ones) were almost completely devoid of leukocytes and bacterial growth was not hindered (Fig. 9-4*b*). At later stages the migration of leukocytes to the lungs ceased almost entirely. Polymorphonuclears which had earlier reached the alveoli and fulfilled their function disintegrated without being replaced. There were also comparatively few leukocytes in the capillaries; they were almost absent in oedematous perivascular spaces and in the lumen of the bronchi. Granular leukocytes were sometimes only seen along the walls of some strongly dilated blood vessels (Fig. 9-5*a*). In the same animals bacterial multiplication was especially intensive in the alveoli (Fig. 9-5*b*). The deficiency of host defence could be detected in some mice as early as after 12 hours, while in others it was only observed later. However, in this series of experiments nearly all the mice died eventually. The individual reactivity of

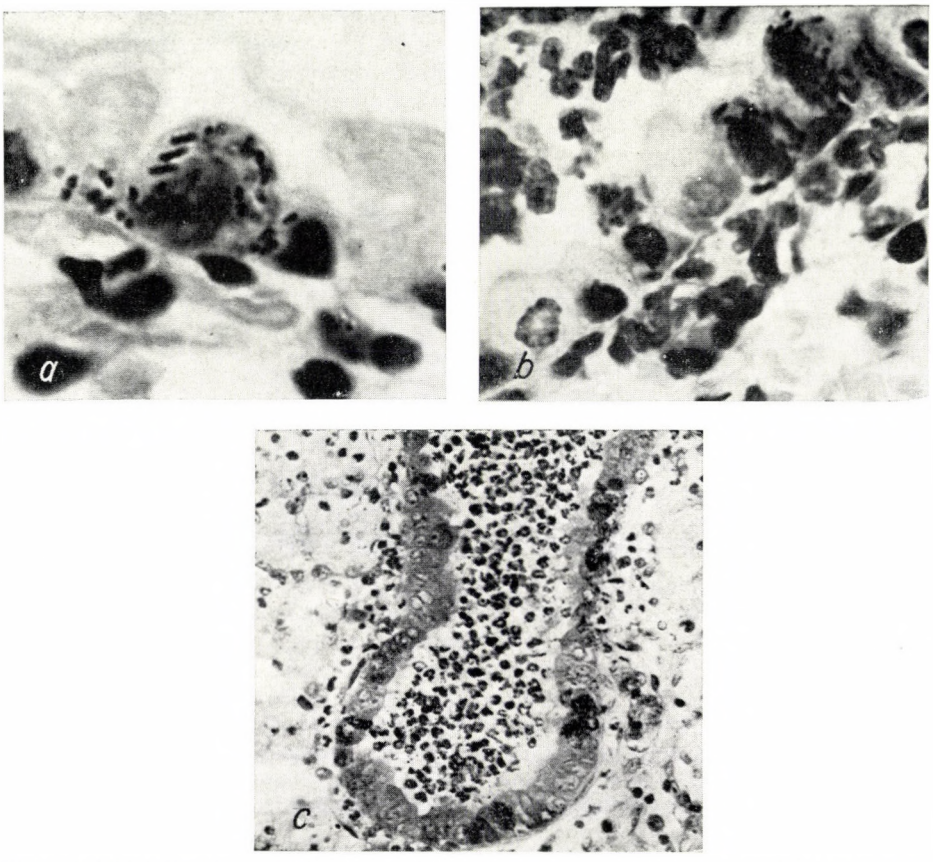


Fig. 9-6. Multiplication of *Sh. flexneri* 2 6 h (*a*) *Sh. sonnei* 9 h (*b*) and *Sh. sonnei* 12 h (*c*) after challenge. *a* Eosin azure, ×2200; *b* thionine, ×1400; *c* eosin azure, ×310

the animals played an essential part in experiments with less pathogenic strains of *Sh. sonnei*, *Sh. flexneri* 2a, *Sh. flexneri* 6 and *Sh. boydii*. In some mice the leukocyte emigration from the blood vessels increased steadily and, 24–48 h after the challenge, organisms that had escaped phagocytosis and destruction could hardly be found in the alveoli filled with exudate. In other animals depression of leukocyte response occurred resulting in unrestrained bacterial proliferation and the death of the animal.

The ability to parasitize in epithelial cells seems to be another important feature of virulent shigellae; this was observed in intranasal challenge of white mice (Fig. 9-6*a–c*). The bacteria which settled in the epithelium of bronchial mucosa appeared to be quite viable on the basis of dividing forms found among them. The epithelial cells seemed to survive for a long time. Their nuclei frequently retained their normal appearance even if the cytoplasm had been densely packed with multiplying organisms. Later on, how-

ever, the cells gradually disintegrated and were shed, and the defects appearing in their place were replaced by polymorphonuclears.

If very intensive growth of shigellae in the alveoli and bronchial epithelium occurred in dying or dead mice, bacterial proliferation could also be observed in the expanded perivascular areas. Though in such cases the branches of lung artery were occasionally surrounded by massive sheaths of microbial colonies, single bacteria were seen in regional lymph nodes extremely rarely.

In experiments with virulent *Shigella* strains more serious lesions of the lung tissues were observed, mainly in the form of vascular disorders (oedema, haemorrhage). No fibrinous deposits were seen.

Outside the lungs an increased leukocyte count was detected in the capillary network of the kidneys and the liver; a breakdown of lymphoid cells (especially in the thymus), indicating considerable general intoxication, took place. Blood cultures from the heart and the suspended spleen pulp revealed moderate bacteraemia in all the experiments (including those with apathogenic strains) directly after inoculation. After 1–3 h the blood cultures were negative and the infectious process was limited to the lungs. Shigellae were cultured in considerable numbers from the blood and the spleen of the majority of dead animals as well as immediately before their death.

As has been mentioned, the ability of shigellae to induce pneumonia was noted in experiments on rabbits. Since the experiments required a great number of animals, all further observations were made on mice. Piéchaud and Szturm-Rubinsten (1959) repeated our first investigations in experiments with intranasal infection of guinea pigs. The results obtained were less reliable, as only half of the animals succumbed to infection. Histological study of pulmonary tissue was not reported.

OBSERVATIONS ON THE PATHOGENESIS OF EXPERIMENTAL SHIGELLA PNEUMONIA

Pathological processes occurring in the lungs after intranasal administration of virulent or avirulent shigellae may be regarded as pneumonia. The focal-type infection develops mostly in the posterior regions of the upper and middle portions of the lungs, i.e. where the microbial bulk seems to settle after intranasal inoculation.* The histological changes are essentially similar to those observed in other types of pneumonia especially if the sections are stained with haematoxylin and eosin. However, the interrelation between shigellae and the various types of host cells in the lung tissue has rather important peculiarities.

* Inoculating mice with aerosol through inhalation (e.g. *Bordetella pertussis* — Voino-Yasenetsky and Khai, 1957) the lower (caudal) portions of the lungs were most severely affected.

First and foremost, shigellae are able to parasitize epithelial cells in the respiratory tract as well as on other mucous membranes. This ability will be discussed in detail after considering other models of dysentery infection and its manifestation under natural conditions. In the lung model the interaction between shigellae and phagocytizing cellular elements is especially noteworthy. This phenomenon is best observed in the lung because the inflammatory exudate and the agents remain in the alveoli, not being excreted as in cystitis and not overflowing as in keratoconjunctivitis. Cough reflexes which would result in expectoration of the exudate are lacking in mice.

As mentioned above, not only virulent shigella but also those which had lost pathogenicity are able to survive and multiply in pulmonary alveoli. Apparently, the substances moistening the alveolar surface and the protein fluid exuding from the blood vessels serve as a nutrient medium. Local macrophages do not interfere with shigellae. However, these cells are capable of clearing the alveoli, of various foreign particles and organisms. Thus, staphylococci injected into the lungs are almost completely engulfed by alveolar macrophages (Laehr, 1887; Vysokovich, 1889; Ilyin, 1958). For some reason, the same cells do not respond to shigellae and the defense against these organisms is mainly accomplished by polymorphonuclears.

In contrast to apathogenic shigellae, virulent ones are not always destroyed by leukocytes. As regards the experiments described above, two possible reasons for polymorphonuclears not being able to interfere with the viability of virulent shigellae, might be suggested. (*i*) Phagocytosis of such bacteria frequently remains unaccomplished, as the phagocyte itself is destroyed. It seems probable that only the least virulent and dying organisms in the inoculum are ingested. (*ii*) Virulent shigellae somehow prevent the emigration of leukocytes into the alveoli. The deficiency of leukocyte response at the initial stages of the infectious process deserves special mention. The suppression of this response, which is almost complete during the terminal period, is less characteristic. It is observed in the final stages of other experimental lethal infections and is obviously an evidence of a dramatic process occurring in the body.

No substances inhibiting chemotaxis have been detected in shigellae so far. There is no reason to connect leukocyte suppression with the products of disintegration, i.e. endotoxins. In additional experiments we administered

TABLE 9-I

Mortality of mice after intranasal administration of 10^9 *killed shigellae*

Strain	Mortality rate after		
	24 h	48 h	72 h
Sh. dysenteriae 2	0	6.0	2.1
Sh. sonnei	0	14.3	27.5
Sh. flexneri 2	0	17.0	34.0

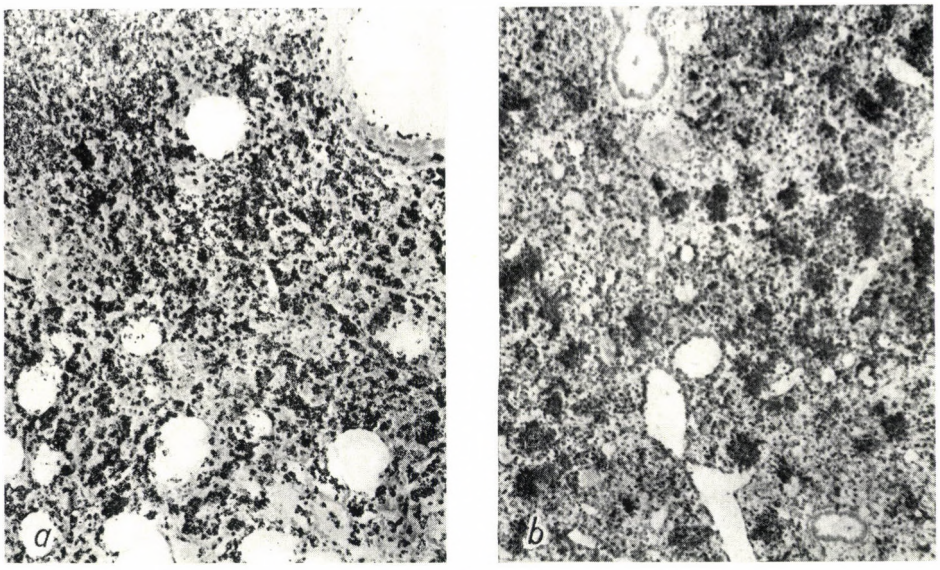


Fig. 9-7. Filling of pulmonary alveoli with granulocytes 24 h (*a*) and 48 h (*b*) after intranasal inoculation with 10^9 killed shigellae. Sudan alpha-naphthol. *a* ×10; *b* ×50

large doses (10^9) of various heat-killed shigellae into the lungs of mice.* It was fatal for some of the animals; *Sh. sonnei* and *Sh. flexneri* 2 displayed higher toxicity than *Sh. dysenteriae* 2 (Table 9-I).

Local response to such large doses of antigen was pronounced. Perivascular oedema developed almost immediately and was promptly followed by perivascular haemorrhage after which granular leukocytes accumulated rapidly in the pulmonary capillaries. They emigrated into some alveoli within 3 h and large pneumonic foci developed (Fig. 9-7*a*) by the 24th h in the majority of animals. Liquid exudate was comparatively scarce, but the filling of alveoli with leukocytes continued up to the end of the second day. This was followed by the onset of clearing of pulmonary tissue, a process frequently lasting for 10–20 days.

There was no essential difference in leukocyte response between various killed shigellae. The most important finding was that leukocyte emigration was in no case inhibited. Even in mice which had succumbed, the pulmonary alveoli were densely filled with cellular exudate (Fig. 9-7*b*).

Although the mechanism of resistance of virulent shigellae to phagocytizing cells is not yet clear, there is no doubt that this property of the microorganisms is of great significance in the onset and development of the

*This amount corresponds to the largest number of shigellae present in the lungs of mice in lethal infection.

infection. The experiments carried out on the lung model enable us to draw the following conclusion: the pathogenicity of shigellae is due to their ability to parasitize in epithelial cells, while the virulence of a pathogenic strain depends on the extent of its resistance to phagocytes

PRACTICAL PROBLEMS OF THE USE OF THE SHIGELLA LUNG MODEL

In an earlier paper (Voino-Yasenetskaya, 1957) it was stated that *Sh. dysenteriae* 2 as well as avirulent strains of *Sh. sonnei* are promptly destroyed in the lungs of mice. *Sh. boydii* is able to resist the host's defence to a certain extent but this property is characteristic only of freshly isolated strains and is lost fairly rapidly in cultures growing on artificial media. In *Sh. flexneri* 2, *Sh. sonnei* and *Sh. flexneri* 6 (Newcastle), the ability to multiply is more stable but it is lost after a certain time though the biochemical and serological characteristics remain unchanged. In general, this fact is in conformity with the observations made during intravesical (Bingel, 1943*a*) and conjunctival (Serény, 1955*b*) challenge of guinea pigs.

A special comparison of the keratoconjunctival test and the lung model was made by Kandyurina (1970*a*, *b*). She drew the conclusion that, while the results almost fully agreed, the lung model permitted to establish a broader spectrum of bacterial virulence. The same conclusion was drawn by Blinova (1970). Petrovskaya and Blinova (1971) tested various classes of hybrids of virulent *Sh. flexneri* 2a and non-pathogenic strains of *E. coli* using them in different models (keratoconjunctival and enteral infection of guinea pigs, intranasal and intraperitoneal challenge of mice, infection of the chorionallantoic sac of chick embryos). The intraperitoneal test was found inconclusive, the chick embryos could only be employed for an approximate differentiation of the strains, while the lung model, the keratoconjunctival test with graded doses and enteral challenge of starved guinea pigs (according to Formal et al., 1958) yielded the most precise results.

It should be pointed out that experiments using the lung model, especially if combined with morphological investigations are rather laborious. In this respect the keratoconjunctival test is more advantageous. The intranasal infection of white mice may help in solving many practical questions (e.g. testing the action of antibiotics—Voino-Yasenetskaya, 1958), but the technique is most valuable in research on the interaction between parasite and host. In the work of Avdeeva (1963, 1964), Blank (1968, 1969), Ivanova (1968), Ivanova and Avdeeva (1968), Chilingaryan (1970), Blank and Avetikyan (1972) the lung model was adopted and successfully employed for studying the immunology of dysentery and the evaluation of shigella vaccine. As it will be shown later in this book, the intranasal infection of mice was useful for the analysis of infectious processes induced not only by shigellae but also by other enteric agents like salmonellae (see Chapter 15) and enteropathogenic *E. coli* (see Chapter 21).

In conclusion, intranasal challenge of white mice with shigellae results in the development of an infectious process mainly limited to the lung

tissue. If a moderate number of shigellae is administered, the course and the outcome of the infection depend on the virulence of the organism; the results of the lung model are in conformity with those of other reliable tests (conjunctival, enteral challenge etc.).

In infectious processes induced by shigellae in mouse lungs, these organisms exhibit not only their capability of parasitism in epithelial cells but also some important peculiarities of their relationship with phagocytes (macrophages and polymorphonuclears).

CHAPTER 10

ENTERAL CHALLENGE OF EXPERIMENTAL ANIMALS WITH SHIGELLAE

by

L. S. BIBINOVA

CHALLENGE WITHOUT PRELIMINARY TREATMENT

Many attempts have been made to render laboratory animals susceptible to shigellae. These attempts included administration of organisms with food, by stomach tube or direct injection into the intestinal tract. All these efforts, however, failed to produce dysentery. Nevertheless, in 1901 Deycke described a fatal dysentery-like condition in cats which had been given food containing a large amount of virulent shigellae. Similar findings were published by other authors (Sergeevich, 1954; Budylina et al., 1957; Belaya, 1958). Several series of experiments were carried out by Sirotinin et al. (1958) to compare the susceptibility of various species and classes of animals to dysentery ranging from invertebrates to monkeys. These experiments enabled them to place cats after monkeys on a scale of models for reproducing dysentery. Sirotinin et al. (1958), as well as other authors mentioned above, emphasized an obvious inconstancy of positive results in rendering kittens and cats susceptible to a fatal enteric infection with shigellae.

Hyperaemia, haemorrhages, oedema and small ulcerations of the colonic mucosa were observed at autopsy in cats that died of shigella infection. The same was noted by other investigators in the mucosa of the small intestine (Lebedeva, 1959; Khomik, 1957)*. Sergeevich (1954) and Lebedeva (1959) described diphtheric colitis in cats. None of the papers mentioned above gave a detailed histological account.

Dogs are usually resistant to intestinal infection with shigellae. The only successful attempt to infect these animals was that of Shiga (1898). After several attempts, he succeeded in producing dysentery only once, by administering undiluted agar culture of dysentery organisms into the stomach; this experiment resulted in a severe condition, with intestinal lesion and a high number of shigellae in the intestinal contents.

Enteric infection could not be, as a rule, caused in small laboratory animals, mostly mice and rats (Floyd and Hoogstral, 1954; Sirotinin et al., 1959; Buchin et al., 1961, etc.). McGuire and Floyd (1958) observed increasing quantities of shigellae in the intestinal tract and their penetration into

* It is noteworthy that non-specific intestinal lesions as well as salmonellosis are often observed in cats. After administration of shigellae, the lesions occasionally develop from a latent state to an acute one.

blood circulation, but they administered extremely high doses (about 5×10^9) of *Sh. flexneri* 3 through a stomach tube. Thirty per cent of the animals succumbed to the disease; they had diarrhoea and died 24–36 h after being infected. This report did not contain histological findings. Klodnitsky and Kats (1936) tried to infect rats, they failed to produce disease, but observed a long-term carriership.

Rabbits are usually resistant to enteral infection, but after intravenous injection with living shigellae or their toxin, intestinal lesions frequently develop. The only description of a dysentery-like condition in these animals comes from Dopter and Repaci (1910), who infected rabbits orally by huge doses of Shiga-dysentery bacilli and observed lesions mainly in the caecum. On histological examination they found catarrhal changes with hypersecretion of glands, hyperaemia, haemorrhages, oedema and inflammatory infiltration at the earliest stages of the disease, whereas later necrosis of the intestinal mucosa and superficial ulcerations occurred. The organisms used for challenge were not found in sections of the intestinal wall.

It appears from these investigations that laboratory animals are virtually resistant to dysentery infection; still there must be some factors—not identified so far—that render them susceptible to the agents. Investigators using very large doses were more successful. It has also been mentioned that the animals were more susceptible if freshly isolated shigellae were used. Moreover, there have been discrepancies in the way of evaluating the results of the experiments. In some of the studies the diagnosis of dysentery was based on symptoms of intestinal disorder or findings of shigellae in excrements, although the animals remained healthy. Only a few papers include a more or less detailed description of morphological changes in the intestinal tract.

Our attempts in producing dysentery in cats and kittens by feeding them *Sh. flexneri* suspended in milk (Bibinova et al., 1960) failed to yield convincing results. Similarly, our first experiments on rabbits, when we administered shigellae directly into different parts of the intestine (Bibinova, 1932*a*, *b*) or orally (Bibinova et al., 1960) were not successful either, except for a single series.

Adult, untreated rabbits were given a highly virulent strain of *Sh. sonnei* intraduodenally, which had been isolated from a patient who had died not long before the beginning of our experiment. Ten out of 11 animals infected developed an acute form of the disease and 4 died during the first day after the challenge. Six of the remaining 7 rabbits had diarrhoea for several days; shigellae were constantly found in their excrement. These rabbits were killed at various stages of the infection. It is worth noting that during the first 4 days shigellae were isolated from various parts of the intestine of all the animals and from the blood of some.

In animals that died within 26 h as well as in a rabbit killed 24 h after the infection, morphological changes occurred mainly in the small intestine. The following vascular disturbances, especially severe in the fatal cases, were found: paretic enlargement of the capillaries of the mucosa with enormously swollen endothelium, vast haemorrhages, thrombi in the veins, oedema of the mucosa and submucosa with an enlargement of the

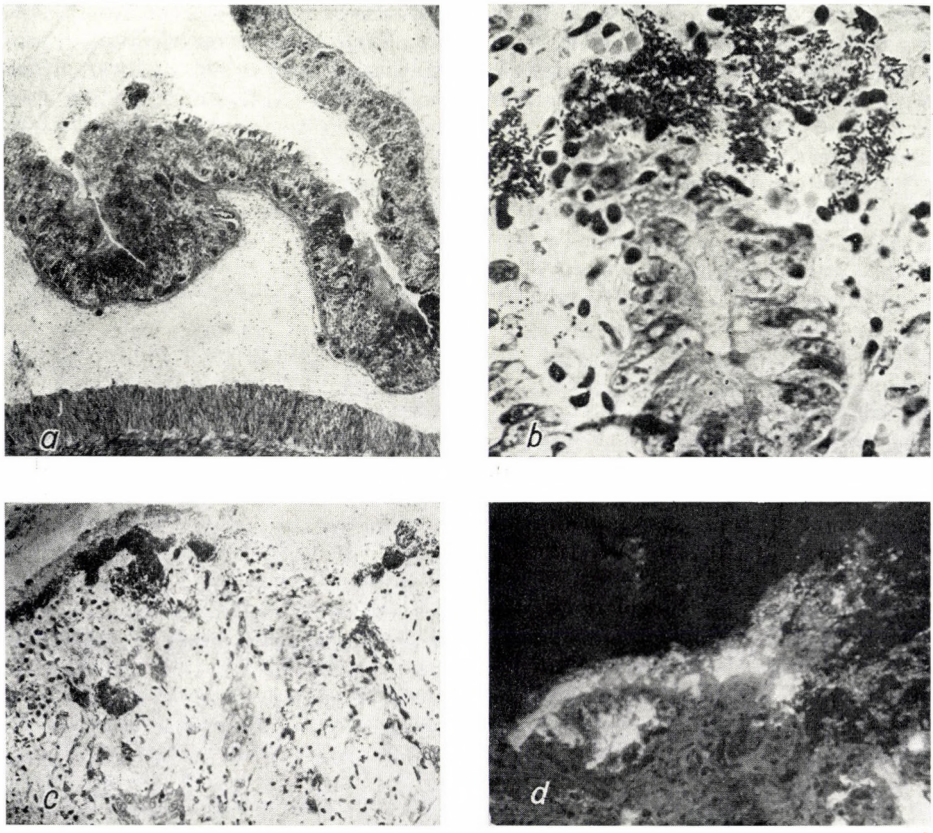
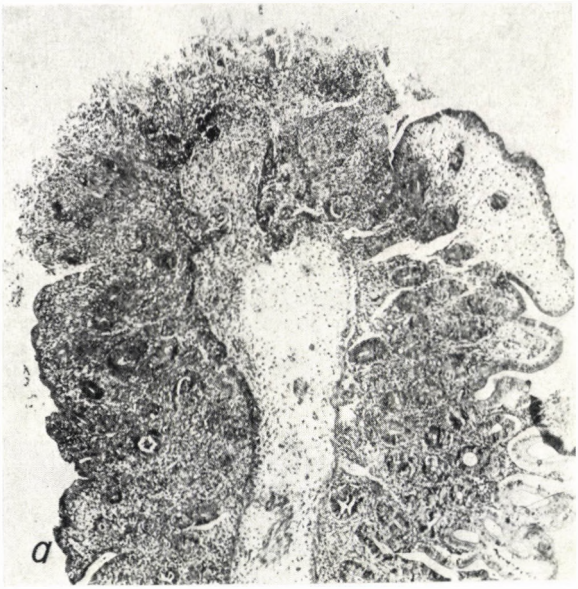


Fig. 10-1. Caecum of rabbit 26 h after infection. *a* Vast haemorrhages in the mucosa, severe oedema in the submucosa. Haematoxylin-eosin, ×40. *b* Partly destroyed crypt with a great number of bacteria. Thionine, ×714. *c* and *d* Necrosis of superficial layers of mucosa with a great number of shigellae. Stained with thionine (*c*) and treated with specific immunofluorescent serum (*d*). ×80

central cavity of villi. At the same time there was an inflammatory infiltration of the mucosa, accumulation of neutrophils in crypts and considerable changes in the content of lymphoid elements. Lymphocytes were numerous both in the epithelium of crypts and in the dilated lymph vessels of the intestinal wall, and an increased rate of destruction of these cells could be observed in Peyer's patches and in the lamina propria. The most severe changes were revealed in rabbits dying 26 h after the infection and examined soon after death. There were lesions not only in the small intestine, but also in the caecum (Fig. 10-1*a*). In addition to the circulatory disturbances and destruction of inflammatory elements in the mucosa, there was a marked necrosis both in mucosal superficial layers and in many crypts which were invaded by a great number of Gram-negative bacteria (Fig. 10-1*b*



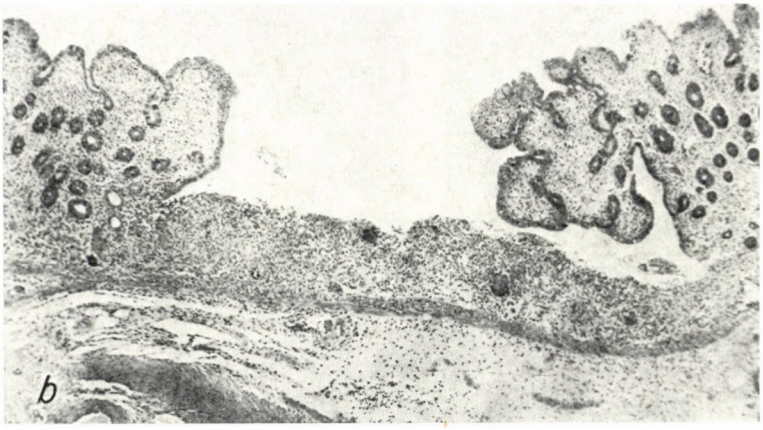


Fig. 10-2. Caecum of rabbit 3–4 days after infection. *a* Necrosis of spiral valve top, oedema and inflammatory infiltration of mucosa and submucosa. *b* Ulcer of caecum, oedema and inflammatory infiltration of submucosa. Haematoxylin-eosin, ×60

and *c*). In supplementary investigations* these bacteria showed a specific fluorescence after treatment with fluorescent gamma-globulin against *Sh. sonnei* (Fig. 10-1*d*).

* For these investigations, carried out together with Dr. Khavkin, we used material embedded in paraffin 6 years earlier.

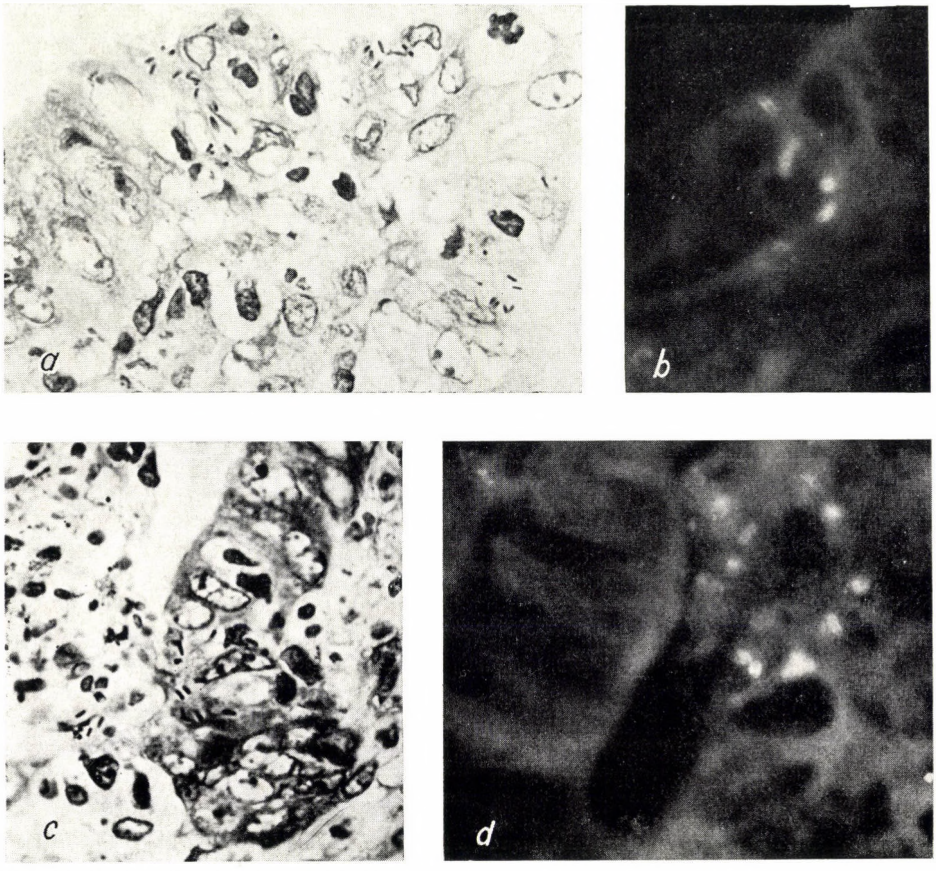


Fig. 10-3. Shigellae in the epithelial cells of superficial layers of the mucosa (*a*, *b*) and crypts (*c*, *d*) of the rabbit caecum. *a*, *c* Stained with thionine; *b*, *d* treated with specific immunofluorescent serum. ×980

In rabbits sacrificed 3–4 days after the infection the small intestine appeared almost unchanged, but in the caecum and in the proximal portion of the colon there was a strong inflammatory reaction with focal necrosis on the top of the spiral valve (Fig. 10-2*a*) and numerous superficial and deep ulcerations (Fig. 10–2*b*); the lumen of some veins was filled with fibrin thrombi. Along the margins of erosions and in the walls of partly destroyed crypts of these rabbits we observed numerous rod-shaped bacteria, arranged inside the epithelial cells and showing bright fluorescence after treatment with specific antibodies (Fig. 10-3*a–d*). Shigellae were phagocytized in great numbers by leukocytes especially at the bottom of the ulcerous defects, and in the preserved intestinal epithelium as well. In the lamina propria bacteria were rarely seen and they were mainly inside the

leukocytes. At later stages of the infection (10 and 14 days) the animals hardly exhibited any intestinal pathological signs and, by this time, they showed no clinical symptoms either.

The data described above show that some very virulent strains of shigellae freshly isolated from man are able to produce enteric disease in rabbits without any pretreatment of the animals. It is worth noting that the high degree of virulence is not stable and decreases very rapidly: our subsequent attempts to repeat the experiment with the same strain failed, although this would have been necessary since it was very difficult to obtain conclusive results due to post-mortem alterations.

The character and localization of intestinal changes in infected rabbits (especially in those killed on the 3rd–4th day of the illness) closely resemble the mild forms of dysenteric colitis in man and monkeys (see Chapters 11 and 12). At the same time, a striking resemblance of some histological findings (circulatory disturbances, necrosis in the area of the spiral valve) exists between these experiments and those in which rabbits were given parenterally viable and killed shigellae or their toxin (Doerr, 1907; Bibinova, 1932*a*, *b*; Letterer, 1944, etc.).

Recently Ogawa and Nakamura (1969*b*) have reported on successful infection of rabbits with shigellae. They introduced bacteria into an isolated segment of the colon after having emptied its contents by washing. A clip at the distal part of the ligated colon loop was removed before the challenge, and 5 h after the inoculation of *Sh. flexneri* 2a suspension the clip at the proximal part was also removed. The animals showed bloody mucous diarrhoea for 3–4 days after inoculation and 4 out of the 18 rabbits died within 2 days. Histological examination of these animals and of 5 others with acute disease killed at the same time, revealed catarrhal purulent colitis accompanied by formation of microulcers and abscesses in the crypts. *Sh. flexneri* was demonstrated by means of the fluorescent antibody technique in the region of ulcers and inside the epithelial cells. The intestine of the rest of the animals killed 5–7 days after the infection was almost unchanged, but some of the epithelial cells contained shigellae. (For the description of similar experiments on isolated segments of the small intestine see Chapter 22.)

In the opinion of some authors, dysentery infection with intestinal lesions can be produced by subcutaneous (Vaillard and Dopter, 1903) intravenous (Besredka, 1925) or subconjunctival (Morgunov et al., 1963) administration of shigellae into laboratory animals. Shiga (1898), Brauer-Eppendorf (1922), Alivisatos and Yovanovic (1926) claimed to have cultured dysentery bacilli from the intestinal contents after massive parenteral administration. Along with other authors we failed to confirm these findings in similar experiments (Bibinova, 1932).

SPECIAL METHODS OF ENTERAL CHALLENGE OF ANIMALS WITH SHIGELLAE

As has been mentioned in Chapter 3, pretreatment reducing the host's resistance may render animals sensitive to experimental dysentery infection. The technique of Formal et al. (1958, 1959), which is a modification of the method used by Koch (1885) and Kazarinov (1904) much earlier, is the one most frequently employed.

Guinea pigs are either deprived of food for 4 days or injected subcutaneously with 0.15 ml carbon tetrachloride 24 to 48 h before challenge. Three hours before the challenge, 125 mg calcium bicarbonate is introduced into the stomach in 5 ml distilled water, and 1 ml of tincture of opium is injected intraperitoneally immediately or 0.5–1 h after infection. For challenge 10^6–10^7 bacteria are introduced into the stomach by means of a stomach tube; this dose produces an acute disease not infrequently becoming fatal within 1–2 days.

Formal et al. (1958, 1959) only performed routine histological investigations mainly in animals that died of the infection. At early stages they found inflammatory changes, focal punctate necroses in the ileum and rather vast ulcerations in the caecum. In animals that had either died or been killed within 2–4 days after the infection there were no changes in the small intestine, but lesions in the caecum and colon were more profound and extensive than earlier. Shigellae were frequently found in the intestinal contents.

Later, using the microtome cryostat and fluorescent antibody technique, LaBrec and Formal (1961) detected shigellae in tissue sections from the intestinal wall of guinea pigs that died of the infection or were killed at an early stage. Shigellae were present not only in the intestinal lumen, but also in the wall, especially in ulcerous regions of the mucosa. Having found shigellae in the lamina propria close to the unchanged epithelium, the authors concluded that the organisms multiplied there having penetrated through the epithelial cells or between them. The authors associated the destruction of epithelium and formation of ulcers with the effect of endotoxin accumulating in the mucosa beneath the epithelium, as a result of a partial destruction of bacteria (see also Formal et al., 1965*d*).

Meanwhile it was shown that in dysentery infection produced in monkeys, shigellae failed to invade the lamina propria but grew in the epithelial cells of the mucosa (Voino-Yasenetsky, 1963; Voino-Yasenetsky and Khavkin, 1964). There is no reason to assume that dysentery infection in guinea pigs differs from that in monkeys, especially in view of the fact that in many different model experiments, described in the previous chapters, shigellae exhibited a peculiar ability to parasitize cells of the epithelial type.

We repeated the experiments of Formal et al. (1958, 1959). In addition, when studying the localization of shigellae in the intestinal wall, we carefully compared the results obtained by fluorescent antibody technique and light microscopy. The same places of the sections, first treated by the method of Coons (1958) and then stained with thionine or according to Leishman's

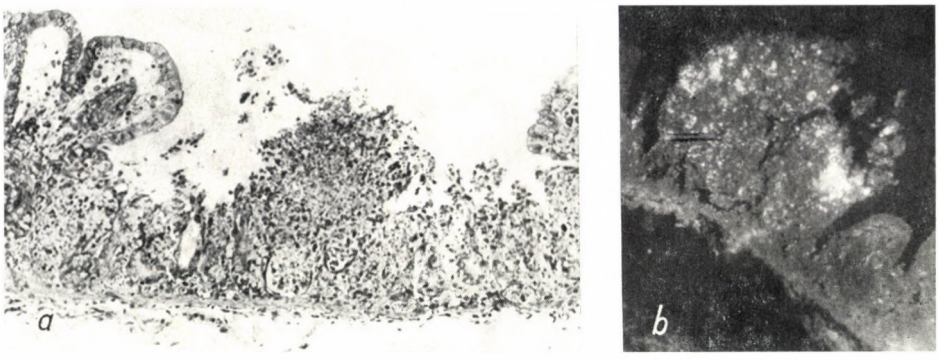


Fig. 10-4. Ulcer of guinea pig caecum with shigella colonies in necrotic tissue 24 h after infection. *a* Stained with thionine; *b* treated with specific immunofluorescent serum. $\times 100$

technique, were examined under fluorescent and light microscope. With this method the localization of Gram-negative bacteria in the tissue could be established more precisely. We investigated only fresh material that had been obtained from animals sacrificed at various stages of the infection in order to exclude any post-mortem changes and multiplication of bacteria in tissues of dead animals.

The results of our experiments, carried out on 100 guinea pigs, generally agreed with those of Formal et al. (1958, 1959, 1965*d*) described above. Animals deprived of food for 4 days fell ill after introducing into their stomach (the contents of which were previously neutralized) 1×10^7–5×10^7 cells of *Sh. flexneri* or *Sh. sonnei*; then tincture of opium was injected intraperitoneally to suppress intestinal peristalsis. Part of the infected guinea pigs died within 1–2 days, the others showed a gradual improvement from the 3rd day on, and finally fully recovered. Histological and microbiological findings in dead animals and in those killed at 3, 6 and 18 h and 1, 2, 3, 4 and 6 days after infection, confirmed the data of LaBrec and Formal (1961).*

Our microscopic investigation—like those of Formal (Formal et al., 1958, 1959, 1963*a*, *b*, 1965*d*, LaBrec and Formal, 1961)—revealed acute enterocolitis with severe lesions in the ileum on the 1st day; changes in the caecum and in the proximal part of the colon occurred later. In the ileum there were very small erosions and ulcers as well as aggregation of polymorphonuclear leukocytes in crypts in the area of Peyer's patches. This latter finding indicated abscess formation as a result of destruction of the crypts. In the caecum more extensive defects of the mucosa were observed, which were covered with membraneous depositions, consisting of leukocytes, erythrocytes, amorphous protein masses and tissue debris.

* The only difference was that in our series shigellae, especially *Sh. sonnei*, were occasionally cultured not only from the intestine, but also from the mesenteric lymph nodes, blood, liver and spleen.

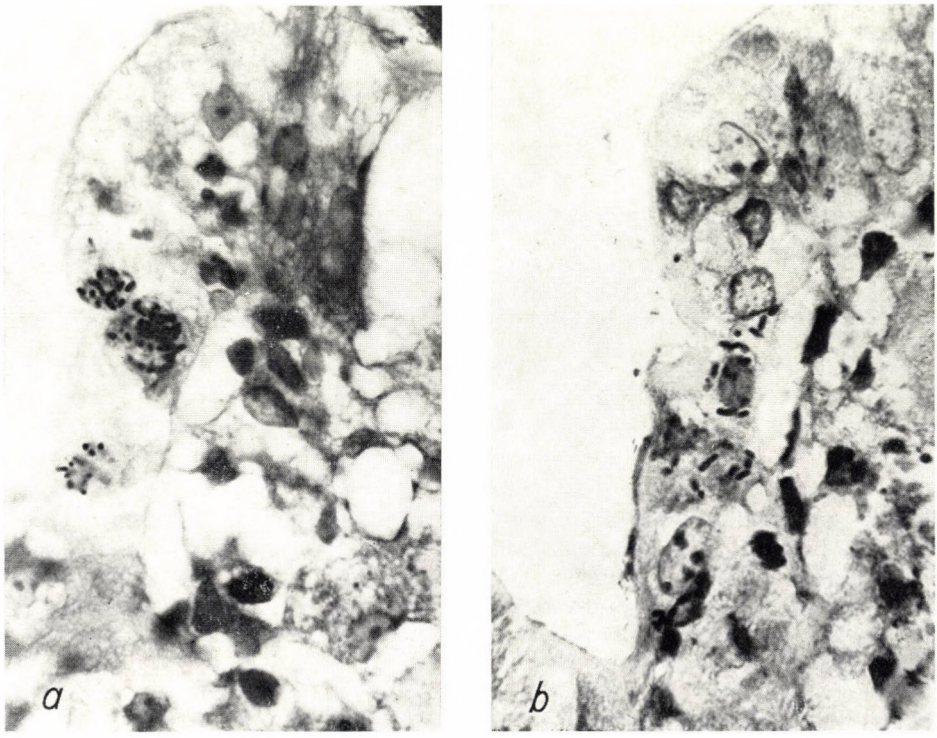


Fig. 10-5 *a* and *b*. Shigellae in epithelial cells of guinea pig caecum 24 h after infection. Thionine, ×980

Like Formal and his co-workers, we also found a great number of shigellae in the intestinal lumen and their accumulation in necrotic debris in the area of ulcerous defects (Fig. 10-4*a*, *b*). The lamina propria of the caecal mucosa showed the presence of few bacteria; they were, as a rule, phagocytized by leukocytes or occasionally by macrophages. In contrast, surface epithelial cells at the edges of erosion sites and ulcers as well as the epithelium of partly destroyed crypts contained large numbers of bacteria (Fig. 10-5*a*, *b*) which became brightly fluorescent after using specific immune sera. Dividing shigellae were demonstrated in the cytoplasm of the epithelial cells. Some of the epithelial cells containing bacteria were sometimes normal in appearance, but frequently displayed some signs of degeneration. In the necrotic tissue, at the base of ulcers and in the lumen of the intestine, desquamated epithelial cells overfilled with shigellae were often found. They constantly appeared inside the leukocytes, but their fluorescence was not so bright as usual; the antigenic material in the leukocytes appeared as fluorescent clumps.

Thus, shigellae in guinea pigs, as in monkeys and rabbits, do invade the epithelial cells of the intestinal wall, but do not settle in tissues under the epithelial lining. Large numbers of shigellae in the lamina propria can only

be detected in dead animals. Obviously, however, penetration of bacteria into circulation is also possible in living infected animals. In some guinea pigs sacrificed on the 3rd–4th day of the illness shigellae were found in the lymphatics of the intestinal wall (in the area of deep ulcerous defects of the caecum) and sometimes in the regional lymph nodes, but without any signs of multiplication.

MICROSCOPIC CHANGES IN THE INTESTINE INDUCED BY ENTERALLY ADMINISTERED DYSENTERY ENDOTOXIN

In view of the discovery of the intraepithelial parasitism of shigellae, earlier suggestions concerning the pathogenesis of dysentery (see Chapter 12) should be revised. The multiplication of bacteria in the epithelial layer of intestinal mucosa as well as the subsequent death of damaged epithelial cells and formation of ulcers are certainly of great significance for the development of the illness. The question arises whether the absorption of toxic substances produced by shigellae only occurs when the epithelial layer is damaged. This problem has not so far been fully solved. Experimental animals can endure, without any noticeable consequences, the introduction of great numbers of killed *Sh. dysenteriae* 1 bacteria or their toxins into the intestinal tract (Doerr, 1907; Flexner and Sweet, 1906; Branham et al., 1949, etc.). Only Dumas and Combiesco (1922) reported positive results in similar experiments. It has been suggested that the intestinal barrier is impermeable to the large molecules of dysentery endotoxin (Clark, 1959; and others). Sanford and Noyes (1958), who administered ^{51}Cr-labelled *E. coli* endotoxin to dogs, observed no absorption through the intestinal wall. Letterer (1944, 1949) observed neither clinical illness nor intestinal lesions in mice after enteral administration of 20 lethal doses of *Sh. flexneri* endotoxin; Formal (cit. LaBrec et al., 1964) fed huge doses of acetone-killed and dried *Sh. flexneri* to monkeys for a month, but failed to record any symptoms.

It is well known, however, that young children may die on the first day of shigella infection, showing signs of severe intoxication, without, however, any intestinal symptoms. Guinea pigs and rabbits often died within several hours after enteral infection with *Sh. flexneri* and *Sh. sonnei*, before intestinal lesions developed. Histological examination of these animals (and of those killed soon after infection) revealed fresh inflammatory changes in the intestinal wall (vascular disturbances, leukocytic infiltration) but an intact epithelial layer. During this period single shigellae were demonstrated within some epithelial cells.

Ravin and Fine (1962) proved the possibility of *E. coli* endotoxin absorption from the intestine after enteral administration of these bacteria to coliform-free rabbits. Grys (1966) introduced *E. coli* endotoxin enterally into sheep and, using the immunofluorescent and radiographic techniques, detected it in the surface epithelial cells of the intestine as well as in the blood and internal organs. There is no direct proof as to the absorption of dysentery endotoxin from the intestine. Nevertheless, Undritsov (1955)

found *Sh. flexneri* endotoxin in the blood of dogs 30 min after enteral administration of heated vaccine prepared from the corresponding bacteria. Göing and Keiser (1966) fed large amounts of *Sh. flexneri* endotoxin (200 to 1000 mg) to dogs and observed the development of a short-term diarrhoea in half of the animals; they performed no histological investigations. Ogawa and Nakamura (1969*a*) observed vascular disturbances and inflammatory changes in ligated colon segment of immunized rabbits after injection of an extract of ultrasonically killed *Sh. flexneri* 2a culture. There were no changes after injecting heat-killed shigellae.

It would appear that the intestinal barrier is not absolutely impermeable to toxic substances which are accumulated in the intestinal lumen due to the presence of shigellae. It is very likely that under certain conditions this barrier may be broken down.

Animal experiments have shown that one of the main factors reducing the resistance to dysentery is long-term fasting. Formal et al. (1960) demonstrated that fasting damaged the detoxifying function of the liver resulting in increased sensitivity to endotoxin. Presumably, long-term fasting weakens the intestinal barrier with the result of an easier penetration of pathogenic agents and their toxic breakdown products into the intestinal wall. There are some data indicating that fasting lessens the resistance of the intestinal epithelium to lesions (Wiebecke et al., 1969) and that the endotoxin of Gram-negative bacteria in itself increases the permeability of the intestinal wall (Sviridova, 1965).

LaBrec and Formal (1961) failed to detect soluble bacterial antigens in the organs of fasting guinea pigs infected with *Sh. flexneri* 2a. They suggested that the product might have been present in amounts not detectable with the technique they used. Besides, they infected the fasting guinea pigs enterally with much larger doses of killed organisms than they used of living bacteria. They reported merely that shigellae failed to penetrate the intestinal wall and no ulcerous defects developed. Thus it is not clear whether the infected animals fell ill and whether or not some other changes had occurred in their intestine.

We tried to obtain indirect evidence by histological investigation of the intestinal wall and internal organs of the animals which, after a long fasting, were given injections of dysentery endotoxin (complete *Sh. flexneri* antigen prepared by the method of Topley and Raistrick) into the stomach.

Control (non-fasting) guinea pigs weighing 350–550 g easily endured the introduction of large doses (200–500 mg) of dysentery endotoxin. Histological examination, however, revealed changes in the wall of the small intestine. Three h after the injection desquamation of surface epithelial cells of the villi was seen (Fig. 10-6*a*) as well as leukocytic infiltration in the Peyer's patches, mucosa and submucosal tissue (Fig. 10-6*b*), disintegration of the leukocytes and their removal into the crypts and lumen of the intestine. An accumulation of leukocytes was also seen in the liver capillaries. These changes were only observed within one day after the beginning of the experiment.

Equal doses of endotoxin given to guinea pigs that had been fasting for 4 days, after the usual neutralization of the gastric juice and suppression

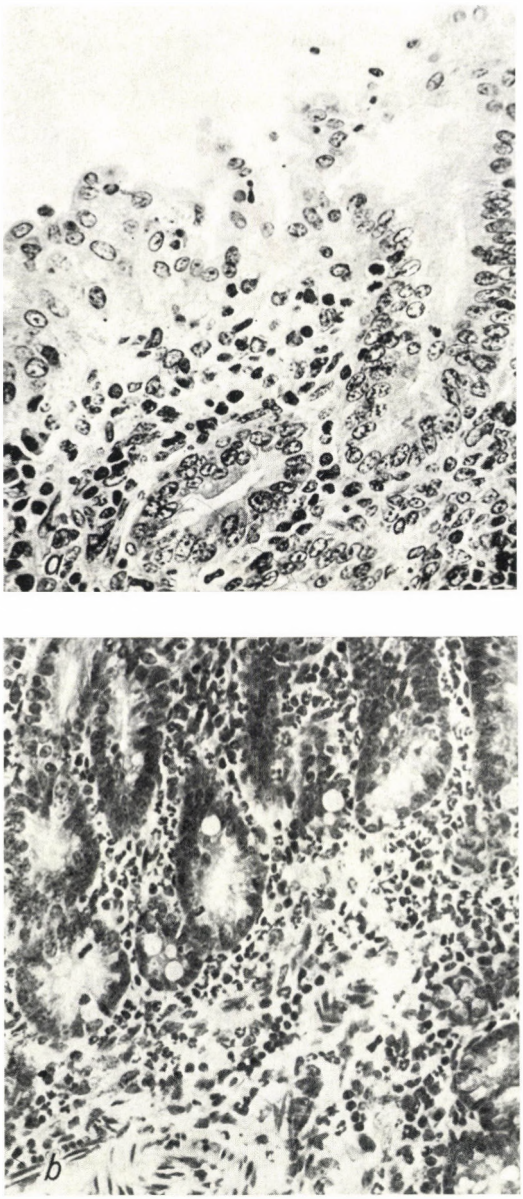


Fig. 10-6. Changes in the mucosa of the small intestine of non-fasting guinea pigs 3 h (*a*) and 8 h (*b*) after endotoxin administration. Haematoxylin-eosin, *a* ×240; *b*×470

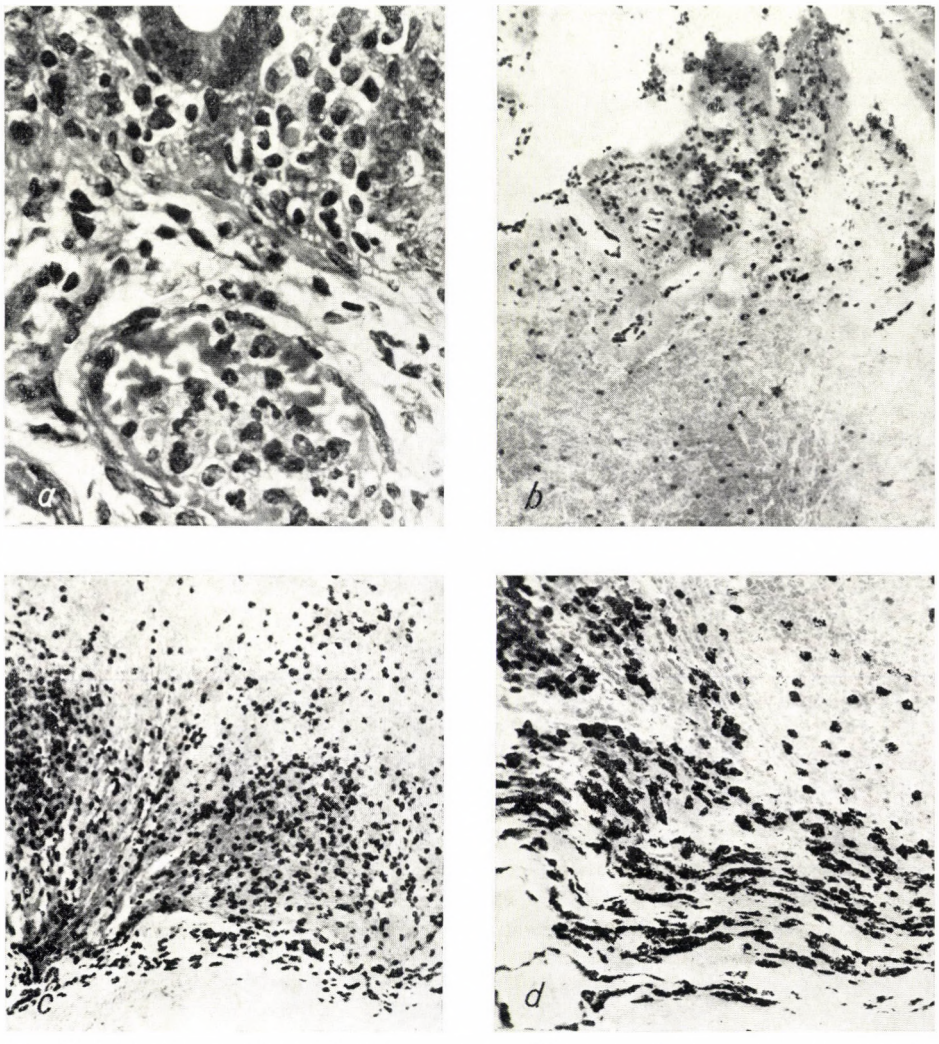


Fig. 10-7. Changes in the wall of the caecum of fasting guinea pigs after endotoxin administration. *a* Thrombus in vein of the submucosa after 18 h. Haematoxylin-eosin, ×470. Abundant leukocytic infiltration of the surface mucosa after 3 h (*b*) and Peyer's patch after 9 h (*c*). *d* Adhesion of leukocytes to the connective tissue fibres of the submucosa after 24 h. Goldmann's Sudan alpha-naphthol staining. *b*, *c* ×116; *d* ×240

of peristalsis by opiate, caused the death of 14 out of 82 animals. The rest, being at first inactive and torpid, became fully normal within 1–2 days. In the animals of this group, histological examination showed more intense changes in the intestine than found in control guinea pigs given endotoxin without any pretreatment. In the small intestine and caecum the following

vascular lesions were seen: hyperaemia, oedema of the intestinal wall, haemorrhages into the follicles of Peyer's patches and the submucosal tissue and occasionally vascular thrombosis (Fig. 10-7*a*). Leukocytic infiltration of the mucosa was especially marked in Peyer's patches where leukocytes were degranulated and destroyed. A pronounced accumulation of these cells was found in the crypts adjoining the patches and in the intestinal lumen (Fig. 10-7*b*, *c*). In some animals leukocytes obstructed the capillarly lumen and accumulated in great numbers in the superficial layers of the mucosa, forming erosions. In others, the emigration of leukocytes was delayed; these cells displayed signs of increased viscosity and accumulated beneath the endothelium of some blood vessels forming clusters of cells in the lumen of veins of the intestinal wall and liver. In these animals there were also large aggregations of leukocytes along the connective tissue fibres giving an extremely characteristic picture in specimens stained with Goldmann's technique (Fig. 10-7*d*). Some animals showed even minute ulcerations in the region of Peyer's patches of the caecum. On the surface of these ulcerations there was a mixed although not abundant bacterial population. Soon (3 h) after endotoxin administration changes in the intestinal epithelium could be observed both in pretreated and untreated animals. The epithelium became loose, uneven and displayed signs of desquamation. Three to four days after the beginning of the experiment the intestinal tract of the majority of the animals was practically free from any lesions. But in one of the guinea pigs that died after 3 days there was acute fibrinous-purulent colitis which developed as a consequence of additional staphylococcal infection.

Histological changes described above seemingly indicate endotoxin absorption from the intestinal lumen. They developed soon after the administration of the endotoxin and were of a transient character. We failed to observe these changes in control fasting animals* and in guinea pigs that obtained nothing but broth after fasting. The absorbed endotoxin probably enters the blood stream, as shown by the symptoms of intoxication (which may be lethal) as well as by vascular changes and histological signs of a general intoxication, i.e. accumulation of leukocytes in the capillaries of internal organs and increased destruction of lymphocytes in lymphoid tissues (Delaunay, 1953; Bibinova and Panova, 1958; Rubenstein et al., 1962).

The experiments failed to solve the problem of the role of endotoxin in the course of dysentery infection. The objection might be raised that the dose of endotoxin administered in our experiments was too high. However, such a high dose is probably necessary to overcome the resistance of the intestinal barrier and the defensive reactions of the host. It is also possible that even more active toxic substances are released in the intestinal tract during dysentery infection. The results obtained show that dysentery endo-

* Using electron microscopy Takeuchi et al. (1965) revealed only insignificant changes in fasting guinea pigs. The number of cells in the lamina propria decreased rather than increased.

toxin (evidently also other endotoxins) can be absorbed by the intestinal wall when its concentration in the lumen is sufficiently high.

Thus, even when using highly virulent organisms, positive results cannot be expected from the enteral challenge of laboratory animals with shigellae without pretreatment of the animals. Following pretreatment of guinea pigs according to Formal's technique experimental dysentery infection accompanied by characteristic lesions in the intestines develops. Shigellae invade and parasitize the epithelial cells of the intestinal mucosa. Fasting and suppression of intestinal peristalsis increase the permeability of the intestinal mucosa to toxic substances of shigellae.

CHAPTER 11

DYSENTERY IN MONKEYS

by

M. V. VOINO-YASENETSKY

In contrast to other animals, monkeys contract dysentery spontaneously without any help of the investigator. It is not known how often it occurs under natural conditions, but the incidence of shigella infection among monkeys kept in breeding farms or animal houses is very high (Lapin, 1961; Lapin and Yakovleva, 1960, 1963). At the same time, artificial inoculation with shigellae is far from being invariably successful.

Troitsky (1958*a*, *b*), summarizing numerous contributions from the Institute of Experimental Pathology and Therapy of the USSR Academy of Medical Sciences at Sukhumi, attributed the failures to infect orally monkeys artificially with *Sh. flexneri* to immunity induced by their very frequent and almost symptomless carriership of the organisms. Feeding *Sh. sonnei* gives more uniform but, as reported by Takasaka et al. (1969), at times negative results. Nevertheless, monkeys are used in experimental studies rather widely, mainly in search for methods of immunization against dysentery. Studies on spontaneously affected or artificially inoculated monkeys have provided valuable information on the pathogenesis of dysentery infections.

In monkeys the disease runs a course very similar to that of human dysentery. Yakovleva (1958*a*, *b*, *c*), and Lapin and Yakovleva (1960, 1963) have also noted the resemblance of microscopic intestinal lesions.

Among their large number of cases (582) these authors observed the same forms of colitis—from catarrhal to diphtheritic and ulcerative—as described in human dysentery. In agreement with many pathologists who studied dysenteric lesions in humans, Lapin and Yakovleva consider desquamative mucous catarrh to be the mildest intestinal lesions. Histologically it appears mainly as separation and death of the mucous membrane epithelium, against the background of an enhanced secretion. The mucous membrane may be destroyed in places, the small defects being filled with exudate containing leukocytes. When the lesions are more severe, the process assumes the form of a muco-purulent inflammation, or of diphtheritic colitis with deep necrosis of the mucous membrane, considerable leukocyte infiltration of destroyed tissues and oedema of the submucosa. In rare cases the main feature is necrosis with slight cellular response. Finally, when the disease has run a protracted course, ulcerative defects tend to clear up with epithelial repair; some abnormalities in the structure of the mucous membrane may persist after termination of the inflammatory

reaction with a tendency to recurrence at places. As in humans, the lesions in monkeys are non-uniform; polymorphous changes may appear in different parts of the same bowel segment.

Lapin and Yakovleva did not investigate the direct part played by shigellae in the development of these lesions. Besides, they studied animals that had died in the course of the disease, so that pathologic intestinal lesions might well have been accompanied by post-mortem changes, tending to develop very rapidly. Other investigators sacrificed monkeys affected with dysentery and studied their organs immediately after death (Voino-Yasenetsky, 1963, 1966*b*; Voino-Yasenetsky and Khavkin, 1964; LaBrec et al., 1964; Levenbuk and Andreeva, 1965*b*; Ogawa et al., 1966*a*; Formal et al., 1966*a*, *b*; Sergeev et al., 1968; Takeuchi et al., 1968; Levenbuk et al., 1970; Ogawa, 1970); these investigations provided more data than the above-described findings. An important advance in this direction is due to the use of Coons' immunofluorescent method whereby shigellae may be demonstrated electively within the affected tissues.

In histological preparations shigellae stain well with basic aniline dyes, particularly with thionine (not all brands, however, are reliable) and azure, which is one of the components of various modifications of the Romanovsky dye (Giemsa, Leishman and others). Good, but rather inconstant results are obtained with the PAS-reaction, demonstrating the wall and the so-called polar bodies of bacteria. Antigenic substances of shigellae are preserved after formalin fixation and paraffin embedding of the specimens. Therefore, routine paraffin sections as well as cryostat sections may be used successfully for Coon's fluorescent antibody method.

Our own studies mentioned above were based on investigation of the intestine and other organs of 15 *Macaca rhesus* monkeys that had contracted dysentery caused by *Sh. flexneri* 4b and, as a comparison, those of 8 normal animals, or animals with diarrhoea caused by other agents.

Six out of the 15 monkeys with dysentery were found to have overt colitis appearing as a thickening of the intestinal wall, with a red and swollen mucous membrane coated with opaque sanguineous fluid; at places a greenish white or grey layer of exudate was also seen in 4 animals. In one of these monkeys the mucous membrane of the descending colon, sigmoid and rectum was almost uniformly coated with 1.5–2 mm thick films of resilient consistency. Another 5 animals had mild colitis; in these animals, it resembled that of the morphological picture seen nowadays at autopsy in children after a protracted course of dysentery: a smooth surface of pink or greyish pink mucosa, a few superficial erosions or small ulcers. Finally, in 4 of the monkeys the intestine was almost normal in appearance.

In healthy monkeys the colonic mucous membrane has a structure similar to that in man. Its lamina propria is relatively poor in cells with few granular leukocytes occurring mainly in basal parts of the mucosa. In cases of non-dysenteric intestinal disorders, a slight leukocyte infiltration of the mucous membrane was occasionally seen, though the epithelial lining remained intact and, as in normal animals, never contained microorganisms.

In dysenteric colitis of moderate severity the colonic mucous membrane showed at most, areas of moderate congestion with numerous goblet cells

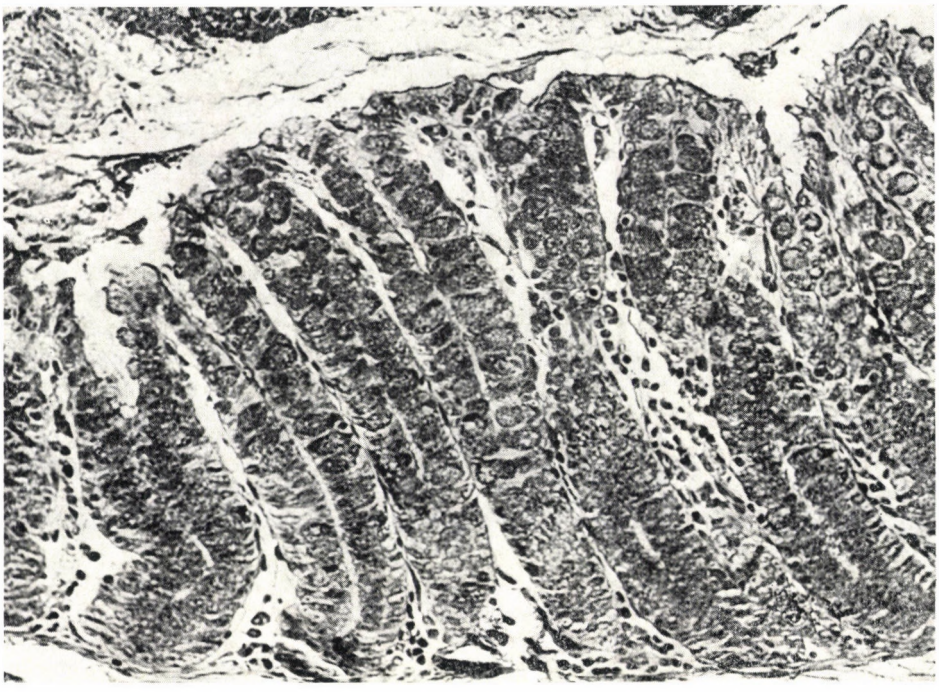


Fig. 11-1. Colonic mucosa of monkey: portion of catarrhal lesion. Monkey No. 7. PAS-reaction (Hotchkiss), ×150

producing copious secretion. However, in contrast to the "desquamative catarrh" described in humans as well as in monkeys examined some time after death, no desquamation of epithelial cells was demonstrated (Fig. 11-1). Bacteria were absent in these areas of the mucosa. At sites of true inflammation, the production of mucus decreased, rather than increased, or had ceased altogether (Fig. 11-2).

Focal accumulation of granular leukocytes* near the mucosal surface (Fig. 11-3*a*) may be regarded as the mildest manifestation of inflammatory response. At these sites the epithelial lining appeared rough or shaggy (Fig. 11-3*b*) while thionine staining showed many epithelial cells, situated between the crypts or at the mouth of the crypts, inhabited by rod-shaped bacteria (Fig. 11-4*a*). These bacteria, with numerous dividing rods among them, produced a bright glow in ultraviolet light after treating the section with fluorescent *Sh. flexneri* antibody (Fig. 11-4*b*). In the lamina propria

* For elective demonstration of leukocyte granules we prefer Sudan alpha-naphthol, as suggested by Goldmann (1929*a*, *b*, 1933), which stains protein-bound lipids. With respect to leukocytes, this method gives results similar to those of the oxidase reaction. Neutrophil and eosinophil stain brown, while common lipid inclusions assume a yellow or orange colour. Safranin may be used instead of Sudan III (Margolin, 1948).

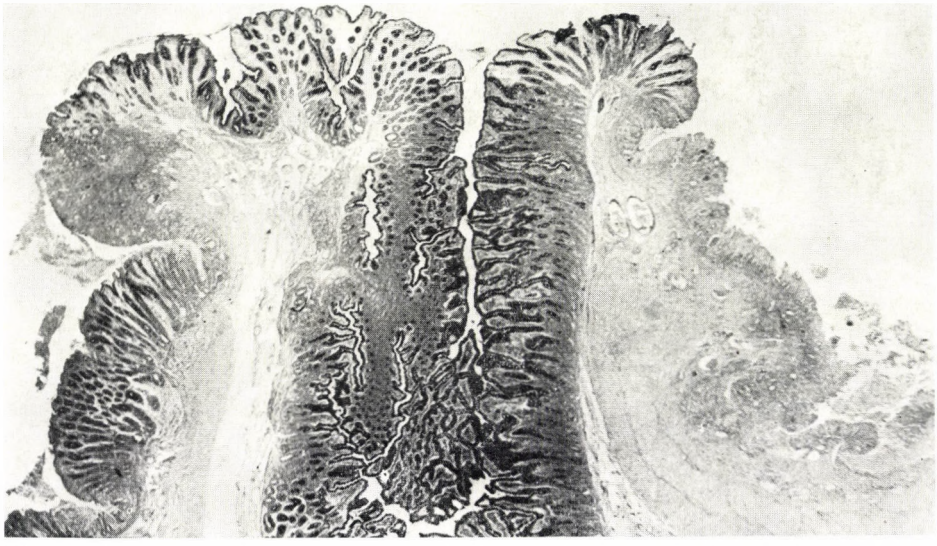


Fig. 11-2. Absence of mucous production in inflammatory foci and hypersecretion in areas of unaffected mucosa. Section through Bauhin's valve. Monkey No. 3, PAS-reaction (Hotchkiss), ×12

of the mucous membrane shigellae occurred much less frequently, usually being engulfed by leukocytes or macrophages. Immunohistochemical investigation of macrophages in the lamina propria also revealed both intact bacteria and products of disintegration of shigellae as indistinct fluorescent forms (Fig. 11-4*c*, *d*). In two monkeys the lesions involved the terminal ileum. Numerous shigellae were seen within the mucous membrane epithelial cells (Fig. 11-5*a*).

Cells infected with shigellae exhibited different conditions. Some of them were apparently slightly injured, while others, containing more bacteria, showed an irregular, often rounded outline and had dark pyknotic or light vesicular nuclei. Vacuoli, however, were seldom seen in their cytoplasm. Epithelial cells infected with bacteria were finally destroyed, but on the free surface of the mucous membrane they were usually cast off while still unbroken (Fig. 11-5*b*) and appeared thus in the intestinal lumen. In non-infected epithelial cells adjacent to those filled with bacteria, certain dystrophic changes were apparent (loss of the brush border, slight swelling of nuclei, etc.) though they did not culminate in cell death.

In addition to the destruction of the superficial mucosa, there were almost invariably deeper defects of the mucosa (Fig. 11-6*a*). In severe colitis these defects reached the muscularis mucosae (Fig. 11-6*b*) without spreading, as a rule, beyond its limits. In these cases shigellae were present in the terminal cells of persisting parts of the crypts (Fig. 11-7*a*, *b*), in the epithelium bordering the ulcers, as well as in leukocytes of the exudate filling the defects. But, whereas within epithelial cells the bacteria appeared as

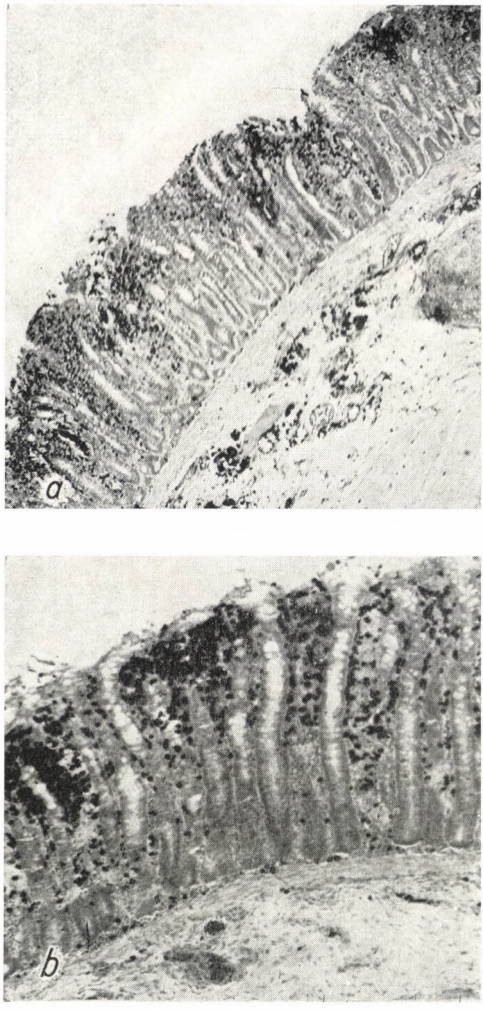


Fig. 11-3. Focal superficial leukocyte infiltration of mucosa. Sudan alpha-naphthol (Goldmann). *a* Monkey No. 3, ×36; *b* monkey No. 1, ×70

regular having a clear outline and staining well, within polymorphonuclears they were mostly seen as shapeless debris (see Fig. 11-4*c*).

Microscopic examination showed the destruction of the crypts to proceed from the surface to the depth of the mucosa with a gradual penetration of shigellae from one epithelial cell into the adjacent one. The epithelial lining of the crypts seemingly melted away and this was followed by a destruction of the remnants of the lamina propria. Much less frequently, simultaneous involvement of many epithelial cells of the crypt could be seen,

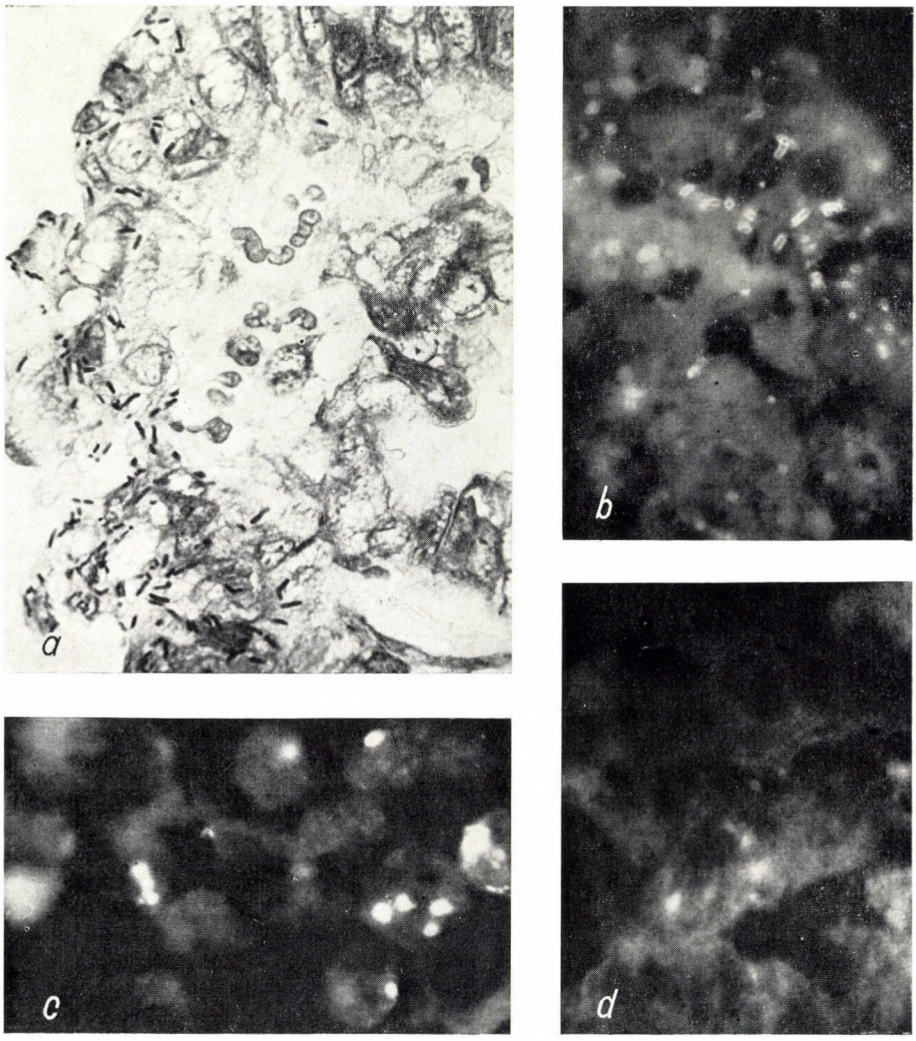


Fig. 11-4. Shigellae in epithelial cells of colonic mucosa (*a*, *b*), their debris in leukocytes of the exudate (*c*) and in macrophages of the lamina propria (*d*). Monkey No. 2. *a* Thionine, ×890; *b*, *c*, *d* sections treated with fluorescein-labelled *Sh. flexneri* antiserum; *b* ×830; *c*, *d* ×1350

mostly when the lumen of the crypt had been blocked and distended (Fig. 11-8).

Particularly deep, narrow, slit-like ulcers were occasionally seen (as in human dysentery) at the sites of follicles within the colonic wall. In these cases, local leukocyte response resulted in what appeared to be purulent

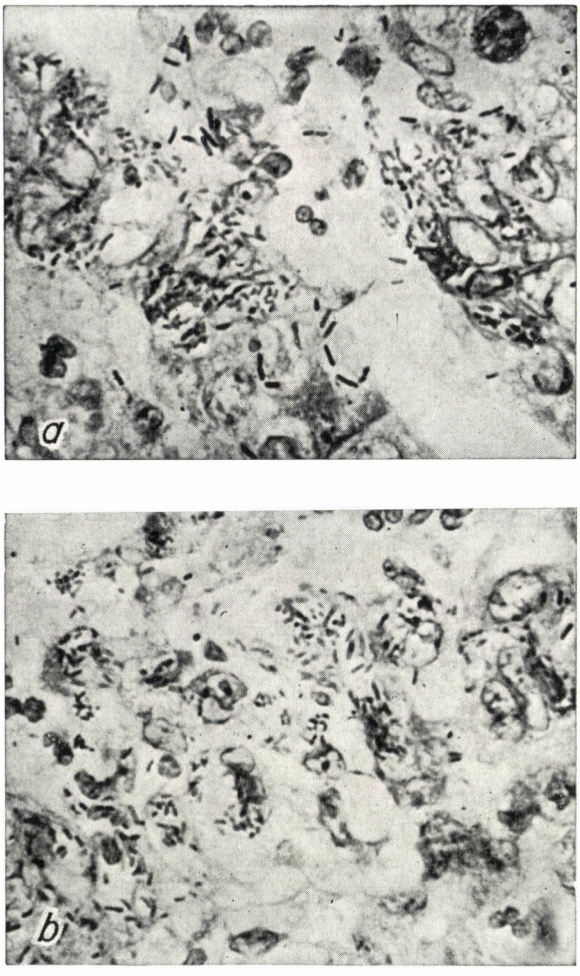


Fig. 11-5*a* and *b*. Shigellae in the epithelium of the ileum terminale. Monkey No. 4, thionine, ×810

decay of individual follicles, although usually they were well preserved even in severe colitis.

Granular leukocytes are the main components of exudative layers covering defects of the mucosa, as demonstrated with the oxidase reaction or by staining the sections with Goldmann's Sudan alpha-naphthol (Fig. 11-6, 11-9*a*), when the specific granules, persisting after degranulation or cytoplasm disintegration, as well as whole cells, are clearly seen. In fresh film-like layers leukocytes are not disposed randomly, but appear to repeat the outlines of destroyed crypts (Fig. 11-6*a*, 11-9*a*); the exudate here is interspersed with homogenous masses showing positive reaction to mucus

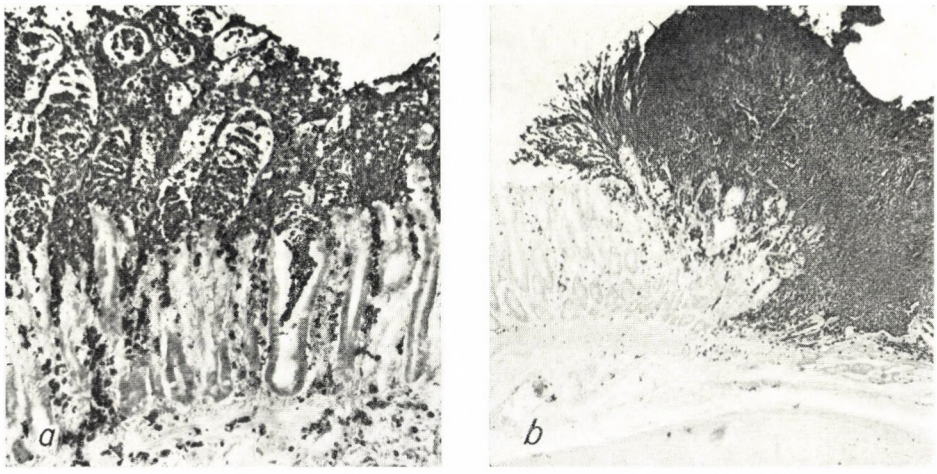


Fig. 11-6. Defects in the colonic mucosa coated with a layer of exudate consisting mainly of granular leukocytes. Sudan alpha-naphthol (Goldmann). *a* Monkey No. 2, ×70; *b* monkey No. 1, ×36

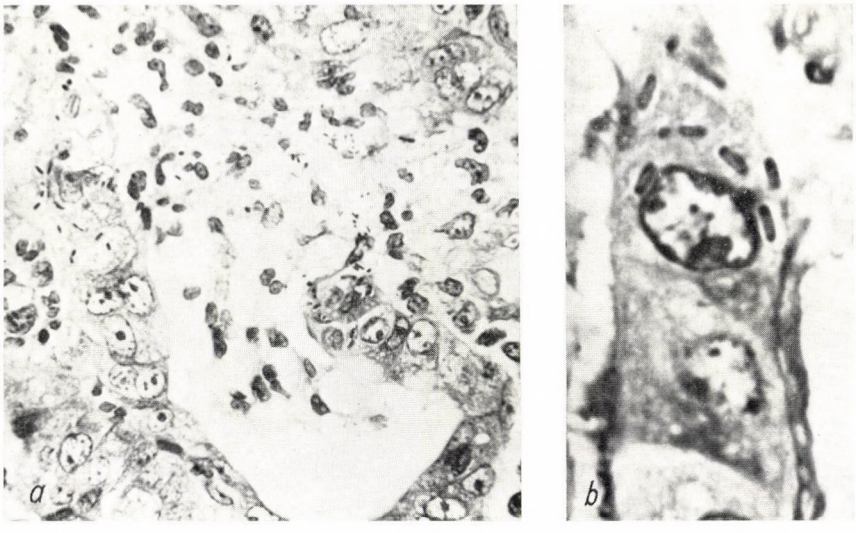


Fig. 11-7. Shigellae in the terminal epithelial cells of crypt remnants. *a* Monkey No. 1, thionine, ×725; *b* monkey No. 2, PAS-reaction (Hotchkiss), ×2200

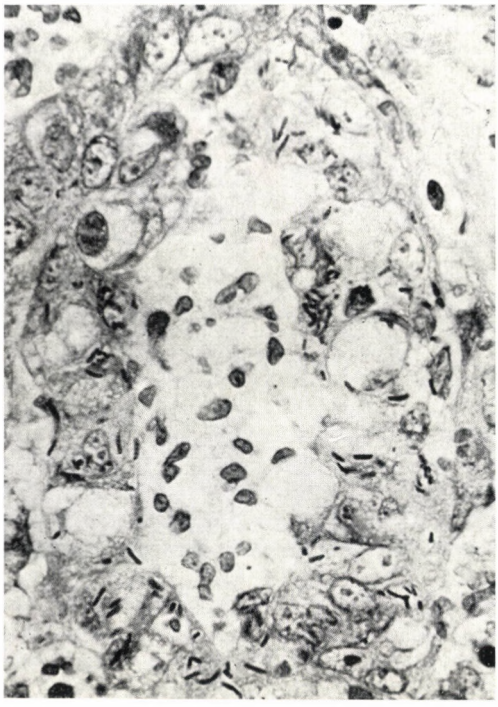


Fig. 11-8. Shigella invasion of numerous epithelial cells in cystic enlargement of crypt. Monkey No. 1, thionine, ×770

(Fig. 11-9*b*). The layers of exudate contain very little fibrin; Van Gieson's or Mallory's stain, or silver impregnation demonstrate the presence of persisting connective tissue fibrils, formerly disposed along the crypts. At the outer parts of these layers some quite structureless sites are found; these assume a uniformly dirty brown colour on staining with Sudan alpha-naphthol, while with the usual stains (haematoxylin-eosin, eosin-azure) they produce the impression of necrosis. On the surface of these "necroses" multitudes of various bacteria may occasionally occur, but these organisms, unlike shigellae, fail to penetrate deeper.

It was noteworthy that the mucous membrane bordering deep defects often remained almost unaltered (see Fig. 11-6*b*) or was just slightly oedematous. Another remarkable finding was the striking adequacy of the leukocyte response: granular leukocytes were seen to fill erosions or ulcers, to infiltrate the lamina propria at areas where the mucous membrane was affected with shigellae, but their number was low in the vicinity. Deeper—in the submucosa—polymorphonuclears were accumulated mainly around the vessels, or were found migrating towards the affected mucosa. Only at sites of deeper lesions of the mucous membrane did the inflammatory infiltration involve the upper layers of the oedematous submucosa.

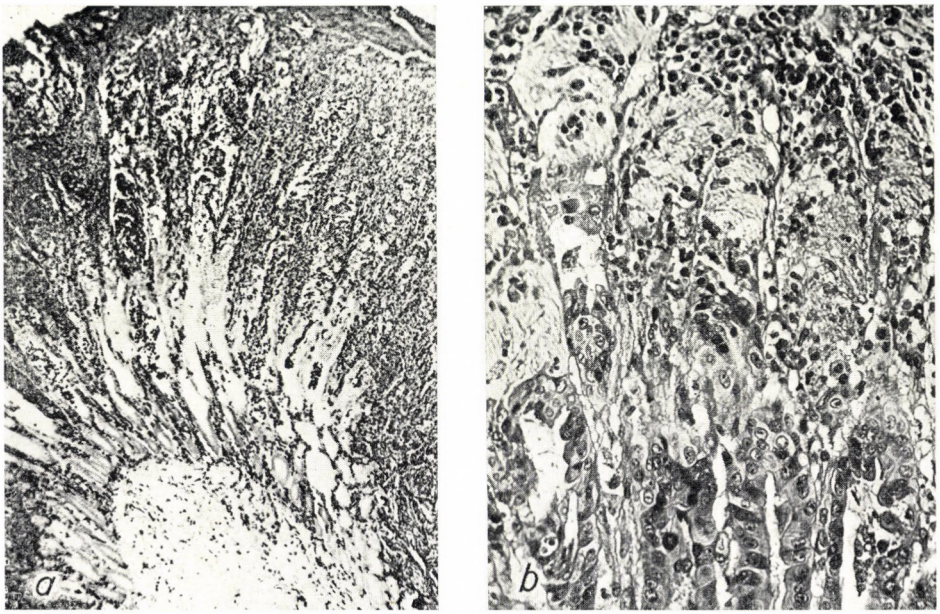


Fig. 11-9. Structure of sloughs in severe dysenteric colitis. *a* Monkey No. 1, Sudan alpha-naphthol, ×50; *b* monkey No. 2, PAS-reaction (Hotchkiss), ×220

In the cases under consideration, even when lesions of the mucous membrane were severe, oedema of the submucosal core was rather moderate and sometimes even slight. Deposition of fibrin in the submucosa was absent, although it has been described as a feature characteristic of the diphtheritic form of colitis in monkeys (Lapin and Yakovleva, 1960, 1963) as well as in man.

Shigellae were usually not so numerous at the bottom of deep erosions or ulcers as in lesions affecting the outer layers of the intestinal mucosa. They were particularly scarce at sites where destruction of the crypts appeared to have been arrested and a new epithelial lining had grown over the surface of the defect. This display of regeneration might have occurred next to crypts where an active infectious process was going on with intracellular parasitic growth of shigellae.

Shigellae were seldom found to inhabit the regenerating epithelium. At the same time, a peculiar and evidently important feature was noted in cases of subacute colitis. In addition to the superficial or deep lesions of the mucosa described above, in 5 cases there was a copious desquamation of superficial epithelial cells without any loss of its continuity (Fig. 11-10*a*). In contrast to post-mortem desquamation, when the detached epithelium (leaving a denuded surface) is hardly altered, or may even retain its usual structure, the rejected cells were rounded or, having assumed irregular or indistinct outlines, appeared to have merged into one another, forming

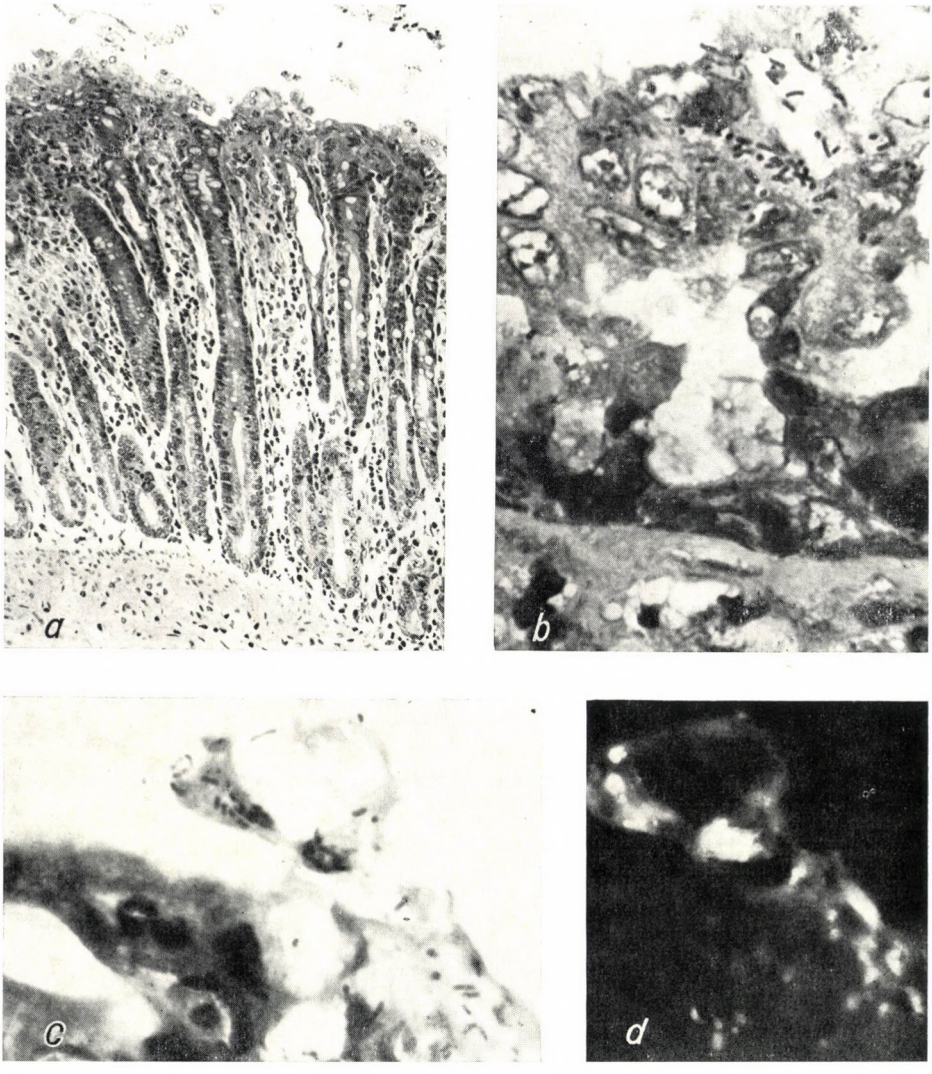


Fig. 11-10. Desquamation of surface epithelium in chronic colitis. Monkey No. 20. *a* Haematoxylin-eosin, ×165; *b* thionine, ×1150; *c* and *d* the same area of the preparation after treatment with fluorescent immune serum (*d*), followed by Leishman's stain (*c*), ×1300

conglomerates. Some of these cells contained only lipid droplets in their clear cytoplasm, while others were inhabited by shigellae (Fig. 11-10*b*, *c*, *d*). Deeper, there were 1–2 layers of flattened epithelial cells with basophilic cytoplasm.

At sites where desquamation of the cover epithelium was intense, the crypts were constricted or, on the contrary, enlarged like cysts, but always with low numbers of goblet cells. Occasionally, the mucous membrane was thin, apparently not having recovered after damage. As a rule, local leukocyte response was insignificant.

In bacteriologically confirmed dysentery, when at autopsy the intestine of monkeys appeared almost normal, histological findings were likewise scanty. There was evidence of earlier lesions (epithelized ulcers, focal atrophy of the mucous membrane, coarseness of the submucosa, etc.). Minute foci of smouldering inflammation with or without shigellae in the epithelial cells could, however, be demonstrated with difficulty, by examining numerous specimens, taken from different sites of the mucosa.

This finding has provided evidence of the ability of shigellae of parasitic existence within epithelial cells of the intestine. It should be stressed that intestinal lesions directly caused by shigellae are limited to the mucous membrane and are of focal nature. In acute colitis this focal nature often appears to be masked by signs of diffuse catarrh, which should in no way be considered an inflammatory process. In dysentery, the true inflammatory response is effected mainly by polymorphonuclears which, accumulating in high numbers, engulf and destroy more or less effectively extra-cellular bacteria.

In addition to the phagocytic activity of leukocytes, which tend to accumulate in excessive amounts, a protective role is apparently played by desquamation—rejection of epithelial cells affected with shigellae or with degenerative changes. This is particularly the case when cells desquamate without any disruption of the epithelial lining, as in our cases of chronic colitis.

In specimens obtained immediately after killing the animals, there were no signs of primary necrosis of the mucous membrane or of epithelial desquamation at sites of simple catarrh. At the same time, additional post-mortem investigation of animals succumbing to dysentery and autopsied after some time (being the usual practice with pathologists) has shown that much of the evidence described above no longer existed by then. Epithelial cells affected with shigellae were destroyed, there was a widespread desquamation of unaffected epithelium, mimicing the picture of desquamative catarrh, while at the sites of local inflammation there were spurious pictures of necrosis of the mucous membrane and underlying tissues due to autolytic processes (Fig. 11-11).

In view of the studies of Ogawa et al. (1964) on the histopathology of dysentery in cynomolgus monkeys *(Macaca iris)*, post-mortem changes in the intestine should be discussed further. They used the fluorescent antibody technique for the examination of mainly dead animals, and came to the conclusion that shigellae multiplied on the surface of the mucous membrane, among necrotized epithelium. They failed, however, to notice any growth of bacteria within epithelial cells, although besides monkeys succumbed to spontaneously contracted dysentery, they investigated 16 animals inoculated artificially with *Sh. flexneri* 2a and killed 3–14 days after the onset of the disease. Later, Ogawa et al. (1966*a*) revised their own experi-

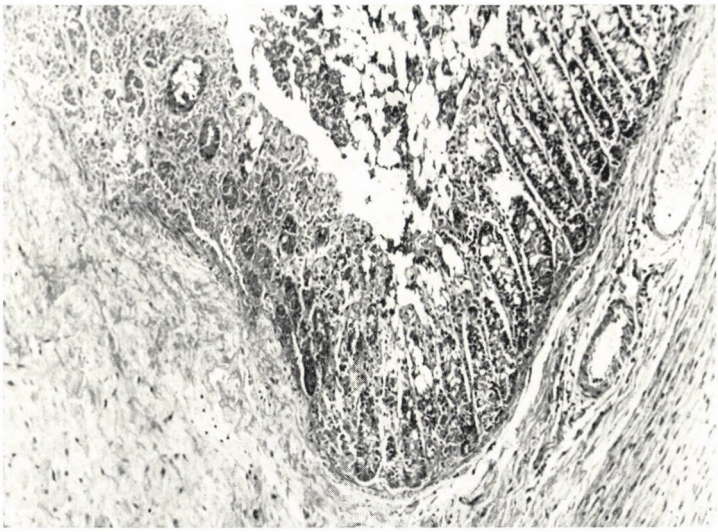


Fig. 11-11. Post-mortem autolysis simulating necrosis (left) and "desquamative catarrh" (right) of the colonic mucosa; autopsy 24 h after death. Haematoxylin-eosin, ×90

mental evidence and, after having examined a few more animals, they assumed that shigellae grew within epithelial cells of the intestine. In the lamina propria the organisms occurred only at the sites of erosions.

Although Ogawa and his collaborators (Ogawa et al., 1966*a*; Ogawa, 1970) maintain that intraepithelial parasitic growth of shigellae plays the leading role in the pathogenesis of dysentery infection, they still assume that intestinal lesions are somehow related to allergic factors. They attribute the inflammatory response in the lamina propria to hypersensitivity involving sometimes the submucosa as well. In their view this does not agree with the conformity between location of bacteria and lesion, which is thought to be the case in other bacterial infections. However, all inflammatory responses, particularly at initial stages, spread beyond the microbial focus, and emigration of leukocytes from blood vessels may frequently be seen at distant locations.

Ogawa et al. (1966*a*) did not study phagocytosis of shigellae by leukocytes and macrophages (in fact, this would hardly be possible using only sections treated with fluorescent serum or stained with haemotoxylin and eosin) and Ogawa (1970) only mentioned its occasional occurrence. At the same time, reporting the results of electron-microscopic studies in his latest contribution, Ogawa noted that shigellae engulfed by phagocytes were seen in membrane-bound vacuoles, while in epithelial cells they lay free in the cytoplasm.

The intraepithelial location of *Sh. flexneri* in the intestine of monkeys was also confirmed in spontaneous infection (Levenbuk and Andreeva

1965*b*) and after artificial inoculation of *Macaca rhesus* for testing the effectiveness of immunization with live dysentery vaccine (Sergeev et al., 1968; Levenbuk et al., 1970). The same conclusion was also drawn by Takeuchi et al. (1968), who formerly believed that shigellae only pass through the undamaged intestinal epithelium, settling deeper in the lamina propria (LaBrec et al., 1964; Formal et al., 1966*a*). In electron-microscopic studies of the intestine of monkeys challenged with shigellae, Takeuchi et al. (1968) made approximately the same findings as they described previously in guinea pigs infected with shigellae (Takeuchi et al., 1965). Shigellae occurred mainly within epithelial cells, where they were seen in the cytoplasm, or were bound to the membrane. These intracellular organisms showed signs of division. Besides, in one of the illustrations given by Takeuchi et al. (1968) passage of bacteria from one cell to another was evident, although not so distinctly as demonstrated in experiments on the cornea of guinea pigs (Tenner et al., 1970) (see Chapter 7). It was also noted by Takeuchi and co-workers that the few shigellae that had penetrated the lamina propria of the monkey intestine were phagocytized by neutrophils, in which they were found within membrane-bound vacuoles. In contrast to observations made by Ogawa et al. (1966*a*), these authors found that the cellular inflammatory reaction was confined to the mucosa, and that there was a distinct correlation between the quantity of bacteria present in tissues and the intensity of the inflammatory response.

Takeuchi et al. (1968) also described dystrophic changes in epithelial cells which were particularly severe in the surface epithelium (located between crypts). These included the shortening of microvilli, dilatation of endoplasmic reticulum, Golgi cisternae, the appearance of membrane-bound phagosomes and accumulation of lipid droplets. It was also stressed, that there were numerous "dark" epithelial cells with pyknotic cytoplasm and undamaged nuclei. In this case severely damaged cells could be found adjacent to seemingly intact ones. It was also noted that various alterations might occur in uninfected cells, as well as in cells infected with shigellae, while the ultrastructure of epithelial cells containing bacteria in their cytoplasm remained frequently well preserved. After detailed examination of the process of separation of injured epithelial cells, Takeuchi and co-workers (1968), as well as ourselves, came to the conclusion that their intense desquamation corresponded to an adequate protective biological response of the host.

Peculiar lesions of the terminal ileum seen in three of our monkeys deserve special mention. Two of these animals were affected with dysentery, while the third had succumbed to diarrhoea of unknown aetiology. In these cases certain epithelial cells of the ileal villi were deformed; they had clear vesicular nuclei while their cytoplasm contained numerous globular inclusions, intensely staining with thionine (Fig. 11-12*a*). Some of these cells (remaining *in situ*, or desquamating) were densely coated with bacteria having shapes of cocci or short rods (Fig. 11-12*b*). These bacteria, as well as shigellae, were Gram-negative but, in contrast to the latter they failed to emit fluorescence after treatment with fluorescent immune sera against *Sh. flexneri* or *Sh. sonnei*. They may have been coliform bacteria, capable

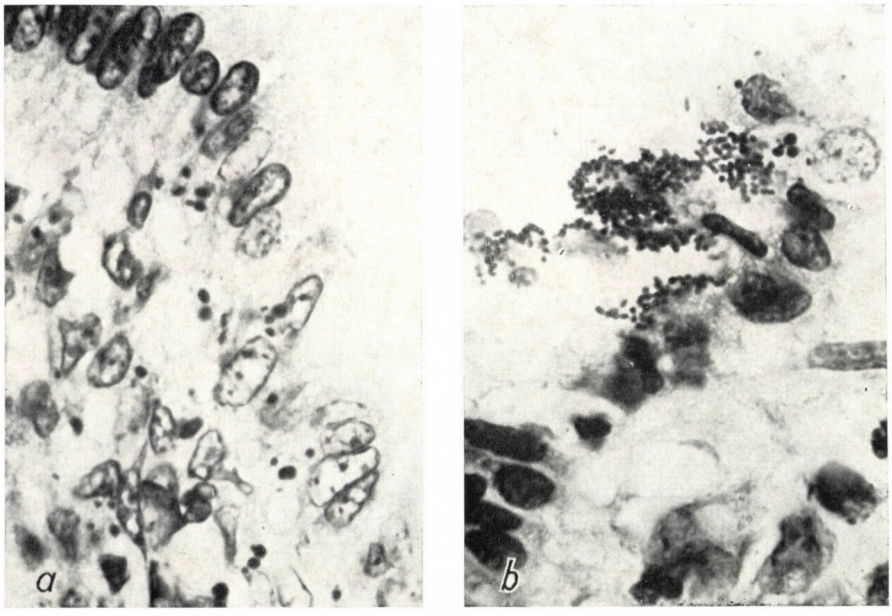


Fig. 11-12. Damage (viral?) of epithelial cells in the terminal ileum (*a*) and growth of undetermined bacteria over the surface of these cells (*b*). Monkey No. 16, thionine, ×1150

of growing on the surface of epithelial cells. Whatever may be the nature of these bacteria or the cause of epithelial damage (resembling that found in virus infections), these observations are undoubtedly of interest. In the first place, they show that damaged cells do not attract shigellae (harboured in great numbers by two of these monkeys). In the second place, as shown by Fig. 11-12*b*, it is apparent that the growth of bacteria over the surface of epithelial cells bears no resemblance to intracellular multiplication of shigellae.

Thus the study of intestinal lesions in dysentery of monkeys, which resemble human dysenteric colitis, contributes to the elucidation of controversial problems of the pathogenesis of dysentery, inasmuch as pathological changes in the intestine of monkeys, autopsied immediately after death, were not distorted by post-mortem alterations.

Earlier theories presuming the purely toxic nature of dysenteric intestinal lesions have not been confirmed. The main peculiarity of this infection is parasitism of its agents in epithelial cells of the colonic mucosa. In conformity with unequal colonization of the mucosa by shigellae, local inflammatory (leukocyte) response is also of focal nature. However, the patchy character of specific lesions can be detected only under the microscope because it is frequently (especially in mild forms of colitis) masked by diffuse common catarrh at gross examination.

CHAPTER 12

PATHOGENESIS OF DYSENTERIC INFECTION IN THE LIGHT OF RECENT OBSERVATIONS AND MODEL EXPERIMENTS

by

M. V. VOINO-YASENETSKY

Investigations of the gut in dysenteric monkeys (see Chapter 11) have shown that the development of colitis depends on the selective damage of epithelial cells of the colonic mucosa by shigellae. The principal morphologic features of acute or protracted dysenteric colitis are due to intracellular parasitic growth of bacteria and to local host responses. These findings have been achieved by examining the intestine before it underwent post-mortem changes occurring promptly after death. Post-mortem investigation of patients who died of dysentery cannot, at present, be performed on a sufficiently large scale in European countries. Still, pathologic patterns established in monkeys may be accepted for humans, provided (*i*) it can be proved that in man shigellae also grow within the intestinal epithelium; (*ii*) the identity of morphologic findings in man and monkeys is confirmed.

Indirect proof of the occurrence of shigellae somewhere within the intestinal wall had been obtained by means of bacteriological studies a long time ago (see Chapter 4). In addition, Lorentzen (1923) revealed organisms within epithelial cells in the colon of two patients who had died of dysentery.

Recently, similar findings have been reported in some fatal cases of dysentery in children (Levenbuk and Andreeva, 1965*b*; Shastina and Leontyev, 1970; Rácz et al., 1973). Bibinova succeeded in finding rod-shaped microorganisms in the intestinal epithelium of children when re-examining earlier preparations. Levenbuk and Andreeva (1965*b*) and Shastina and Leontyev (1970), using the fluorescent antibody technique, identified the intracellular organisms as shigellae. However, Shastina and Leontyev (1970) have noted that small groups of bacteria are mainly observed in desquamated epithelial cells. They have not reported on the time elapsing between the death of patients and autopsy. Tsinzerling (1973*a*, *b*) did not consider the invasion by shigellae to be a regular phenomenon that would play an essential part in the pathogenesis. But these autopsies were made at intervals of up to 24 h or more (personal communication, not mentioned in his paper); however, as we have pointed out, it is useless to study the intestinal epithelium after such a long interval. That is why the post-mortem observations of Rácz et al. (1973) in the intestine of three patients who died of dysentery, are of particular interest. Autopsy in these cases was performed no longer than 30 min after death. The connection between shigella intracellular parasitism and the intestinal lesions was quite distinct, and the

authors confirmed our conclusions based on the investigations of dysenteric monkeys (Voino-Yasenetsky, 1963, 1966*a*, *b*).

The analogy between dysenteric colitis in man and monkeys may appear easy to prove. The similarity of manifestations is doubtless, but there are controversial descriptions of microscopic events (see Chapter 4). Some reported data confirm while others contradict the findings obtained in animals. For example, some pathologists consider dysenteric colitis in man to start with common catarrh and epithelial cell desquamation, while others believe the origin of the lesion to be the exudative (purulent) inflammation of mucosa resulting, as in monkeys, in the formation of erosions and small ulcers. The same holds true for such important details as mucous membrane necrosis, the presence or absence of fibrin in fibrinous colitis, etc.

The disparity and some vague points of the microscopic anatomy of dysentery make the comparison of colitis in man and monkeys, based only on literary data, pointless. Direct comparison of histologic specimens obtained by similar methods is necessary. This has been accomplished by a kind of excursion into the past.

COMPARISON OF MICROSCOPIC ANATOMY OF DYSENTERIC COLITIS IN MONKEYS AND IN MAN

By courtesy of Dr. Yu. N. Darkshevich, Chief of the Pathological Department of the Botkin Memorial Hospital in Leningrad, we cut some pieces out of specimens of the gut from 23 patients who died of dysentery some 20–50 years ago. All essential forms of colitis were represented ranging from slight catarrhal to the most severe diphtheritic, gangrenous or ulcerous ones. From this material, stored in Kaiserling solution, satisfactory sections were obtained in most cases (although the paraffin embedding of tissues resulted in a more intense shrivelling than usual). Staining histochemical reactions and even the fluorescent antibodies could be applied for these sections.*

Some of the specimens showed considerable additional alterations, evidently not associated with their prolonged storage, but rather with the delay before autopsy. The post-mortem origin of these alterations (Fig. 12-1) is confirmed by their close similarity to the histologic picture observed in monkey gut after similar delay of autopsy (see Fig. 11-11, p. 153). As a rule, cadaveric autolysis is most pronounced in tissues damaged or infiltrated by leukocytes. This sometimes leads to the false impression of focal necrosis of mucosa with demarcated inflammation.

Artifacts simulating necrosis were rare in old sections, but the loss of epithelial cells on the mucous membrane surface was almost a constant feature. The epithelium was only well preserved in the depth of the crypts.

* Ogawa et al. (1964) could not investigate children's dysenteric gut specimens stored in formalin for more than 1 year. Nevertheless, in solutions used for preserving museum specimens, the histologic structure of the tissues and their sensitivity to staining are maintained for many years (Voino-Yasenetsky, 1940).

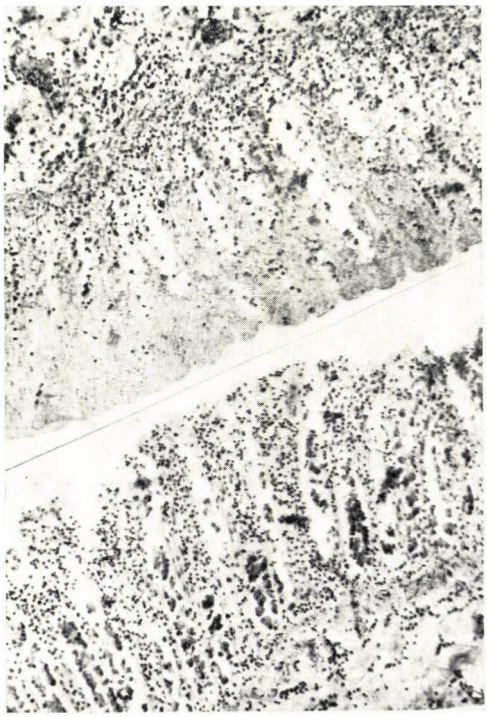


Fig. 12-1. Post-mortem changes of human colonic mucosa in acute colitis: epithelial desquamation (bottom), and total autolysis in areas of former inflammation (top). Dysentery *(Sh. sonnei)*. Death on the 7th day of illness (in 1970). Haematoxylin-eosin, × 90

This alteration greatly limited the investigation of specific lesions which occurred first in the superficial epithelium or in the crypt mouths.

The general characteristics of pathological processes observed in the human intestine coincide, to a great extent, with findings in monkeys. A perfect coincidence could be observed in mild colitis. In these cases solitary, either almost continuous erosions or small ulcers of different depths covered by a layer of exudate consisting mainly of neutrophilic leukocytes and products of their disintegration were revealed in both human and monkey preparations (Fig. 12-2). The fibrin content of this layer was low; solitary strands forming a delicate network only in a few places were revealed by Mallory's phosphotungstic acid haematoxylin. The layer could easily be removed (as evidenced by splits seen in Fig. 12-2*b*) and was not consistently preserved in the specimens studied.

In the mucous membrane under the exudate layer rod-shaped bacteria were demonstrated, which stained with thionine (Fig. 12-3) and showed bright fluorescence after treatment with specific fluorescein-labelled serum (Fig. 12-4). Shigellae were located at the same sites as in monkeys, viz. on

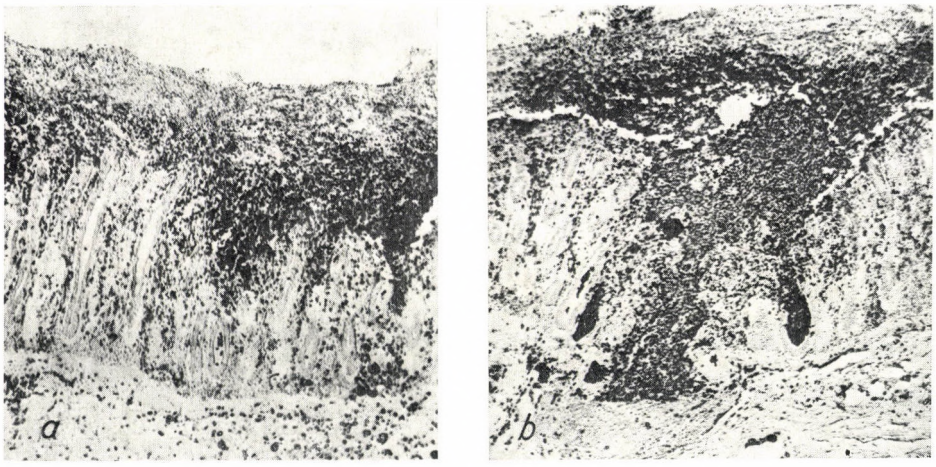


Fig. 12-2*a*, *b*. Defects of colonic mucosa (particularly deep over the follicle) covered by a layer of exudate consisting mainly of granular leukocytes. Death on the 2nd day of illness (in 1950). Goldmann's Sudan alpha-naphthol, ×48

the mucous surface between the crypts or at crypt mouths (Fig. 12-3*a*, *b* and Fig. 12-4*a*, *b*, *c*), as well as in the remnants of crypts in the depth of erosions (Fig. 12-3*c*, *d*, *e* and Fig. 12-4*d*). The only difference was that the epithelial cells, damaged by the bacteria, were destroyed or desquamated. Besides shigellae were considerably less numerous in human mucous membrane than in monkeys. In monkeys the intra-epithelial localization of shigellae, as well as other fine details of morphologic alterations, were well observable in sections taken immediately or not later than 1–2 h after the animals' death. Similar findings are not available in human specimens owing to the reasons referred to above.

As pointed out in Chapter 10, abundant post-mortem multiplication of shigellae on the colonic mucous membrane surface was observed in rabbits that had died after effective inoculation with *Sh. sonnei*. In man with dysentery caused by *Sh. flexneri* or *Sh. dysenteriae* 1, there were no signs of multiplication of these bacteria in the cadaveric gut. With rare exceptions, long rods characteristic of bacterial growth under unfavourable conditions (Hughes, 1956) were observed in epithelial cells (see Fig. 12-3*d*). Shigellae (usually small numbers) were dispersed among the exudate cells in damaged areas, but they did not grow into micro-colonies.

A far greater variety of organisms appeared on the external surface of the exudate layers covering the mucous membrane. Bacteria not reacting specifically after treatment by fluorescent anti-shigellae globulin were growing in dense colonies but usually did not penetrate deeply. This should not be considered a solely post-mortem event, since the same phenomenon, although to a lesser extent, could be observed in monkeys autopsied directly after death.

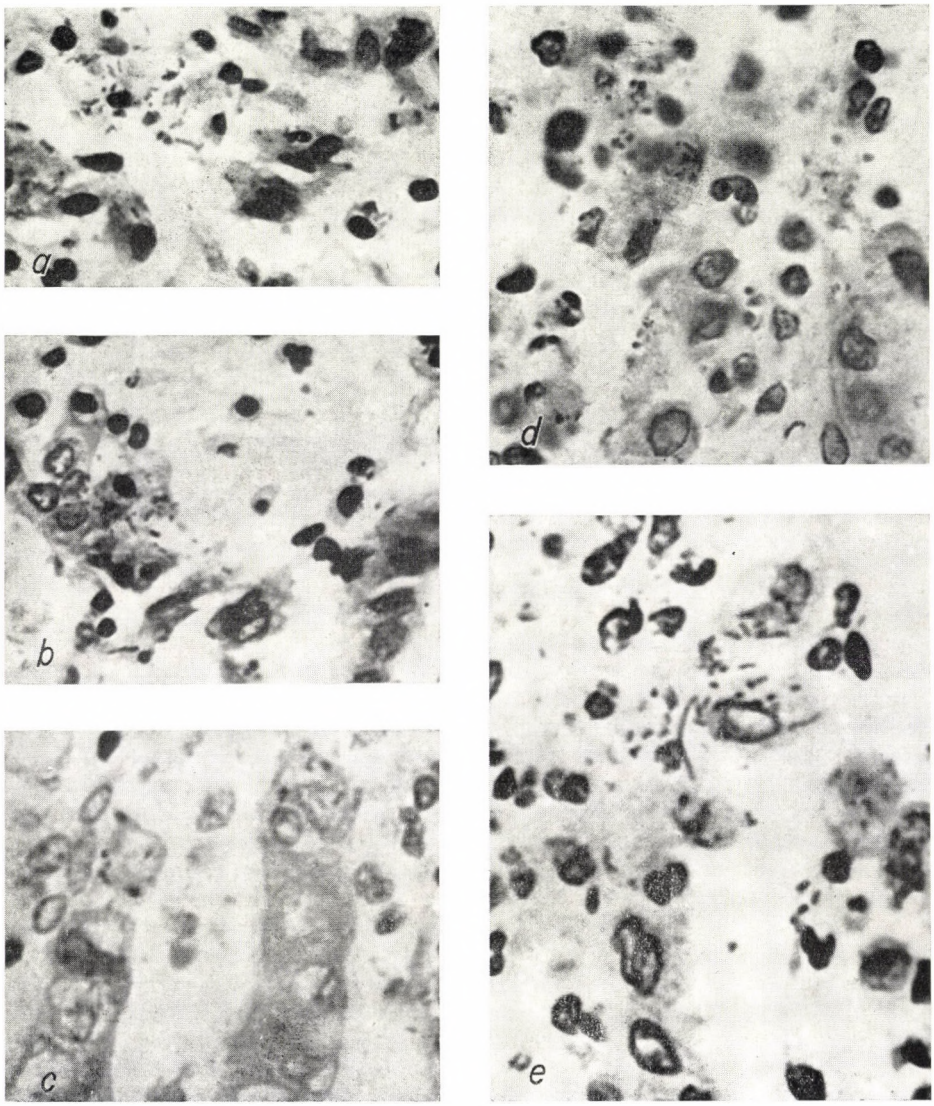


Fig. 12-3. Bacteria in epithelial cells on the surface of mucous membrane (*a*, *b*) and in crypt remnants (*c*, *d*, *e*). Dysentery *(Sh. flexneri)*. Death on the 2nd day of illness (in 1935 and 1949). Thionine, *a*, *b* ×830; *c*, *d* ×1100; *e* ×1200

Evidently it is the emergence of foreign flora that accounts for the indistinct structure of the external part of the exudate layers. As a rule the granulation demonstrable with Sudan alpha-naphthol staining disappeared gradually, without clear boundaries; however, in one case, along with a well-

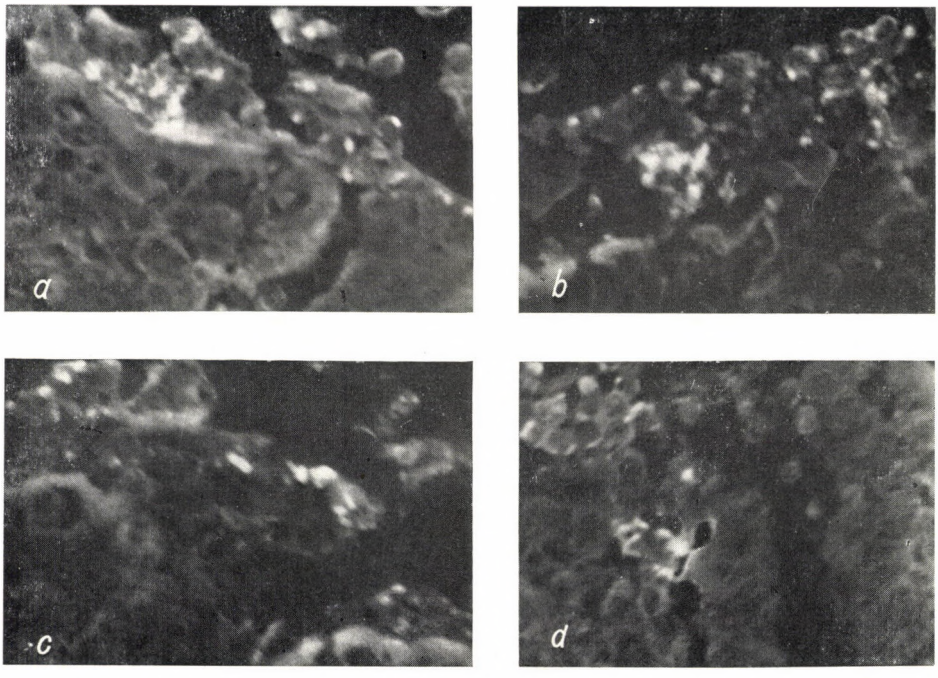


Fig. 12-4*a–d*. Bacteria in disintegrated or desquamated epithelial cells and their remnants in exudate leukocytes after treatment of sections with fluorescent immune serum against *Sh. flexneri*. The same case as shown in Fig. 12-3*a*, *b*, *c*

preserved leukocyte coat (Fig. 12-5*a*), the outer layers of exudate resembled a rather clearly demarcated necrotic zone in some of the intestinal specimens studied (Fig. 12-5*b*). It seemed probable that part of the mucous membrane had perished and then an inflammatory reaction arose. The development of necrosis appeared to be due to a vascular thrombosis; this is, however, an unusual event as other, rather big necrotic areas of the gut were not associated with any ischaemic infarction. Besides, in an almost structureless part of the exudative layer the traces of exudate cells (their previous components) could still be discerned, and thionine or Gram-Weigert's stain revealed many different Gram-positive or Gram-negative bacteria. It is quite probable that such alterations in layers of exudate coats (if they have not been detached for a long period) may occur even when the patient is still alive.

In 4 out of 9 cases of *Sh. flexneri* or *Sh. sonnei* colitis, the lesions of deep layers of the intestinal wall were not considerable. Manifestations included moderate oedema of the submucosa (more pronounced in severely damaged areas of the mucous membrane), dilatation of blood vessels or slight, mainly perivascular leukocytic infiltration. Haemorrhages, if present, were insignificant; fibrin was almost never detected.

Fig. 12-5. Massive layer of exudate on damaged mucous membrane, consisting mainly of leukocytes and products of their disintegration (*a*). Loss of structure in the outer part of the exudate layer (*b*). Death on the 11th day of illness (in 1936). *a* Goldmann's Sudan alpha-naphthol, ×48; *b* Dominici's stain, ×60

Diphtheritic colitis was caused by *Sh. dysenteriae* 1 in all six cases studied. In areas that were the most seriously damaged (and in two cases over the whole intestinal section studied), the intestinal surface was covered with a dirty, dry, greenish-brown crust which could not be removed. The gut wall, having a purple-red cut surface, was 6–10 mm thick; the mucous membrane was almost totally absent. More often the intestinal surface was structureless with disintegrated leukocytes in deeper layers (Fig. 12-6*a*, *b*) and a number of various organisms on the surface. In the considerably thickened submucosa there was evidence of oedema, haemorrhage and abundant fibrin deposition (Fig. 12-6*c*, *d*).

The walls of small blood vessels close to the considerably damaged intestinal surface often seemed unevenly thickened showing staining for fibrin or PAS-positive reaction. The vessels located deeper in the tissue, which was soaked by fibrin or blood, were only markedly congested and contained no thrombi.

Apparently, the severe alterations observed in the submucosa do not signify its final destruction. When dysentery did not rapidly lead to the patient's death, a partial removal of fibrin strands by macrophages took

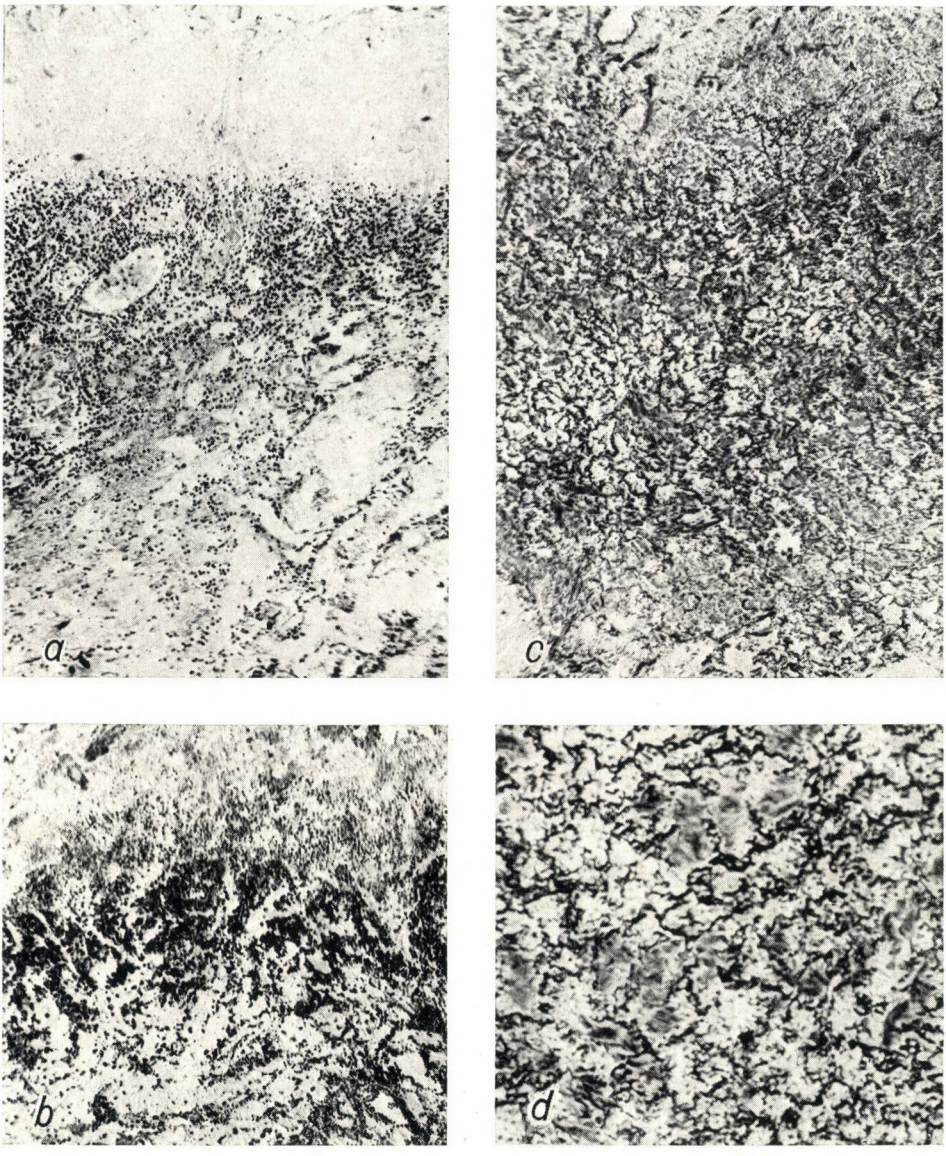


Fig. 12-6. Intestinal lesions in diphtheritic colitis caused by *Sh. dysenteriae* 1. Death on the 7–9th day of illness (in 1932 and 1936). *a* Dominici's stain, ×66; *b* Goldmann's Sudan alpha-naphthol, ×52; *c* (×46) and *d* (×135) Mallory's phosphotungstic acid haematoxylin

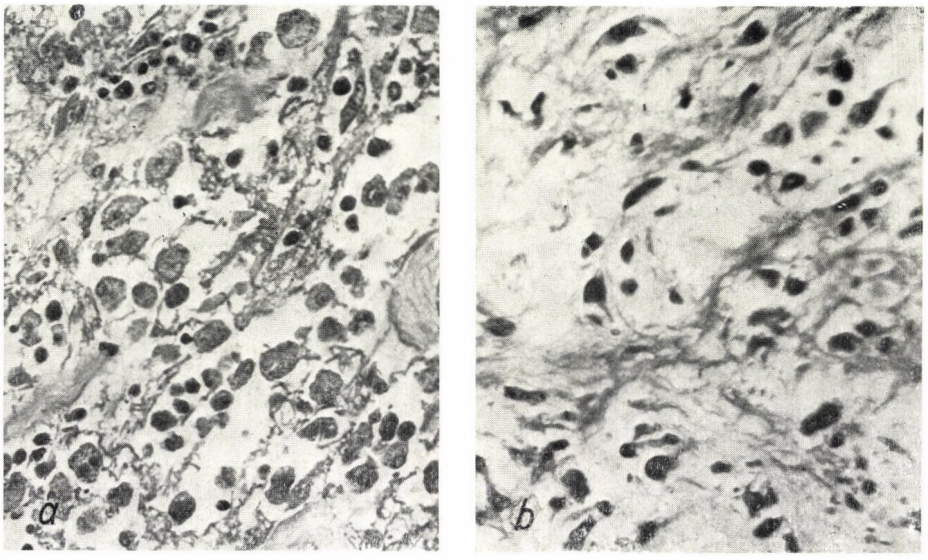


Fig. 12-7. Macrophages (*a*) and fibroblasts (*b*) between submucosal fibrin deposits. *a* The same case as shown in Fig. 12-6*a*. Hotchkiss' PAS reaction, ×460. *b* Ulcerative colitis (in 1930). Thionine, ×390

place (Fig. 12-7*a*) or else signs of connective tissue formation were observed in areas of fibrin deposition (Fig. 12-7*b*). In most of the specimens studied, only the mucous membrane was completely destroyed, though at sites it remained preserved in spite of considerable alterations in the submucosa (Fig. 12-8*a*). This was most frequently seen at sites bordering on less damaged intestinal portions. Yet, occasionally, deeper necrosis extending to the muscularis externa was recorded (Fig. 12-8*b*). In such patchy areas, there was a real inflammatory demarcation with penetration of well-preserved leukocytes into the necrotic zone. The direct cause of the development of focal necrosis was not established. These phenomena probably lead to the development of rather deep ulcers penetrating under the mucous membrane, formerly frequently observed in chronic dysentery.

In almost all cases of diphtheritic colitis there were areas (mainly in the ascending or transverse colon) where the colonic mucous membrane was not so severely damaged. In appearance these areas conformed to the above-mentioned, less severe forms of acute colitis. Only the oedema was more considerable, or at some sites there were fibrinous meshes of haemorrhages in the submucosa. In the poorly preserved mucous membrane epithelium, intracellular bacteria were also found but, for lack of a specific *Sh. dysenteriae* 1 fluorescent serum, they could not be identified.

These investigations confirmed the close similarity between the main (catarrhal and croupous) forms of dysenteric colitis in man and in monkeys. Some differences, especially in the occurrence of epithelial cells affected by shigellae, are mostly due to post-mortem changes present in the intestine

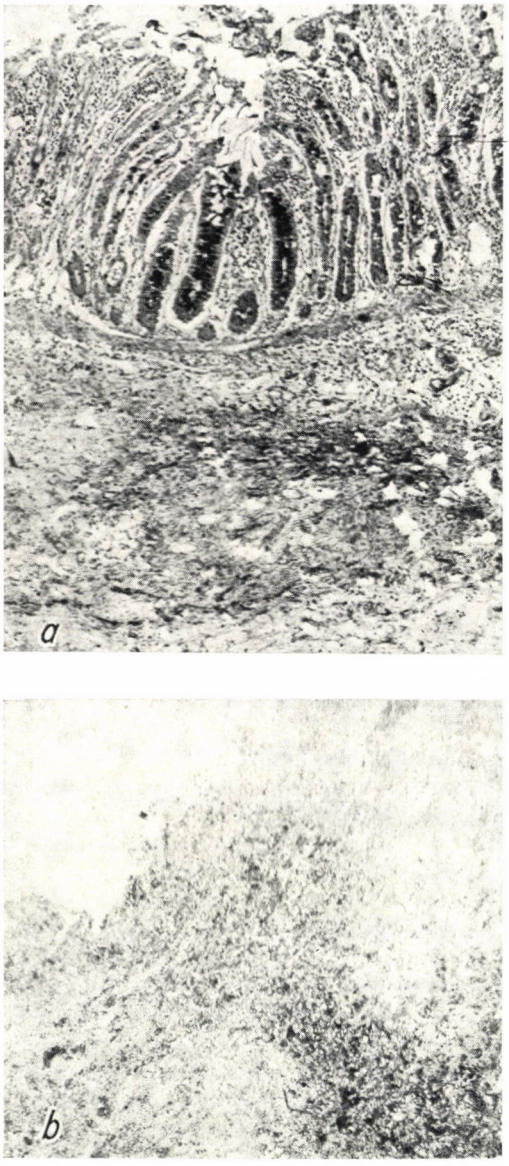


Fig. 12-8. The mucosa is partly preserved over a markedly changed submucosa (*a*); deep necrotic focus demarcated by a band of inflammation with haemorrhages (*b* upper right corner). Dysentery (*Sh. dysenteriae* 1). Death on the 8th day of illness (in 1935). *a* Hotchkiss' PAS reaction, ×50; *b* Goldmann's Sudan alpha-naphthol, ×17

of dead humans and absent in monkeys. Judging by literary evidence (Lapin and Yakovleva, 1960, 1963), diphtheritic forms of colitis, which we have not yet encountered, can be observed in dysenteric monkeys.

THE MECHANISM OF INTESTINAL DYSENTERIC LESIONS AS REVEALED BY RECENT STUDIES

Having established the identity of the most frequent forms of colitis occurring in dysentery of monkeys and man, one may feel more assured in using the data presented in Chapter 11 to explain the pathogenesis of dysentery infection. As it has been noted there, shigella settling in epithelial cells of the colonic mucosa may be held responsible for the development of colitis. The infected cells gradually disintegrate and desquamate producing the characteristic intestinal lesions (which have previously been attributed to bacterial toxins). The same conclusions were drawn by Ogawa (1970) who proposed a scheme for the various stages of the destruction of the intestinal epithelium by shigellae. However, other important pathologic processes observed in various forms of colitis were not included in his scheme (neither in a similar scheme by Rácz et al., 1973). Figure 12-9 shows our attempt to classify the main components of morphologic intestinal changes in dysentery. They are as follows: (*i*) direct involvement of the epithelium by shigellae, (*ii*) inflammatory leukocyte response, and (*iii*) sequelae of toxic vascular lesions.

It should be emphasized again that specific lesions of the colonic mucosa are of local nature. In moderate forms of colitis such foci may be quite small; the signs of diffuse catarrh are evident only from an increased secretory activity of the epithelium and from a more pronounced congestion. Our studies do not confirm the view of certain authors considering such catarrh to be the initial stage of more severe forms of colitis (see Chapter 4). Catarrhal phenomena cannot be generally called inflammatory (Davydovsky, 1961). Increased mucus secretion is one of the defensive responses of the body to prevent lesions of the epithelial lining (Florey, 1962). Desquamation of cells described in the catarrhal stage of dysentery is thought to be a postmortem artifact (Voino-Yasenetsky and Zhabotinsky, 1970).

In accordance with observations made by Heubner (1886), Bibinova (1954, 1967) and Aschoff (1928) (see Chapter 4), the inflammatory response accompanied by emigration of polymorphonuclears to those areas of mucosa where shigellae have settled, has been found already at the very onset of colitis in dysentery. In the affected areas leukocytes accumulate in excessive numbers. The leukocytes and cellular debris connected not only with fibrin but even more with mucus and connective tissue fibre remnants constitute those rather easily detachable filmy layers which are characteristic of more severe (croupous) forms of colitis. Such layers may be rather thick and may occasionally show considerable elasticity. Obviously, purulent liquefaction does not occur inside them as leukocyte lysosomes containing proteolytic enzymes remain intact. Specific leukocyte granules corresponding to lysosomes are detectable with oxidase reaction or with

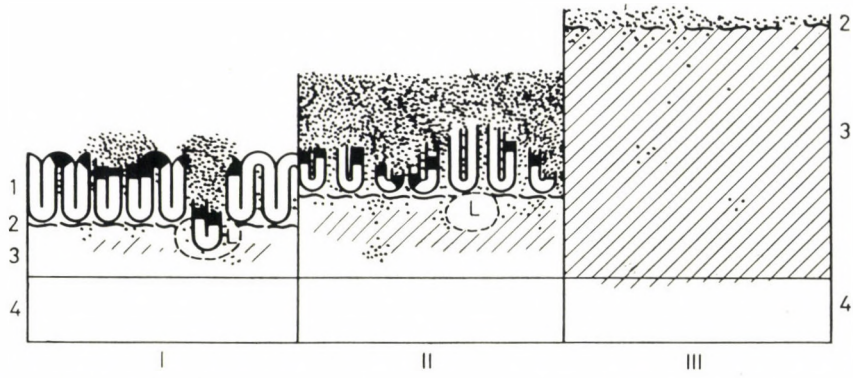


Fig. 12-9. Scheme of intestinal lesions in dysentery. 1 — Tunica mucosa, 2 — tunica muscularis mucosae, 3 — tunica submucosa, 4 — tunica muscularis externa. L — lymph follicle. I — moderately pronounced catarrhal colitis. II — more severe colitis with filmy layers (croupous form), III — diphtheritic colitis. Black areas indicate epithelial cells infected with shigellae; dots show granulocytes; cross-striation indicates oedema, deposition of fibrin and haemorrhages

Goldmann's Sudan alpha-naphthol staining, even after a complete destruction of these cells. However, as mentioned above, in superficial layers of the films the granules may become indistinguishable due to the growth (intravitam or post-mortem) of some extraneous microorganisms.

Many earlier investigators found that severe colonic lesions in dysentery started with focal mucosal necrosis caused by toxins of shigellae present in the intestinal lumen. The results of experiments made on various experimental models (see Chapters 5 and 7–10) and the data obtained by studying the natural course of dysentery in monkeys are in disagreement with this statement. Changes resembling such primary necrosis were only observed in autopsy materials obtained with a delay. It should be stressed again that post-mortem autolysis, as a rule, is most marked in tissues that had been affected and infiltrated by leukocytes. As a result, findings simulating not only necrosis but even inflammatory demarcation around it are occasionally seen in colitis.

The possibility of the noxious effect of toxic breakdown-products of Gram-negative organisms cannot be ruled out with certainty. Ultrastructural changes in superficial layers of the corneal epithelium in experimental conjunctivitis (see Chapter 7) as well as the signs of dystrophy in certain non-infected epithelial cells in monkey intestines can be probably accounted for by shigella endotoxins. However, small mucosal defects only appeared alongside leukocyte infiltration, when huge doses of endotoxin were inoculated into the gastrointestinal tract of starved animals with impaired intestinal peristalsis (see Chapter 10).

The action of bacterial toxins on blood vessels of the intestinal wall seems to be more convincing. Local circulatory disturbances manifested as congestion, oedema and moderate haemorrhages can be observed in early stages of various forms of colitis. This cannot be considered an exclu-

sive sign of toxic vascular lesion since similar phenomena may occur in the presence of any inflammatory reaction. In colitis of the diphtheritic type, however, an impairment of vascular permeability was quite evident. It led both to marked oedema with abundant haemorrhages and to deposition of a thick network of fibrin filaments in the involved areas of the intestinal submucosa.

Obvious lesions of intestinal blood vessels revealed in human diphtheritic colitis cannot be definitely ascribed to shigellae themselves. After destruction of the epithelial barrier by shigellae growing inside it, shigella toxins as well as other noxious substances (or microorganisms) always present in the intestinal lumen, may gain access to the intestinal wall.

Having studied the intestinal lesions in animals poisoned by corrosive sublimate, Kaufmann (1888) considered diphtheritic inflammation of the colon to be a secondary phenomenon caused by the multiplication of certain bacteria in the affected tissues. Jaffé and Laing (1934) found the above-mentioned fibrinoid necrosis (more precisely fibrinoid with fibrin) of blood vessels directly underlying the destroyed colonic surface in dysenteric as well as in uraemic colitis. In their opinion, in both cases such lesions were secondary, being apparently connected with the abundant proliferation of various bacteria inhabiting the intestinal surface after the destruction of its epithelial lining. If this is the case, the apparent similarity between the types of diphtheritic colitis having different aetiology can be explained. This resemblance had earlier led to the supposition of the same mechanism of development for uraemic sublimate and dysentery colitis.

Secondary infection seems to play a rather important part in the development of ulcerative colitis in cases of dysentery with a protracted course or with complications. The most striking (but, fortunately, rather rare) complication is colonic gangrene caused by anaerobic bacteria.

CONTROVERSIAL AND UNSOLVED PROBLEMS OF DYSENTERY PATHOGENESIS

In discussing the pathogenesis of dysentery we only considered intestinal lesions, which are the most characteristic findings. Nevertheless, as it is the case with other infectious processes having a characteristic target organ, the disease, in fact, affects the whole body. Also, the general state of the body influences the development and outcome of the infectious process in the intestines. Immune processes connected with the infection are of particular interest. Symptoms of general intoxication infrequently observed in dysenteric patients should not be neglected either.

Toxic agents may easily enter the blood circulation from the colon impaired by shigellae; moreover, the blood flowing from distal parts of the gastrointestinal tract, most severely affected in dysentery, enters the inferior vena cava. Consequently, it by-passes the liver where the toxins, coming through the portal vein from other sections of the intestine, are detoxicated. Far less probable is the passage of dysentery endotoxins (in amounts dangerous for the body, at least) through the intact epithelial

barrier (see Chapter 4). However, small children infected with shigellae may die within a very short time, amidst signs of severe general intoxication. The morphologic changes in such cases are moderate and mostly restricted to the small intestine.

In shigella-infected, starved guinea pigs pretreated with opiate injection, initial lesions appeared in the small intestine. Formal et al. (1963*a*) established that multiplication of the inoculated organisms, obviously enhanced by the suppression of peristalsis occurred in this case. Eight hours after infection they found signs of circulatory disturbance (congestion, moderate oedema) in the small intestinal mucosa. Leukocyte infiltration and the onset of shigella penetration through the mucosa was noted.

From the above-mentioned facts it would appear that dysentery infection arises first in the small intestine even if the disease runs its common course and, maybe, this stage cannot be observed in patients dying some time later. This suggestion is erroneous because as soon as on the second day after the onset of dysentery pronounced lesions are revealed in the colon, but not in the small intestine (see Fig. 12-2). They are directly connected with the vital activity of shigellae (see Figs 12-3 and 12-4). The results of artificial shigella infection of monkeys are no less convincing: as soon as after 12 h the pathologic processes are limited to the colon (Ogawa et al., 1966*a*).

For the development of shigella lesions in the small intestine, special conditions are obviously necessary: first of all, a sudden disturbance of peristalsis. The fact that in infants an early absorption of dysentery toxins becomes possible under such conditions cannot be ruled out.* However, there is no reason to believe that the same phenomenon can be observed when dysentery infection takes its common course.

This statement does not exclude the possibility of alterations occurring in the small intestine in dysentery. Along with the functional disturbances (also observed in the stomach) which have long been known, certain structural damages (Bluger et al., 1973*a*, *b*) have been reported.

The recognition of intracellular shigella parasitism as the most important factor in the pathogenesis of dysentery poses several questions.

It is not known how non-motile organisms penetrate the epithelial cells. The most evident suggestion is that the cells themselves engulf dysentery agents. Some authors believed that epithelial cells might be able to phagocytize various small particles, including microorganisms. Most investigators deny this possibility. However, even if the phagocitizing ability of epithelial cells is proved it remains to be explained why the cells only engulf shigellae when the intestine contains a wide variety of bacteria. Moreover, even among shigellae they must find the most virulent ones, capable of intracellular growth. It is more probable that parasites themselves settle in the cells they need.

* In newborn mice and guinea pigs substances like bile, colloid dyes, particles of Indian ink (Möllendorff, 1925) and horse radish peroxidase penetrate the ileal and duodenal epithelial cells.

As is known, severe lesions of dysenteric colitis appear most frequently (though not necessarily) in the mucosa of the rectum and sigmoid as well as on top of the folds of the descending colon. Formerly it was believed that at these sites the mucosa is more prone to the toxic effect of the intestinal contents, which moves slowly here. But these factors must also contribute to the penetration of shigellae from the intestinal lumen into mucosal epithelial cells.

Shigella invasion of the epithelium is undoubtedly also facilitated by the rough mucosal surface showing not only folds but also small depressions and protrusions. Therefore, the foci of inflammation caused by shigellae most frequently appear in cryptal orifices or near the prominences (with a pit at the top) projecting over lymph follicles in the submucosa.

Findings dealt with in the preceding chapters suggest the possibility of active migration of living shigellae. For example, Fig. 7-5 shows the passage of an organism from one epithelial cell into another. The interaction between parasites and cells will be discussed in detail in the last chapter after having reported on data obtained in other intestinal infections.

COMPARISON OF DIFFERENT EXPERIMENTAL DYSENTERY INFECTION MODELS

Experimental models of dysentery infection may be classified into two groups:

1. Models of conjunctival (Chapters 6 and 7), intranasal (Chapter 9), intravesical (Chapter 8) and enteral (Chapter 10) infection as well as experiments on isolated intestinal segments (Chapter 22) on mice, guinea pigs and rabbits. Some of the experiments on tissue cultures also belong to this group. Comparative investigations have shown these models to yield similar results (Stenzel, 1960, 1962*b*, *e*; LaBrec et al., 1964; Formal et al., 1965*d*; Andreeva, 1965; Rauss et al., 1967; Bakhutashvili, 1968; Andreeva and Bakhutashvili, 1968; Kandyurina, 1970; Petrovskaya and Blinova, 1971). Moreover, shigellae that had proved to be pathogenic when tested in any of these models were capable of evoking typical dysentery in monkeys (Watkins, 1960; LaBrec et al, 1964; Nakamura, 1967; Ogawa et al., 1967*a*; Mikhailov et al., 1968) and in man (Istrati, 1961; Rédey, 1964, 1970 cit. by Bakács et al., 1970).

All these artificial pathologic processes have a single and highly important common feature: in spite of the difference in location and course of the infection, they invariably affect epithelial tissue. Assuming that intraepithelial parasitic growth of shigellae is the most important pathogenic factor in dysenteric infection, these models conform to the conditions outlined in Chapter 3. Indeed, they reflect the most essential aspect of the interaction between shigellae and the host.

The results of experiments with tissue culture, also included in this group, present some contradictions. In these tests virulent shigellae display the capacity to multiply within cells of different origin, in epithalial

cells as well as in fibroblasts and monocytes. Isolated cells, however, growing on artificial media acquire peculiar properties and should not be compared indiscriminately to their parent cells *in vivo* (Willmer, 1960).

2. The second group includes one of the most currently used methods for testing shigellae in animals, namely intraperitoneal inoculation into white mice. It is assumed that the virulence of the organisms may be assessed in terms of the death and survival rates of the mice. As shown in Chapter 5, however, a fatal infectious process in the peritoneal cavity develops not only with pathogenic, but also with non-pathogenic shigellae which cannot penetrate into epithelial cells and are perfectly innoxious to monkeys and man. Besides, the process is not specific. It cannot, therefore, serve as a model of dysentery infection.

Apparently, intravenous or subcutaneous administration of shigellae also belong to the second group since, using these routes, the bacteria do not come in contact with epithelial cells. The significance of experiments with chick embryos has been disputed e.g. by Stenzel (1962*b*, *c*), who claimed that in the embryo, as well as in the peritoneal cavity, shigellae cause a non-specific general infection. Nevertheless, Belaya (1959) and Petrovskaya and Blinova (1971) obtained a satisfactory correlation between the results of the keratoconjunctival test and experiments in chick embryos inoculated with shigellae into the yolk sac (see Chapter 5).

The fact that certain experimental procedures proved to be inadequate in research on shigellosis does not imply that intraperitoneal, intravenous and other similar methods of animal inoculation should be rejected. In a number of other infections (including salmonellosis) they are quite acceptable. It cannot be denied that these methods may yield information on the properties of shigellae, or on host responses.

Thus, re-examination of earlier human intestinal samples taken from dysenteric colitis cases yielded results similar to those obtained in monkeys. In the catarrhal and croupous forms of colitis, the morphology of intestinal lesions mostly only differed due to post-mortem changes in man, which are absent in monkeys. The pathogenesis of the severest, fibrinous form of colitis, most frequently induced in man by *Sh. dysenteriae* 1, is not yet sufficiently clear.

By correlating the results obtained in natural dysentery cases with those of model experiments, the practical value of the latter as well as the scope of their application can be assessed.

PART III

CHAPTER 13

TYPHOID FEVER AND EXPERIMENTAL SALMONELLA INFECTION

by

M. V. VOINO-YASENETSKY

Among numerous species of the *Salmonella* genus, *S. typhi*, the agent of human typhoid, is of peculiar interest. The aetiology of this disease was established long ago but its pathogenesis is still unclear.

In the clinical picture of typhoid fever general symptoms of infection prevail, such as gradual rise of body temperature, weakness, headache, etc. Pea soup-like stools which were earlier considered to be typical, are rarely observed at present, constipation being more frequent (Bilibin, 1962). However, occasional haemorrhages and intestinal perforation point to serious bowel lesions, which are fully revealed at autopsy.

MORPHOLOGY

Along with inconsistent signs of a mild diffuse intestinal catarrh, the most characteristic lesions appear in the ileum, mostly in its distal portion. Lymphatic structures, i.e. the Peyer's patches and solitary follicles, swell considerably during the first week of the disease. The hyperplastic tissue is soft, succulent and reddish-grey in colour ("medullary swelling"). During the second and at the beginning of the third week, necrosis develops in the tissue of swollen patches. After sloughing during the third and early in the fourth week of the disease, ulcers may extend deep into or through the muscle coat; there is danger of profuse haemorrhages from the affected vessels as well as of intestinal perforation. If the course of the disease is favourable, the ulcers heal gradually without noticeable traces.*

Pathologic phenomena occurring during typhoid fever in the intestinal walls are due to the appearance of granulation tissue consisting of large, mononuclear, pulpish cells called "typhoid cells" (Fig. 13-1). They have phagocytic properties and frequently contain engulfed erythrocytes and debris. They develop from reticular cells and histiocytes and are, in fact, common macrophages. Their massive accumulation in the form of granulomas and whole fields extending partly beyond the outlines of follicles and

* According to Christeller (1928), the duration of the individual stages mentioned is observed only in one half to two-thirds of fatal typhoid cases. Complete necrosis of Peyer's patches is not usually observed in thyphoid of children and is not a consistent finding in adults either (Gräff, 1918; Davydovsky, 1956; Abrikosov, 1957).

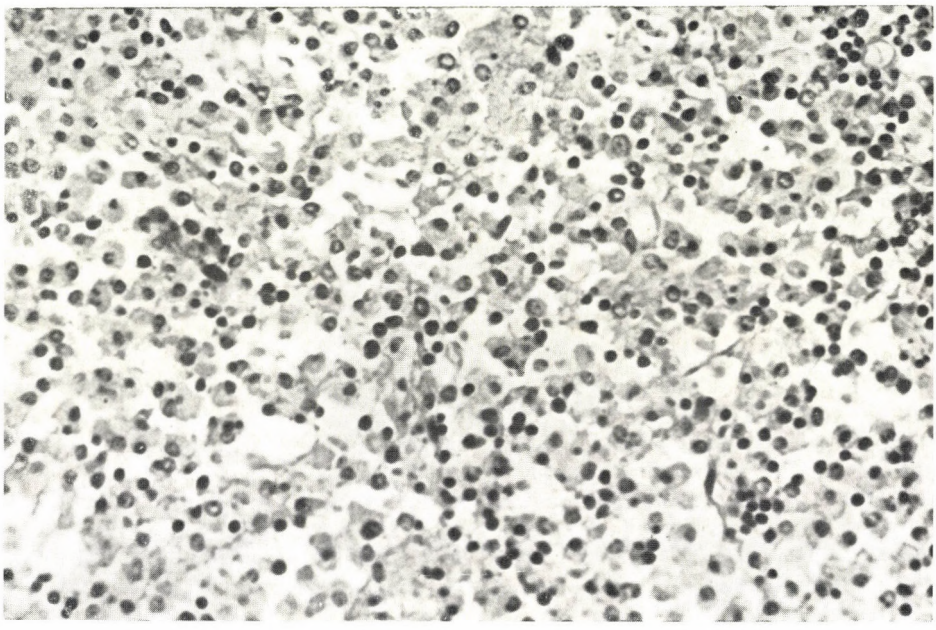


Fig. 13-1. Typhoid cells in Peyer's patches of a patient who died on the 14th day of the disease. Haematoxylin-eosin, ×320

the Peyer's patches is particularly characteristic. In contrast to dysentery, pathologic processes in typhoid fever take place in the lamina propria and not in the epithelium. For some time before the onset of necrosis of the patches, the epithelial coat over the foci of the lesions remains seemingly intact.

Another important feature of typhoid is its generalized character. Lesions similar to those seen in the Payer's patches also occur in the mesenteric lymph nodes (Fig. 13-2*a*). In the liver, spleen, and occasionally in the bone marrow and some other organs, focal lesions (single granulomas) appear (Fig. 13-2*b*).

The mechanism of typhoid granuloma formation has not been sufficiently studied. Death hardly ever occurs earlier than the end of the first week. At this stage granulomas consisting almost exclusively of "typhoid cells" are disclosed. It is generally thought that local inflammatory response is of productive nature from the very beginning, i.e. it is displayed by focal growth of macrophage-type cells and leukocytes do not participate in the process. Granulocytes only appear when granulation tissues for some reason become necrotized during the second week of the disease. Some investigators consider this response to be the sequence of secondary non-specific infection of necrotized areas.

Granulomas formed in the liver during typhoid occasionally reach the size of 1–2 mm in diameter, but usually they are small and can only be revealed

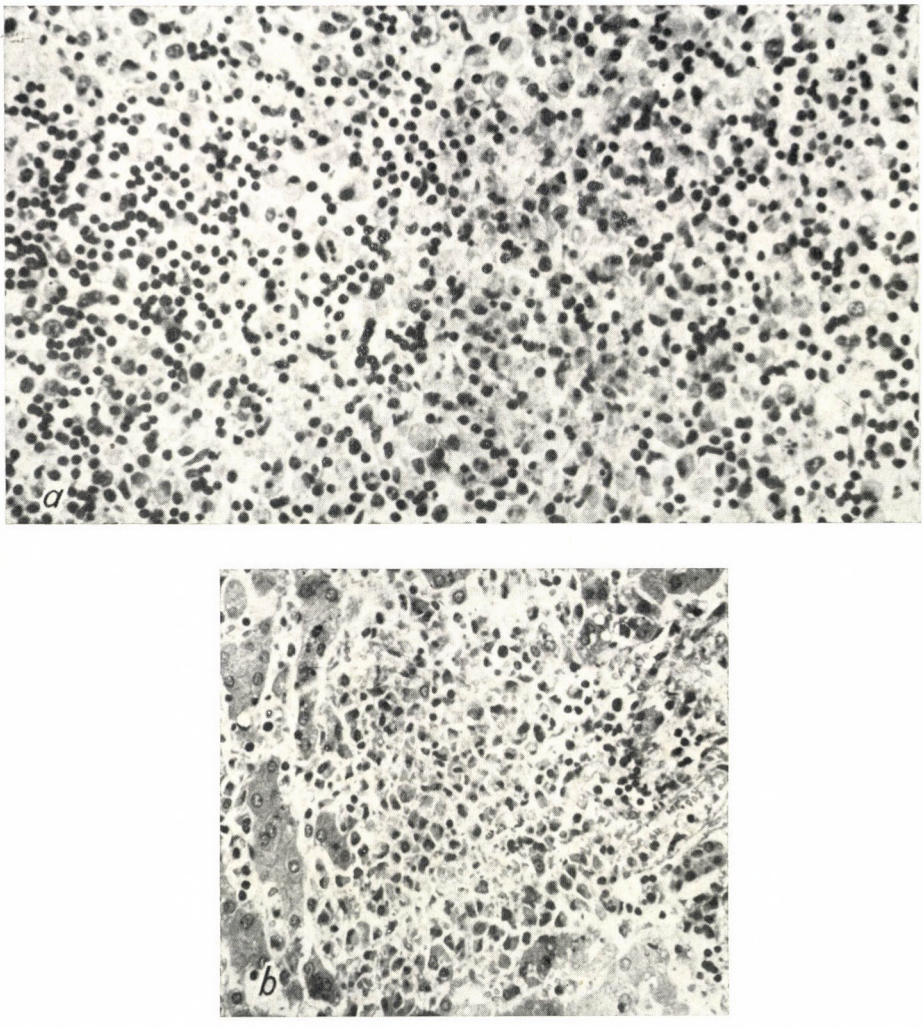


Fig. 13-2. Granulomas in a mesenteric lymph node (*a*) and the liver (*b*) of man in typhoid. Haematoxylin-eosin, (*a*) ×290; (*b*) ×240

microscopically. Being most frequently round, they are arranged in the liver lobules irregularly, nearer either to the periphery or to the centre. Two kinds of "typhoid nodules", necrotic and cellular, are distinguished.*

* Occasionally "lymphomas", i.e. aggregations of round cells in the interlobular connective tissue, are considered as such nodules. But, being encountered in many infectious diseases they are not peculiar to typhoid fever (Faber, 1921; Mestitz, 1923; Gruber, 1930). Their incidence in these diseases was not higher than in healthy persons who died accidentally (Voino-Yasenetsky, 1950).

Only Mestitz (1923), Gruber (1930) and, to some extent, Jaffé (1920) believe that these forms of local lesions are different, arising independently. Other investigators consider them to be manifestations of an advanced stage of typhoid granuloma formation.

According to Fraenkel and Simmonds (1886), Reed (1895), Gräff (1918) and Faber (1921), formation of typhoid granulomas in the liver begins with the appearance of foci of necrosis resulting in local cellular response. Other morphologists hold the opposite view, considering nodule formation from the reticuloendothelial cells (histiocytes) — that are destroyed later — to be the primary phenomenon (Schmidt, 1907; Joest, 1914; Jaffé, 1920; Christeller, 1928; Lennert, 1961).

Christeller (1928) and MacCallum (1945) emphasized that typhoid granulomas in the liver are unique examples of macrophage response without the participation of polymorphonuclears from the very beginning. Having applied histochemical techniques, Faber (1921) and Mestitz (1923), however, found a certain number of granulocytes to be present at every stage of granuloma development in the liver. Schmidt (1907) and Gräff (1927) have also mentioned the leukocyte response (as if it were related to necrosis).

Mallory (1898) proved that typhoid granulomas form in the liver as a result of occlusion of liver capillaries (sinusoids) by macrophages which get there from the spleen on haemogenous route. Gruber (1916) disproved this supposition by showing that mice challenged with salmonellae developed granulomas in the liver even in cases when the spleen had previously been removed. However, MacCallum (1945) repeated Mallory's hypothesis, without stating the origin of the occluding cells. Having noted that animals challenged with salmonellae exhibit many large mononuclears in the blood vessels of different organs, McGuire et al. (1968) concluded that macrophages revealed in the liver and the spleen seem to originate from different parts of the body. The question, however, arises why granulomas then fail to appear in these organs in malaria when, as shown in Chapter 1, large macrophages may be found in the blood flow in high numbers.

There is an almost unanimous agreement of authors that granuloma formation in the liver is due to the effect of salmonella endotoxins. Endotoxins, however, must accumulate at later sites of granuloma formation. Circulating endotoxins cause a different kind of liver damage, which is usually more diffuse or localized in the centre of the lobules. It is easier to assume that granulomas arise in places of salmonella colonization (or destruction) as suggested by the frequent positivity of bacteriological cultures made from the impaired organs.

Salmonellae have not yet been revealed microscopically in the hepatic foci, but they have been found in granulomas of other organs. Moreover, *S. typhi* is known to have been discovered while studying the impaired tissues morphologically, and many years had to pass till the direct role of salmonellae in the development of lesions was understood.

SALMONELLAE IN FOCAL LESIONS IN TYPHOID FEVER

In histological preparations from mesenteric lymph nodes and the spleen of 23 patients who had died of typhoid fever, Eberth (1880) found microcolonies of rods with rounded ends in 12 cases. For lack of a good staining method, he used unstained sections. He only found colonies intercellularly (Fig. 13-3*a*), and rather infrequently, i.e. from 1–2 per several sections up to 2 and more in one section. Eberth mentioned a number of authors who had previously reported on the presence of some organisms in the tissues during typhoid, nevertheless, his description is regarded as the discovery of typhoid agents.

Nobody doubts that the bacteria that Eberth observed and described were *S. typhi*. However, soon afterwards, Reher (1885) suspected that colonies of *S. typhi* in the dead tissue had formed during the post-mortem growth of a few or even single organisms. Pictures similar to those described by Eberth (1880, 1881) and later by Gaffky in 1884 (who was the first to isolate *S. typhi* in pure culture) were found by Reher (1885) only in one case, when autopsy was made 42 h after the patient's death. At autopsies made soon after death, accumulation of bacteria was not revealed.* Later Fraenkel and Simmonds (1886) came to the same conclusion. Having studied a considerable amount of autopsy material and having carried out many experiments on animals, they had been convinced of the multiplying ability of typhoid organisms in dead tissues (Fig. 13-3*b*). They even suggested to grow the organisms artificially by leaving pieces of organs unfixed for 24 h prior to histological study. Later Fraenkel (1900) successfully applied preliminary tissue incubation for studying typhoid roseolas. The technique of "additional growing" of salmonellae is also useful in experimental investigations (see Chapter 17), but the relations preexisting between organisms and cells of the affected host cannot be judged in this way.

Christeller (1928) pointed out that post-mortem growth of salmonellae may also be observed because bacteraemia is frequently present in this infection. In dealing with the pathology of typhoid, however, microcolonies of rather large rods in the Peyer's patches, lymph nodes and splenic pulp are usually mentioned. But mostly it is not stated that bacteria multiplying at these sites are responsible for the specific lesions. In hypotheses on typhoid pathogenesis and the causes of morphological changes in the intestine and other organs, the main role is attributed not to salmonellae themselves but to their endotoxins.

Meanwhile, Goodpasture (1937) showed the possibility of a new approach to study the mechanism of typhoid infection. In testing various organisms in experiments on the chorio-allantoic membrane of chick embryos, Goodpasture and Anderson (1937) showed *S. typhi* to possess a peculiar feature.

* In the cases described by Eberth in 1880 and 1881 where he found colonies of organisms in mesenteric lymph nodes, autopsy was usually made as late as 18–46 h after the patients' death. In the organs obtained at the three earliest autopsies (2, 3, 6 h after death) he failed to detect any organisms. Gaffky (1884) did not report on the time that had elapsed between death and autopsy.

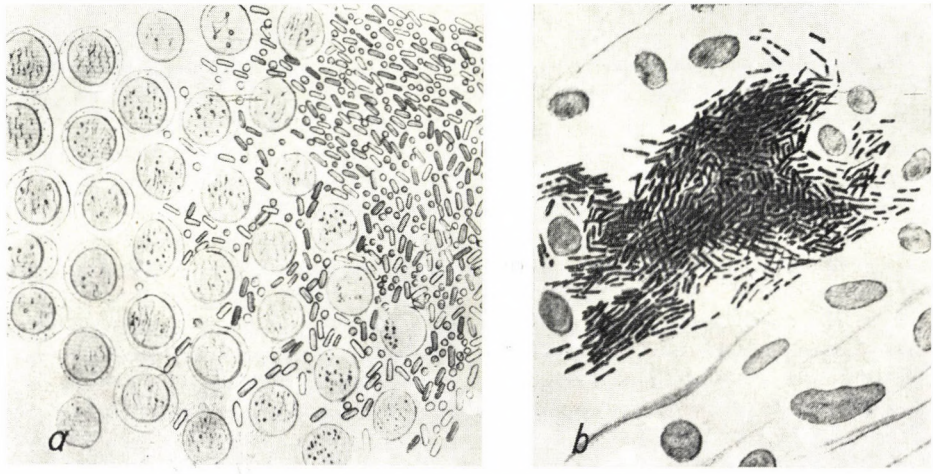


Fig. 13-3. Microcolonies of typhoid bacteria in histological preparations. *a* Unstained section (drawing by Eberth, 1880); *b* methylene blue stain (Fraenkel and Simmonds, 1886)

They grew in entodermal epithelium where they were very small, while in necrotic tissues they appeared as rather large rods similar to *S. typhi* grown on artificial nutrient media. Having examined, 90 min after death, the intestine of a girl who had died on the eleventh day of the disease, Goodpasture (1937) stained the sections as described by Wright (Romanovsky's technique modified). This enabled him to find in the affected Peyer's patches and mesenteric lymph nodes small rods and coccoid organisms resembling those detected in chick embryos. These organisms, however, were not lodged in the epithelial coating preserved above the patches but in cells having a rather large cytoplasm with some degree of basophilia. In these cells, which Goodpasture (1937) considered to be young plasma cells, numerous organisms formed aggregates in circumscribed light areas of the cytoplasm. Such cells containing organisms were, in general, not numerous but were encountered in each section.

Goodpasture's observations were confirmed by Adams (1939); he studied the organs of six patients who had died of typhoid. Using a modified staining technique, Adams observed small bacteria in cells resembling plasma cells or lymphoblasts (Fig. 13-4). He considered the organisms to be inside the vacuoles, their number amounting to 100 or more in each vacuole. At the same time the infected cells appeared viable, occasionally displaying signs of mitotic division.

Pointing out the undoubtful importance of Goodpasture's investigations one should note that he, as well as Adams, were evidently wrong in considering the cells which contained small forms of *S. typhi* to be plasma cells. As Planelyes and Forshter (1946, 1947) stated, these were young reticular cells or histiocytes also distinguished by their basophilic cytoplasm.

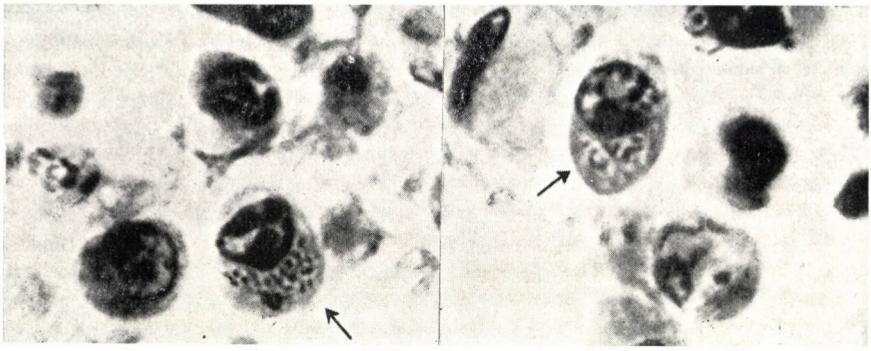


Fig. 13-4. Small organisms (arrows) in the cytoplasm of cells of a Peyer's patch in typhoid fever. Basic fuchsin–light green stain, ×2500 (Adams, 1939)

Unfortunately, the work of Goodpasture (1937) and Adams (1939) has not been continued and no further data on intracellular parasitism of typhoid agents in human intestine are available.

Sprinz et al. (1966) took repeated biopsies from volunteers infected with *S. typhi*. Pieces of mucosa were taken from the initial portion of the jejunum. The findings were rather scanty: only signs of enteritis such as "focal granulomatous changes" were described; the presence (or absence) of bacteria in the tissues was not mentioned.

Keiserling et al. (1972) studied a mesenteric lymph node removed from a child with typhoid fever. In necrotic areas rods strongly staining with thionine were prevalent. In the cytoplasm of certain macrophages there were faintly-staining small organisms; as shown by electron microscopy they were inside membrane bound vacuoles.

Considering salmonella infection not to be limited to the alimentary tract, Bilibin et al. (1970) studied the bone marrow of typhoid patients as well as of *S. typhi* and *S. paratyphi-B* carriers. The bone marrow culture obtained by biopsy from the iliac crest was investigated. In 13 out of 20 cases there was a macrophage infection with typical salmonellae; heteromorphic and L-forms of the agents exhibited a specific reaction after treatment of the preparations with fluorescent antisera.

INFECTIOUS PROCESSES CAUSED IN MAN BY VARIOUS *SALMONELLA* SPECIES

In addition to *S. typhi*, the agent of "classical" typhoid fever, other members of the *Salmonella* group are pathogenic to man. On the basis of literary data, Shur (1970) enumerated 30 types of these bacteria as having an importance in human pathology, but in Dack's (1963) opinion, each serotype (more than 1600 are known at present) may be considered a potential menace.

Based on epidemiologic considerations, *S. typhi* and *S. paratyphi-A*, *B* and *C* found only in humans have been grouped separately. Serotypes

affecting both humans and animals have been called "bipathogenic". From the pathogenic point of view this grouping is not adequate because *S. paratyphi-A* causes diseases which are almost indistinguishable from typhoid fever, while the course of infection caused by *S. paratyphi-B* and especially by *S. paratyphi-C* is very frequently quite different. In this respect *S. paratyphi-B* and *S. paratyphi-C* stand closer to "bipathogenic" salmonellae.

In most schemes classifying clinical features of salmonella infection gastrointestinal, typhoid-like, septic forms and various focal lesions, more frequently occurring as secondary complications of some other diseases, are stressed. In Table 13-I data on 7779 cases of salmonella infection published by Saphra and Winter (1957) are given. These clinical data are probably not quite precise, since salmonella infection frequently remains unrecognized (according to Tarasov, 1971, in 54 per cent of the cases), they nevertheless show the similarities and differences in the pathogenic properties of individual salmonella types.*

As shown in Table 13-I, certain clinical symptoms are not always associated with a definite type of *Salmonella*. Acute gastroenteritis is the most typical manifestation of the majority of these organisms (except *S. paratyphi-A* and *B*, and *S. cholerae-suis*). As a rule, the disease appears in population groups having eaten some food contaminated with salmonellae. The comparatively rapid appearance of enteric disorders accompanied by symptoms of general intoxication suggested poisoning with toxic substances produced by salmonellae; in fact, the term food poisoning is still used for these cases (Prost and Reimann, 1967). However, similarly to shigellae, large doses of killed salmonellae or endotoxin extracted from them has been found harmless when introduced into the alimentary tract of experimental animals. At the same time, clinical and epidemiological data prove salmonella gastroenteritis to be undoubtedly an infectious disease (Dack, 1956, 1963; Novgorodskaya, 1960; Ralovich et al., 1968; Arbuzova, 1968*b*; Chakhutinskaya, 1968; Gurevich, 1970; Bluger et al., 1975).

In acute gastroenteritis caused by *S. paratyphi-B* and *S. typhimurium*, signs of catarrh and of vascular disturbances with occasional superficial erosions were found in the intestinal mucosa. Lesions of lymph follicles and Payer's patches characteristic of typhoid fever were absent or these lymphoid structures only showed slight non-specific swelling (Huebschmann, 1913; Pick, 1928; Kiparisov, 1956, 1964; Story and Hansbury, 1957). Inflammatory changes were mainly observed in the small intestine, particularly in its distal portion. Pick (1928) was of the opinion that the lesions of the colon were the result of dysentery infection associated with salmonellosis. However, in

* The data of Saphra and Winter (1957) do not reflect the temporal and geographical fluctuations in the importance of certain salmonella types in human pathology (Dack, 1963). *S. typhimurium* has long been the prevalent serotype all over the world; in Leningrad *S. heidelberg* was previously ranked the second (Novgorodskaya, 1960) while in 1969 the incidence of diseases caused by *S. panama* and *S. derby* was the highest (Balabanova et al., 1970). According to the data of Krasnitskaya et al. (1970) in the USSR 60 per cent of toxinfection outbreaks are caused by *S. typhimurium*, 20 per cent by *S. enteritidis*, 4.5 per cent by *S. cholerae-suis* and 3.5 per cent by *S. dublin* (see also Bluger et al., 1975).

TABLE 13-I

Clinical manifestations of salmonellosis
(according to Saphra and Winter, 1957)

Most important and frequent *Salmonella* types	Number of cases	Clinical manifestations (per cent)				Death (per cent)
		Gastro-enteritis	Typhoid or septic syndrome	Focal manifes-tations	Carrier conditions	
Total, averages	7779	68.3	8.8	7.4	15.5	4.1
S. paratyphi-A	40	7.5	67.5	7.5	17.5	—
S. paratyphi-B	349	37.0	41.3	5.4	16.3	1.1
S. san-diego and *S. saint-paul*	133	74.4	7.6	6.0	12.0	0.8
S. derby	182	65.4	6.0	6.6	22.0	2.2
S. typhimurium	2385	80.8	4.1	4.9	10.2	4.1
S. bredeney	82	53.7	4.9	19.5	22.0	3.7
S. cholerae-suis	359	15.6	47.6	35.7	1.1	20.3
S. montevideo	659	70.7	3.5	5.5	20.3	2.4
S. oranienburg	641	65.2	7.6	8.7	18.5	3.6
S. bareilly	236	66.9	3.0	6.3	23.7	4.2
S. tennessee	739	55.6	5.0	3.3	36.0	1.3
S. newport	701	77.3	4.3	4.6	13.8	2.7
S. muenchen and *S. manhattan*	254	76.8	3.1	5.5	14.5	2.0
S. enteritidis	240	75.8	5.0	9.6	9.6	5.8
S. panama	234	55.5	16.7	12.0	15.8	4.3
S. anatum and *S. newington* etc.	346	68.2	2.3	4.0	25.4	2.3

salmonellosis, too, the colonic mucosa may be involved in the pathological process as indicated by the presence of mucus and blood in the stools. "Colitis syndrome" was most frequently observed in diseases caused by *S. panama*. In these cases the inflammation of colonic mucosa was confirmed by sigmoidoscopy (Kasatkina et al., 1970).

A case of fulminant infection with *S. saint-paul* described by Hartz et al. (1950) is of interest. Autopsy was made 30 min after the patient's death (girl, aged 2 years), and histological study detected acute inflammation with superficial erosions limited to the colon.

In man, infections induced by *S. typhimurium* and by most other kinds of salmonellae rarely become septic or cause focal lesions in some organs. In contrast, such a course (frequently fatal) of disease is almost invariably seen in infections caused by *S. cholerae-suis* (see Table 13-I). Gurevich (1956) found that damage of intestinal lymphoid structures was completely absent in diseases caused by salmonellae of group C (among them *S. cholerae-*

suis). At the same time, Gurevich noted the complexity and diversity of the pathologic picture in these diseases since in the majority salmonellosis is associated with other infections or pathologic states. The same is true for complications of other kinds of salmonellosis as well. In some clinical classifications they are called special "nosoparasitic forms".

There was a time when certain organisms, very frequently joining spirochetae in recurrent typhus, aroused great interest. Isolated by Kulesha and Titova (1922, 1923), and Ivashentsov (1923, 1926), they were formerly called "B. paratyphi N_1" and "N_2", at present they bear the name of *S. paratyphi-C* and *S. moscow*. In a vast post-mortem material of 268 cases of recurrent typhus, Kulesha and Titova only detected signs of catarrh in the intestines; secondary salmonella infection was mainly associated with lesions of the renal cortex resembling granulomas or focal infiltrations containing diverse cells with small colonies of rod-like organisms among them.

In the statistics of Saphra and Winter (see Table 13-I) salmonella infections with typhoid course were assessed together with those having a septic course. No information is available on the occurrence of peculiar typhoid lesions of the lymphoreticular apparatus of the intestine in any type of salmonellosis, except in infections caused by *S. typhi*, *S. paratyphi-A*, and occasionally by *S. paratyphi-B*. On the other hand, the lesions induced by *S. typhi* are, in cases, limited to the intestinal mucosa, leaving follicles and Peyer's patches intact (one case was described by Balogh, 1922).

EXPERIMENTAL STUDY OF SALMONELLOSIS

The aim of the initial animal experiments was to confirm the aetiological role of organisms isolated in typhoid fever in man. However, efforts to infect animals by the natural route, i.e. by feeding them *S. typhi* or introducing the agent into the alimentary canal, proved to be futile. The only successful attempt was that by Remlinger (1897) who succeeded in causing severe infection in four rabbits and ten rats which had been fed for a long time (up to 10 days) on greens infected with large amounts of typhoid bacilli. He noted swelling and even ulceration of the Peyer's patches in dead animals. Apparently, these experiments have not been repeated, and the opinion prevails that laboratory animals are completely resistant to *S. typhi* when challenged orally.

Lower primates are also resistant to *S. typhi*. Successful experiments were only reported on the chimpanzee (Grünbaum, 1904; Metchnikoff and Besredka, 1911; Gaines et al., 1968). Some of the animals receiving the agent with food developed a mild disease and some died.

The main purpose of a long series of experiments on the chimpanzee (Edsall et al., 1960; Gaines et al., 1968; and others) was to find out whether vaccination against typhoid was possible; in addition, certain information on the course of experimental infection in monkeys was also expected.

In spite of an extremely massive challenge (monkeys received bananas infected with 10^{11} viable *S. typhi*), the response ranged from a moderately severe disease to the absence of symptoms and bacteraemia. However,

specific antibodies appeared in the blood in all cases. Alterations in the Peyer's patches and lymph nodes, similar to those seen in typhoid fever were only observed in a few of the animals sacrificed at various intervals after challenge. At the same time, conspicuous responsive follicular hyperplasia of lymphoid tissue of the mesenteric lymph nodes and of the spleen was noted in all monkeys. (In contrast, in human typhoid fever such follicles are markedly decreased, they almost disappear and are replaced by typhoid cells.) In all the animals the authors diagnosed a diffuse enteritis characterized by infiltration of the lamina propria with lymphocytes, monocytes, plasma cells and macrophages. However, similar cells are found in a variable (occasionally considerable) amount in the intestinal lamina propria of healthy monkeys and man (see Chapter 1). The monkeys used by Edsall and others could hardly be considered healthy as their intestines were abundantly invaded by helminths. The presence or absence of typhoid organisms in various organs was only judged by bacteriological examination.

Thus, enteral challenge of monkeys has so far contributed little to the understanding of human typhoid. The same holds true for the results of parenteral introduction of *S. typhi* to various animals.

Having failed in the oral challenge of animals with *S. typhi*, Fraenkel and Simmonds (1886) experimented with a whole series of other techniques on rabbits, such as the introduction of bacteria into the duodenal lumen by means of laparotomy, inhalation of microbial suspension and injection of organisms directly into the lungs, subcutaneous and peritoneal injection of salmonellae and their injection into the ear vein. Intravenous inoculation alone resulted in an infection with a septic course and lethal outcome. The same result was obtained when they employed peritoneal challenge of mice. At autopsy, enlargement of the spleen, Peyer's patches and mesenteric lymph nodes was observed; histological investigation was not made.

Avtsyn and Berezina (1958, 1971) made successful intranasal challenge of mice with very large doses of *S. typhi* (see Chapter 15). The authors recommended the use of the infectious process induced in the lungs as an experimental model for testing antibiotics.

Takaki (1927), Vedibeda et al. (1972), and some other workers cited by them reproduced typhoid carrier state of a very long duration (up to 2 years and longer) by introducing *S. typhi* into the bone marrow of animals, usually rabbits. Bacteriological studies made by Vedibeda and co-workers showed that during the first 2–4 months, typhoid organisms could be isolated not only from the bone marrow but also from various viscera, faeces and urine.

The experiments carried out by Planelyes and Forshter (1946) attracted attention in their time. Using the method described by Berman et al. (1939), they introduced 200–500 virulent typhoid organisms subcutaneously into the front footpad of mice. Then, studying imprints made from the sites of injection and regional lymph nodes, they concluded that these organisms grew embedded in lymphocytes where they penetrated the nucleus. When multiplying salmonellae formed central or eccentric colonies which gradually ingested the cell nucleus, leaving of the latter something like a ring or

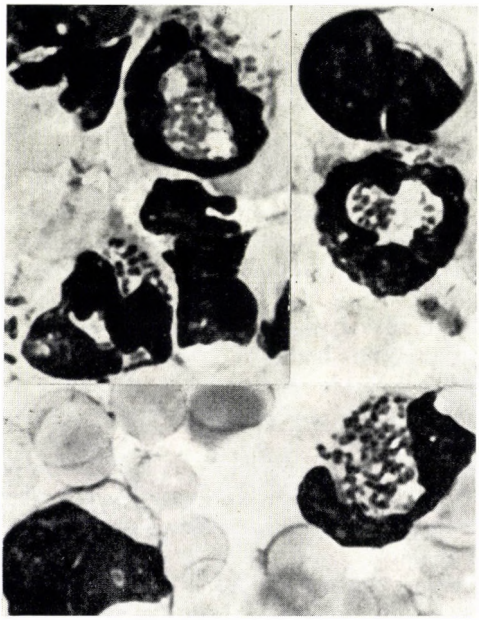


Fig. 13-5. Mouse polymorphonuclears filled with organisms. Imprint of the lung made 6 h after intranasal administration of 1.5×10^9 heat-killed *Sh. dysenteriae* 2. Eosin-azure, $\times 600$

a narrow circle. Photomicrographs recording the authors' conclusions are very similar to that shown in Fig. 13-5. Nevertheless, our micrographs do not show bacterial growth inside the lymphocytes, merely phagocytosis of heat-killed shigellae by mouse polymorphonuclears. A false impression of cell nucleus erosion (the cell is the same size as a lymphocyte) is obtained because in mice young polymorphonuclears, corresponding to human band leukocytes, have frequently ring-shaped nuclei. Even Planelyes and Forshter, who were not morphologists, did not repeat their statement about salmonellae penetrating lymphocytes in their later publications (Planelyes and Forshter, 1950; Forshter, 1957). Recently, Bondarenko et al. (1970), however, have made an attempt to confirm the statement that *S. typhi* is capable of parasitizing lymphocytes. They observed the growth of these bacteria in lymphoid cells cultivated *in vitro*. Without disputing their statement, we must emphasize once more that cells growing isolated on artificial nutrient media differ considerably from parent cells in the organism.* This is evidently the reason why bacteria capable of intracellular growth may parasitize various cells *in vitro*, including those which they never inhabit *in vivo* (see Chapter 5). This is, essentially, stated in Bondarenko's

* Bondarenko et al. (1970) reported that lymphocytes cultivated by them were readily transformed into polyblasts.

earlier work (1967) where the ability of *S. typhi* to grow on fibroblasts, HeLa cells, and cells of certain other tissue cultures was demonstrated. This had also been reported previously by Shepard (1959) and Smadel (1963).

Unlike *S. typhi* and *S. paratyphi-A*, other kinds of salmonellae causing human disease, are pathogenic for most laboratory animals. Of special interest is the fact that enteral challenge of mice and rabbits with *S. typhimurium*, *S. enteritidis*, and some other members of the *Salmonella* genus may cause disease closely resembling typhoid but not toxinfection (Valdman, 1928*a*, *b*, 1930, 1931*a*, *b*). The peculiarities of these experimental diseases should, however, be taken into consideration when assessing the results.

The results of the experiments performed by Valdman (1955, 1964) summed up in two monographs have provided a sound basis for the understanding of the pathogenesis of salmonelloses. However, she did not succeed in establishing the relations between certain morphologic alterations and the activity of pathogens. Having failed in the bacterioscopy of histological preparations and smears, she concluded that salmonellae were present in the tissues in a peculiar invisible form. Meanwhile, the above-mentioned observations of Goodpasture (1937) and Adams (1939) show that, though *S. typhi* may undergo considerable changes in a host, they still can be detected even by light microscopy.

Using eosin-azure staining, Oakberg (1946) was able to reveal small, faintly staining rods in reticuloendothelial cells of the liver and spleen of mice infected with *S. typhimurium*. He assumed them to be degenerative forms of organisms perishing in phagocytes. By applying fluorescent antibodies, Tanaka et al. (1960*a*, *b*) detected organisms of various sizes and shapes in the same cells of mice challenged with *S. enteritidis*. However, they did not consider the heterogeneity to be a sign of microbial destruction, assuming that the status of the bacilli in the animal was substantially different from that observed *in vitro*. The same conclusion was also drawn by Pritulin (1963) who investigated the smears from the subcutaneous tissue of various animals after local injections of salmonellae.* The characteristics of intracellular salmonella growth can be best studied in the lung model described in Chapter 15 (Voino-Yasenetsky, 1964*b*).

For a detailed study of the interrelation between salmonellae and host cells the use of enteral challenge as well as other models may be advantageous. In contrast to experiments with shigellae, the subcutaneous, intravenous (see Chapter 18) and intraperitoneal (see Chapter 14) modes of challenge are occasionally useful. Only some *Salmonella* strains are capable of inducing experimental conjunctivitis in guinea pigs, but the results of such experiments as well as of those introducing salmonellae into the bladder are of undoubted interest (see Chapter 16).

Obviously, not all salmonellae are equally capable of attacking laboratory animals. The usual assessment of pathogenicity based on death rate is

* Mitsuhashi et al. (1959) have also established indirectly (by means of centrifugal fractionation) that, 24 h after storage, *S. enteritidis* in the mouse viscera become smaller than while growing on nutrient media.

insufficient, since the infection does not invariably result in death. Its development may be detected by means of bacteriological investigations of the viscera (Klyachko, 1947; Arbuzova, 1960; Chakhutinskaya, 1960).

Strains showing various degrees of pathogenicity are commonly encountered within the same serological type. In a study of 862 cultures of *S. typhimurium* isolated from humans, Arbuzova (1957, 1960) showed that less than half of them were able to kill mice within 6–12 days which had been given 5×10^2 bacteria per os. Even 20×10^9 bacteria of most of the remaining cultures caused but a prolonged infection, leading to death in a smaller part of the cases. Such strains were characterized by a higher enzyme activity. Differences in the virulence of certain *S. typhimurium* strains were evidently associated with genetic factors. The attempt to increase the virulence by passages in mice was not successful.* However, in contrast to shigellae, the virulence of more pathogenic strains did not diminish with prolonged cultivation on artificial medium. To highly pathogenic *S. typhimurium* strain No. 4669 and strain No. 4801 with low pathogenicity have been successfully used in experimental work. The difference between these two strains was fully exhibited only after enteral challenge. When mice were injected intraperitoneally with only several scores of cells, both strains caused a fatal infection (Arbuzova, 1957).

The great difference in the dose of bacteria necessary for enteral and parenteral challenge of mice is striking. The same was observed by Klyachko (1947) in studying *S. heidelberg*, and by Planelyes and Forshter (1950) who compared the response to oral and intradermal infections in mice. Obviously, only very few out of hundreds of millions and even billions of salmonellae getting into the alimentary tract succeed in penetrating the inner medium of the body. These start the infectious process while all others perish or are rapidly eliminated from the intestines. This was also confirmed by bacteriological studies (Ørskov et al., 1928; Planelyes and Forshter, 1950).

The infecting dose of salmonellae may be decreased 10–20 thousand times in oral challenge of mice if the organisms are introduced with water or milk and not with solid food (e.g. bread) (Pshenichnov, 1936; Planelyes and Krasinskaya, 1950). The organisms in the liquid rapidly reach the intestine without being exposed to the damaging effect of saliva and gastric juice (Planelyes and Krasinskaya, 1950). After oral introduction of microbial suspensions to mice, no lesions of the Peyer's patches are commonly observed (Valdman, 1935, 1955), and salmonellae may first penetrate the mediastinal lymph nodes (Planelyes and Krasinskaya, 1950). In rabbits and guinea pigs the same mode of challenge is responsible for a rather characteristic infection involving the intestinal and mesenteric lymphoid structures (see Chapter 17).

The introduction of salmonellae into the duodenal lumen of rabbits produces various effects depending on the injected dose. Small doses cause a disease similar to typhoid fever, while massive doses induce a severe dif-

* Markov et al. (1966) reported on an increase in the virulence of *S. typhimurium* as a result of a 10–15 h growth in dead mice or minced meat at 37 °C.

fuse enteritis (Valdman, 1931*a*, *b*, 1955; Bibinova, 1939). In guinea pigs fatal enteritis may be produced without changing the oral dose, by inhibition of the elimination of the organisms by means of suppressing the intestinal peristalsis (Kent et al., 1966*a*).

To imitate natural conditions as closely as possible the minimum amount of organisms causing the infection should be applied. In bacteriology high doses are frequently used. This accelerates the development of the infection and thus makes the evaluation of the results easier. Massive challenge (parenteral particularly) is far less adequate for clarifying the pathogenesis of infectious processes. When there are many free organisms in the infectious focus, it is difficult to decide whether the organisms engulfed by phagocytes are capable of multiplying. Under such conditions, the increase in the number of organisms in the macrophage's cytoplasm may be due to the multiplication of organisms previously ingested as well as to continued phagocytosis. In addition, a considerable part of the organisms introduced subcutaneously into the blood stream, respiratory tract, etc. is usually rapidly destroyed. This may result in a poisoning of the experimental animal with toxic microbial breakdown products.

Very high doses of microorganisms can even be the cause of contradictory results. Infecting mice with pneumonia virus, Gogolak (1953) noted a reduction of animal mortality in cases when the concentration of virus administered was exceedingly high. We observed that intranasal administration of 1.2×10^7–1.3×10^9 *B. pertussis* rods induced rapid death in a great number of white mice while in surviving animals the further course of the infection was milder than after challenge with smaller doses (Voino-Yasenetsky and Khai, 1960). This finding may be explained by the so-called non-specific resistance appearing under the effect of bacterial endotoxins.

As in experimental study of other infections, the course of salmonellosis may be changed by exposing the animals to various influences (Sarkisov and Remezov, 1960; Valdman, 1964; Smirnov, 1966; Wundt, 1966; Boros and Gerichter, 1968; Reade and Bateman, 1969; Olitzki, 1972). Interventions decreasing (vitamin deficiency, cooling, cortisone, blockade of the reticuloendothelial system, etc.) or increasing (non-specific resistance induced by endotoxin pretreatment) the defensive functions of the host are mostly assessed in terms of the death rate and of the time elapsing until death. Direct observations on the changes in the infectious process itself are rather scanty.

Mouse strains characterized by an increased resistance to salmonellae have been obtained by inbreeding. The resistance is principally due to successful phagocytosis and digestion of organisms by reticuloendothelial cells (Gowen, 1960), though certain other unclarified non-specific factors may also be of importance (Boehme, 1970).

The mechanism of specific resistance arising after the termination of salmonella infection or vaccination is not quite clear either. The problems of immunity in enteric diseases are extensively studied but these are beyond the scope of this paper. For the understanding of the pathogenesis of salmonellosis it is important that, according to the present view, an increased ability of phagocytes to digest the organisms engulfed underlies acquired resistance. Macrophages are believed to play the main part here (Collins,

1971). Meanwhile, the activity of polymorphonuclears is significantly increased in the recovering and immunized host (Metchnikoff, 1905; Berman and Slavskaya, 1958; Podlevsky, 1962; Pokrovskaya et al., 1963).

Immunity in salmonellosis (as well as in certain other infections caused by intracellular parasites) provides safe protection against new infection by similar microorganisms, but does not interfere with the persistence of the agents of the previous infection in the body, which is a rather frequent phenomenon. These organisms seem to dwell in reticuloendothelial cells without losing their virulence (Hobson, 1957), and a relation, resembling symbiosis, is established between them and the host.

DIFFICULTIES ARISING IN ANIMAL EXPERIMENTS

As is known, occasional epizootics caused by *S. typhimurium*, *S. enteritidis* or other salmonellae occur among laboratory animals. Apart from causing economic losses, they create considerable difficulties and may even be responsible for errors in experimental results. In this respect the prolonged carrier state developing in part of the animals that had suffered from the insidious form of the disease is especially dangerous. The balance between the host and the parasite may be disturbed in such cases during the experiment. Then an outbreak of the infection (easily transferred to other experimental animals) will interfere with the experiment, or, if it remains unnoticed, will distort the results.

Other latent infections are also rather widespread among laboratory animals (Cohrs et al., 1958; Cotchin and Roe, 1967; Voino-Yasenetsky and Zhabotinsky, 1970). They may, in their turn, interfere with salmonella experiments. Thus, Wundt (1966), referring to some former observations, remarks that the high mortality rate of mice infected with salmonellae and exposed to cold may possibly be accounted for by an activation of latent viral infection induced by cooling.

Spontaneous disease of the animals may be an impediment of all experimental investigations. In studying salmonellosis such intervening circumstances are especially important. It is, for instance difficult to study granuloma development in the liver if similar hepatic structures are frequently encountered in healthy mice (see Chapter 18). It has been mentioned that helminth invasion of monkey intestine complicated some experiments. In our laboratory Polotsky has sometimes experienced much difficulty when using the intestinal loop technique in rabbits infected with coccidiae (see Chapter 22). In addition, not all investigators are aware of the fact that, as a rule, certain organisms, which may occasionally be rather numerous, are present in lymphoid structures of the rabbit's intestine (see Chapter 17). Inconstancy of certain physiological signs in laboratory animals, leukocyte count in the circulating blood, in particular, may cause trouble in experimental work. The obstacles mentioned cannot be considered unsurmountable, but they should be known and borne in mind.

In conclusion, it may be stated that salmonella infection in man appears mainly as typhoid fever or gastroenteritis. Though typhoid and paratyphoid

fever are usually accompanied by bacteraemia, the major pathologic processes occurring in them are of focal nature, manifesting themselves in the development of specific granulomas in lymphoid structures of the intestine or mesentery as well as in the organs of the reticuloendothelial system. The formation of such granulomas appears to be related to the ability of salmonellae to parasitize in macrophage-type cells. However, autopsy materials do not make a thorough study of the pathogenesis of typhoid and paratyphoid fever possible.

Since laboratory animals are resistant to enteral challenge with *S. typhi* and *S. paratyphi-A*, other kinds of salmonellae pathogenic for mice, rabbits and guinea pigs are used for research. To assess the results and the significance of these experiments correctly, it is necessary to understand the development of the processes in the experimental animals.

CHAPTER 14

INTRAPERITONEAL CHALLENGE WITH SALMONELLAE, EXPERIMENTS ON ISOLATED CELLS AND CHICK EMBRYOS

by

T. N. KHAVKIN and V. L. BELYANIN

As it was shown in Chapter 5 the intraperitoneal challenge of animals with shigellae seems inexpedient; nevertheless, the use of the same method proves justifiable for salmonellae. The peritoneum, especially the omentum and the peritoneal cavity itself, abounds in reticuloendothelial cells: macrophages and histiocytes, in which parasitism of these organisms may be observed. Similarly, shigellae, as a rule, do not invade the blood either after peritoneal injection or under natural conditions; in contrast, experimental and natural salmonella infections are accompanied by bacteraemia and specific lesions of various organs.

Intraperitoneal salmonella injection is used for different purposes such as testing drugs and vaccines, checking immunity, studying visceral lesions (particularly, those of the liver) and for determining the virulence of the pathogenic agent. It is true, the assay of virulence of salmonellae by administration of rather large amounts of organisms is of doubtful value. Rapid death of the animals observed at that time mainly reflects toxic properties of salmonellae (Wundt, 1966), which do not necessarily correlate with virulence (Rowley, 1971). Administration of relatively smaller amounts of organisms is more acceptable. Recently this method has been successfully used in the analysis of the dynamics of salmonella dissemination in the host (Johnson et al., 1974). However, the nature of the pathogenic process occurring in the peritoneal cavity has not yet been elucidated.

There are important new data on the interaction between salmonellae and macrophages and leukocytes of the peritoneal fluid as well as on the inflammatory exudate taken from the peritoneal cavity. However, the results (to be dealt with later) are not sufficient to explain the infectious process. To obtain further data, we carried out joint morphological and bacteriological studies together with Arbuzova (1972, 1973), employing *S. typhimurium* cultures of moderate and high virulence in various doses.

The omentum, smears of the peritoneal exudate, and the washing fluid from the peritoneal surface were the subject of the study. For morphological investigations, the specimens were fixed with acetone and treated with rabbit fluorescent gamma-globulin according to the direct Coons' method or stained with the modified Giemsa–Leishman technique employed in our laboratory.*

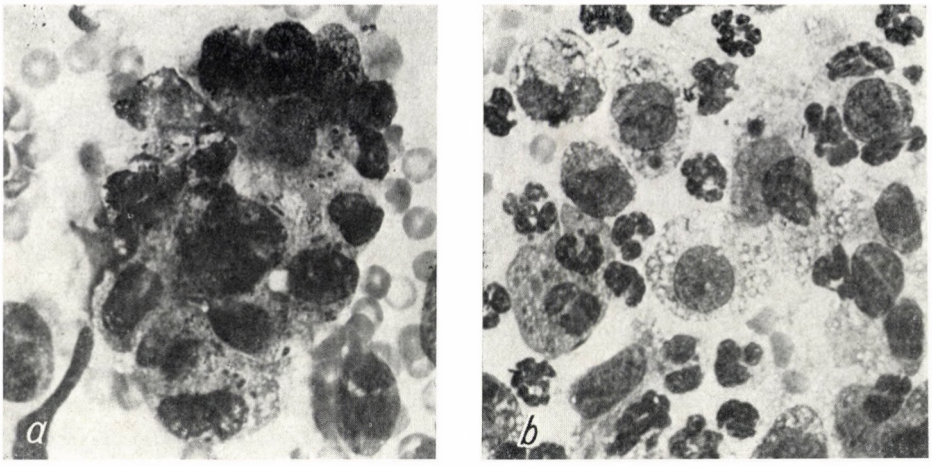


Fig. 14-1. Salmonella phagocytosis by histiocytes of the omentum and of the exudate. *a* Organisms in histiocytes of a milk spot 90 min after challenge; ×1450. *b* Salmonella phagocytosis by leukocytes and macrophages of the exudate 24 h after challenge; Giemsa-Leishman stain, ×960

Omental spreads proved to be a convenient object for phase-contrast microscopy in the native state. To investigate the omentum for microbial count, it was preliminarily rinsed to remove the organisms which were loosely adsorbed to it. Special experiments showed that it was sufficient to rinse the omentum in 10 portions of saline.

The two different salmonella cultures used induced fatal generalized infection in white mice. Virulent culture No. 28,747 caused death within 72–96 h independent of the dosage, while in challenge with moderately virulent culture No. 4838 an infectious process could be observed from 4–5 days up to 1 week depending on the dose of organisms. The peritoneal infectious process could be best studied with doses of $3–5 \times 10^6$ organisms of culture No. 4838; in this case the bacteria were detected in the omentum immediately after challenge also microscopically and the infectious process lasted about five days.

Bacteriological studies (Arbuzova, 1972, 1973) showed that part of the organisms invaded the blood in the peritoneal cavity immediately after challenge. Only 0.03–0.09 per cent of the inoculated bacteria remained in the omentum, then after a short interval, the salmonella content of the

* The specimens are placed into the initially undiluted solution of Leishman's stain for 5 min and then transferred into an aqueous solution (4:100) of Giemsa-Romanovsky stain without rinsing. Staining lasts for 1–2 h at 37 °C and for 16–18 h at room temperature. If necessary, the intensity of staining may be reduced by means of citrate buffer (pH 3.5). This is followed by dehydration in two portions of acetone, clarification in acetone-xylol and pure xylol; afterwards the specimens are embedded in polystyrol or Canada balsam.

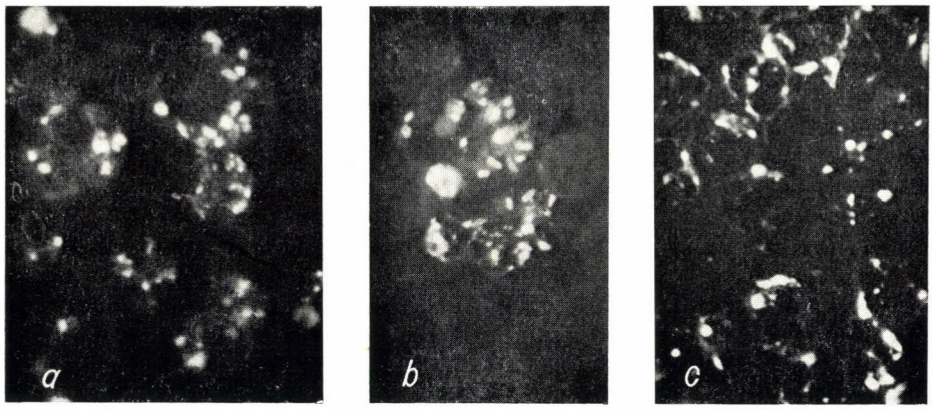


Fig. 14-2. Specific fluorescence of salmonellae in omentum spreads 24 h after challenge. Bacteria scattered in the cytoplasm of macrophages (*a*) or as small aggregates (*b*). *c* Amorphous masses of antigen material show fluorescence in polymorphonuclears. Coons' method, × 700

omentum and the peritoneal fluid began to increase. Three h after challenge there was an approx. 17-fold increase in the number of viable organisms in the omentum and in the peritoneal cavity. After 6 h the increase was 60-fold and after 24 h 50,000-fold.

Similarly to shigellae, salmonellae caused an exudative inflammation of the peritoneum with leukocytes in the exudate. Leukocytes and histiocytes (before the appearance of the former) ingested organisms (Fig. 14-1*a*). Unlike shigellae, salmonellae continued multiplying not only in the peritoneal fluid but also in macrophages and omental histiocytes.

Leukocytes migrating into the peritoneum first concentrated around the milk spots of the omentum, but later completely infiltrated the whole of its tissue. Three h after challenge signs of macrophage response started to become apparent and after 24 h the macrophage content in the inflammatory exudate increased considerably. Organisms were present in many of these cells as well as in leukocytes (Fig. 14-1*b*). They were scattered in the cytoplasm or formed small aggregations (Fig. 14-2*a*, *b*). In addition, amorphous granules of antigen, evidently remnants of destroyed organisms, were detected by means of the fluorescent antibody technique (Fig. 14-2*c*).

Salmonellae growing in the abdominal cavity exhibited polymorphism and considerable difference in the intensity of staining. In the liquid exudate and leukocytes, especially in damaged cells, there were many large rod-like, intensively staining forms (Fig. 14-3*a*). Small, poorly staining rods or small coccoid forms, approximately the same as in alveolar macrophages, prevailed in macrophages (see Chapter 15). In addition gigantic thread-like forms strongly staining with aniline dyes and brightly fluorescent when treated with Coons' method (Fig. 14-3*b*, *c*) were sometimes seen in peritoneal macrophages. The intensity of specific microbial fluorescence may be considerably different. By means of microfluorimetric photography (Barsky

and Khavkin, 1969) it was demonstrated that the brightness of organisms ranged within 10 to 15-fold limits.

Beginning with 48 h after challenge, some animals died. At this time the omentum of most animals exhibited great numbers of organisms residing extracellularly. As before, polymorphonuclears, many of them overfilled with phagocytized organisms and evidently destroyed, prevailed in the inflammatory exudate (Fig. 14-4*a*). At the same time, in some mice sacrificed when still in a comparatively good condition, macrophages were prevalent in the inflammatory exudate but the number of leukocytes considerably diminished. The agents were localized mostly in macrophages and histiocytes (Fig. 14-4*b*) in the omentum; they were rarely seen extracellularly. Histiocytes with organisms could be best detected in native preparations of the omentum with phase-contrast microscope (Fig. 14-5*a*). Many organisms were dividing; these bacteria exhibited slight fluorescence (Fig. 14-5*b*).

A similar, apparently favourable, course of the local process in the peritoneum was frequently seen when mice were challenged with a smaller number ($3–5 \times 10^5$) of salmonellae of the same moderately virulent strain, though these animals also died of generalized infection. In contrast, larger doses ($5 \times 10^7–5 \times 10^8$) resulted in death in 24–48 h. The organisms invaded the whole body and settled mostly in the omentum extracellularly. The inflammatory response was suppressed here, and phagocytosis appeared only during the first hours after challenge.

When mice were challenged with the highly virulent *S. typhimurium* strain No. 28,747, the infection had a severe course regardless of the dose

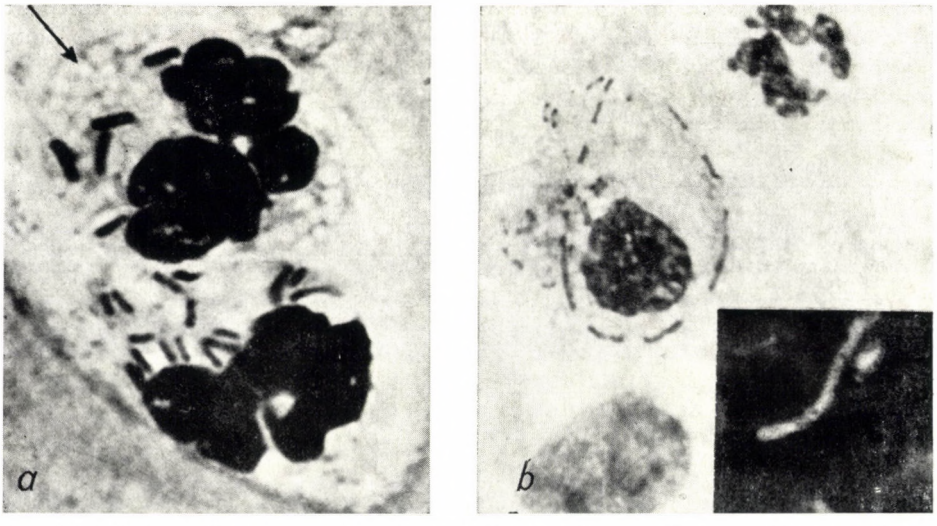


Fig. 14-3. Salmonella polymorphism in an infectious focus. *a* Large rod-like organisms in a polymorphonuclear cell (arrow) and smaller ones in a macrophage with lobulated nucleus. Giemsa-Leishman stain, ×2400. *b*, *c* Gigantic thread-like salmonellae. *b* Giemsa-Leishman stain, ×1000, *c* Coons' method, ×2000

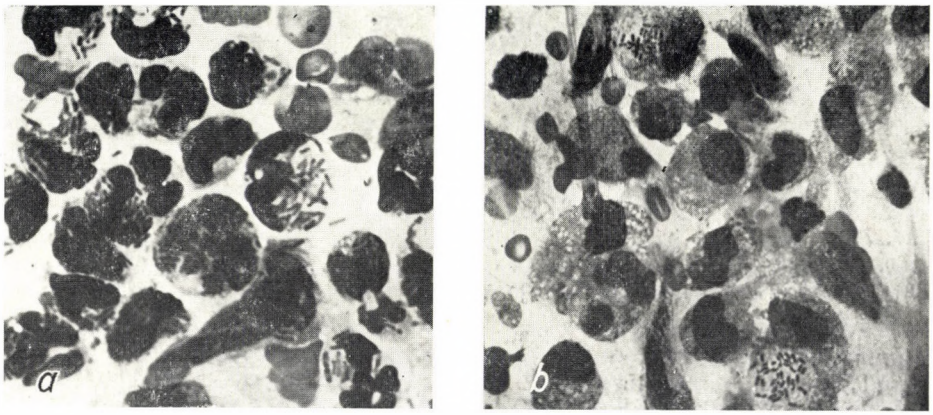


Fig. 14-4. Varieties of the inflammatory exudate in the peritoneum. *a* Polymorphonuclears taking up bacteria, abundant extracellular salmonella multiplication (48 h after challenge). *b* Macrophages were most numerous in the exudate after clearing extracellular organisms from the peritoneum (72 h after challenge). Giemsa-Leishman stain, *a* ×1450, *b* ×960

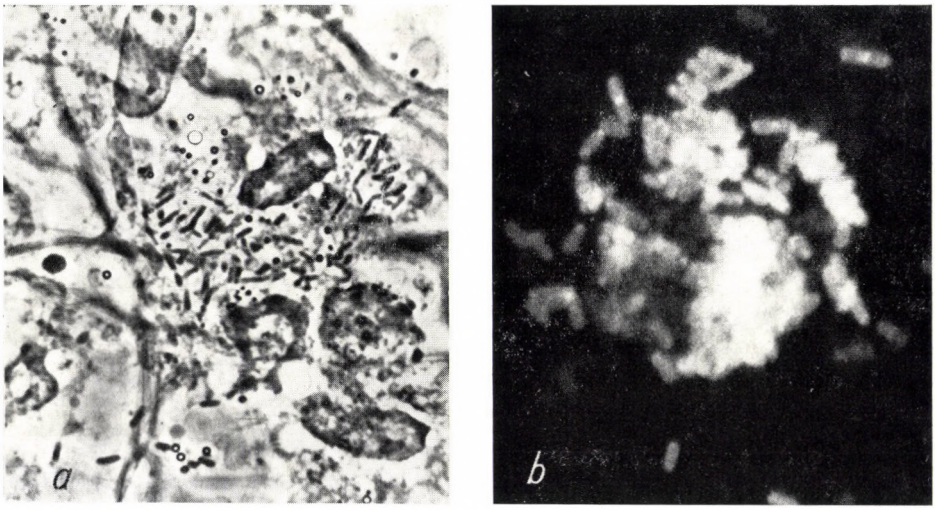


Fig. 14-5. Salmonellae in omental histiocytes *a* under phase-contrast microscope (×1000) and *b* after treatment with Coons' method (×1800)

(3–5 $\times 10^5$ to 3–5 $\times 10^6$). Culturing from the omentum and the peritoneal fluid yielded approximately the same results as in case of challenge with a less virulent strain (Arbuzova, 1972, 1973). However, as shown by morphological investigations, many organisms in the omentum were not phagocytized. Challenging mice with 3–5 $\times 10^5$ organisms of a highly virulent

strain, the inflammatory response was marked, but with 3–5 $\times 10^6$ organisms it was obviously inhibited.

It has been found that macrophages and histiocytes of the omentum are the first to interact with the organisms. The most active part in the body defence is played by polymorphonuclears which rapidly emigrate from the blood vessels. Leukocytes digest the phagocytized organisms and are not infrequently destroyed themselves. A conspicuous salmonella parasitism is observed in macrophages, as the infected cell obviously remains viable for a short time; while the cell is intact, leukocytes do not respond to the organisms lodging in it.

Microbial polymorphism is apparent both in the peritoneum and other organs during the infectious process. Unlike with intranasal challenge of animals (see Chapter 15) the transformation of salmonellae in the omentum cannot be followed. The reason for the appearance of different forms is not quite clear; the influence of various environmental conditions inside and outside the cells, as well as their different multiplication rates may be involved. Large, intensively staining and strongly fluorescent bacilli grow in the exudate and in the debris of dying-off cells (Berman and Slavskaya, 1958, 1959). The low specific fluorescence of dividing organisms may suggest the deficiency of O-antigen in the microbial cell (Kovaleva et al., 1967), which is characteristic of rapidly multiplying salmonellae (Collins, 1964). The thread-like organisms may, at the same time, be the result of an inhibitory effect on the agent's growth (Kourany and Kendrick, 1966).

There are some doubts whether it is expedient to use excessively high doses of salmonellae for peritoneal challenge. Body defence is evidently impaired when salmonellae are inoculated in a number causing the death of all or most of the animals within a short time. Under such conditions infection loses its specific features and acquires a septic character. Critical doses of salmonellae depend on the virulence of the strain used and can only be determined empirically.

EXPERIMENTS ON ISOLATED CELLS

Experiments on free cells of the peritoneal cavity and the peritoneal exudate belong to the same group because they yield results that are similar in significance. The most important results are those which confirm that salmonellae are capable of parasitizing macrophages and those characterizing factors involved in the interaction between salmonellae and the host cells. From the technical point of view the experiments with isolated cells may be roughly divided into three groups.

1. *Studying cells of the peritoneal exudate taken from the peritoneal cavity at different intervals after challenge.* Experiments of this kind allow insight into the infectious process, primarily into the inflammatory response in the peritoneum, the fate of the organisms in the exudate and the effect of the host's condition on the processes.

The exudate obtained by puncturing the same animal at different times after challenge (Valdman, 1955) is suitable for a morphological study immediately after centrifugation. Yamamoto and Hampton (1966) successfully applied a cellular precipitation for electron microscopy. According to their observations, in peritoneal macrophages salmonellae are arranged inside the phagosomes serving as the site of destruction for slightly virulent organisms; while virulent bacteria remained intact and even divided.

It is difficult to differentiate microscopically viable organisms from dead ones. Therefore, bacteriological cultures of the liquid fraction and homogenized cells were made at the same time (Collins, 1969). Berman and Slavskaya (1958, 1959) used the technique of "mirror impressions". The inflammatory exudate was spread on agar plates and incubated for 1–2 h. Then glass impressions were made from the agar surface and stained according to the Romanovsky-Giesma technique. In the authors' opinion signs of division and gigantic salmonellae in the cells, have shown that the organisms retained their viability.

To determine the bactericidal action of exudate cells on salmonellae, Blanden et al. (1969) employed the modified Maaløe's technique (1964). A mixture, containing a definite number of salmonellae and other microorganisms more resistant to intracellular digestion, was administered into the peritoneal cavity and at intervals bacterial cultures of the exudate cells were made. Bactericidal cell activity was evaluated in terms of the change in the interaction between salmonellae and microorganisms after their settling in the cells. It was shown that in animals which had suffered from infections whose agents may parasitize macrophages, the peritoneal macrophage activity markedly increased. This phenomenon known as acquired cellular resistance is non-specific. In the opinion of Mackaness (1970), it appears when phagocytic and bactericidal cell activities increase. According to his observations, macrophages of a normal mouse do not always engulf *S. typhimurium* whereas macrophages of mice with listeria infection engulf salmonellae even in the absence of opsonizing antibodies. An example for the high activity of such macrophages is their ability to destroy 98 per cent of salmonellae within 15 min while macrophages of a normal mouse kill not more than 50 per cent of the organisms they took up.

It is difficult to establish the quantitative ratio between the organisms and the cells interacting in the abdominal cavity. While the number of organisms inoculated can be standardized, the quantity of cells migrating into the abdominal cavity may vary considerably. The avidity of salmonella phagocytosis and the rate of destruction of salmonellae by the cells depend on the organism/cell ratio. In order to decide whether there is an intracellular multiplication or destruction of salmonellae, it is necessary to establish the number of organisms per one exudate cell and to determine how this ratio changes in time. To obtain this index, Olitzki et al. (1964) suggested a complex method allowing the calculation of the number of exudate cells in the counting chamber, the determination of bacterial counts in the cells and in the liquid exudate fraction by cultivation and studying the cells which have engulfed the organisms in stained smears. They have come to the conclusion that organisms of the virulent *S. typhi* Ty2 strain adminis-

tered into the mouse peritoneal cavity are poorly phagocytized, but if they do penetrate the cells, they can multiply there. At the same time, organisms of the avirulent strain O901 are rapidly engulfed by peritoneal macrophages in high numbers, under the same experimental conditions, and are destroyed within several hours.

The technique elaborated for studying exudate cells is of limited value because it does not reflect the host's humoral factors influencing host–organism interaction and also because the fate of salmonellae can be followed microscopically during the first hours after inoculation only with difficulty if applying small doses of bacteria.

2. *Interaction between salmonellae and isolated cells of the peritoneal cavity of non-infected animals in vitro.* In these investigations the abdominal cavity of non-infected animals serves as a source of cells which can be mixed with organisms in a test tube in different proportions; various substances (electrolytes, enzymes, proteins, etc.) may be added to the mixtures, and other conditions (e.g. temperature) may also be arbitrarily chosen.

The experiments last, as a rule, from several minutes to 1–2 h. After incubation the smears or suspensions are studied unstained or stained and bacteriological cultures are made simultaneously from the homogenized cells and the liquid mixture fraction to count the number of viable organisms. The viability of the cells is determined by staining with eosin, trypan blue, and neutral red; dead cells stain diffusely.

Experiments with microbial-cellular suspensions *in vitro* are especially convenient for studying early stages of cell–organism interaction. Moreover, also the phagocytic activity can be expressed in terms of quantitative indices (engulfing index, phagocytic quantity).

To isolate free cells, Jenkin and Rowley (1963) recommended the use of abdominal cavity fluid of animals not treated with stimulating agents. Intraperitoneal administration of saline, polysaccharides, proteins, oils and especially bacterial endotoxins elicits an inflow of cells to the abdominal cavity and, at the same time, affects the macrophages and leukocytes. This process is accompanied by the phenomena of acquired cellular resistance manifested first of all by phagocytic and bactericidal cellular activity. It has been reported (e.g. Mackaness and Blanden, 1970; Mackaness, 1970) that intraperitoneal administration of bovine gamma-globulin to white mice 48 h before sampling for peritoneal macrophages, increased the activity of these cells to a degree comparable to that of macrophages in immunized animals.

Although *in vitro* experiments, by necessity, fail to reproduce conditions prevailing in the animal body, highly standardized procedures may yield important information on the effect of various humoral factors, on the relation between salmonella virulence and phagocytic activity as well as on the fate of the organisms engulfed. In this manner, it has been shown that non-specific and specific opsonization of organisms plays an important role in their phagocytosis and subsequent digestion, but also the heterogeneity of both the macrophage and microbial populations has been demonstrated. Some macrophages were unable to destroy even well opsonized salmonellae and in contrast, part of the microbial population failed to adhere to macro-

phages even after opsonization with specific antibodies (Auzins and Rowley, 1963; Jenkin et al., 1964; Blanden et al., 1966; Rowley, 1971).

3. *Interaction of salmonellae with peritoneal macrophage culture.* Macrophages in cellular suspensions removed from the peritoneal cavity readily adhere to glass surface forming a monolayer culture. The latter may be infected with salmonellae by adding a preselected number of organisms to the nutrient medium (Mitsuhashi et al., 1961; Arbuzova, 1964*a*; Krotkova, 1971). The macrophage culture of Whitby and Rowley (1959) contained exudate cells and bacteria which had been withdrawn together from the peritoneal cavity of challenged mice. Hsu and Radcliffe (1968) cultivated infected macrophages in suspension.

Macrophage cultures, especially those growing as monolayers are suitable for cytological and cytochemical investigations. In the presence of antibiotics live cultures remain suitable for microscopical observation for several days. The antibiotics are applied to suppress the growth of extracellular organisms. Arbuzova (1964*a*) reported on the effect of various antibiotics and media on the activity of macrophages.

Like other cells cultured *in vitro*, macrophages, too, differ from the parent cells. Accordingly, all restrictions, mentioned in Chapter 5 for shigellae, pertain to macrophage cultures. In obtaining peritoneal cells for the purpose of cultivation, as mentioned above, it is necessary to consider the possible effect of stimulating agents on the cellular activity. In spite of their limitations, peritoneal macrophage cultures may be advantageously applied in the study of acquired cellular resistance, the role of humoral and cellular factors, and the importance of microbial antigens in the interaction between salmonellae and macrophages and leukocytes (Jenkin and Rowley, 1963; Ushiba, 1965; Olitzki, 1970, 1972; Roantree, 1971; Rowley, 1971).

As mentioned, experiments with free cells of the peritoneal exudate carried out with various methods have confirmed that salmonellae facultatively parasitize reticuloendothelial cells. Disagreements regarding the details of this process are due to differences in technique. Thus, Furness (1958), Furness and Ferreira (1959), Arbuzova (1964*a*) state that virulence does not influence the phagocytic activity of macrophages but is responsible for the agent's resistance to the bactericidal activity of the cells. In the opinion of Pike and Mackenzie (1940), Ushiba (1962), Jenkin and Rowley (1963), Kamzolkina et al. (1966), and Olitzki (1972) under similar conditions virulent cultures are more resistant to phagocytosis than weakly virulent ones and also, virulent cultures are more resistant to bactericidal macrophage activity. Furness (1958) has reported on the complete destruction of avirulent salmonella strains in macrophages, while Hsu and Radcliffe (1968) have claimed that avirulent salmonellae are only partly destroyed in macrophages, and the remaining organisms multiply inside the cells.

There are also differences in opinion, which may likewise be attributed to divergent techniques, regarding the fate of salmonellae pathogenic for man *(S. typhi, S. paratyphi-A, B)* in the peritoneal macrophages of rodents for which these serotypes are weakly pathogenic. Olitzki et al. (1964), Kamzolkina et al. (1966) and Kourany and Kendrick (1966) reported on

intracellular multiplication of these serotypes while Saito et al. (1960), Furness (1958), Ushiba (1962, 1965) and Arbuzova (1964*a*) did not observe the same. Unfortunately, opinions on the fate of salmonellae are supported only by insufficient ultrastructural studies. Thus, parasite–cell relationship during prolonged parasitism of salmonellae in macrophages and the relation of the agent to intracellular structures are still obscure.

Experiments with free peritoneal cells have also furnished some additional information on the role of polymorphonuclears in salmonella infection. According to Gershon (1965), contact with salmonellae enhances the metabolic and digestive activity of polymorphonuclear cells. The latter, as observed also in macrophages (Pokrovskaya et al., 1963; Petrovskaya, 1967), markedly increases in the presence of specific antibodies. In opinion of Gowen (1960) the intensity of the leukocytes' entering the peritoneum is one of the inherited factors of natural individual resistance to salmonellae.

EXPERIMENTS ON CELL CULTURES OTHER THAN PHAGOCYTES

Salmonellae can multiply in various established monolayer cell cultures (HeLa, Hep-2, mouse and chick fibroblasts, etc.), eventually inducing lesions of the infected cells and the destruction of the cellular monolayer (Furness, 1958; Shepard, 1959, etc.). The technique of challenge and investigation of cell cultures is similar to that used in the work with shigellae (see Chapter 5).

However, the ability of salmonellae to inhabit, similarly to shigellae, various cell lines *in vitro*, again indicates a change in the properties of cells growing outside the body. At the same time, this capacity of intracellular multiplication corresponds to the pathogenic properties of salmonellae. Thus Giannella et al. (1973*b*) showed a correlation between the ability of *S. typhimurium* strains to invade HeLa cells *in vitro* and their capacity to invade rabbit ileal mucosa *in vivo*. Strains failing to penetrate HeLa cells lacked the capacity to invade rabbit mucosa. They observed no correlation between the murine LD_{50} value and the capacity to invade either HeLa cells or rabbit mucosa. Kvitashvili and Bakhutashvili (1972—cit. by Bluger et al., 1975) supposed that pathogenic strains of *Salmonella* produce substances which may damage the lysosomes of the infected cell. They believe that this feature can help to distinguish pathogenic and non-pathogenic organisms.

Salmonellae usually begin to multiply in the cells within 3–4 h after inoculation. The process leads to destruction of the cell monolayer within 24–48 h. The multiplication rate *in vitro* of various salmonellae is variable (Bondarenko, 1967). Giannella et al. (1973*b*) showed a difference in the rate of cell monolayer destruction by various salmonella strains, and concluded that cytotoxic and invasive capacities may be different factors of virulence. They, in agreement with Ivanenko (1962) and Gavrilyuk (1966), considered the HeLa cell model a useful tool for studying the pathogenic and virulence properties of salmonellae.

EXPERIMENTS ON CHICK EMBRYOS

Challenge of chick embryos with salmonellae was first carried out by Goodpasture and Anderson (1937). They performed comparative studies of infectious processes induced in chick embryos with a variety of organisms including *S. typhi*. The inoculum was spread on the surface of the chorio-allantoic membrane.

S. typhi multiplied and usually caused the death of the embryo. Histological investigations showed that the inflammation developed at the site of inoculation and dystrophic alterations appeared in the viscera. Salmonellae were encountered inside exudate cells; this finding was considered a sign of phagocytosis. In addition, the organisms accumulated on the surface of the chorio-allantoic membrane as well as inside the ectoderm cells. Arrangement in ectoderm cells of bacteria in groups suggested, according to many authors, an intracellular growth of *S. typhi*. These organisms were smaller than the bacteria growing on necrotic debris. These observations inspired Goodpasture (1937) to search for *S. typhi* arranged intracellularly in the Peyer's patches of typhoid patients (see Chapter 13).

The chick embryo model has been applied to determine the virulence of *S. typhi* (Batson et al., 1950), to study the effect of immune sera (Grabar and Le Minor, 1951) as well as to culture *S. typhi* on embryos serving as a nutrient medium (Vilner, 1958, 1960). The technique of challenge and the mode of assessing the results were almost similar to those used for shigella tests (see Chapter 5). Histological studies carried out by Vilner (1960) demonstrated intracellular accumulation of organisms in the reticuloendothelial cells of the liver. Little is known about the infected avian egg used as a model of salmonella infection. Some observations have shown that difficulties may arise when using this model, due to the fact that in the course of embryo harvesting its defence systems change. Such a change is known to occur in the resistance of avian embryo against some salmonellae (Board and Fuller, 1974). Furthermore, one should consider the possibility of natural infection of avian eggs with some kind of salmonellae (Haglund et al., 1964; Shur, 1970; Bluger et al., 1975).

Thus, in contrast to shigellae, salmonellae induce in the peritoneum a specific process, the course of which depends on the virulence and dose of the agent. Salmonellae exhibit facultative parasitism in mobile and fixed cells of the reticuloendothelial system, peritoneal macrophages and histiocytes. Leukocytes ingest and destroy the organisms. The relationship between salmonellae and leukocytes and macrophages can be analysed *in vitro* on cells obtained from the peritoneum of infected and non-infected animals. In chick embryos salmonellae multiply in ectodermal cells of the chorio-allantoic membrane. This finding confirms the conception of facultative intracellular salmonella parasitism.

CHAPTER 15

INTRANASAL CHALLENGE OF ANIMALS WITH SALMONELLAE

by

M. V. VOINO-YASENETSKY

Intranasal inoculation of animals with shigellae (see Chapter 9) may at first sight seem somewhat unnatural, while similar experiments with salmonellae do not arouse any objection. Though certain peculiar "pneumonic" forms of salmonella infection were assumed to have occurred earlier, such cases are not encountered at present. Nevertheless, the possibility of specific lung affection as a complication of typhoid fever has been proved (Christeller, 1928; Lazovsky et al., 1947, 1948). Olitzki (1972) is of the opinion that infection via the respiratory tract with *S. typhi*, particularly in children, is possible.

Since animals are resistant to *S. typhi*, experimental pneumonia can be produced in them only by injecting the agent in very high numbers into the respiratory tract (Sprunt et al., 1935; Neufeld and Kuhn, 1936; Ermolyeva et al., 1954; Avtsyn and Berezina, 1958, 1971). Even under these conditions Sprunt and co-workers (1935) failed to detect any multiplication of the injected bacteria in the pulmonary tissue. The local inflammatory reaction was mostly produced by macrophages; the pulmonary alveoli contained great numbers of large pulpish cells. The authors considered this response nonspecific, as the same finding was revealed in experiments with *B. pertussis*. Similar morphological changes were described by Avtsyn and Berezina (1958), who inoculated up to 7.5×10^9 *S. typhi* into the lungs of white mice. They believed, however, that mononuclear cells accumulating in the alveoli (especially those after 5–7 days) were similar to "typhoid" cells, which are characteristic of typhoid fever. They mentioned that a great number of rod-like bacteria were detectable in the cytoplasm of these cells, as well as extracellularly, with the silver impregnation method of Levaditi. Part of the organisms seemed to grow in the pulmonary tissue as treatment with antibiotics prevented the rapid death of mice. Avtsyn and Berezina (1958, 1971) suggested to use the lung model for research on the therapy of typhoid fever.

Still, as mentioned above, in experimental infections *Salmonella* serotypes pathogenic for animals are mostly used. Inhalation of *S. typhimurium* aerosol or dripping the suspension of this organism into the nostrils was used by a number of workers to infect white mice (Trillat and Kaneko, 1921; Lange and Keschischian, 1924; Etinger-Tulczynska, 1932; Neufeld and Kuhn, 1936; Darlow et al., 1961; Tannock and Smith, 1971). The aim of such experiments, however, was only to study the possibility of infection

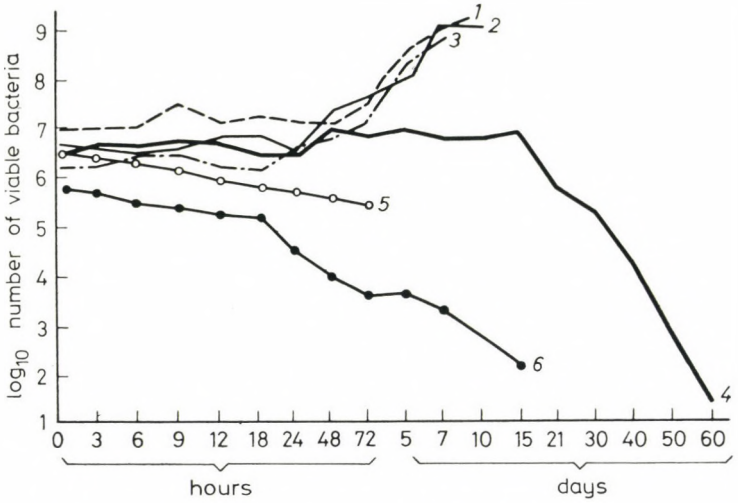


Fig. 15-1. Salmonella content in the lungs of white mice at different intervals after challenge. 1 *S. typhimurium* (strain No. 4801), 2 *S. heidelberg*, 3 *S. typhimurium* (strain No. 4649), 4 *S. paratyphi-B*, 5 and 6 *S. typhi*

with salmonellae via the respiratory tract. It was confirmed that on entering the lungs, even a very small dose of *S. typhimurium* (10^2–10^4) can induce a fatal generalized infection. The changes occurring in the pulmonary tissue itself have not been studied.

Arbuzova (1964*b*) tested the various salmonellae on the lung model by microbiological means. She used the same technique as we employed in experiments with shigellae. The results of certain quantitative bacteriological studies are presented in Fig. 15-1. Judging from the dynamics of microbial cultivation, it may be assumed that *S. typhi* were gradually eliminated from the lungs while other kinds of salmonellae persisted in an almost unchanged number for some time, after which they either started to multiply *(S. typhimurium, S. heidelberg)* or died *(S. paratyphi-B)*. In fact, the data represent the results of a rather complex interaction between parasites and the host immediately after infection.

Morphological studies (Voino-Yasenetsky, 1964*b*) which were carried out simultaneously as presented in Fig. 15-1, showed the salmonellae to elicit the same cellular response in the lungs of mice as that produced by shigellae. Beginning with the third hour, the alveoli gradually became filled with polymorphonuclears, and by the end of the first day pneumonia developed (Fig. 15-2). In the alveoli there was a slight amount of liquid exudate; haemorrhages were infrequently observed. The interrelation between salmonellae and the phagocytic cells was different in this case from that seen with shigellae.

Already at the earliest stages, salmonellae which had reached the lungs were partly engulfed by alveolar macrophages (Fig. 15-3). The rest of the

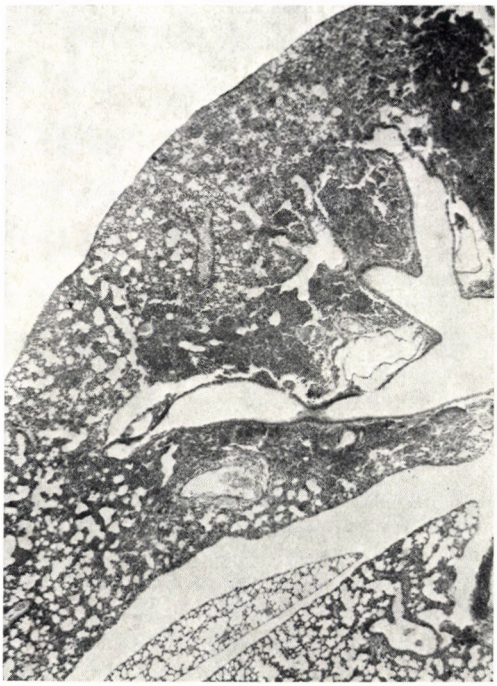


Fig. 15-2. Lung section of a mouse killed 24 h after challenge with *S. typhimurium*. Thionine, ×24

bacteria were taken up somewhat later and were almost completely destroyed by polymorphonuclears arriving from the blood vessels. Salmonellae residing in macrophages remained alive and were seen to multiply, filling up the cytoplasm (Fig. 15-4a, *b*). Soon afterwards the histological picture of the lungs showed a definite change: most of the infected macrophages were destroyed and the bacteria disseminated in the alveoli were phagocytized by polymorphonuclears. Then the salmonellae that had inhabited other macrophages continued to grow along with a gradual disappearance of organisms in the leukocytic exudate.

The multiplication of salmonellae in pulmonary macrophages of mice was more or less (though not completely) synchronous. However, the duration of the intracellular growth cycles of various salmonellae (completed by the destruction of the macrophage and the release of bacteria) was different. When the animals were infected with *S. typhimurium*, macrophages overfilled with bacteria, and even disintegrating ones, were found as soon as 9 h after the beginning of the experiment. The most intensive accumulation of *S. paratyphi-B* occurred in these cells after 12 h, whereas that of *S. heidelberg* only after 18 h. Then the period during which salmonellae remained in macrophages increased up to 1–2 days.

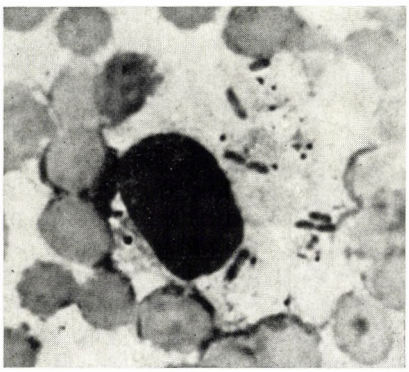


Fig. 15-3. *S. typhimurium* in pulmonary macrophage 15 min after challenge. Imprint; eosin-azure, ×1600

The morphology of parasites changed with the development of the infection. At the beginning of the experiments the organisms in imprints appeared as rather thick rods, 2–3 μ in length (see Fig. 15-3). With eosin-azure they stained violet except for some small oxyphilic areas at the ends and in the middle of the cell. In sections they stained well with thionine. Later, especially in subsequent intracellular generations, the organisms looked different. In macrophages where the number of bacteria was still rather low, they often appeared as slender, thin rods (Fig. 15-5*a*), frequently staining reddish-violet (darker at the poles) with Giemsa's stain, and often in the stage of division. Such forms of salmonellae could be revealed in lung sections only after trying different brands of thionine (combined with previous methylene blue staining as recommended by Nicolle for poorly staining Gram-

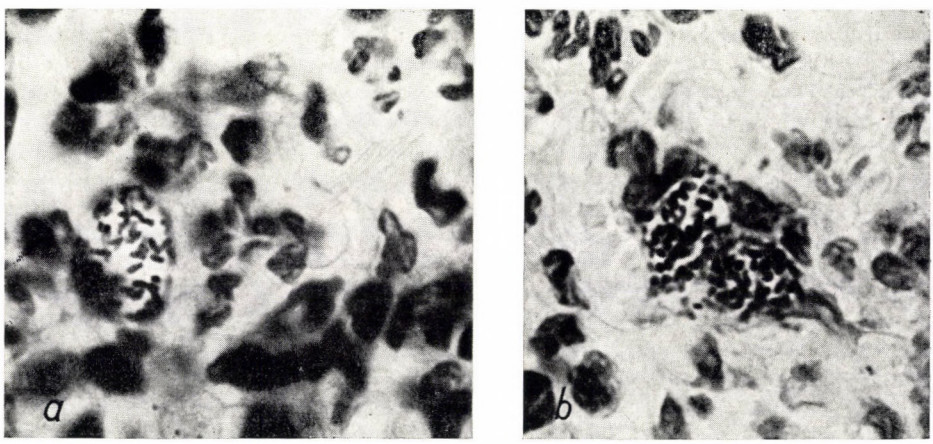


Fig. 15-4. *S. typhimurium* in pulmonary macrophages 6 h (*a*) and 9 h (*b*) after challenge. Section; thionine, ×1600

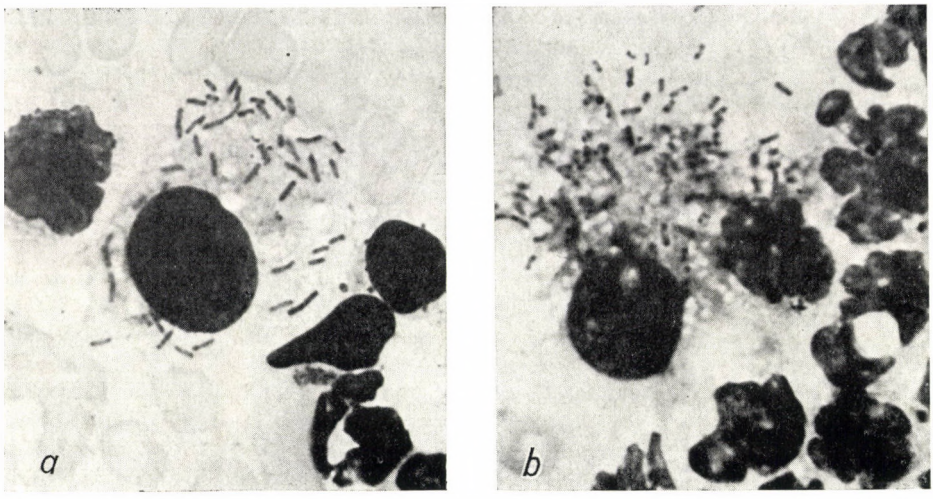


Fig. 15-5. *S. typhimurium* in pulmonary macrophages 12 h (*a*) and 48 h (*b*) after challenge. Imprints; eosin-azure, × 1600

negative organisms). When stained well, they exhibited some metachromasia. In macrophages abundantly filled with organisms rather small rods prevailed. They were almost twice as short and thin as the initial ones (Fig. 15-5*b*). These rods stained intensively with azure and thionine, but the staining was not uniform, being more definite mainly at the ends; thus, the rods resembled diplococci. They were especially small, occasionally hardly discernible in histological sections where all the cells showed a considerable decrease in size (see e.g. Figs 15-4 and 15-5 showing the same magnification).

Salmonellae revealed in the leukocyte exudate differed considerably in appearance. Among free and phagocytized bacteria there were both small and large rods; the above-mentioned thin, faintly staining organisms were almost absent. Occasionally the bacteria presented signs of lysis and disintegration. At the same time, cellular elements participating in salmonella phagocytosis themselves frequently underwent degenerative changes. First and foremost this refers to polymorphonuclears in which fragmentation or nuclear lysis as well as the appearance of vacuoles and lipid droplets in the decomposing cytoplasm were observed. Especially large and intensively staining organisms were seen in dead leukocytes (Fig. 15-6). Macrophages containing bacteria were preserved better and, evidently, for a longer time. Even when the whole cytoplasm was filled with bacteria, the cell nuclei frequently had a distinct chromatine pattern and a regular outline.

The cells belonging to the group known as macrophages are not quite uniform. In the lungs the so-called alveolar phagocytes, arranged singly on alveolar walls, belong to this group. They engulfed salmonellae immediately after challenge. During the first two days the number of these cells did not increase, or only slightly. Already during the first hours, lympho-

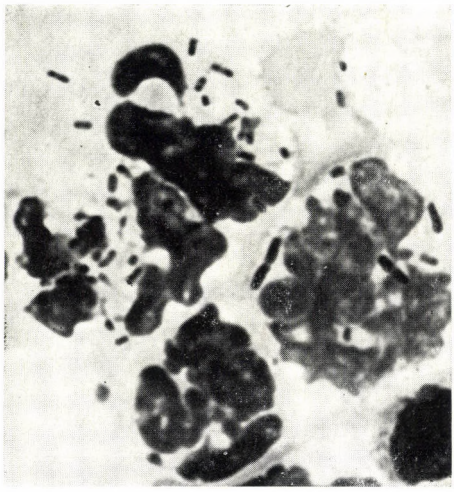


Fig. 15-6. *S. heidelberg* in leukocyte exudate 10 days after challenge (on the right large organisms in the destroyed polymorphonuclear cell). Imprint; eosin-azure, ×1700

cyte-type and polyblast-type cells appeared; their cytoplasm was markedly basophilic and a whole series of transitional forms were seen. The cells being intermediates between lymphocytes and macrophages occasionally exhibited phagocytic properties. In common lymphocytes bacteria were not observed in a single case.

Parasitism in macrophages was a common property of all salmonellae studied, but the outcome of the infection they induced was different. A protracted but uneventful course was most often seen in experiments with *S. paratyphi-B*. In mice infected with these bacteria, pneumonia (severe at the beginning) resolved slowly but almost completely. First, after 2–3 weeks, the alveoli became free from granulocytes and their breakdown products, though some small fresh focal polymorphonuclear accumulations, seemingly connected with disintegration of infected macrophages, regularly appeared at sites. From the 5th–10th day, and later (2–3 weeks) in the alveoli that had not yet expanded, proliferation of large cells with round nuclei and large light cytoplasm was noted (Fig. 15-7). At this time (2nd–3rd week) only some of these cells contained bacteria (Fig. 15-8*a*); in addition to the growth of surviving rods, there was evidence of digestion and destruction of salmonellae by macrophages (Fig. 15-8*b*). Later (after 30–60 days) bacterioscopy of sections and imprints yielded negative results.

In three other series of experiments *S. heidelberg* and *S. typhimurium* multiplied rapidly and, beginning with the second or third day, pneumonia extended to large areas of the lungs. Infection induced by a rather virulent strain of *S. typhimurium* (No. 4649) took a particularly severe course. Morphological investigations showed these agents, as well as other salmonellae to grow chiefly in macrophages at first. However, within 2–3 days they were

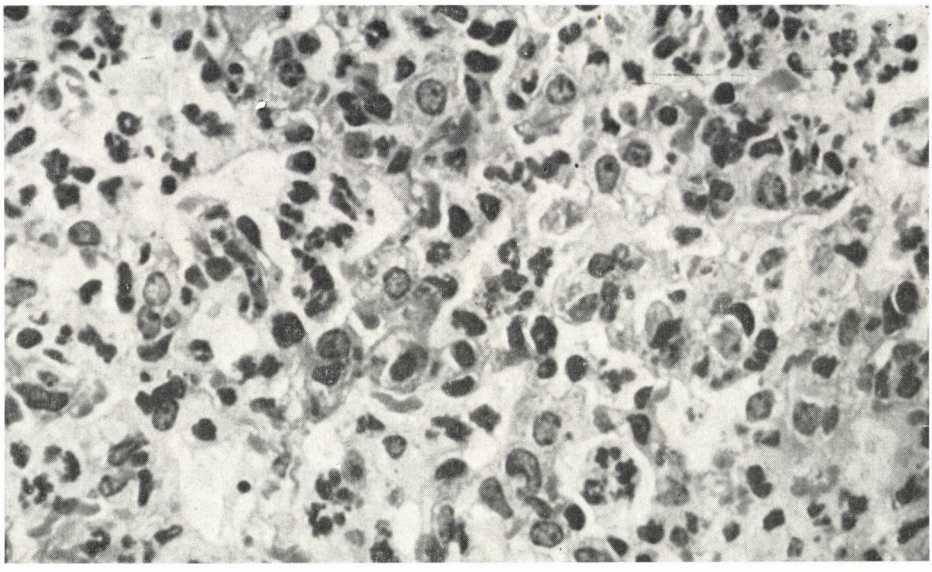


Fig. 15-7. Large cells in collapsed pulmonary alveoli 5 days after intranasal inoculation of *S. paratyphi-B.* Eosin-azure, ×830

found in great numbers among leukocytes filling the alveoli. By this time it was difficult to find out about the interrelation between bacteria and cells as most of the cellular elements in the alveoli (polymorphonuclears in particular) were destroyed or changed beyond recognition. Many bacteria also bore signs of death and digestion.

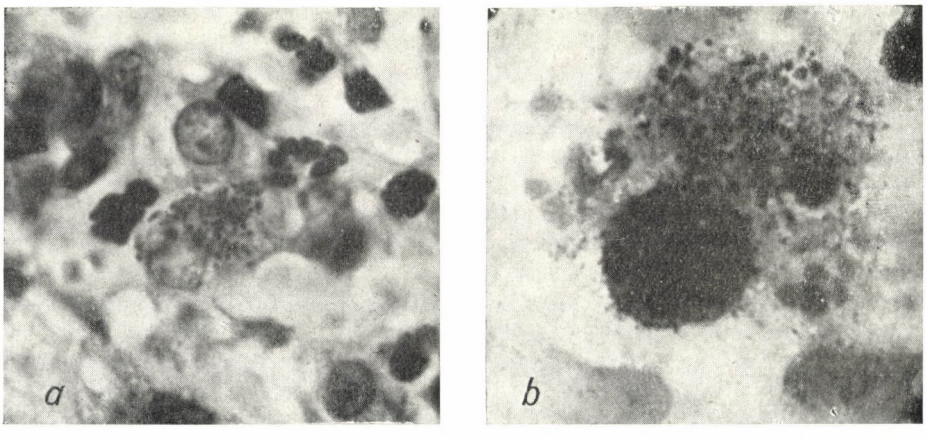


Fig. 15-8. Living (*a*) and killed (*b*) bacteria in pulmonary macrophages 8 days after challenge. *a* Section; thionine, ×1600; *b* Imprint; eosin-azure, ×1600

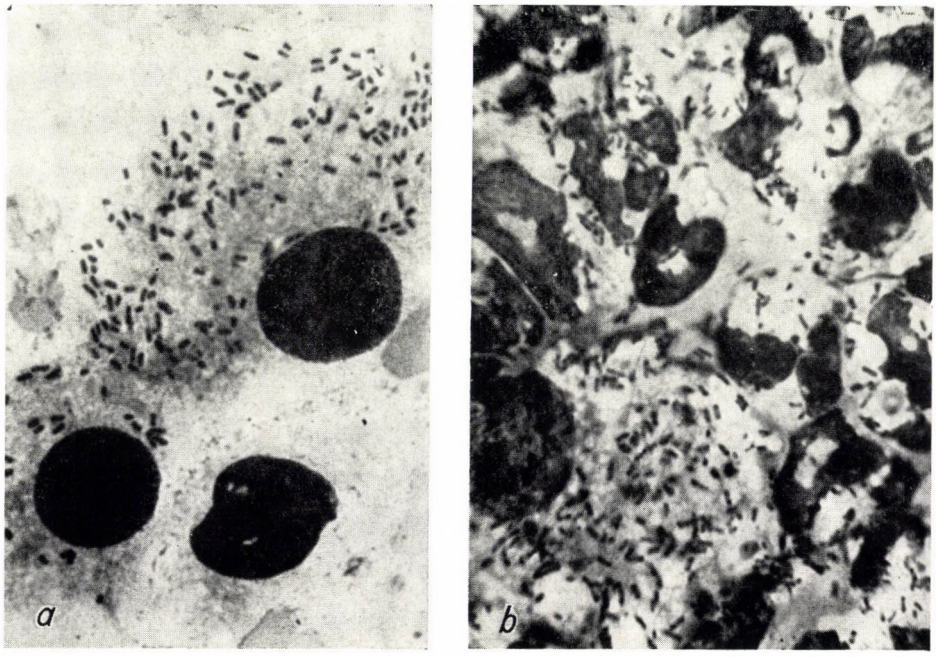


Fig. 15-9. *S. heidelberg* growing in macrophages (*a*) 5th day after challenge, and (*b*) engulfed by leukocytes during the period of macrophage desintegration (7th day). Imprints; eosin-azure, *a* ×1500; *b* ×1300

A less virulent strain of *S. typhimurium* (No. 4801) retained its cyclic development up to the completion of the experiments. Periods during which the bacteria could be detected only exceptionally inside the macrophages alternated with periods of disintegration of these cells and phagocytosis of free bacteria by polymorphonuclears. A similar cyclic development of organisms associated with selective parasitism in macrophages (Fig. 15-9*a*) was observed with *S. heidelberg*. After six days there were great numbers of salmonellae not only in the macrophages but also in leukocytes which seemingly did not fulfil their function efficiently enough (Fig. 15-9*b*).

In intranasal challenge of mice with salmonellae there was no paralysis of the leukocytic response, which frequently occurred in similar experiments with shigellae. Even in animals infected with highly virulent strains of *S. typhimurium* and which were in a very serious condition when sacrificed, pulmonary alveoli were abundantly filled with mostly disintegrating granulocytes (Fig. 15-10). Liquid exudate was scanty, but at some sites in the alveoli thick protein masses with fibrin threads could be found. Rarely, small parietal thrombi were seen. On prolonged observation, large areas of necrosis with massive thrombi inside the blood vessels were seen in pneumonic foci in some animals (Fig. 15-11*a*, *b*). Bacteria remaining at the pe-

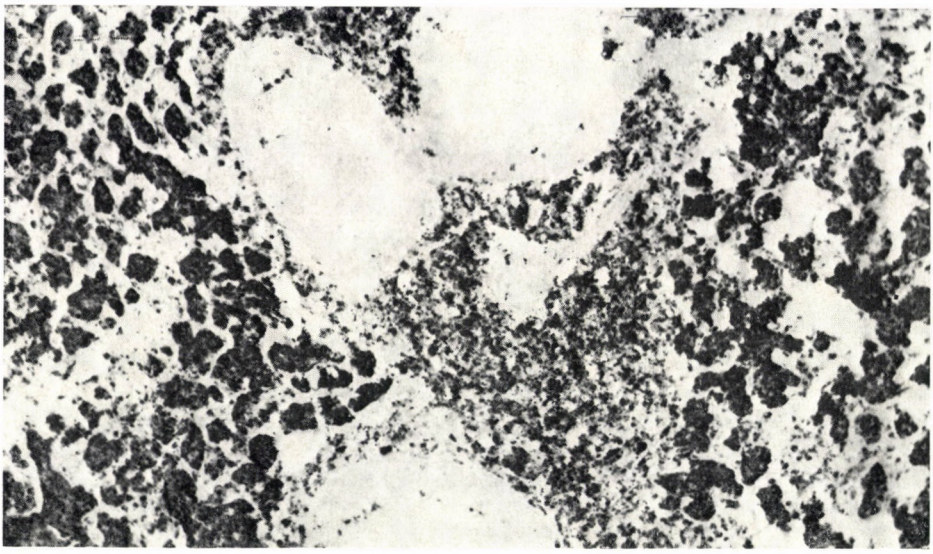


Fig. 15-10. Granulocytes in the alveoli and around the branch of pulmonary artery (in the centre); 5 days after challenge with a highly virulent strain, *S. typhimurium* No. 4649. Goldmann's Sudan alpha-naphthol, × 150

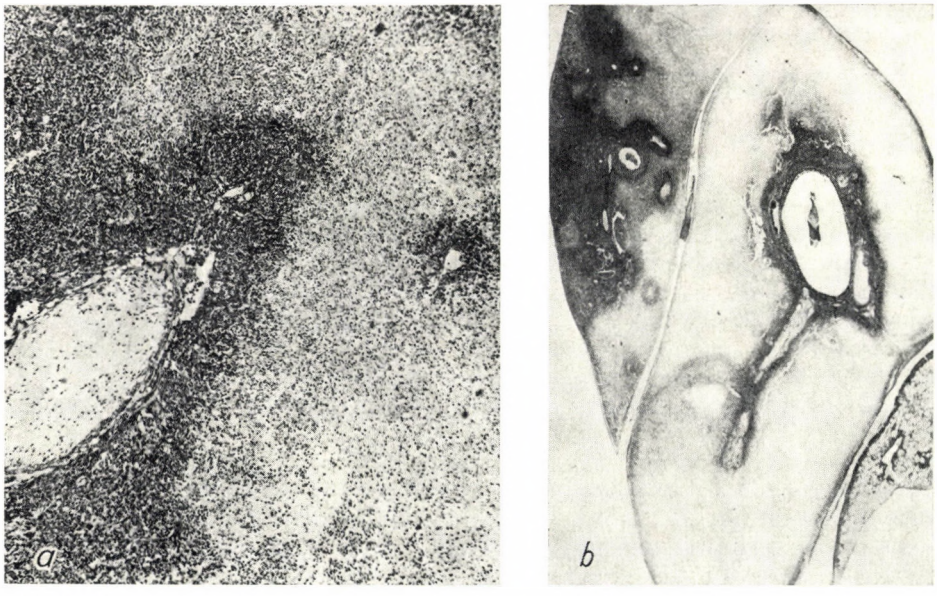


Fig. 15-11. Necrotic focus with thrombus in the blood vessel (*a*, to the left) and almost total necrosis (*b*, light areas) of the lung filled with exudate; 10th and 15th days after challenge with *S. typhimurium* No. 4801. Thionine, *a* × 70; *b* × 12

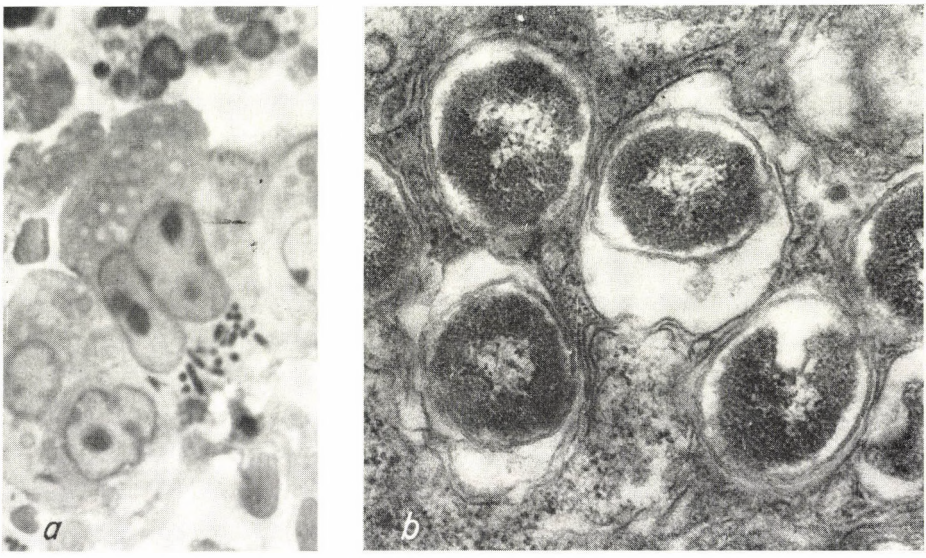


Fig. 15-12. *S. typhimurium* (strain No. 4649) in the basal part of epithelial cells of mouse bronchi. *a* Toluidine blue, ×1700; *b* electron micrograph of the same; ×58 000 (Ariel and Bernovskaya, 1970)

riphery of necrotic zones in destroyed macrophages probably survived, as they became larger and stained more intensively.

Ariel and Bernovskaya (1970) occasionally found bacteria in certain epithelial cells of mice killed 1–2 days after challenge with *S. typhimurium*. The bacteria were lodged in the basal portions of the cells (Fig. 15-12*a*), and, as shown by electron microscopy, every salmonella was surrounded by a membrane (Fig. 15-12*b*). Between the membrane and the outer surface of the rods there were crevices (some of them probably artefacts) without any contents capable of absorbing the electron stream. At the same time the bacteria engulfed by polymorphonuclears were enclosed in typical phagosomes, displaying signs of decay. Similar phagosomes were also encountered in certain macrophages, whereas in others the salmonellae formed bulky accumulations in the cytoplasmic matrix.

Thus, intranasal challenge of mice with salmonellae is suitable for the bacteriological and morphological study of *in vivo* activity of these microorganisms. The main pathogenic properties of salmonellae could thus be demonstrated, e.g. parasitism in macrophage-type cells, and the ability to pass through the epithelium without damaging it appreciably. Various degrees of resistance of certain salmonella strains to polymorphonuclears were also revealed; the virulence of the organisms apparently depended upon this factor to a considerable extent. In addition, the changes that salmonellae themselves underwent in the living body were also seen. Differences in the appearance of bacteria in the lungs of infected animals

suggested the presence of some secondary infection which, however, had not been confirmed by bacteriological examination. As shown also in Chapters 13, 16 and 17, the morphology of salmonellae is highly variable depending on the period of experiments.

The ability of salmonellae to parasitize macrophages and to multiply inside these cells is beyond doubt.* However, one should keep in mind that cells possessing phagocytic properties are capable of engulfing a high number of live or dead bacteria. Therefore, when millions and even milliards of certain microorganisms are introduced into the lungs pulmonary macrophages become frequently overfilled with them even at early periods of observation. With dosages used in our experiments (some millions of salmonellae), a rather small number of bacteria settled in single pulmonary alveoli. Part of them (usually not more than two-fifths) was taken up by macrophages and the rest was destroyed by polymorphonuclears during the first hours. Consequently, the increase in the number of salmonellae in macrophages, occurring when free salmonellae have already disappeared from the alveoli (see Fig. 15-4), can only be explained by the fact that bacteria engulfed earlier multiply in the cell. The intracellular growth is also confirmed by morphological changes: homogeneity of the bacteria and the presence of dividing forms.

In conclusion, it must be noted that although salmonellae pathogenic to mice are able to induce very severe damage in lungs, death is not invariably and exclusively due to the development of pneumonia. In contrast to pneumonia induced by shigellae, experimental salmonella pneumonia is accompanied by a generalized infection from the very onset. Along with bacteraemia, in some organs (mostly in the liver and the spleen) there appeared small foci of specific lesions (granulomas). Quite unexpectedly, however, there were no considerable changes in the regional lymph nodes of the lungs, and organisms could be detected extremely rarely in them. In mice that died on the 7th day after infection with a highly virulent strain of *S. typhimurium*, tracheo-bronchial nodes were almost devoid of lymphocytes, while hyperplastic reticular cells contained many well-staining organisms. In such experiments an abundant accumulation of the rapidly disintegrating granulocytes was observed about the fine branches of the pulmonary artery where lymphatic vessels are known to pass (see Fig. 15-10). If the course of infection was milder, lymphoid and plasma cells accumulated sheath-like in this area, from the 2nd–3rd day on. This was obviously connected with local antibody production.

Thus, after intranasal administration of a moderate amount (some millions) of salmonellae to white mice, a specific infectious process developed in the animals' lungs. In contrast to similar experiments with shigellae, these organisms colonized macrophage-type cells. During their growth cycle salmonellae underwent essential changes, becoming smaller than their extracellular

* Similar results were obtained in experiments with guinea pigs (Baskerville et al., 1972, 1973). These animals, in contrast to mice, survived even after the intranasal inoculation of an enormous number (4×10^9) of *S. cholerae-suis*.

forms. Organisms outside the cells are ingested by polymorphonuclears, more or less effectively depending on the virulence of the strain. Leukocyte response is usually strong even in dying animals though in such cases polymorphonuclears cope with the organisms less successfully.

The lung model supplemented with quantitative bacterial analysis permits fairly precise determination of the virulence of salmonellae as well as of the resistance of the host. Morphological studies followed by bacterioscopy yield important data for the understanding of the pathogenesis of salmonella infection.

CHAPTER 16

CONJUNCTIVAL AND INTRAVESICAL CHALLENGE OF GUINEA PIGS WITH SALMONELLAE

by

M. V. VOINO-YASENETSKY and T. BAKÁCS

CONJUNCTIVAL TESTS

Having observed corneal ulceration and hypopyon induced by *S. paratyphi-A* in a patient, Sedan and Herrman (1924) attempted to reproduce such damages in guinea pigs. Diffuse inflammatory corneal infiltration and slight keratitis were noted after local inoculation of *S. typhi* to the eye surface treated previously with sterile bovine bile. Similar results were obtained by Zoeller and Bastouil (1924) who rubbed *S. typhi*, *S. paratyphi-A* and *S. paratyphi-B* cultures onto the conjunctiva of guinea pigs. These cultures failed to cause any apparent changes without pretreatment with bile.

In later studies, the guinea pigs were, as a rule, not pretreated. Serény (1957, 1960*a*, *b*) and a number of other authors using his technique (Siroko, 1957; Moore, 1957; Yakhnina and Kuznetsova, 1959; Katsnelson, 1961) reported that different salmonellae were capable of evoking conjunctivitis in guinea pigs but, in contrast to shigellae, did not attack the cornea. Bayramova (1963), Stenzel (1962*b*, *c*), Istrati et al. (1963*b*) found several strains of *S. typhimurium* and *S. enteritidis* that induced not only conjunctivitis but also keratitis after having been dripped into the conjunctival sac. According to Bayramova, anaerogenic *S. typhimurium* strains behave in this manner. The ability of some salmonellae to damage the cornea is gradually lost under laboratory conditions, though the organisms continue to induce conjunctivitis.

It has been shown (Bayramova and Gleiberman, 1963; Gleiberman et al., 1964) that conjunctivitis with serous and sero-purulent exudation arises after introducing $2–4 \times 10^9$ cells of aerogenic *S. typhimurium*. In smears single reticular elements containing Gram-negative organisms in the cytoplasm were seen together with numerous polymorphonuclear leukocytes. In their histological investigations these authors used no stains for revealing organisms in tissues. They reported that during the first days after challenge conjunctivitis was characterized by moderate congestion and oedema as well as by a considerable productive response displayed as large cell infiltration consisting of histiocytic, epithelioid and reticular elements and a small number of polymorphonuclear leukocytes. Large lymphoid follicles were found as soon as on the fourth or fifth day (Gleiberman et al., 1964). After 3–4 weeks the inflammatory infiltration of the conjunctiva and eye-lids disappeared (though the follicles remained). The process did not involve the cornea and the tissues surrounding the eye, but there was a general infection with specific changes in the viscera, and some of the animals died.

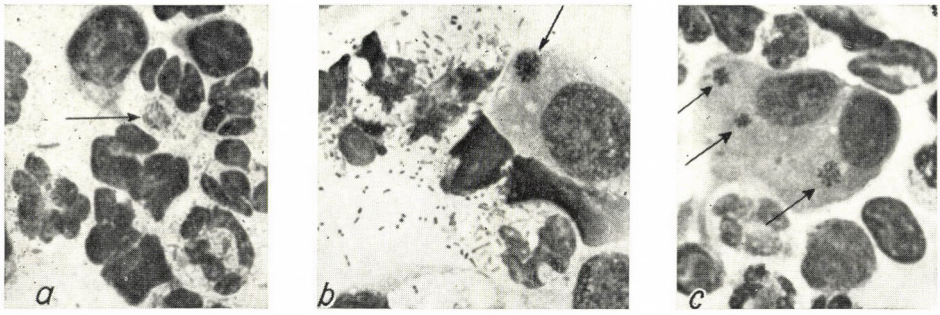


Fig. 16-1. Salmonellae in conjunctival epithelial cells and in polymorphonuclear leukocytes (*a*), 9 h (*b*) 24 h, (*c*) after challenge. Arrows show the accumulation of small organisms in the cytoplasm. Exudate smears. May-Grünwald-Giemsa stain, ×1200 (Tenner et al., 1971*a*)

When challenging with an anaerogenic variant of *S. typhimurium*, the inflammatory response in the conjunctiva was more pronounced. Infiltrating cells, including many polymorphonuclears, disintegrated at certain sites. The conjunctival epithelium became ulcerated. On the second week the cornea also ulcerated and sometimes even perforated. Oedema and inflammatory infiltration with necrosis spread to the ocular muscles and orbital connective tissue, and most of the animals died.

Severe keratoconjunctivitis was induced in about half of the guinea pigs infected with Gärtner's bacilli *(S. enteritidis)*; the rest of the animals developed only conjunctivitis.

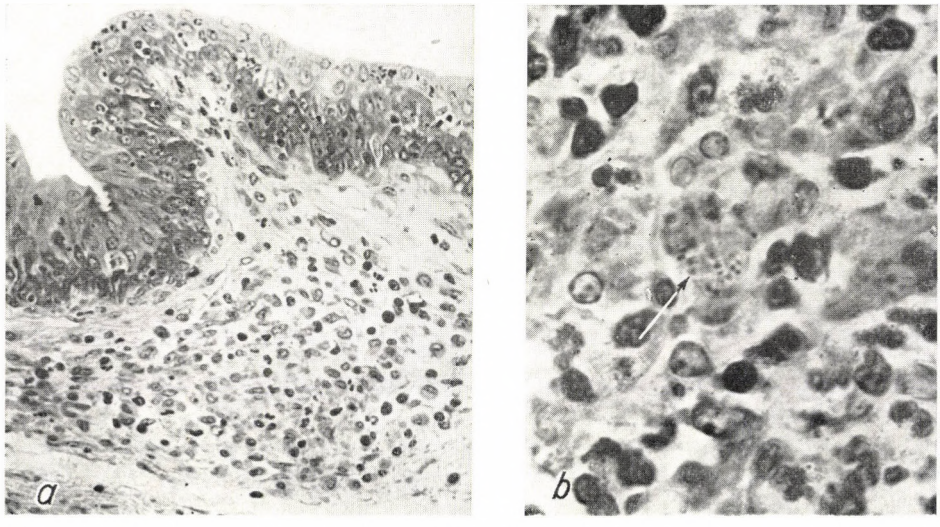


Fig. 16-2. Focal accumulation of large light cells in the conjunctiva 6 days after challenge (*a*, ×240) and salmonellae in the cytoplasm of such cells (*b*, ×1060) after 9 days. Eosin-azure

In the studies of Tenner et al. (1971*a*) all the *S. typhimurium* and *S. enteritidis* strains tested produced only conjunctivitis. They paid special attention to the interaction between the organisms and the epithelial cells. Similarly to shigellae (see Chapter 7), the earliest alterations caused by salmonellae appeared in the region of the conjunctival fornix. Here, one hour after challenge, single epithelial cells containing 1–2 short rods in their cytoplasm could be observed. Under the epithelium small numbers of leukocytes were frequently seen. The inflammatory infiltration increased 6–12 hours later, often involving the whole epithelial lining, or else, appearing as separate microabscesses. Meanwhile, the number of infected epithelial cells also markedly increased. The study of histological sections and smears of the exudate obtained from the conjunctival sac showed that salmonellae in epithelial cells were usually small in size and accumulated in small isolated groups (Fig. 16-1*b*, *c*). In several polymorphonuclears the agent preserved its common form and was apparently vacuole-bound (Fig. 16-1*a*). Other leukocytes were diffusely filled with organisms and destroyed (Fig. 16-1*b*). Rather early (12 h after challenge) macrophage-type cells, the number of which had increased especially by the end of the first day, appeared among the leukocytes infiltrating the conjunctival epithelial lining.

Salmonellae did not reside in the conjunctival epithelium long: 48–72 h after the beginning of the experiment the number of infected cells markedly increased, but by that time a considerable number of organisms appeared under the epithelial lining, mostly in macrophages of lymphatic follicles. Part of the infected macrophages disintegrated, releasing clusters of free organisms; the place of these macrophages was occupied by polymorphonuclears that have accumulated here.

In some animals, a number of large cells with massive, light cytoplasm and rounded or slightly oval nuclei with loosely arranged chromatin, appeared in lymphoid follicles and in other areas of the conjunctival substantia propria after 8–10 days. A picture characteristic of salmonella granulomas was seen in these areas.

In a series of experiments (Voino-Yasenetsky et al., 1977) we studied the results of challenging guinea pigs with various *Salmonella typhimurium* strains using Serény's technique. All induced conjunctivitis of varying severity corresponding to the above descriptions. A peculiar feature in these experiments was the formation of granulomas consisting of large light cells resembling "typhoid" cells in the conjunctival subepithelial tissue (Fig. 16-2*a*). In some of these cells small organisms could be revealed (Fig. 16-2*b*). However, new formation of lymphoid follicles mentioned by Gleiberman et al. (1964), has not been confirmed. Such cells are also found under the conjunctiva (at the fornix) in normal guinea pigs, and at the climax of salmonella conjunctivitis they may be partly substituted by proliferation of macrophage-type cells.

Among the *S. typhimurium* strains studied there was one which could induce keratitis in guinea pigs. This (gas-producing) strain has been isolated from a patient with dysentery-like salmonellosis (Ismailov, 1974). Unfortunately, it lost some of its virulence in the course of time and corneal lesions could only be observed in some of the challenged animals. These lesions were

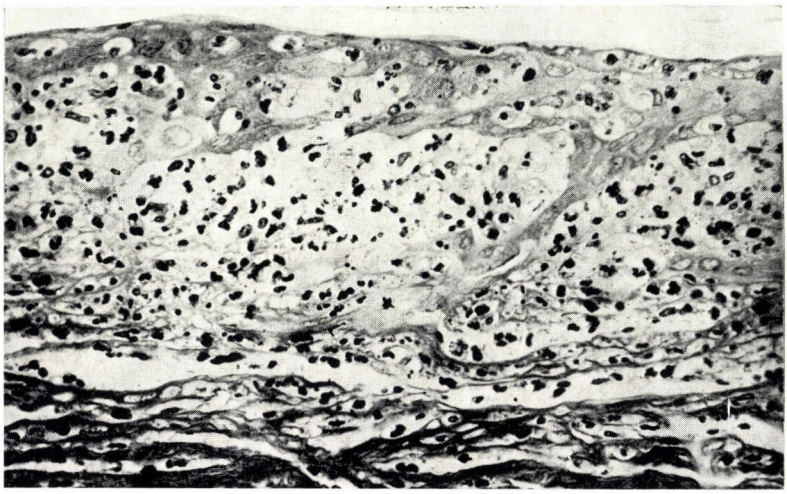


Fig. 16-3. Inflammatory process in the cornea 13 days after challenge. Eosin-azure, ×360

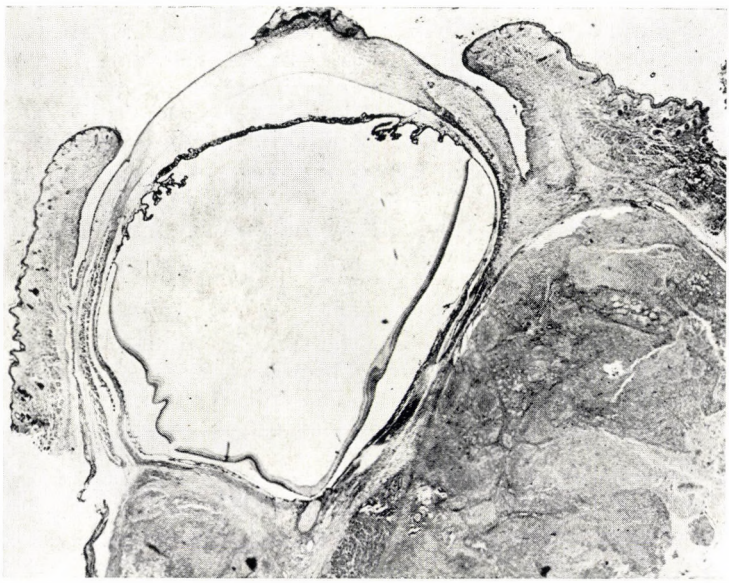


Fig. 16-4. Marked enlargement of inflamed lacrimal glands causing extrusion of the eye-ball and leaving the eye unshielded by eyelids. The lesion is covered by a slough in the centre of the cornea. 13 days after challenge. Eosin-azure, ×8

essentially different from those caused by shigellae in similar experiments. They developed much later (not earlier than 5 days after challenge), starting usually not from the periphery but in the central parts of the cornea. Here, along with the small superficial ulcerations and focal inflammatory infiltration, a peculiar inflammatory epithelial cell proliferation appeared (Fig. 16-3). The involvement of the cornea was evidently due to exophthalmus that developed in many animals as a result of severe inflammation and a marked enlargement of the lacrimal glands in the posterio-lateral part of the orbit (Fig. 16-4). Nevertheless, eye protrusion had not invariably preceded keratitis. At any rate, the possibility of infection spreading to subepithelial tissues and to deeper lying parts of the orbit show the strong invasive power of salmonellae; such an invasiveness has not been observed in the same experiments with shigellae (see Chapter 7).

INTRAVESICAL CHALLENGE WITH SALMONELLAE

Dealing with shigella cystitis in Chapter 8 we referred to Bingel's (1943*a*) result of salmonella infection in the guinea pig's bladder. According to his observations (made on single animals), *S. typhi* and *S. paratyphi-B* induce an insignificant, only microscopically appreciable inflammatory response, i.e. leukocyte accumulation under the preserved bladder epithelium.* Intravesical introduction of *S. typhimurium* and especially *S. enteritidis* caused more severe local alterations (phlegmonous inflammation spreading over the bladder musculature and even over the pelvic connective tissue) followed by bacteraemia and the death of the animal. Braun et al. (1953) also mentioned the possibility of experimental reproduction of salmonella cystitis. Studying different *Enterobacteriaceae* Stenzel (1960) established that in such experiments *S. enteritidis* and *S. java* were the most pathogenic and *S. typhimurium* and *S. newington* were next in order; freshly isolated salmonellae were much more virulent than laboratory ones.

Tenner et al. (1971*c*), used a *S. typhimurium* strain freshly isolated from a patient suffering from enterocolitis. The technique employed was the same as in shigella challenge: 1 ml of broth culture containing 10^9 microbial cells was introduced into the bladder; 56 guinea pigs weighing 200–250 g and 20 animals weighing 700–800 g were challenged. The latter were used in view of the fact that among young guinea pigs a considerably high death rate was observed as soon as 24 h after challenge. But usually, as noted also by Stenzel (1960), the severity of the disease varied even in animals of the same age group.

In young guinea pigs swelling of the perineum was noted 12 h after challenge; from 24 h incontinence and ruffling of the fur all over the body were observed. At necropsy of sacrificed animals oedema of the bladder wall, later extending to the connective tissue of the small pelvis, could be seen after 6 h. Punctate haemorrhages appeared on the congested bladder mucosa

* As shown in Chapter 8, the same response is induced when sterile broth is introduced into the bladder.

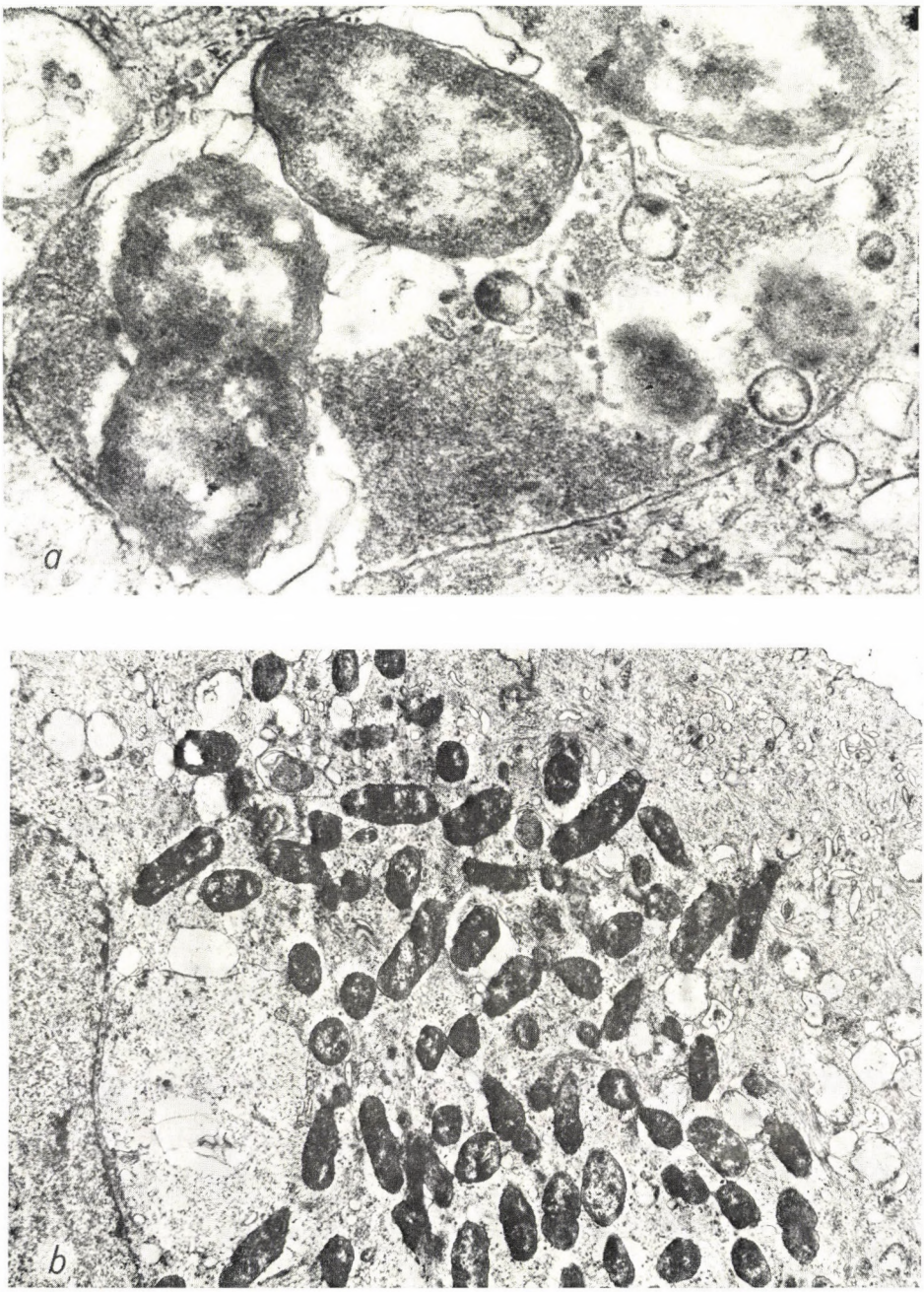


Fig. 16-5. Salmonellae in the epithelial cells of the guinea pig bladder. *a* Vacuole-bound, ×46,000; *b* multiplying freely in the cytoplasm, ×8300

(occasionally also on the serosa) after 1–6 days, but there were no ulcers. After 24 h enlargement of the spleen became evident [this finding, as emphasized by Bingel (1943*a*) and Stenzel (1960), is never found in experimental shigella cystitis]. Bacteriological studies of the viscera (kidneys, liver and spleen) revealed generalized infection.

Histological investigations showed that one hour after the beginning of the experiment salmonellae penetrated the epithelial cells of the bladder mucosa. In the next hours the number both of the infected cells and of the organisms they contained, increased.

According to unpublished electron-microscopic studies of Tenner, part of the intraepithelial salmonellae in the bladder are enclosed in vacuoles, whereas the cells containing them show no apparent sign of degeneration. The cytoplasm of other cells (many obviously destroyed) is filled with diffusely scattered organisms. In the same epithelial cell, free organisms as well as vacuole-enclosed ones may be present and occasionally larger rods occur together with very small ones.

The structure of vacuoles bordered by simple (ordinary) membranes is shown in Fig. 16-5*a*. In addition to bacteria (well-preserved or seemingly destroyed), these vacuoles also contain particles of cytoplasmic organelles and an amorphous substance of moderate electron density. They closely resemble the phagosomes in polymorphonuclears and macrophages engulfing bacteria or other small particles. In the most severely damaged epithelial cells very active salmonella multiplication is evident (Fig. 16-5*b*).

The fate of epithelial cells infected by salmonellae varies. Some superficial cells (Fig. 16-6*a*) are sloughed into the bladder lumen. Others, residing somewhat deeper, begin to disintegrate 9 h after challenge. Then polymorphonuclears, ingesting the released bacteria, occupy their place (Fig. 16-6*b*).

Unlike in experimental shigella cystitis, the spread of infection to the epithelial lining of the bladder mucosa was not observed. In the course of the disease some other cells were damaged; these, however, had obviously been infected from the lumen, where salmonellae were always present. Neither signs of salmonella transfer from one infected epithelial cell to the other, nor erosions and ulcers were observed. Several microabscesses, vacuoles with liquid content, and traces of debris sometimes remaining in their place were seen as signs of inflammatory infiltration.

The number of infected epithelial cells increased during the first day of the experiment and decreased gradually later. At the same time, as it was the case in conjunctival challenge, salmonellae extended into the deep layers of the bladder wall. By the end of the first day separate histiocyte-type cells with minute organisms in their cytoplasm were seen. Subsequently, true granulomas formed out of large cells with light cytoplasm in the bladder wall (Fig. 16-7*a*, *b*). Some of them contained organisms.

Several animals developed more serious phlegmonous inflammation in the deep layers of the bladder wall, with a large number of rod-like organisms in the exudate. In one of these cases mechanical injury of the mucosa was seen, which had undoubtedly been caused by the glass catheter during the challenge. Obviously, part of the bacteria introduced through the catheter directly entered the deep tissues of the bladder wall.

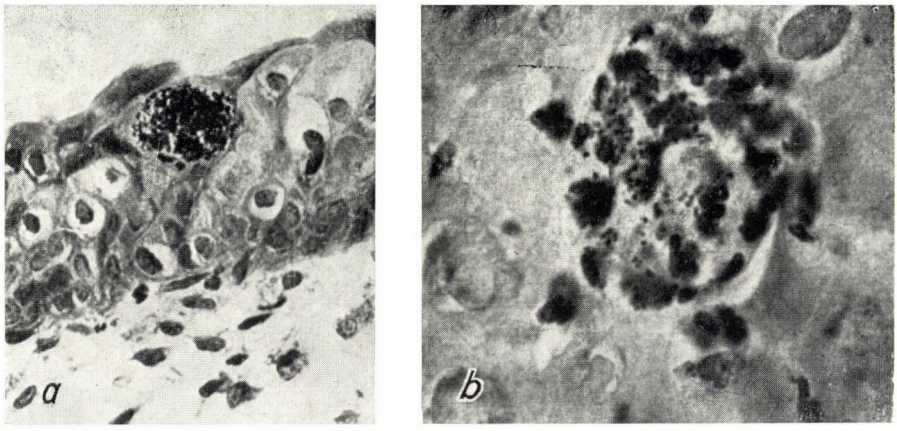


Fig. 16-6. *a* Salmonella multiplication in the bladder epithelial cell. *b* Accumulation of polymorphonuclears after the destruction of the damaged cell 9 h after challenge. Thionine, *a* ×480; *b* ×1200

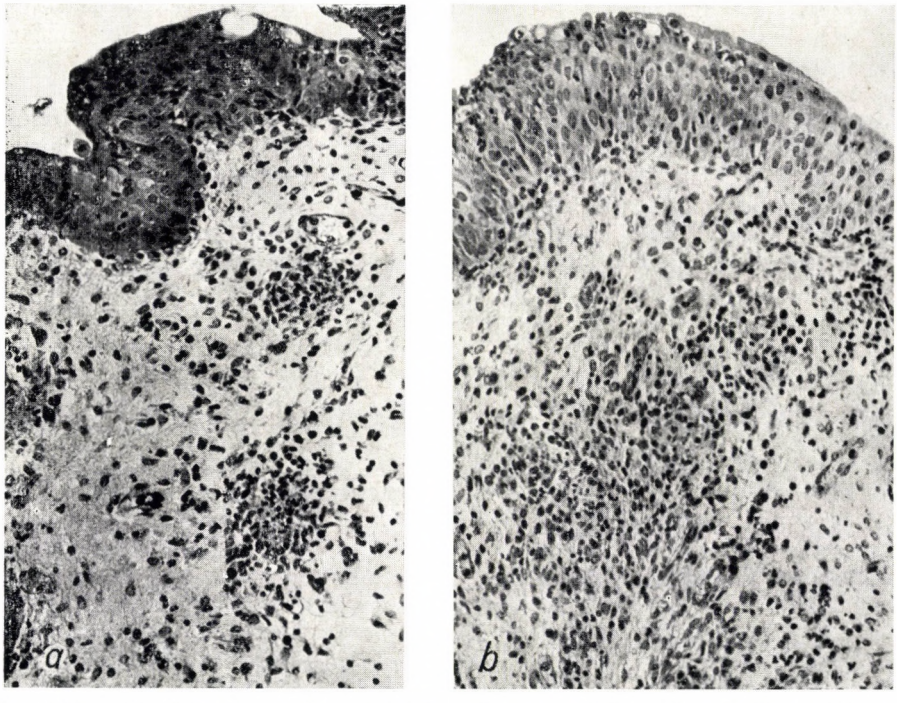


Fig. 16-7*a* and *b*. Granulomas in the bladder submucosa 4 days after challenge. Haematoxylin-eosin, ×450

SIGNIFICANCE OF CONJUNCTIVAL AND INTRAVESICAL SALMONELLA INFECTION

Serény's keratoconjunctival bioassay, if applied to salmonellae, is mainly of theoretical interest. The same may be said of intravesical challenge.

Salmonella parasitism in macrophages, demonstrated on the lung model, was also revealed in conjunctival and intravesical challenge of guinea pigs. As also observable in other organs, part of the salmonellae engulfed by macrophages seemed to be destroyed whereas others, not enclosed in phagosomes multiplied. Bacteria growing in the cytoplasm of living cells showed a considerable decrease in size.

The initial stage of salmonella infection in the bladder and the conjunctiva differs from that in the intestine. At both sites, in order to enter the macrophages in deeper layers, the organisms must break through the intact epithelial barrier. While passing through the intestinal monolayer columnar epithelium, salmonellae are surrounded by membranes or osmiophilic substances (Takeuchi and Sprinz, 1967); and they neither multiply nor induce cellular lesions during their passage. The mechanism of the passage of salmonellae through the stratified conjunctival and transitional vesicular epithelium is less clear.

In the epithelial cells of the conjunctiva the organisms of common *S. typhimurium* strains also seem to be surrounded by membranes isolating them from the cytoplasm. This can be judged from the clear-cut border and the round form of aggregations of small, obviously multiplying organisms. The possibility of salmonella growth in such vacuoles has been confirmed by electron-microscopic investigations of the bladder showing that the bacteria in fact grow in abundance directly in the cytoplasm. Salmonellae, however, have not shown the ability to pass from one epithelial cell into another, as seen with shigellae. In spite of that (and again in contrast to shigellae) salmonellae were found to penetrate subepithelial tissues of the conjunctiva and of the bladder, and not infrequently to cause fatal, generalized infection.

In summary it should be stressed that salmonellae induce experimental conjunctivitis and occasionally also keratitis in guinea pigs. These organisms penetrate the conjunctival epithelial cells and may multiply there, however, without passing from one cell into another. Therefore, in contrast to shigellae, salmonellae do not usually cause a severe lesion of the epithelial lining but penetrate deeper, lodging in the macrophages of the connective tissue and conjunctival lymphoid follicles where characteristic granulomas develop. Orbital lacrimal glands may also be affected, leading to inflammation and exophthalmus.

Experimental salmonella infection has a similar course in the guinea pig bladder. Epithelial lesions are more marked here but are confined to certain cells. In the same way as in the conjunctiva, salmonellae penetrate deeper tissues of the bladder wall where typical granulomas are formed.

CHAPTER 17

ENTERAL CHALLENGE OF LABORATORY ANIMALS WITH SALMONELLAE

by

V. L. BELYANIN and M. V. VOINO-YASENETSKY

Since challenge of laboratory animals with *S. typhi* is unsuccessful (see Chapter 13), other members of the *Salmonella* genus are used for modelling typhoid fever. The reason for this substitution is, principally, the similarity of appearance between morphologic alterations observed in human typhoid fever and the disease developing in white mice, guinea pigs and rabbits after enteral challenge with *S. typhimurium* or certain other kinds of salmonellae. The bacteriology and pathological anatomy of these experimental infections have been studied in detail, but information on the interrelation between the host and the causative agent has begun to accumulate only recently.

ENTERAL CHALLENGE OF WHITE MICE

To produce characteristic intestinal lesions solid food infected with salmonellae was given to mice, since oral administration of salmonellae in liquids gave quite different results (see Chapter 13). Dry bread usually soaked in water or milk containing 10^7 to 3×10^9 salmonellae* was given to mice caged individually and fasted for about 12 h.

Challenge with a highly virulent culture of *S. typhimurium* caused a severe, gradually developing infection in all animals. Apparent signs of the disease could be noticed only 3–4 days after challenge. The activity and appetite of the animals diminished and ruffling of the fur could be observed; diarrhoea was generally absent (Valdman, 1955). The first deaths occurred within 5–6 days, and by the middle of the second week almost all the animals died.

Bacteriological investigations carried out by Ørskov et al. (1928), Sollazzo (1929), Valdman and Rostova (1936), Arbuzova (1960) and many other researchers have shown that bacteria reaching the alimentary tract are rapidly cleared from the bowel. By the end of the first day a few salmonellae may occasionally be cultured from the intestinal contents, Peyer's patches and mesenteric lymph nodes. Then the amount of organisms in these lymphoid structures rapidly increases. After 2–3 days salmonellae are present

* The number of salmonellae ingested in this case is of little importance (Chapter 13).

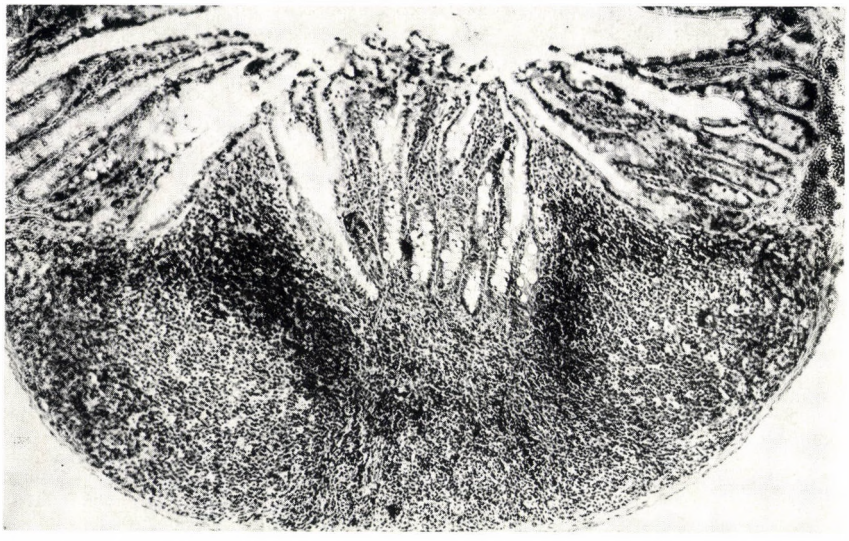


Fig. 17-1. Peyer's patch of a healthy mouse. Haematoxylin-eosin, ×100 (courtesy of A. A. Valdman)

in increasing numbers in the liver and spleen; after 3–4 days they appear in the circulation. At about the same time morphological changes in the above organs, but mainly in the intestinal lymph nodes, become conspicuous.

The intestinal lymphoid apparatus of the healthy mouse is usually represented by 8–9 Peyer's patches situated in the jejunum and ileum. They extend over the entire thickness of the mucosa (Fig. 17-1) and consist of 4–6 lymphatic follicles indistinctly bordered by thin layers of connective tissue with vessels. The top of the follicle faces the intestinal lumen and is dome-shaped. The outlines of rounded areas, secondary nodules with light reactive centres in the middle, are indistinctly seen in the follicles. The domes are covered with a layer of prismatic epithelial cells. Mesenteric lymph nodes appear as a strand consisting of three nodules situated in the mesenteric ileocaecal corner.

Three to four days after enteral challenge with salmonellae, swelling of the intestinal Peyer's patches and mesenteric lymph nodes is noted. The spleen and the liver are enlarged; on the surface of the latter small yellowish spots are detectable at autopsy.

In agreement with Valdman's description (1955), our investigations showed that microscopic intestinal alterations are detectable as soon as after 2–3 days (Belyanin, 1968, 1969). At this time focal aggregations of granulocytes appear in the Peyer's patches of the distal ileum (Fig. 17-2). The epithelium over the follicles at such sites is mostly undamaged, but there are rather numerous polymorphonuclears among its cells, sometimes accumulating in the form of microabscesses. Slight leukocytic infiltration of the lamina

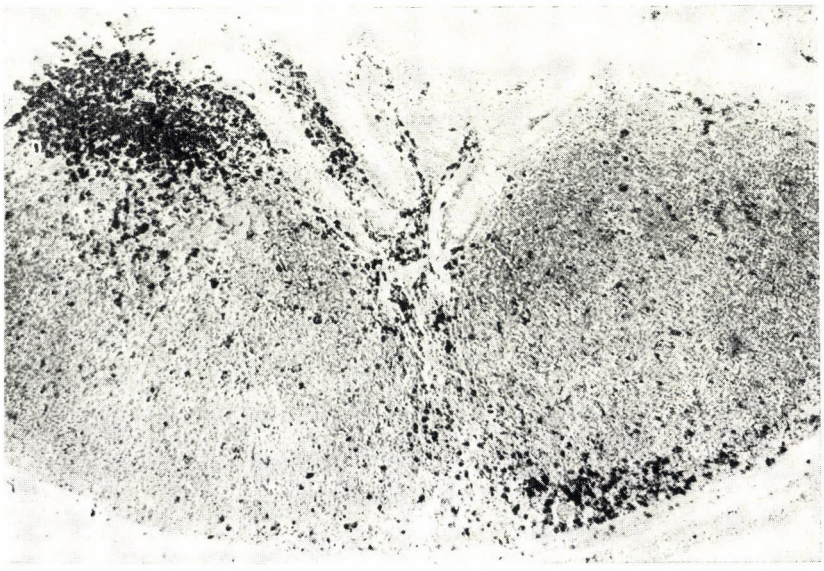


Fig. 17-2. Aggregation of leukocytes in lymphatic follicles of a Peyer's patch. Sudan alpha-naphthol, ×85 (courtesy of A. A. Valdman)

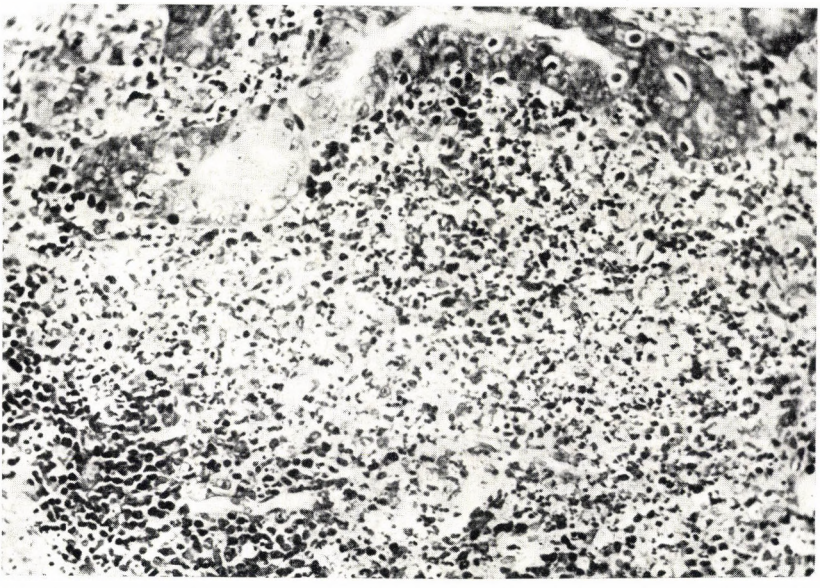


Fig. 17-3. Leukocyte disintegration and beginning of reticular cell growth in a Peyer's patch on the 5th day after challenge. Methylene blue-thionine, ×265

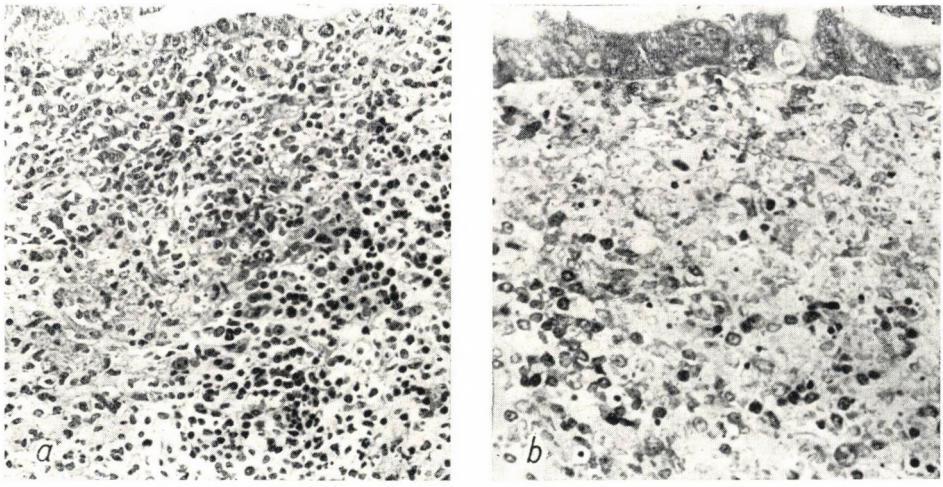


Fig. 17-4. *a* Aggregation of epithelioid cells in a Peyer's patch on the 6th day after challenge. Haematoxylin-eosin, ×225. *b* Their partial disintegration on the 7th day. Methylene blue-thionine, ×390

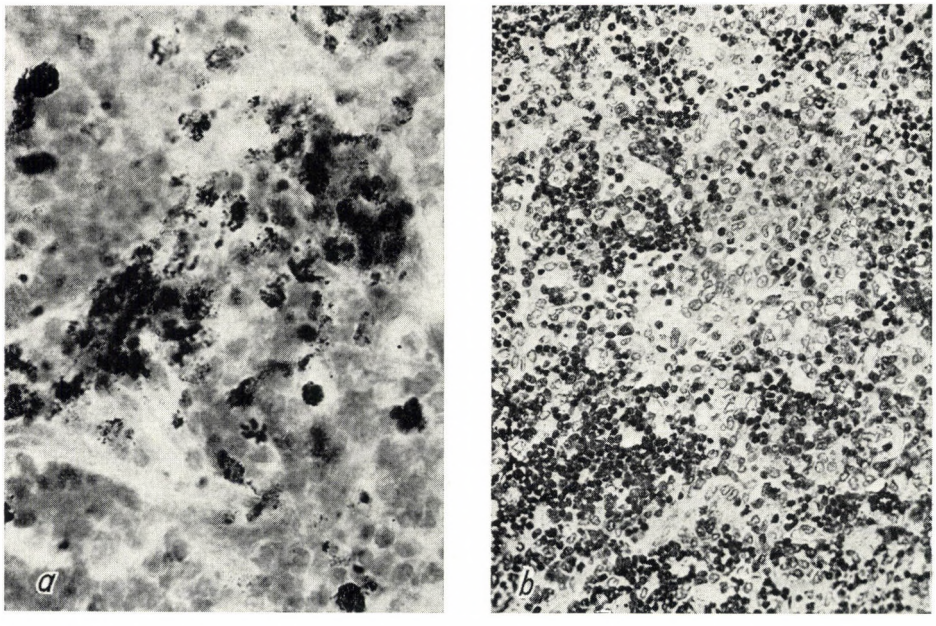


Fig. 17-5. *a* Accumulation and disintegration of granulocytes, on the 3rd day after challenge; Goldmann's Sudan alpha-naphthol, ×240. *b* Growth of light cells resembling typhoid cells in mesenteric lymph node; sixth day, methylene blue-thionine, ×140

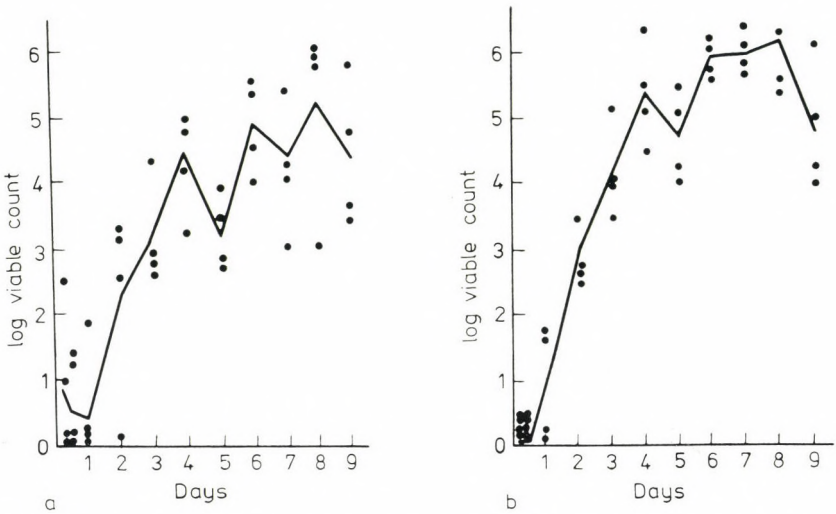


Fig. 17-6. Results of bacterial counts from Peyer's patches (*a*) and mesenteric lymph nodes (*b*) of mice after oral challenge. Dots mark microbial contents in individual mice. Curves show mean logarithm values characteristic of each time interval of the experiment

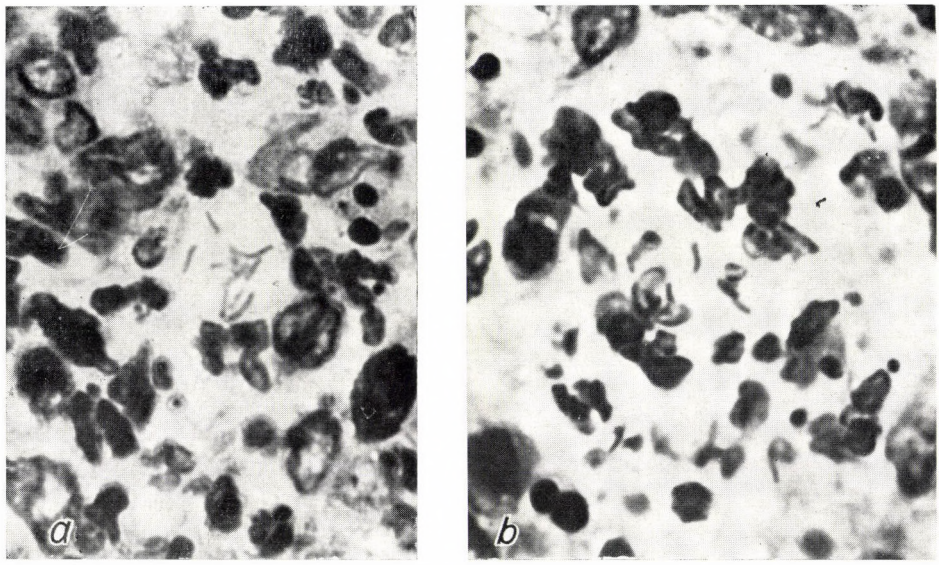


Fig. 17-7. Salmonellae in a macrophage (*a*) and among leukocytes (*b*) in a Peyer's patch 3 days after challenge. Methylene blue-thionine, *a* ×1600; *b* ×1900

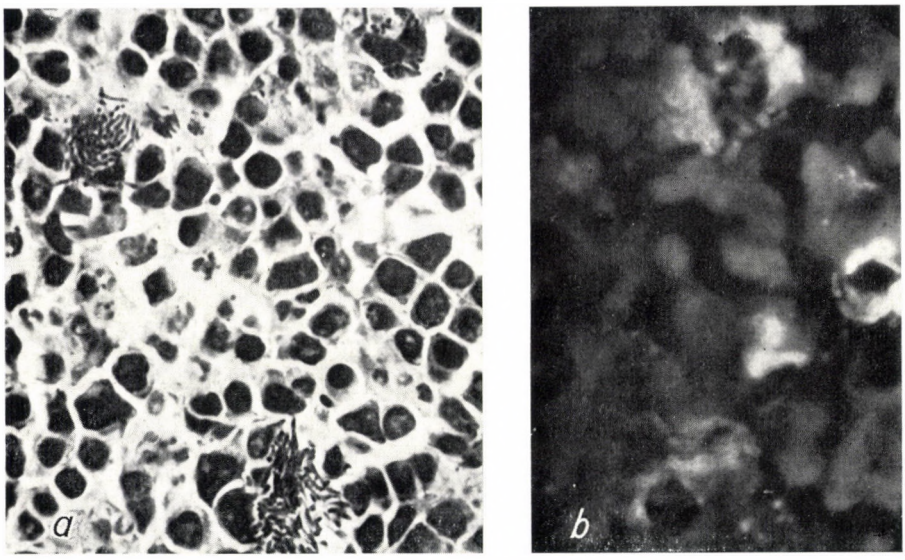


Fig. 17-8. Salmonellae in a Peyer's patch after incubation for 4.5 h at 37 °C. *a* Leishman and Giemsa stain; *b* treatment with fluorescent immune serum against *S. typhimurium*, ×1200

propria of occasional villi may be seen among the follicles; this finding is less frequent in other portions of the mucosa.

After 4–5 days, the foci of inflammation are characterized by an appreciable disintegration of granulocytes and appearance of large light cells (Fig. 17-3). From the sixth or seventh day onward, inflammatory foci become still larger, consisting mainly of epithelioid cells (Fig. 17-4*a*); leukocytes, predominantly disintegrating ones, may be seen in varying numbers. In some mice small necrotic areas developed under the preserved epithelial coating (Fig. 17-4*b*) or small superficial ulcerations appeared about the domes of lymphatic follicles.

In mesenteric lymph nodes the morphological changes are, as a rule, similar to those encountered in the intestinal Peyer's patches. They start as focal aggregation and disintegration of granulocytes (Fig. 17-5*a*) with subsequent growth of cells resembling typhoid cells (Fig. 17-5*b*). No distinct evidence of necrosis of such granulomas has been observed. Three to four days after challenge small granulomas are formed in the spleen and liver.

Bacteriological studies of the Peyer's patches and mesenteric lymph nodes of mice infected with *S. typhimurium* No. 4649 (courtesy of E. M. Dragunskaya) showed very low salmonella counts up to the third day of the experiment (Fig. 17-6*a*, *b*). In agreement with this finding even on the third day only a few macrophages containing a small number of organisms were found in sections stained with thionine, methylene blue and Leishman's method (Fig. 17-7*a*); several of the infected macrophages were already

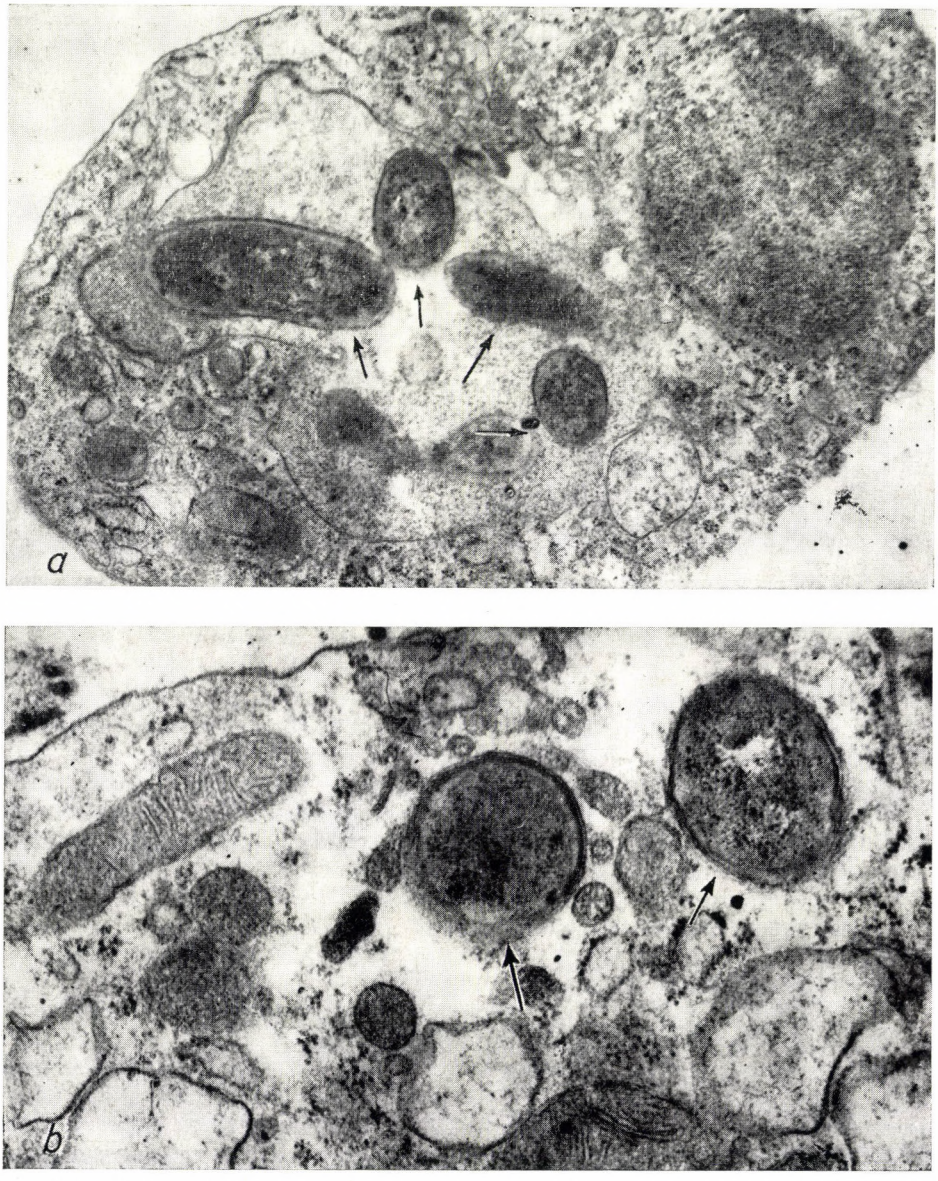


Fig. 17-9. Salmonellae (arrows) in macrophages of a Peyer's patch on the 6th day after challenge. *a* In phagosome, ×8000; *b* in the cytoplasm, ×34 000. Electron micrographs

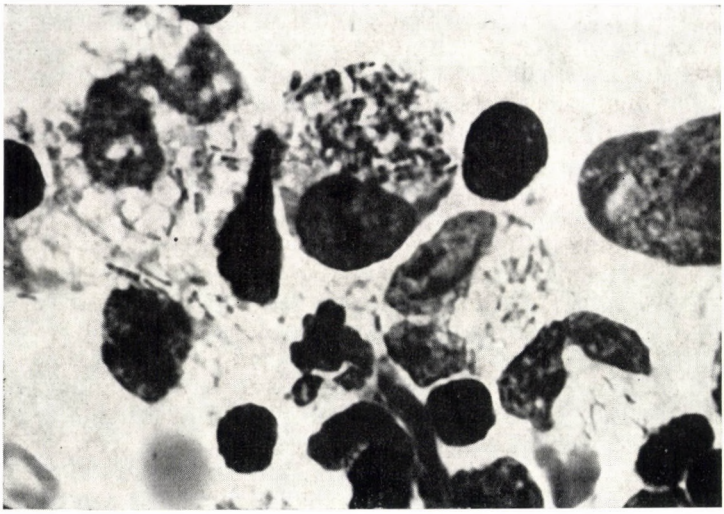


Fig. 17-10. Intracellular bacteria in mesenteric lymph node on the 5th day after challenge. Imprint, Giemsa, ×1265

disintegrating and were surrounded by polymorphonuclears engulfing free salmonellae (Fig. 17-7*b*). In tissue pieces incubated at 37 °C (see Chapter 13) the bacterial count considerably increased (Fig. 17-8*a*, *b*). Judging from the shape of microcolonies (as well as from the cell debris around them) salmonellae had formerly been lodged within macrophages. In such microcolonies the organisms were larger and stained more intensively than previously.

On the following days bacterial count and microscopy showed an increased number of salmonellae, especially in the animals sacrificed shortly before death.

Electron-microscopic studies (courtesy of B. M. Ariel) indicated that in macrophages bearing no signs of lesions, salmonellae were lodged in membrane-bounded vacuoles (Fig. 17-9*a*); macrophages, in which the organisms were found directly in the cytoplasm, showed signs of destructive alterations (Fig. 17-9*b*).

It is impossible to assess the actual contents and diversity of intracellular forms of bacteria in paraffin sections, where the size of cells and microorganisms decreased almost to half and salmonellae were poorly stained. Imprints give much more reliable results (Fig. 17-10).

At sites where granulomas were chiefly composed of macrophages (epithelioid cells) and polymorphonuclears were scare, the number of salmonellae was considerably lower than in foci with active leukocyte response. In addition, in the early periods of the experiment it appeared to be easier for polymorphonuclears engulfing bacteria to digest them, than towards the end of fatal infections. If during the first days deformed or swollen and almost unstained rods were frequently detected in leukocytes, later, on the contrary,

the phagocytes themselves died 6–7 days after challenge, containing large, intensively staining bacteria growing in them.

Thus, after enteral challenge with a highly virulent strain of *S. typhimurium* an infectious process developed in white mice, which was similar to human typhoid fever. Though the earliest stages of the process in man are not known (see Chapter 13), the macrophage granulomas formed in mice by the end of the first week after challenge were very similar to those seen in typhoid patients, especially in children in whom granulomas do not always necrotize. The course of infection caused by *S. typhimurium* in mice and *S. typhi* in man is, however, obviously different, especially in view of the fact that all the mice died during the second week of the disease. In our experiments carried out in cooperation with A. A. Vikhman, a less pathogenic strain of *S. typhimurium* (No. 4801) failed to cause a fatal infection after enteral challenge even with enormous doses (several milliards of bacteria); no characteristic intestinal lesions developed although the organisms had been cultured from the viscera for a long time.

S. cholerae-suis and *S. cholerae-suis* var. kunzendorf produced the same intestinal lesions in mice after enteral challenge as did a highly virulent strain of *S. typhimurium* (Ugleva, 1953), while *S. heidelberg* evoked similar but much milder lesions (Valdman and Mendelson, 1953). After dripping into the mouth or injecting into the duodenum a liquid suspension of *S. enteritidis*, a general fatal infection developed with marked hepatic and splenic involvement but only slight intestinal alterations (A. M. Smirnova, 1955).

ENTERAL CHALLENGE OF RATS

According to the observations of A. M. Smirnova (1950) and Valdman (1964), as compared with mice, rats are more resistant to *S. typhimurium* and *S. enteritidis*. After oral administration their bacteria induced a short-term infection recorded only by the results of bacteriological studies and morphological alterations in the lymphoid system of the intestine, liver and spleen. The changes were similar to those observed in mice but considerably less extensive. Evidence of diffuse enteritis was absent.

Maenza et al. (1970) succeeded in causing enteritis in starved rats by intragastric administration of 2×10^9 *S. typhimurium*. Half of the animals developed prolonged diarrhoea and some of them died. Inflammation involved principally the mucosa of the ileum and the caecum and was characterized by local leukocyte aggregation with the subsequent appearance of histiocyte-type cells. The method used by Maenza et al. did not allow the detection of salmonellae in the tissues.

ENTERAL CHALLENGE OF GUINEA PIGS

Valdman (1964) reported that mainly leukocyte infiltration could be observed in intestinal lymphoid patches of guinea pigs following intraduodenal administration of 10^5–5×10^5 salmonellae. However, typical granulo-

mas composed of epithelioid cells formed in mesenteric lymph nodes. In contrast to mice and rats, guinea pigs also displayed diffuse inflammatory alterations of the ileal mucosa, congestion, leukocyte infiltration and oedema of the villi.

Kent et al. (1966*a*) challenged guinea pigs intragastrically with 10^8 *S. typhimurium*; tincture of opium (1 ml) was administered to some of the animals intraperitoneally immediately after challenge. Non-opiated guinea pigs developed infection gradually and died in 4 to 10 days. The morphological changes were of focal granulomatous character, appearing first in the intestine and mesenteric lymph nodes, and later in the spleen and liver. In granulomas consisting of histiocyte-type cells (with polymorphonuclears admixed in the intestine), necrotic foci were commonly present. The fluorescent antibody technique showed salmonellae to clear rapidly from the intestinal lumen, while the focal lesions contained an increasing number of organisms. In patches of necrosis fluorescent antigenic debris was detectable.

Guinea pigs, the intestinal motility of which was decreased with opium, died within 24–72 h. Besides focal lesions of lymph nodes, spleen and liver, diffuse enteritis developed. Fluorescent bacteria were present in the lumen of the ileum all the time. At 12 h and later they were demonstrated in the epithelial cells and in the lamina propria; in the submucosa they showed a focal arrangement. After 48–72 h large numbers of salmonellae were present throughout the mesenteric lymph nodes and in the spleen.

In electron-microscopic experiments of Takeuchi (1967), the guinea pigs had not only been pretreated with opium but they had also been starved for 4 days. The lesions developing after intragastric administration of salmonellae were especially severe. Takeuchi observed that 12 h after challenge, leukocytes migrated into the intestinal lumen where they engulfed salmonellae. Some of the polymorphonuclears, which were present among the epithelial cells of intestinal villi, also contained bacteria. Salmonellae lying near the surface of the mucosa induced no alteration of the epithelial lining until they reached the "critical proximity" (less than 350 Å). Then, at the point where the organisms adjoined, degeneration ensued and a cavity was formed. Subsequently, this cavity became closed and a vacuole containing organisms, some particles of microvilli and dead cytoplasm appeared. Surmounting the junction complex, salmonellae could also penetrate through the crevice between the epithelial cells thus gaining entrance to one of the adjacent cells through its lateral surface. Irrespective of the mode of invasion, the organisms inside the epithelial cells were usually found in membrane-bound vacuoles (frequently even containing dense osmiophilic substance), and, in Takeuchi's opinion, migrated through the cells penetrating deeper and finally entering the lamina propria. During the passage through the epithelial cells, neither the organisms nor the cytoplasm of the cells underwent any changes. In the lamina propria of intestinal villi salmonellae were engulfed by phagocytes and, as further studies indicated (Takeuchi and Sprinz, 1967), they partly died and partly multiplied in these cells.

Takeuchi's investigations confirm and specify the conclusions of Florey (1933) who made experiments on isolated intestinal loops of guinea pigs

(see Chapter 22). According to Florey *S. typhimurium* may pass through the epithelium (or among the epithelial cells) into the lamina propria, but he thought them to be killed within the epithelial cells. Takeuchi was unable to confirm Florey's statement that salmonellae are carried into the tissues by leukocytes, phagocytizing them near the intestinal surface.

We do not know whether it is justified to correlate the development of fatal enteritis in guinea pigs with the relatively mild salmonella-induced human gastroenteritis. However, it is of considerable interest and importance that diffuse inflammatory processes in the animal gut are associated with the activity of salmonellae penetrating the unaffected mucosa. Only later, 24–48 h after challenge did Takeuchi and Sprinz (1967) find some extensive, but usually not well-pronounced, dystrophic alterations in the cells of the epithelial lining.

EXPERIMENTS ON RABBITS

As shown in Chapter 3, Valdman (1930, 1931*a*, *b*) was the first to challenge rabbits with salmonellae. Having established that the stomach in these animals raised an unsurmountable barrier for *S. typhimurium*, which were administered by stomach tube, she started injecting bacterial suspension into the duodenum. Later, Kucheryavy (1956) simplified Valdman's method requiring laparotomy, and thus after a short deprivation of fluid, the organisms suspended in milk could be given orally or introduced intragastrically.* For challenging rabbits with this technique, 5×10^8 salmonellae are required. Intraduodenal challenge is successful with 10^6–10^7 organisms. The animals become ill after 2–3 days as shown not only by a decrease in activity, loss of appetite and weight, but also by a gradual rise in body temperature (falling again at the terminal stage) and leukopenia. Some of the animals die at the end of the first week, the rest remain carriers for a long time (up to 3 months according to Smirnov, 1966). Pathological changes mainly occur in the intestinal lymphoid structures.

The structure of the lymphoid system of the rabbit bowel is very similar to that in other laboratory animals and in man. The follicles, however, are more markedly separated by connective tissue layers. The large, light, central areas—"reactive centres"—are more conspicuous in the basal portion of the pear-shaped follicles. At the same time the distribution of lymphoreticular tissue shows certain peculiarities. Along with the Peyer's patches in the small intestine, there is a large aggregation of lymphoid tissue with large follicles in the so-called sacculus rotundus or lymphatic sac—a thick-wall projection observed in rabbits at the junction of the ileum with the caecum. There are also many lymphatic follicles in the long appendix.

* Prokopowicz and Tomaszko (1968) have suggested a similar method. The only difference was that they first administered milk into the stomach by a stomach tube then the salmonella suspension in saline, followed by milk again. In addition, tincture of opium (3 ml per kg) was injected intraperitoneally before challenge. Ingestion of salmonellae mixed with 100 ml of milk gives also quite reliable results.

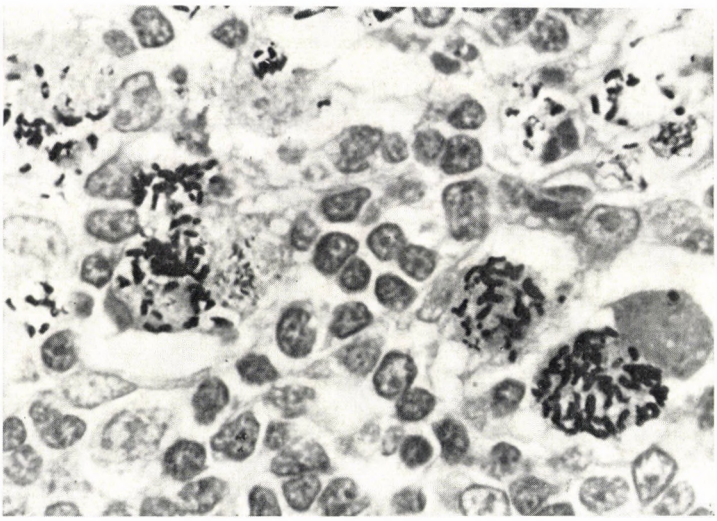


Fig. 17-11. Symbiotic organisms in lymphoid tissue of rabbit appendix. Gram $\times 1600$

The follicles of the rabbit sacculus and appendix (occasionally also of the Peyer's patches) are regularly inhabited by certain anaerobes (Ribbert, 1885; Bizzozero, 1885), but their importance is not clear (Policard, 1963). They are frequently arranged within macrophages (Fig. 17-11), and may disturb experiments with salmonellae, although, unlike the latter they stain well with Gram but not with methylene blue and thionine, and are not detectable with specific fluorescent sera.* These organisms do not multiply when isolated pieces of intestine are incubated in a thermostat (Belyanin, 1969) and are probably killed when engulfed by reticular cells. These rods do not evoke a leukocyte reaction.

According to Valdman's observations (1955, 1964) granular (pseudoeosinophilic) leukocytes started accumulating under the epithelium covering the Peyer's patches 30 h after intraduodenal administration of 10^7–10^8 *S. typhimurium*. By the end of the second or at the beginning of the third day, leukocyte infiltration extended widely to outer portions of the follicles, and later to their deeper parts, too. The infiltrating cells rapidly disintegrated and large foci of necrosis arose at the site of the follicles. Subsequently, at the border of the preserved lymphoreticular tissues, epithelioid cells appeared. When the follicles were entirely necrotic, non-specific granulation tissue started to grow around them. In mesenteric lymph nodes the pathological process was different. First there were focal leukocyte accumulations, followed after 3–4 days by granulomatous growth of epithelioid cells, simi-

* As a rule, in macrophages of the Peyer's patches of the rabbit intestine there are also many granules of brown pigment giving bright scarlet fluorescence.

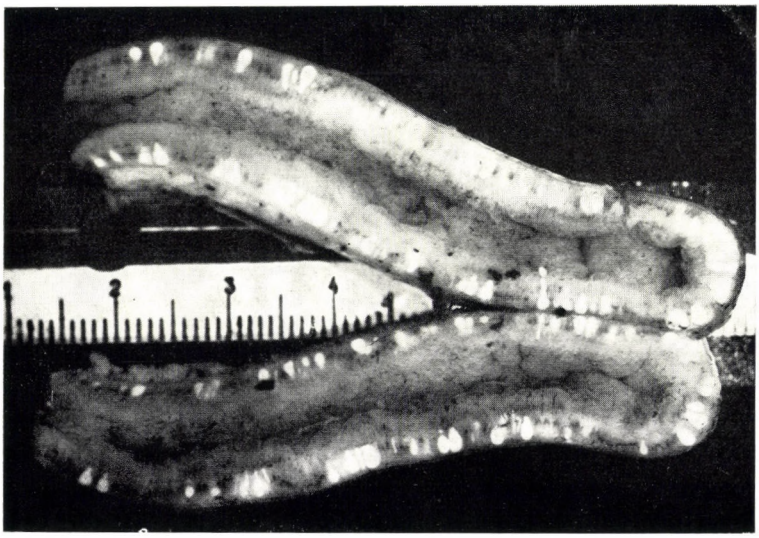


Fig. 17-12. Lesions of appendicular lymphatic follicles 7 days after challenge

larly to findings in mice and guinea pigs. Later these changes became partly necrotic. In the liver and spleen of adult rabbits no focal lesions could be observed, regardless of the presence of salmonellae detected by bacteriological sampling. In newborn rabbits challenged with salmonellae such lesions may be found.

Valdman (1955) did not consider the differences in the course of intestinal alterations due to salmonella infection in rabbits and mice essential. There is some contradiction in her statement that microorganisms participate in salmonella-induced intestinal lesions in rabbits. She maintained earlier that macrophages adjacent to areas of necrosis contained "phagocytized rods" (Valdman, 1955, p. 52), but later she denied the presence of detectable salmonellae in imprints and sections of the viscera. Meanwhile, she was able to culture the organisms from the foci of lesions.

In our studies (Belyanin, 1968, 1969) rabbits were challenged with *S. typhimurium* as described by Kucheryavy (gastric administration of 5×10^9 organisms with milk). Clinical manifestations and intestinal lesions developed similarly as in Valdman's experiments. At autopsy of animals sacrificed at various intervals (2–3 days), there was a conspicuous swelling of intestinal lymphoid structures, and yellowish white areas of necrosis appeared somewhat later (Fig. 17-12). Histological studies showed that, following a rather early and abundant leukocyte infiltration (Fig. 17-13*a*), histiocytic elements (epithelioid cells possessing macrophage properties) started to grow at certain places in affected areas of follicles (Fig. 17-13*b*). The reactive phenomena are evidently associated with an invasion of the lymphoid tissue by salmonellae.

It is difficult to follow the passage of salmonellae through the epithelial lining, since they are rapidly eliminated from intestines with peristalsis.

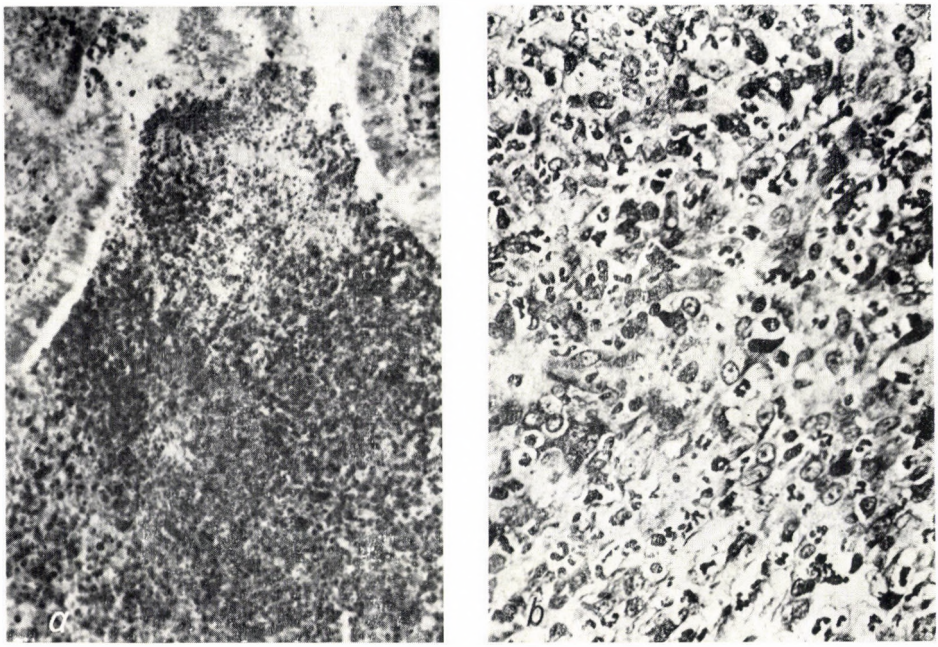


Fig. 17-13. *a* Aggregation and disintegration of granulocytes 3 days after challenge; Goldmann's Sudan alpha-naphthol, ×116. *b* Growth of large cells with light nuclei 6 days after challenge in rabbit Peyer's patch; methylene blue-thionine, × 185

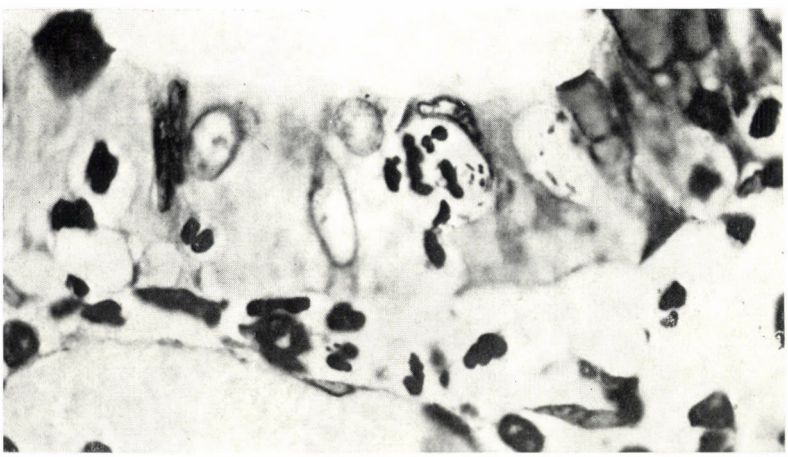


Fig. 17-14. Microorganisms and polymorphonuclears among epithelial cells in the sacculus rotundus. Methylene blue-thionine, ×1265

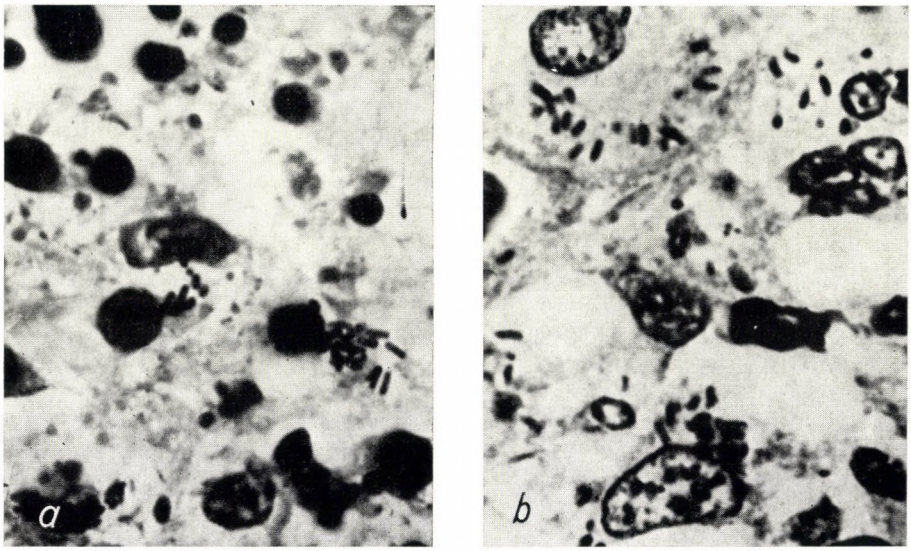


Fig. 17-15. Salmonellae in a necrotic area (*a*) and in adjacent macrophages (*b*) in the sacculus rotundus 72 h after challenge. *a* Methylene blue-thionine, ×1750; *b* Leishman-Giemsa stain, ×2000

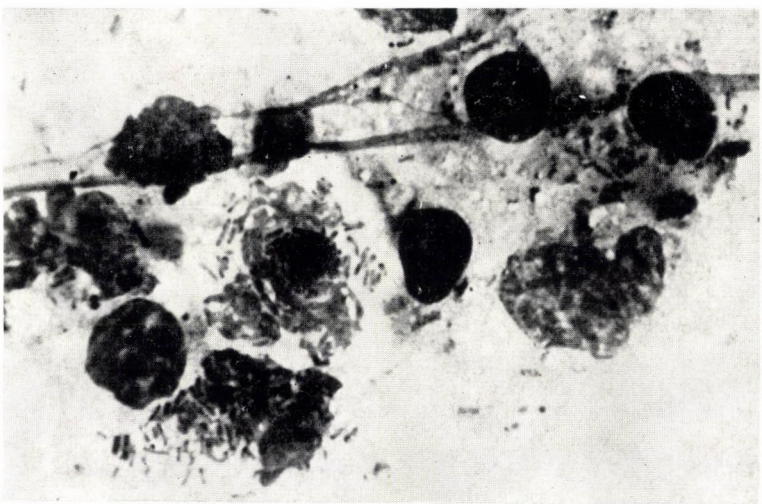


Fig. 17-16. Salmonellae in dying cells 72 h after challenge. Imprint from sacculus rotundus. Giemsa, ×1450

Only rods lying among the epithelial cells of the lymphatic sac and engulfed by polymorphonuclears, are demonstrable (Fig. 17-14). Histological investigation of the lymphoreticular tissue underlying the epithelium has shown it to be free of necrosis at the time of the early leukocytic infiltration of intestinal lymphatic structures. Only the lymphocytes disappeared (or were reduced in number), while histiocytes displayed signs of hyperplasia. Inside these cells there were rod-shaped organisms sometimes filling the whole cytoplasm. In contrast to the saprophytes mentioned above, these organisms were Gram-negative, multiplied in tissue samples incubated at 37 °C and reacted with fluorescent immune serum against *S. typhimurium*.

At early periods (20–24 h), only few macrophages contained salmonellae (1–12 per section from the injured follicle); 48 h after the beginning of the experiment there were several of them in almost every high-power field. The infected macrophages frequently disintegrated and organisms were seen within the polymorphonuclears as well. It should be noted that while incubating intestinal samples at 37 °C, organisms within the macrophages multiplied and formed microcolonies, but those inside polymorphonuclears remained few in number and stained faintly, that is, they were obviously dead.

After an almost complete leukocyte disintegration and occurrence of necrotic areas in the follicles, piles of well-stained rods were encountered among dead cells (Fig. 17-15*a*); the organisms were most numerous in histiocytes near the necrotic area (Fig. 17-15*b*). Salmonellae were especially well demonstrated in imprints (Fig. 17-16).

The above facts prove that pathologic processes occurring in the intestine of rabbits challenged with *S. typhimurium* are directly related to the activity of these organisms. This relation may be schematically expressed as follows. Salmonellae (seemingly in small numbers) invade lymphatic intestinal patches through the intact epithelial coating. The resulting leukocyte response causes the partial destruction of the invading organisms, but those engulfed by local macrophages (reticular cells) multiply until the cell sheltering them dies. As disintegration of such cells increases and local leukocyte response becomes more intensive, many of the multiplying organisms are destroyed and their endotoxins produce follicle necrosis. Toxic lesions of tissues cause hyperplasia and transformation of macrophages into epithelioid or "typhoid" cells. In these cells surviving salmonellae may find shelter again.

Injection of 5×10^8–10^9 virulent *S. typhimurium* directly into the duodenal lumen results in the development of a very severe illness, usually causing death 3–4 days after challenge. Signs of general intoxication (dyspnoea, weakness, weight loss) prevail; diarrhoea develops only in part of the rabbits shortly before death. However, at autopsy of sacrificed and dead animals prominent diffuse lesions of intestinal mucosa could be detected (Bibinova, 1939). In Bibinova's experiments enteritis extended to the entire small intestine, especially to its distal portions. At autopsy swelling and congestion of the mucosa were revealed; histological studies showed generalized and focal infiltration by pseudoeosinophilic leukocytes. Leukocytes migrated into the intestinal lumen passing the well-preserved epithelium of the villi;

they showed evidence of disintegration at the earliest periods of observations. Hyperplastic histiocytes were readily seen among them. Bibinova also noted the presence of small Gram-negative rods among disintegrating cells as well as within macrophages. From pieces of the intestinal wall salmonellae were cultured. Thus, the experimental salmonella enteritis of rabbits described by Bibinova (1939) is rather similar to the lesion of the small intestine in guinea pigs reproduced by Kent et al. (1966*a*).

In addition to *S. typhimurium*, in enteral challenge of rabbits, *S. paratyphi-B* and *S. heidelberg* (Valdman, 1955), *S. enteritidis* (Smirnova, 1950; Rejniak et al., 1970) and *S. cholerae-suis* (Ugleva, 1953) were also pathogenic.

CHALLENGE OF MONKEYS WITH SALMONELLAE

Although challenge with *S. typhi* was only successful in primates (see Chapter 13), other kinds of salmonellae may attack lower simians experimentally. In monkey nursery gardens and animal houses these animals often become infected spontaneously (apparently from human carriers).

Making long-term observations on large numbers of monkeys in the animal house in the city of Sukhumi, Aksenova (1958) isolated *S. typhimurium* the most frequently; *S. paratyphi-B*, *S. paratyphi-A* and *S. enteritidis* were next in order. According to Rowe (1969), during sporadic diseases and outbreaks of enteritis in monkeys observed in research institutes and zoos of Great-Britain, *S. stanley* was the most frequent causative agent, followed by *S. typhimurium*, *S. senftenberg*, *S. newport*, *S. anatum* and others.

Lapin et al. (1956) succeeded in challenging *Macaca rhesus* with *S. paratyphi-B*. Stasilevich (1958) challenged the same species with *S. heidelberg* by administering first 4 ml of bovine bile and then 1 ml of suspension, containing $3–5 \times 10^{10}$ organisms. In these experiments the disease developed rapidly: already on the second day diarrhoea appeared, the animals rejected food and became weak; most of them recovered without treatment in 6–8 days. Excretion of salmonellae with faeces continued for 10–21 days. The course of infection was much more severe if *S. enteritidis* was used (Stasilevich, 1961): 5 out of 8 monkeys died in 3–12 days. From samples taken at autopsy, *S. enteritidis* was isolated in pure culture from the blood, liver, spleen, mesenteric lymph nodes and from the lungs showing pneumonic foci. The same mode of challenge in baboons caused but a mild form of the disease.

Yakovleva (1966) revealed similar pathologic alterations, mainly catarrhal enteritis and reticular cell proliferation in mesenteric lymph nodes in monkeys that died or were sacrificed after challenge with *S. paratyphi-B*, *S. heidelberg* and *S. enteritidis*. Several animals had foci of pneumonia which was, apparently, a specific reaction as there were accumulations of macrophage-type cells and bacterial sampling revealed salmonellae in the lungs.

Kent, Formal and LaBrec (1966*b*) obtained rather different results in experiments on *Macaca rhesus* by using *S. typhimurium*. The organisms caused

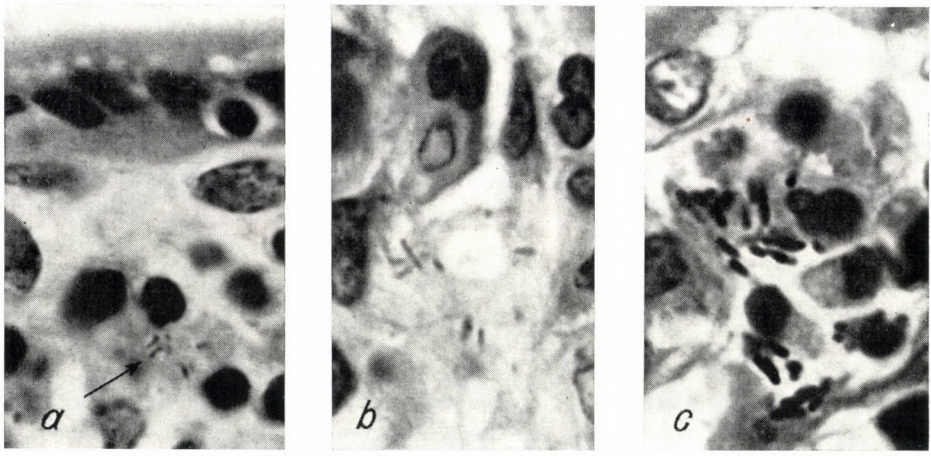


Fig. 17-17. Salmonellae in macrophages of the lamina propria of the jejunal villi (*a*), and in the Peyer's patches (*b*, *c*) of monkeys. Eosin-azure, *a* 24 h after challenge, ×1600; *b* after 48 h, ×1900; *c* after 48 h (and 3 h of incubation of the samples at 37 °C), ×1750

acute inflammation of the intestinal mucosa, which was less pronounced in the jejunum than in the colon especially in its proximal part. A very large number (3×10^{10}) of bacteria suspended in 20 ml of broth was administered intragastrically to monkeys (weighing 2–3 kg) through a stomach tube. Using fluorescent antibodies and Giemsa staining, they occasionally found salmonellae in epithelial cells of the mucosa; the bacteria were mainly present in macrophages of the lamina propria.

Kent et al. (1966*b*) considered the experimental infection in monkeys very similar to acute salmonella gastroenteritis in man. However, they emphasized that there was a considerable difference in severity and spread of intestinal lesions among the experimental animals.

In our experiments (Belyanin et al., in press) made on 18 rhesus monkeys results, similar to those of Kent et al. (1966*b*), were obtained. After administration of some bile through a stomach tube followed by challenge with 3×10^{10} *S. typhimurium* or *S. cholerae-suis* organisms, the animals developed acute gastroenterocolitis subsiding within 7–10 days. At autopsy of sacrificed monkeys, in addition to congestion and swelling of the mucosa of the alimentary tract (especially in the terminal ileum, caecum and the proximal part of ascending colon), there was enlargement of Peyer's patches and mesenteric lymph nodes but not so marked as in typhoid fever in humans. Bacteriological studies showed salmonellae to be present not only in the intestinal contents but also in the intestinal wall tissue. Twenty-four, but especially 48 h after challenge, organisms were found in many macrophages (histiocytes) of the lamina propria of the jejunal villi and in the Peyer's patches (Fig. 17-17*a*, *b*). Intracellular salmonellae stained weakly, however, in tissue samples previously incubated in a thermostat (cf. experiments on

mice and rabbits) they became not only more numerous but also larger and thicker, staining strongly with basic stains (Fig. 17-17*c*). At the same time salmonellae found in polymorphonuclears remained small, pale and did not show any signs of multiplication. Obviously they were dead. Leukocyte infiltration of the intestinal mucosa was focal at early stages, more diffuse at 2–3 days and then subsided, being replaced by moderate accumulation of macrophage-type cells. There was no necrosis or ulcer formation.

EXPERIMENTAL STUDY OF SALMONELLA GRANULOMAS IN THE LIVER AND SPLEEN

Challenging of animals with salmonellae via the gastrointestinal tract or any other route usually results, except for adult rabbits, in a generalized infection. As in man, bacteraemia develops and specific granulomas are formed in the organs of the reticuloendothelial system (spleen, liver and bone marrow). The mechanism of development and direct causes of such focal lesions in typhoid fever are not known (see Chapter 13) and, therefore, the results of experiments with other salmonellae should be considered in more detail.

According to Valdman (1955, 1964), focal changes in the mouse liver become conspicuous 3 days after enteral challenge with *S. typhimurium*, i.e. almost simultaneously with similar processes arising in the intestinal Peyer's patches and in mesenteric lymph nodes. In both structures there is a strong leukocyte response at the beginning, then the polymorphonuclears rapidly disintegrate and disappear, being replaced by epithelioid cells. Valdman mentioned no necrosis in such granulomas, but pointed out that, in addition to granulomas, large necrotic foci resembling anaemic infarctions are frequently formed under the hepatic capsule.

Akazaki et al. (1956) also demonstrated that salmonella granulomas in the mouse liver and spleen began with focal leukocyte infiltration. They emphasized that leukocytes in such accumulations were usually deformed and displayed signs of degeneration. Other authors failed to recognize this process correctly. Thus, when describing "necrotic nodules" in the liver, Mestitz (1923) noted "peculiar patterns of polymorph nuclei arranged in the granular necrotic substrate", apparently not suspecting that these were merely neutrophils in the state of being destroyed. Joest (1914) suggested that lymphoid cells were accumulated in salmonella granulomas and then were destroyed within them, but he rejected the possibility of leukocytes participating in granuloma formation. At the same time, figures shown by Joest as well as by Gruber (1916) and Böhme et al. (1959), who have described hepatic "focal necrosis" in experiments with salmonellae, suggest that polymorphonuclears disintegrated in these areas.

In our own experiments (Voino-Yasenetsky, 1964*b*) it was revealed that the development and morphology of focal lesions in the liver of mice arising after intranasal challenge depended on the virulence of salmonellae (Table 17-I).

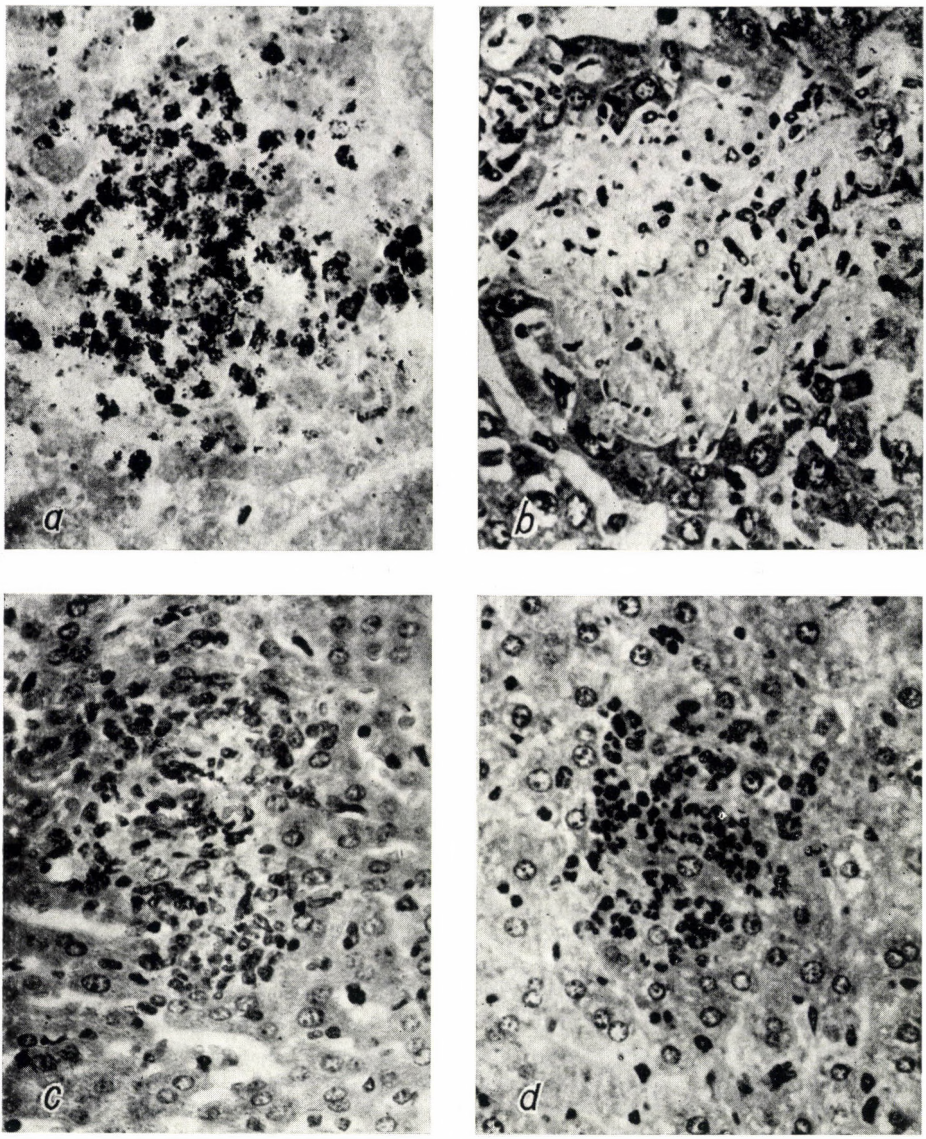


Fig. 17-18. Focal lesions in the liver of mice after challenge with various salmonella strains. *a S. typhimurium* No. 4801, 3 days, Goldmann's stain. *b S. typhimurium* No. 4649, 5 days, thionine. *c S. typhimurium* No. 4801, 15 days, haematoxylin-eosin. *d S. paratyphi-B*, 15 days, haematoxylin-eosin, ×430

TABLE 17-I

Average number of cellular foci in the liver after intranasal challenge with various salmonella strains

Organism	Days after challenge									
	1	2	3	5	7	10	15	20	30	50
S. typhimurium No. 4649	0.3	2	103 necrosis							
S. typhimurium No. 4801	0.3	2	4	40	40	45	248			
S. heidelberg	0.0	0.7	16	23	116	204				
S. paratyphi-B	0.3	1	1	6	26	101	72	54	26	22

Two to three days after challenge, small accumulations of polymorphonuclears appeared in the liver. Already at this time many of these cells showed a considerable deformation, but were clearly recognizable when stained by Goldmann's method (Fig. 17-18*a*). In mice challenged with a highly virulent *S. typhimurium* strain (No. 4649), the number of small leukocyte foci was 60–127 in every section made across the right hepatic lobe. Then polymorphonuclears completely disintegrated, and round, almost structureless areas reaching 80–110 μ in diameter formed in their place 4–5 days after challenge (Fig. 17-18*b*). With Mallory's phosphotungstic acid haematoxylin stain they displayed a thick fibrin network. Adjacent liver cells seemed to be compressed, and appeared to be pushed aside. Numerous parietal thrombi formed simultaneously in hepatic blood vessels, wedge-shaped infarctions being apparent under the capsule of the organ.

Hepatic lesions developed later after challenge with *S. heidelberg* and with a less virulent *S. typhimurium* strain (No. 4801) (see Table 17-I), but the disintegration of focal leukocyte accumulations was not followed by such marked local lesions. Slightly stretched cells with basophilic cytoplasm, later transforming into lighter epithelioid cells, appeared in the foci after 5–7 days. These foci, not exceeding 50–80 μ in diameter resembled granulomas (Fig. 17-18*c*). In mice challenged with *S. paratyphi-B*, the granulomas were quite small (30–40 μ); frequently they seemed to consist of transient polymorphonuclear accumulations in liver sinusoids (Fig. 17-18*d*) not causing any further damage in the structure of the organ.*

Quite naturally, the question arises as to the cause of focal leukocyte accumulations and subsequent macrophage growth. In the opinion of Stuart et al. (1969) and Stuart (1970), both responses are directed against the liver cells that have suffered some kinds of injury. However, leukocytes may primarily be attracted by the salmonellae themselves.

Only in mice sacrificed during agony did Valdman (1964) find large rods intensively staining with Loeffler's methylene blue; the bacteria

* Granulomas resembling those elicited by salmonellae are frequently revealed in the liver of apparently healthy white mice not infected with salmonellae (Voino-Yasenetsky and Zhabotinsky, 1970). In view of this finding, special groups of animals in which these spontaneous hepatic alterations were relatively rare had to be selected for the experiments.

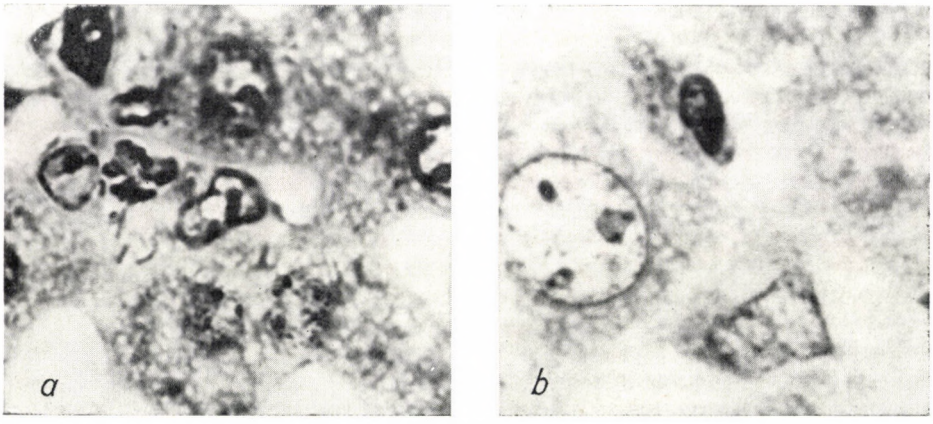


Fig. 17-19. Salmonellae in the liver. *a* In a mouse sacrificed when critically ill 5 days after challenge with *S. typhimurium* No. 4649, ×1265. *b* Very small organisms in a Kupffer cell, 3 days after challenge with *S. heidelberg*; methylene blue-thionine, ×2280

were arranged in small groups both inside and outside the cells. This finding was confirmed in our experiments (Fig. 17-19*a*). Staining by methylene blue and thionine permitted to find, though not without difficulty, very small forms of organisms in the cytoplasm of Kupffer cells early after challenge (17-19*b*). Single larger rods could also occasionally be detected among focal leukocyte accumulations.

Using fluorescent antibodies Tanaka et al. (1960*b*) found rod-like bacterial antigen of various size in the liver of mice challenged intraperitoneally with several scores of *S. enteritidis*; the amount of the antigen was first insignificant, then, after the third day, it began to increase. After 5–8 days, the antigen concentrated in cells of granulomas formed by that time, though rod-like inclusions and particles became gradually less numerous and were even entirely absent in some granulomas.

As mentioned above, Kent et al. (1966*a*) using fluorescent antibodies also revealed a great number of salmonellae (and, later, their breakdown products) in granulomas formed in guinea pigs' liver after challenge with *S. typhimurium*.

As has been seen, enteral challenge of animals with salmonellae pathogenic for them results in the development of infection, leading not infrequently to death. Depending on challenge conditions pathologic processes similar in localization to typhoid fever or salmonella gastro-enteritis may be reproduced in such experiments. Both kinds of lesion are directly related to a deep penetration of the organisms into the intestinal wall. However, the sequence in the development of local pathologic processes caused by salmonellae in lymphatic intestinal structures is not the same in different animals.

CHAPTER 18

PROBLEMS OF THE PATHOGENESIS OF SALMONELLA INFECTION

by

M. V. VOINO-YASENETSKY

Clinical observations and post-mortem studies have so far failed to provide satisfactory answers to questions related to the essence of the interaction between the host and salmonellae in human typhoid fever, as well as to the immediate role of salmonellae in the development of intestinal and other lesions. The resistance of laboratory animals to *S. typhi* and *S. paratyphi-A* has hindered experimental work in this field. In model experiments, therefore, not only the host but also the pathogenic agent is usually substituted. The results of investigations dealt with in Chapters 14–17 show that the course of salmonella infection induced in different ways in various animals cannot directly be compared with human typhoid. There were, however, important common traits, of which the capability of all kinds of salmonellae (including *S. typhi*) of intracellular parasitism as well as the similar location of specific lesions are the most important.

INTRACELLULAR SALMONELLA PARASITISM

Similarly to the causative agents of dysentery, salmonellae are able to penetrate the mucosal epithelium; however, in contrast to shigellae, they do not settle there but pass into deeper lying tissues. Electron-microscopic studies have shown that salmonellae are separated from the cytoplasm by membranes and do not cause any apparent lesions in the epithelial cells. Only a limited (to single cells) multiplication of *S. typhimurium* was observed in the stratified transitional epithelium of the urinary bladder (see Chapter 16). Parasitism in macrophage-type cells should be considered an essential biological property of salmonellae. This feature, also noted in human typhoid fever (see Chapter 13), was clearly demonstrated in all experimental models.

Groups of salmonellae revealed in macrophages are usually inside the so-called phagosomes, i.e. vacuoles which, besides bacteria, may contain an electron-dense substance and some debris. This fact in itself, however, does not prove that the organisms have merely been engulfed and are successfully ingested. Such obvious macrophage parasites as *Coxiella burneti*, ornithosis virus and *Francisella tularensis*, and some other organisms grow inside the membrane-bound vacuoles. In the opinion of C. A. Brown et al. (1969) mycobacteria inhabiting phagosomes paradoxically use defensive substances deriving from lysosomes as nutrients.

It has been shown that some of the salmonellae taken up by macrophages are killed, while others multiply in them exhibiting a specific life cycle. Their morphological changes are quite regular and can be best demonstrated in the lung model. The structure of other organisms may also change due to the environmental influences, but the difference between small organisms filling the macrophages and large rods growing extracellularly and/or on the dead debris, is especially great for salmonellae. The fact that extracellular organisms stain more intensively and their antigenic substances are more readily revealed by fluorescent antibodies, also indicates a difference between the two forms.

Shigella antigens are preserved after formalin fixation and even after paraffin embedding, whereas salmonellae must be studied in cryostat sections or fixed in cooled ethanol (Sainte-Marie, 1962), the second being a less adequate technique. Salmonellae growing on dead tissues may be visualized by Coons' technique also in specimens fixed in formalin (Belyanin, 1969; Yagud and Barshtein, 1971).

In vivo salmonellae found outside macrophages are mostly destroyed by polymorphonuclears, depending on the virulence of the organism and the state of the host. All kinds of salmonellae evoke a rather rapid and intensive leukocyte response, but this is fully efficient only against organisms of low pathogenicity for experimental animals, e.g. *S. typhi*. Under similar conditions *S. typhimurium* and some other highly virulent salmonellae may survive and destroy the phagocyte. The latter organisms usually cause the animals' death. During the terminal period the infection shows a septic course with an almost complete lack of leukocyte emigration from blood vessels. Preliminary specific immunization markedly enhances the leukocyte response (Berman and Slavskaya, 1958, 1959; Pokrovskaya et al., 1963). A gradual increase in leukocytic activity is also noted in human typhoid fever running a favourable course (Podlevsky, 1962).

Macrophages obtained from immunized animals are also capable of destroying the ingested salmonellae. The significance of specific antibodies is not quite clear in this case. According to investigations of Mitsuhashi et al. (1961) and Sato et al. (1962), the increase in macrophage activity is of systemic nature, involving the reticuloendothelium and histiocytes of various organs. At the same time macrophages do not behave in the same way even in a single organ, e.g. in the convalescent stage after intranasal challenge, some pulmonary macrophages destroyed the engulfed salmonellae, while in others the bacteria remained intact (see Chapter 15).

LOCALIZATION OF INFECTIOUS FOCI IN SALMONELLOSES

The earlier haematogenic hypothesis of the pathogenesis of typhoid fever, (Sanarelli, 1894, 1926; Schottmüller, 1902) has been refuted in view of recent experimental results. The hypothesis had been founded on bacteraemia regularly observed in early stages of the disease as well as on the occasional absence of intestinal manifestations. In addition, certain experimental studies seemingly indicated that salmonellae having a peculiar "enterotrop-

ism" could enter the intestine from the blood via the bile ducts or directly through vessels of the intestinal wall (Besredka, 1925; Sanarelli, 1926).

In fact, bacteraemia very frequently occurs in typhoid fever, especially during the first week. But this does not prove that the disease commences with bacteraemia and that intestinal lesions are still absent at the time when clinical manifestations of the disease become apparent. The haematogenic hypothesis has not been confirmed by experiments on animals either.

When salmonella suspension is administered orally to mice, the organisms can be recovered from the heart blood and some viscera as soon as after 5 min (Klemparskaya, 1952), or even after 20 sec (Gerichter, 1960) but, as a rule, not after 1–2 hours. Ralovich and Emődy (1969) as well as Ralovich and Rauss (1969) proved that this short-term primary bacteraemia is merely an experimental artifact. It depends on the conditions of administration (number of bacteria and volume of suspension) and may be observed in experiments with a variety of organisms including harmless ones. When challenging mice with salmonellae pathogenic for them, a lasting bacteraemia only appears after 3–5 days. By this time there are marked pathological alterations in the intestines (see Chapter 17).

A very rapid spread of organisms to the circulation after enteral challenge has been noted, suggesting that the organisms enter the blood stream through the intestinal mucosa. However, it is difficult to assume that this might occur within 20 sec. In our laboratory early short-term bacteraemia was observed not only in cases of enteral, but also of intranasal challenge. In all cases the organisms obviously entered the blood stream in the fauces or airways where they are frequently found also after oral challenge.

Valdman (1955, 1964) has established that the site of the lesions in experimental salmonelloses depends on the method of challenge. When injecting a salmonella suspension into the lumen of rabbit intestine at different sites, lesions, as a rule, only arose in lymphatic structures of portions located distally to the site of administration. Intracutaneous injection of salmonellae into the fore or hind footpad of mice, invariably resulted in the development of specific changes in regional (axillary or inguinal) lymph nodes. Generalized infection setting in later affected the liver and the spleen; other lymphatic structures, including the mesenteric nodes and intestinal Peyer's patches, remained intact.* Intravenous injection of salmonellae also failed to cause intestinal lesions in these animals.

Rabbits challenged intravenously with very large doses of organisms rather frequently developed purulent cholecystitis together with focal inflammatory changes in the spleen and liver. Single small foci of lesions in the follicles of the lymphatic sacculus and the appendix (but not in the Peyer's patches and mesenteric nodes as after enteral challenge) were noted only in part of the sick animals. The possibility of inducing a disease similar to human typhoid fever by challenging rabbits parenterally has not been confirmed with adequate morphological investigations (Valdman, 1955). Valdman, as well as Planelyes and Krasinskaya (1950), came to the

* Intestinal lesions were also absent after intranasal challenge of mice with salmonellae, which usually induced not only pneumonia but also a generalized infection.

conclusion that pathologic processes arising after enteral challenge in lymphatic structures of the intestine and the mesentery are primary and are due to the portal of entry of the infectious agent.

The question, however, arises why the intestinal lesions are selectively localized in lymphatic follicles and ileal Peyer's patches. It is certain that the passage of salmonellae is the most effectively delayed by these structures projecting somewhat over the mucosal surface. But why then do shigellae pass follicles and Peyer's patches in the colon? If salmonellae occasionally affect the colon (the so-called colotyphoid), they appear mainly in mucosal lymphatic follicles but not in the mucosa as shigellae do. Hence, some tropism of salmonellae to lymphoid structures or, more precisely, to their reticular cells must be postulated. This phenomenon is all the more interesting as intestinal follicles and Peyer's patches are situated under the epithelial lining which the organisms have to pass.

THE DEVELOPMENT AND THE FATE OF SALMONELLA GRANULOMAS

The settling of salmonellae in macrophages (histiocytes) of the intestine and of other organs is harmless as long as the cell which has sheltered the parasites is living. The development of focal lesions always follows the leukocyte response occurring after the destruction of infected macrophages. The rapid destruction of polymorphonuclears engulfing extracellular salmonellae as well as local tissue damage (usually limited to small areas) could be explained by the toxic action of breakdown products. But the matter is not as simple as that.

In experiments on tissue cultures the endotoxins of Gram-negative organisms either caused insignificant injuries or none at all (Delaunay et al. 1948; Gabliks and Solotorovsky, 1962; Heilman, 1965; Yabrov et al., 1967; Ambarnikov, 1967; Wiener and Levanon, 1968; Bergman and Weibull, 1969) though macrophages proved more sensitive than fibroblasts or epithelial cells (Heilmann, 1964, 1965).

The cytotoxic effect of endotoxins is attributed by many authors to lymphocyte depletion in the spleen, thymus and other organs after parenteral administration (Kind et al., 1964; Zemskov, 1972). According to Selye (1950), atrophy of lymphoid organs is not directly caused by the endotoxins, but rather by adrenocortical hormones secreted in response to their administration. This was confirmed by Bibinova et al. (1962*a*) who have shown that though mouse sensitivity to endotoxins markedly increased after removal of the adrenals, decomposition of lymphoid cells occurred far less actively than in the controls.

Hepatic and splenic tissue damage is not significant at the sites where salmonella granulomas develop. Mallory (1898) even suggested that liver cells were only compressed by macrophages accumulating in the sinuses, and thus became gradually atrophied. As shown in the previous chapter, this is not quite the case. Formations giving the impression of focal necrosis are in fact not liver cells but disintegrating polymorphonuclears accumulated

to destroy the bacteria. Apparently, breakdown products of the latter also lead to the death of some local tissue elements.*

The macrophage response is probably in connection with the effect of breakdown products which leads to the accumulation of epithelioid or typhoid cells, and the localization of these foci reflects some degree of specificity, a similar response is caused by breakdown products of other organisms. Thus, Chistovich (1906) observed the appearance of epithelioid cells in response to substances obtained from tubercle rods. Investigators in our laboratory demonstrated an increased accumulation of macrophages and polyblasts at the sites of administration of pertussis and dysentery endotoxins (Bibinova and Osipova, 1959), killed rickettsiae (Shifrin and Khavkin, 1962) and dead *Candida albicans* (Tsinzerling, 1958). It has also been reported that endotoxins stimulate macrophages *in vitro* (Heilman, 1964) and increase the number of monocytes in tissue cultures of human blood (Oppenheimer and Perry, 1965).

Thus, the salmonella granulomas appear to be due not so much to vital activity of the pathogens as to their destruction. Faber (1921), Christeller (1928), Goodpasture (1937) were also of this opinion. This assumption may also explain the low number or even absence of organisms in infectious granulomas consisting predominantly of macrophages. The granulomas do not increase in size, and undergo a gradual involution in healed cases.

S. typhi penetrating the Peyer's patches and mesenteric lymph nodes in man are partly destroyed by leukocytes at the very beginning of the development of intestinal lesions. Consequently, also in typhoid fever, a focal macrophage growth may start under the local effect of salmonella endotoxins. However, the development of typhoid granulomas has some peculiarities which have not yet been reproduced in animal experiments.

First of all, "medullary" swelling of intestinal lymphatic structures is not so marked and typhoid cells are not so abundant in animal salmonelloses. In addition, in the animal granulomas the severe stage of necrosis known to occur in humans is absent. In mice challenged with *S. typhimurium* or other salmonellae, necrotic alterations are, as a rule, not appreciable in the Peyer's patches and mesenteric lymph nodes. In rabbits necrotic changes are marked but appear in the first days and the progress of microbial growth proceeds uninhibited. Typhoid cells appear simultaneously or following necrosis (see Chapter 17). In human typhoid fever, in contrast, granulation tissue, consisting chiefly of macrophages by this time, begins to necrotize only during the second week of the disease.

The necrotic stage occurring in the development of typhoid granulomas cannot at present be explained. As mentioned in the previous chapter, salmonella parasitism is observed in some typhoid cells of intestinal lymphatic structures and in the mesentery. It is possible that formation of specific antibodies during the second week of the disease contributes to the destruction of parasites by macrophages themselves, or by polymorphonuclears

* True necrosis of liver tissue occurs as small infarctions occasionally observed (along with granulomas) both in the liver of animals and man with salmonella infection; these develop as a result of blood vessel thrombosis.

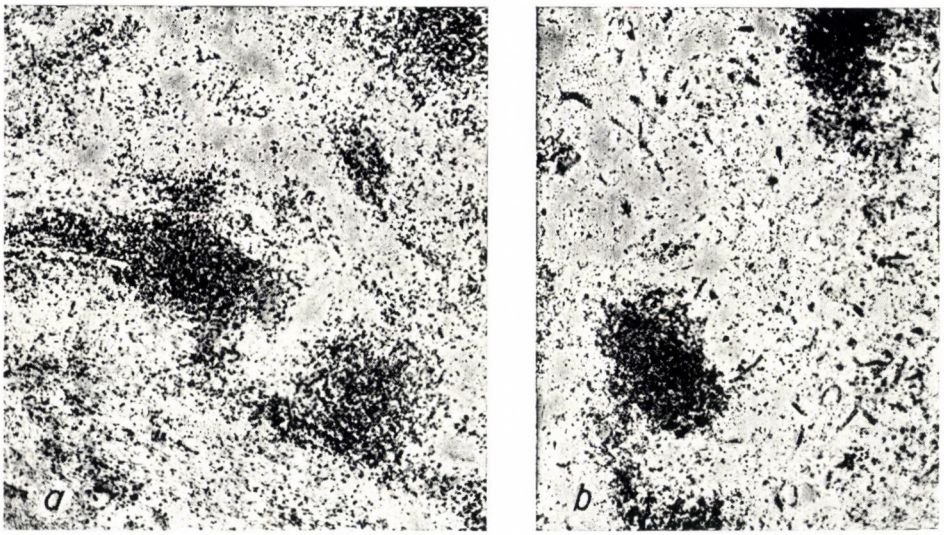


Fig. 18-1. Focal accumulation of granular leukocytes at the stage of necrosis in Peyer's patches (*a*) and in mesenteric lymph nodes (*b*) in typhoid fever. Goldmann's Sudan alpha-naphthol, *a* ×49; *b* ×44

appearing here. Many authors have observed great numbers of leukocytes in necrotizing granulomas, but they explained this finding as a response to necrosis which had already occurred or attributed it to extraneous organisms penetrating the destroyed tissue from the intestine. A secondary infection, especially in surface layers of Peyer's patches may obviously be assumed. Nevertheless, even in partial necrosis involving only single groups of cells, one can see focal aggregations of intact and disintegrating granular leukocytes deeply in the Peyer's patches and mesenteric lymph nodes (Fig. 18-1*a*, *b*). Such infiltrates are dissimilar to those of inflammatory demarcation, but greatly resemble local leukocyte response occurring when macrophages disintegrate after infection by some other parasitic organisms (see Chapter 1 — Fig. 1-3).

Thus, there is reason to believe that in typhoid fever the stage of necrosis following medullary swelling of intestinal lymphatic structures is in some way related to the destruction of *S. typhi*. It is not clear, however, why tissue damage is especially severe and well-pronounced in such cases, though according to Goodpasture (1937), Adams (1939) and Keiserling et al. (1972) (see Chapter 13), there are relatively few organisms in typhoid granulomas. It may be assumed that some kind of sensitization of the granulation tissue to breakdown products of salmonellae, occurs at this stage of the disease.

This hypothesis does by no means suggest the revival of earlier arguments that typhoid bacteria are merely allergens (Lavergne, 1923) and granuloma formation is an allergic phenomenon (Rössle, 1948). Allergy may, in fact, not be involved at all. Braun and Kessel (1964) have established that

endotoxins as such are harmless in tissue cultures, but the cells are killed if immune serum is added. These observations are of interest since "the necrotic stage" of the development of typhoid granulomas, as already mentioned, corresponds to the appearance of specific antibodies in the patient's blood. In the experiments of Braun and Kessel, the cytotoxic action of endotoxin was pronounced only in the presence of immune serum but even under these conditions it developed slowly, becoming apparent only 24–48 h after the addition of the endotoxin. In contrast to allergic responses, granulation tissue necrosis in typhoid fever develops gradually, first involving only single granuloma areas (evidently where salmonellae, released from macrophages, were destroyed).

PATHOGENESIS OF SALMONELLA ENTERITIS

As shown in Chapter 13, acute salmonella enteritis should be considered an infectious disease and not the mere consequence of poisoning with food contaminated with salmonella toxins. Clinical and epidemiological observations leading to this conclusion have been confirmed by the results of experimental studies. However, the question of the pathogenesis of these diseases must not be regarded as solved.

In experiments using oral challenge of guinea pigs and monkeys with large doses of *S. typhimurium* and *S. enteritidis* (see Chapter 17), a good model of salmonella enteritis has been obtained. Unlike in experiments when smaller doses of salmonellae were used for challenge, now the organisms did not prevail in Peyer's patches but penetrated throughout the intestinal mucosa. Here they usually lodged in macrophage-type cells, but then died off fairly rapidly.

These experimental data are supported by observations reported by Giannella et al. (1971) that in man, too, very large infecting doses are, evidently, involved in the development of salmonella enteritis. Enteritis was especially severe in patients with decreased gastric juice secretion, in whom the organisms passed into the intestines more easily then in subjects with normal gastric function, where a considerable part of salmonellae were destroyed. Giannella et al. (1971) also referred to data concerning a marked susceptibility to salmonella enteritis after gastrectomy.

Biopsy specimens were obtained from the initial segment of the small gut when salmonella gastroenteritis was already subsiding (Giannella et al., 1971). No noteworthy mucosal alterations could be seen in the specimens indicating a short-term microbial parasitism. Studying specimens of small intestinal mucosa obtained by suction biopsy from patients with salmonellosis, Bluger et al. (1973*a*, *b*) have not noted any peculiarity of the inflammatory infiltration of the lamina propria either. However, in a later work (Bluger et al., 1975), they stressed the abundance of macrophages at the climax of infection. Not infrequently the macrophages contained intact or destroyed organisms. In the opinion of these authors, the local as well as general symptoms are due to endotoxin liberated when macrophages and leukocytes destroy salmonellae.

However, since the experiments with enteral administration of salmonella endotoxins gave no results, attempts have been made to detect other noxious substances secreted by salmonellae into the nutrient medium which might be dangerous when administered orally. Substances fulfilling to some extent the requirements mentioned, were described by several investigators (Branham et al., 1928; Savage, 1933; Raynaud, 1946, 1947; Mesrobeanu et al, 1961) though the authors themselves do not insist on their immediate importance in the pathogenesis of salmonella enteritis. Pointing out that such enteritis closely resembles cholera in its clinical manifestations, Giannella et al. (1973*a*) prove that salmonellae do not produce any enterotoxins which might be correlated with the major signs of cholera infection. Meanwhile the experiments performed on rabbit intestinal loops (see Chapter 22) suggest the possibility of salmonellae producing substances closely resembling enterotoxins in their effect. Sakazaki et al. (1974*c*) suggest that the enterotoxic activity of culture filtrates is commonly recognized in *Salmonella* strains as well as in noninvasive enteropathogenic *E. coli*, and that the mechanism of producing gastroenteritis is similar in both groups of organisms. It is difficult to accept this view, since other biologic properties of the organisms mentioned playing important parts in the pathogenesis of the diseases are not similar.

Thus, the pathogenesis of typhoid and other salmonelloses is not fully understood but the results of experiments on animals have supplied important data. Any comparison with human pathology, however, should be made with great caution.

Different ways of challenge dealt with in the preceding chapters, result in salmonella parasitism in macrophage-type cells. The majority of such experiments permit also to study both salmonella penetration through the epithelial lining and the activity of polymorphonuclears destroying these organisms more or less successfully.

The interrelation between salmonellae and phagocytes (macrophages and polymorphonuclears) can be best studied after intranasal challenge of white mice. Although the lung model also demonstrates the passage of organisms through the epithelial cells, this phenomenon is most conspicuous with enteral challenge and in experiments on isolated intestinal loops. Conjunctival challenge, successful in studying shigellae, is less suitable in salmonellosis because only occasional salmonella strains are capable of inducing conjunctivitis and keratitis in guinea pigs.

Enteral challenge of animals with different kinds of salmonellae is suitable for reproducing characteristic lesions of human typhoid fever. However, the development of intestinal lesions in mice, rabbits and other animals has a somewhat different course. This difference makes the understanding of some important peculiarities of typhoid fever rather difficult.

As regards salmonella enteritis, it might be regarded as an infectious disease rather than the outcome of intoxication. The ability of salmonellae to penetrate deep into the mucosa from the intestinal lumen might be in the background of such an infection. At the same time salmonellae are likely to secrete substances with actions similar to those of enterotoxins.

PART IV

CHAPTER 19

ENTEROPATHOGENIC *ESCHERICHIA COLI*

by

E. M. NOVGORODSKAYA and YU. E. POLOTSKY

Coliform bacteria were first described by Escherich (1885, 1886) as microorganisms prevailing in the intestinal flora of diarrhoeal and healthy infants. Owing to significant improvement of bacteriological culture techniques, it has become known that non-sporing, strictly anaerobic bacteria predominate in the human faecal flora, accounting for more than 99 per cent of the total organisms (Hill and Drasar, 1975). Among aerobic bacteria, which constitute a small proportion (1 per cent) of the intestinal flora, *E. coli* organisms are the most numerous. Despite their relatively scanty population they seem to play a very important role in human physiology and pathology (Hill and Drasar, 1975).

As shown in Chapter 1, the intestinal flora is favourable and even necessary for the host. It contributes to the development of the normal morphological structure and activity of the intestine; it also helps the development of non-specific immunity which is necessary for host defence against infectious agents. According to many investigators, *E. coli* organisms, normally inhabiting the gut, are the main source of antigenic stimulation of the host; they promote the production of "normal" antibodies, cause the hyperplasia of the lymphoreticular tissue, increase the phagocytic mobilization and digesting power of leukocytes and macrophages (Šterzl et al., 1960; Ravin and Fine, 1962; Horowitz et al., 1964; Landy and Weidanz, 1964; Miller et al., 1964; Schaedler and Dubos, 1964; Abrams and Bishop, 1965; Dubos, 1966; Horowitz, 1966; Zykov, 1968; Tlaskalova et al., 1969, 1970; Midtvedt and Trippestad, 1970, 1971; Foo et al., 1974).

At the same time, many intestinal organisms especially *E. coli* may be dangerous for the host under certain conditions. Thus, it has long been known that *E. coli* frequently causes appendicitis, peritonitis, urinary, genital and biliary infections, pneumonia, etc. (Gabrichevsky, 1894; Gilbert, 1895; Orlovsky, 1897; Vincent, 1928; Kauffmann, 1948, 1954; Minkevich, 1950; Tillotson and Lerner, 1966, 1967*a*, *b*; Lachowicz, 1968; Lerner, 1969; Šťastna, 1971, etc.). In animals it causes mastitis, arthritis, salpingitis, pneumonia, etc. (Frey, 1953, 1955; Radostits, 1961; Renk, 1962; Sojka, 1965; Barnum et al., 1967). These diseases are not communicable and develop, as a rule, due to some defect of the anatomical or functional barriers at the expense of the propagation of the "indigenous" microflora; that is why they are called autoinfectious diseases (Anitschkow, 1960).

Similarly, *E. coli* has long been known to cause acute diarrhoeal diseases of human and animal newborns (Hueppe, 1887; Jensen, 1892, 1913; Gilbert, 1895; Christiansen, 1917; T. Smith and Little, 1922, 1927; Adam, 1923, 1927; T. Smith and Orcutt, 1925; Goldschmidt, 1933; Lovell, 1937; Minkevich, 1950, etc.). However, for lack of knowledge of its antigenic structure and in the absence of methods for identification and classification of this vast group of microorganisms, *E. coli* strains isolated from diarrhoeal and healthy humans and animals could not be differentiated. Therefore, the "endogenic coli-infection and coliascension" theory dominated for a long time (Medovikov, 1910; Moro, 1916; Bessau and Bossert, 1919; Bessau, 1928). It was suggested that the resistance of the small intestine to infection diminished as a result of various emaciating diseases or under the influence of overheating, malnutrition etc., and coliforms invaded the upper portions of the small intestine giving rise to characteristic infantile diarrhoea (*"toxische Dyspepsie"*).

A new era began when Kauffmann (1944, 1947, 1954, 1966) and his team had elaborated serological identification methods as well as an antigenic classification scheme for the *Escherichia* genus. It has been stated that *E. coli* strains possessed three kinds of antigens present in their cell wall: (*i*) surface somatic heat-stable O antigen, (*ii*) heat-labile K antigens (encountered in the form of capsules or envelopes classified according to their physicochemical properties as A, B and L antigens);* they cover O antigens which can be revealed only after inactivation of K antigen by heating; (*iii*) flagellar H antigen. Like with *Salmonella*, the unit of *E. coli* classification is a serotype which is determined by comparing the O, K and H antigens of the strain under study with those of an international collection of *Escherichia* type cultures. By the year 1975, 163 O, 99 K and 56 H antigens have been established (I. Ørskov et al., 1973, 1975*a*, *b*, *c*).

Representatives of the first 25 *E. coli* O serogroups as well as some others like O50, O75, O81, O83 etc. are mostly found in the healthy human intestine (Kauffmann, 1954; Taylor, 1966; Bettelheim et al., 1974; F. Ørskov and Sørensen, 1975). Inasmuch as these normal inhabitants of the gut are unable either under normal conditions or in volunteers to induce enteric diseases (Ferguson, 1956; Ewing and Davis, 1961; Ewing et al., 1963; Ralovich et al., 1974; Sakazaki et al., 1974*b*), they are called non-enteropathogenic *E. coli* (NEEC) though representatives of the same O serogroups are commonly isolated in autoinfectious diseases (Kauffmann, 1954; Leppänen, 1958; Ujváry, 1958; Turck et al., 1962; Rantz, 1962; Vosti et al., 1964; McGeachie, 1965; Pryles and Glagovsky, 1965; Taylor, 1966; Drach and Cox, 1967; Borowski et al., 1970; Šťastna, 1971; Brede et al., 1974).

* On the basis of immunoelectrophoretic studies of extracts from all *E. coli* O and K antigen test strains, F. Ørskov et al. (1971, 1972) have revised previous statements about *E. coli* K antigens. They consider B antigens not to be a separate type but a special form of the O antigen lipopolysaccharide molecule. Therefore, it has been proposed that the numbering of new K antigens should be limited to those which are serologically easily differentiated from the lipopolysaccharide antigens (I. Ørskov et al., 1973).

The establishment of the serological classification scheme of *Escherichia* helped the elucidation of the aetiological role of some representatives of this genus in the development of epidemic human enteric (diarrhoeal) diseases. These are called enteropathogenic *E. coli* (EEC), however, this sometimes may cause misunderstanding.

Enteric (diarrhoeal) diseases caused by the organisms of the *Escherichia* genus are heterogeneous both as regards their clinical and epidemiological characteristics as well as the biological properties of their pathogens. Therefore, it is useful to distinguish two categories, namely EEC-I, including pathogens causing diarrhoea in infants, and EEC-II, the causative agents of diarrhoeal diseases of adults and children (Novgorodskaya, 1966*a*, *b*, 1968, 1970, 1973). Similar subdivision was proposed by Japanese investigators (Sakazaki et al., 1967, 1974*a*, *b*), however, they call EEC-I the salmonella-like group of EEC, while the agents of dysentery-like diseases of adults and children are named the shigella-like group of EEC.

INFANTILE *E. COLI* ENTERITIS

This disease (also called *Colidyspepsie*, *Säuglingsenteritis*, epidemic infantile gastroenteritis, infantile diarrhoea, enteric coli infection, etc.) may be varied in its clinical appearance. In severe forms the onset is acute with a sudden rise of temperature, vomiting and profuse, protracted diarrhoea resulting in dehydration and emaciation. Toxicosis is usually present with central and peripheral nervous symptoms and circulatory involvement (*"toxische Dyspepsie"*). In mild forms of *E. coli* enteritis there are only mild general symptoms, there may be no rise of temperature and no vomiting, while diarrhoea is less persistent and protracted; toxicosis is also absent. The clinical course and the treatment of *E. coli* enteritis is dealt with in detail in the literature (Adam, 1923, 1927, 1956; Neter, 1959, 1965; Maslov, 1962; Tsimbler, 1962; Nisevich, 1964; Riley, 1971, etc.). Its epidemiology and the biological properties of the pathogens have also been studied (Novgorodskaya, 1948, 1953*a*, *b*, 1957, 1958, 1959, 1966*a*; Braun, 1953, 1956, 1957, 1968, 1971; Kauffmann and Ørskov, 1956; Ewing, 1956, 1962, 1966, 1968, 1969; Ocklitz, 1956; Taylor, 1959, 1961, 1966; Khazenson, 1960, 1964, 1968, 1970; Ewing and Davis, 1961; Sedlak and Rische, 1961; Edwards and Ewing, 1962, 1972; Yakhnina et al., 1962; Ewing et al., 1963, 1972; Golubeva, 1964; Lachowicz, 1968). A number of serotypes of EEC-I have been recognized to be the causative agents of infantile *E. coli* enteritis (Table 19-I).

It is noteworthy that O antigens of EEC-I are similar to somatic *Salmonella* antigens in chemical structures as well as serologically (Braun et al., 1954; Kauffmann, 1954; Westphal et al., 1960; Lüderitz et al., 1966; F. Ørskov et al., 1967). Thus, EEC O44 bear an antigenic relationship with *Salmonella* of O6 serogroup; and EEC O111, O55 and O86 O antigens are identical with *Salmonella* O antigens of O35, O50 and O43 serogroups, respectively. In a number of other EEC-I antigenic relations to *Salmonella* are less evident.

TABLE 19-I

Enteropathogenic E. coli of the first category (EEC-I) causing infantile E. coli enteritis

Most frequent	Less frequent	Rare
O111 : K58(B4)	O18 : K76(B20)	O4 : K3(L)
O55 : K59(B5)	O20 : K84(B)	O6 : K13(L)
O26 : K60(B6)	O33 : K(B)*	O25 : K3(L)
O127 : K63(B8)	O44 : K74(L)	O25 : K11(L)
O119 : K69(B14)	O86 : K61(B7)	O91 : K2(L)
	O86 : K62(L)	O114 : K90(B)
	O125 : K70(B15)	O142 : K86(B)
	O126 : K71(B16)	O146 : K87(B)
	O128 : K67(B12)	O153 : H7^{+}
	"408"**	O158 : H23^{++}
		O159 : H20^{++}

* Serotype "9" (Novgorodskaya et al., 1960) was identified as 033: K (B) by F. Ørskov (personal communication).
** Serotype "408" (Novgorodskaya, 1948, 1953*a*, *b*), still unidentifiable in available OK antisera.
$^{+}$ F. Ørskov et al. (1972).
$^{++}$ I. Ørskov et al. (1975*a*).

Not so long ago *E. coli* enteritis was the most widespread enteric disease in infants, frequently giving rise to epidemic outbreaks in maternity homes, children's hospitals and nurseries. In the 'forties and the 'fifties the lethality in *E. coli* enteritis reached 5 per cent (Novgorodskaya, 1959), even amounting to 50–80 per cent during some outbreaks (Gulkevich, 1966; Taylor, 1966). However, in recent years the morbidity and especially the lethality of *E. coli* enteritis decreased markedly and the disease usually runs a milder course (Taylor, 1966, 1971; Braun, 1968, 1971; Khazenson et al., 1968; Neter, 1971). The reason for this phenomenon might be found in the combined effect of antiepidemic and hygienic measures and rational antibiotic therapy (Khazenson et al., 1968). At the same time the virulence of circulating agents has apparently also decreased (Taylor, 1966). However, severe cases still occur, as e.g. the outbreak of infantile *E. coli* O142 enteritis with fatal cases described by Kennedy et al. (1973).

Comparative studies have shown that the severity of the clinical course of *E. coli* enteritis is connected with the general condition of the infant and with the pathogenic serotype. The most severe form of the disease was caused by EEC O111 : K58(B4) : [H2], O55 : K59(B5) : [H6], O127 : K63(B8) : H–, especially in premature infants, in those weakened by various somatic diseases and in bottle-fed babies (Braun, 1956, 1957; Novgorodskaya, 1958; Taylor, 1959, 1961, 1966; Khazenson, 1960, 1964, 1970; Tsimbler, 1962; Golubeva, 1964). Serotypes EEC O111 : K58(B4) : H12, O20 : K84(B): H34, O26 : K60(B6) : [H11], O33 : K.(B) : H6, O44 : K74(L) : H34 and a number of other serotypes were mostly associated with the mildest forms of the disease (Taylor, 1959; Novgorodskaya et al., 1960, 1963, 1964*b*, 1970; Khazenson, 1970).

Beside the essential differences some authors have pointed out similarities between *E. coli* enteritis and salmonellosis in clinical manifestations, in the positivity of tests for demonstration of the pathogens in the blood and viscera, and in some other respects (Novgorodskaya, 1953*a*, *b*, 1959; Braun, 1956; Cruickshank et al., 1966; Sakazaki et al., 1967, 1974*a*, *b*, *c*).

In infants who died of *E. coli* enteritis bacterial counts revealed enormous amounts of EEC in the upper portion of the small intestine (Moro, 1916; Adam, 1923, 1927, 1956; Novgorodskaya, 1948, 1953*a*, *b*; Thomson, 1955). Multiplication of EEC in the small intestines was demonstrated by Thomson (1955) by means of tubing of affected infants. The aetiological role of EEC in the development of *E. coli* enteritis in infants has also been proved by challenging infants—a rather hazardous method (Neter and Shumway, 1950; Hiroki, 1953). EEC-I caused enteric diseases in adult volunteers, but as will be shown later, the symptoms did not resemble infantile *E. coli* enteritis.

At autopsy of infants who died of *E. coli* enteritis, either no evident intestinal alterations are found or occasionally there are slight signs of catarrh in the small intestinal mucosa with erosions and even small ulcers. The findings are regarded as non-specific since similar lesions are present in other intestinal and non-intestinal diseases (Vishnevetskaya et al., 1960; Mironchik, 1961; Essbach, 1961; Rho and Josephson, 1967, etc.). Most pathologists still believe *E. coli* enteritis to be a focal recurrent inflammatory process in the small intestine (and even in the proximal colon) with a protracted persistent course (Adam and Froboese, 1925; Ilgner, 1956; Polonskaya and Darkshevich, 1959; Zarudin, 1961; Tarasova, 1962, 1963; Alferyeva and Zakharova, 1964; Tikhonova and Nedozorova, 1965; Tsinserling et al., 1968; Kennedy et al., 1973, etc.). Intestinal vascular lesions (oedema, haemorrhages) are also considered to be characteristic. During protracted *E. coli* enteritis sclerosis and thinning of the mucosa, and occasionally intestinal pneumatosis may be seen. Marked purulent inflammation is not commonly observed in *E. coli* enteritis unless secondary infection is superimposed.

Rapidly developing post-mortem changes destroying the surface epithelium and altering the histological picture prevent the histological study of the bowels and particularly revealing the pathogen in the gut (Adam and Froboese, 1925; Ilgner, 1956; Voino-Yasenetsky and Zhabotinsky, 1970). At early autopsies of infants who died at the initial stage of *E. coli* enteritis, Adam and Froboese (1925) and Ilgner (1956) revealed large accumulations of Gram-negative bacilli adhering to the epithelial lining of the small intestinal mucosa forming a border.

Ilgner (1956) who had a vast autopsy material at his disposal pointed out that superficial multiplication of organisms resulted in the destruction of the brush border and apical cytoplasm. At subsequent stages of the disease he noted epithelial derangement, infiltration with polymorphonuclears as well as shedding or destruction of epithelial cells. In such cases the organisms were found in deeper layers of the mucosa where marked oedema and moderate infiltration with leukocytes, lymphocytes and histiocytes were apparent.

Similar changes were reported by Afanasyeva (1959, 1962, 1964). Drucker et al. (1970) using the fluorescent antibody technique proved that organisms in histological sections belonged to the same serogroups of *E. coli* as had been isolated from the corresponding intravital faecal cultures. Drucker and co-workers were even able to recover the pathogenic agent from sections of infants not yielding positive faecal cultures ante mortem. Moreover, these investigators frequently found EEC not only on the epithelial lining and in the lamina propria, but also in the mesenteric lymph nodes, the blood and the viscera of infants who died of *E. coli* enteritis.

Most workers attach great importance to the ability of EEC-I to grow on the surface of the intestinal epithelium.* This phenomenon has been the subject of numerous experimental studies dealt with in Chapter 22.

Newborns and infants are most susceptible to both *E. coli* enteritis and salmonellosis (Novgorodskaya, 1953*a*, *b*, 1959). In adults, as a rule, the disease does not develop in response to infection with EEC; this apparently is due to a specific immunity that had developed in early childhood (Braun, 1957; Taylor, 1959; Sedlak and Rische, 1961; Winter, 1974).

There are, however a few descriptions of food- and water-borne outbreaks of acute gastroenteritis in adults caused by EEC O86, O111, etc. (Costin, 1963; Costin et al., 1964; Sakazaki et al., 1967, 1974*a*; Schroeder et al., 1968). Occasionally EEC O111, O55, O26 and others were also revealed in sporadic cases of acute enteritis in adults (Stevenson, 1950; McNaught and Stevenson, 1953; Perepelkin et al., 1963; Sakazaki et al., 1967, 1971, 1974*a*).

When feeding adult volunteers with very large doses of EEC O111, O55, O86, O127, O44 and O142 (Kirby et al., 1950; Braun and Henckel, 1951, 1952; Braun and Resemann, 1952; Ferguson and June, 1952; June et al., 1953; Fukumi and Kosakai, 1953; Koya et al., 1954*a*, *b*; Wentworth et al., 1956; Ralovich et al., 1974; Sakazaki et al., 1974*b*) the development of acute gastroenteritis (or enteritis) with symptoms of intoxication varying in severity (occasionally absent) was observed. The infection had a dramatic course at first but then rapidly subsided. Challenge with the same amounts of NEEC or even larger doses of killed EEC did not induce any diseases in volunteers. Administration of EEC into various portions of the colon had no effect either (Koya et al., 1954*a*). According to Ferguson (1956), experiments with EEC in adults resulted in a disease resembling "food toxinfection" but not infantile *E. coli* enteritis.

Obviously, imperfect defence mechanisms of infants (Berman, 1957, 1961; Maslov, 1962), the "immaturity" of their intestine and insufficient non-specific immunity (Ilgner, 1956; Sprinz, 1962; Sunshine et al., 1971) play an important part in the development of *E. coli* enteritis. Passive immunity, i.e. antibodies obtained from the mother, is of great importance in the defence of newborns against pathogenic microorganisms. Before delivery antibodies are passed to the foetus through the placenta, after

* Tsinzerling et al. (1968) showed that in one of their micrographs fluorescent bacteria markedly adhered to the epithelial surface. In spite of that they consider EEC to multiply only inside the gut lumen.

birth with the mother's colostrum and milk. The role played by maternal antibodies has been shown by epidemiological and immunological studies (Khazenson, 1967, 1968). In breast-fed infants exposure to EEC does not necessarily result in illness (Mata and Urrutia, 1971).

Khazenson (1967, 1968) as well as Khazenson and Gennadyeva (1969) have shown that maternal blood serum contains 7S IgG, corresponding to antibodies against agents of air-borne infections and dysentery, and 19S IgM corresponding to antibodies against EEC. However, while IgG immunoglobulins can pass the placental barrier and then to the intestine from the infant's blood, IgM macroglobulins are unable to pass through the placenta.

Powerful defensive antibodies, "secretory" 11S immunoglobulins of the IgA class, providing passive immunity of infants against EEC-I, shigellae, viruses and other microorganisms, enter the infantile alimentary tract with maternal colostrum and milk (Tomasi, 1970; South, 1971; Michael et al., 1971; Wernet et al., 1971; Tomasi and Grey, 1972; Gennadyeva et al., 1973; Khazenson et al., 1973). They are not broken down by digestive processes and do not pass into circulation but are adsorbed onto the epithelial cell surface (South, 1971; Gennadyeva and Khazenson, 1973; Gennadyeva et al., 1973; Khazenson et al., 1973).

IgA molecules consist of the main secretory 3S–4S component determining their antigenic specificity and produced by epithelial cells and two 7S proteins produced by plasma cells of the subepithelial lamina propria. The secretory component of IgA is observed immediately after birth but whole IgA molecules are only seen after plasma cells begin to appear in the lamina propria by the end of the third week of life. Simultaneously with the increase of the plasma cell number, IgA level in the intestine rises during the first year of life and becomes still higher in the second and third years. Later this process is slowed down and the adult level is reached during puberty (South, 1971). Tomasi (1970) and other authors believe that this process reflects the development of the response to intestinal microflora, especially to *E. coli*. However, South (1971), Khazenson et al. (1973) and others emphasize that both IgA of maternal milk and IgA of the infantile intestine possess a specific anti-EEC-I effect.

Thus, the infant only becomes able to defend itself against EEC-I after the maturation of intestinal defence mechanisms, i.e. when IgA antibodies have formed and accumulated by the end of the first year of life.

DYSENTERY-LIKE DISEASES OF ADULTS AND CHILDREN

These diseases are far less known than infantile *E. coli* enteritis and have been studied only recently. In contrast to *E. coli* enteritis, they occur in infants infrequently and like dysentery mostly affect adults and children over one year of age (Novgorodskaya et al., 1964*a*, 1966*a*, *b*; Kolta, 1965; Trifonova, 1965; Novgorodskaya, 1966*a*, *b*, *c*, 1968, 1970; Sakazaki et al., 1967; Trabulsi et al., 1967; Naftulyeva et al., 1970). Frequently they show the symptoms of enterocolitis and are clinically indistinguishable from

mild forms of dysentery but are less contagious than *Sh. sonnei* dysentery (Novgorodskaya, 1970).

Epidemiologic studies have shown that *Escherichia* enterocolitis also spreads by contact but not so often as dysentery. Infections conveyed by water or food are more common indicating the importance of huge doses of the pathogen (Kolta and Deák, 1962; Novgorodskaya,1968, 1970; Sapozhnikova et al., 1970; DuPont et al., 1971; Marier et al., 1973).

The intestinal alterations are not known because fatal outcome has not been described. Sigmoidoscopy of patients and infected volunteers has revealed changes similar to those found in mild forms of dysentery (Novgorodskaya et al., 1964*a*; Formal et al., 1971*a*; DuPont et al., 1971; Marier et al., 1973).

The agents of *E. coli* enterocolitis differ from pathogens of infantile *E. coli* enteritis in their biological properties. However, infectious pathology for the former has not so far been worked out. Information on these kinds of organisms is rather scanty; the best known type is EEC O124.

In Great Britain a severe food-borne outbreak of acute diarrhoeal disease was reported by Hobbs et al. (1949) among schoolchildren. The organism named "paracolon 411" caused disease in adult volunteers after intake of contaminated food, and an unintentional laboratory infection was also noted. Ewing (1953) determined "paracolon 411" as *E. coli* O124 : K72 (B17); he had previously isolated six cultures of the same organism from adult patients with diarrhoea (Ewing and Gravatti, 1947). The fact that antigen O124 proved to be identical with the somatic antigen of *Shigella dysenteriae* 3 (Wheeler and Stuart, 1946; Ewing, 1953) deserves attention.

Severe water- and food-borne outbreaks of dysentery-like diseases of adults and children induced by EEC O124 were described in Hungary (Kétyi et al., 1958; Borián et al., 1959; Kubinyi, 1959, 1960; Lányi et al., 1959; Rédey and Csizmazia, 1960; Kolta and Deák, 1962; Hanny and Horváth, 1963; Kolta, 1965, 1967), in the USSR (Novgorodskaya et al., 1964*a*, 1966*b*; Novgorodskaya, 1970; etc.) and in the USA (Marier et al., 1973). Sporadic cases and restricted small-scale outbreaks of dysentery-like enterocolitis caused by EEC O124 were also observed in Japan (Sakazaki and Namioka, 1957; Matsumoto and Hara, 1959; Suzuki, 1959; Sakazaki et al., 1967), in Rumania (Costin, 1963; Costin and Olinici, 1965), in Czechoslovakia (Aldova et al., 1968), Bulgaria (Manolov, 1968), Brazil (Trabulsi et al., 1967), India (Sakazaki et al., 1971), in the United Kingdom (Rowe et al., 1974) and in a number of other countries.

It has been shown that EEC O124, similarly to shigellae, induce keratoconjunctivitis in guinea pigs (Rédey and Csizmazia, 1960; Stenzel, 1962*b*, *c*, 1965; Serény, 1963; Novgorodskaya et al., 1964*a*; Trabulsi, 1965). Rédey and Csizmazia (1960) even suggested to use Serény's test for isolation of EEC O124 and other similar organisms from patients.

EEC O124 is the most common agent of dysentery-like diseases and is spread throughout the world. But, in addition to EEC O124, a number of other EEC-II inducing diseases similar to dysentery are known. They belong to 11 other serologic groups of *Escherichia* (Table 19-II). These EEC-II, similarly to shigellae, produce a positive keratoconjunctival test in guinea

pigs* (Vörös et al., 1964; Novgorodskaya, 1966*b*, 1968, 1970; Sakazaki et al., 1967, 1974*b*; Trabulsi et al., 1967; Aldova et al., 1968; Ciufeco, 1970; Polotsky et al., 1971).

Many of these EEC-II (like EEC O124) possess antigens related to those of shigellae. Thus, the identity of O antigens of EEC O112a112c and *Sh. dysenteriae* 2 (Ewing et al., 1952), EEC O129 and *Sh. flexneri* 5 (Seeliger, 1955; Grinberg, 1968), EEC O135 and *Sh. flexneri* 4b (Rauss and Vertenyi, 1956), EEC O143 and *Sh. boydii* 8 (Okada et al., 1958; Hara and Matsumoto, 1959; Vörös et al., 1964; Novgorodskaya et al., 1968*a*) has been established.

The following organisms have been isolated and described as enteropathogenic agents after the establishment of the extended *E. coli* antigenic scheme: O115 (Trabulsi et al., 1965, 1967; Trabulsi and Fernandes, 1969)**, O129 (Seeliger, 1955), O135 (Rauss and Vertenyi, 1956), O136 (Sakazaki and Namioka, 1957), O143 (Okada et al., 1958; Hara and Matsumoto, 1959; Rédey and Csizmazia, 1960; Vörös et al., 1964), O144 (Yamagata et al., 1956; Harada et al., 1959; Aldova and Lazničkova, 1967; Fernandes and Trabulsi, 1967; Sakazaki et al., 1967; Aldova et al., 1968), O152 (Sakazaki et al., 1974*a*, *b*).

However, EEC O25, O28a28c, O32 and O112a112c had been known long before the modern techniques of *Escherichia* identification were developed. In view of their close resemblance to dysentery agents in pathogenic properties and less active fermentative capacities, it is not surprising that they had initially been described as shigellae. Thus, EEC O25 (serotype 147, or 145–46, or Alkalescens-Dispar 02) had been described as *Shigella alkalescens* Andrews or *Sh. tieté* (De Assis, 1939*a*, *b*, 1948*b*); EEC O28a28c (serotype 792) was referred to as "Katwijk" type of shigellae or *Sh. scholtensii* (Scholtens, 1940); EEC O32 was reported as serotype A-12 of shigellae (Sacks, 1943), later it was even called *Sh. boydii* 14 (Carpenter, 1961, 1966; Trifonova, 1965), and finally, EEC O112a112c was termed *Shigella guanabara* (De Assis, 1948*a*). Meanwhile detailed serological and biochemical studies of the properties of all these strains have shown them to be *Escherichia* (Ewing and Hucks, 1950; Ewing and Kauffmann, 1950; Ewing et al., 1950, 1952, 1958, 1963; Seeliger, 1952, 1954; Ewing and Tanner, 1955; Taylor and Charter, 1955; Nakanishi et al., 1956, 1958; Hara and Matsumoto, 1958; Avdeeva et al., 1966; Ewing, 1966; Sakazaki et al., 1967, 1974*a*; Trabulsi et al., 1967; Avdeeva, 1968; Smirnova et al., 1975).

Nevertheless, up to now opinions have differed as to the taxonomic position of several organisms, in particular of EEC O25 and O28a28c which occur not infrequently. Manolov (1958, 1959), Trifonova (1960, 1963, 1965) and Stenzel (1962*a*, 1964) believe that epidemiological and clinical data and especially the ability to elicit keratoconjunctivitis in

* Except for EEC O129 and O135, but these organisms have not been tested when freshly isolated (Vörös et al., 1964; Grinberg, 1968).

** This organism has recently been proved to belong to *E. coli* O152 (Stenzel, 1975).

TABLE 19-II

Enteropathogenic E. coli of the second category (EEC-II)—agents of dysentery-like diseases of adults and children

Epithelial parasites (Shigella-like)	Showing no epithelial parasitism
O124 : K72(B17)	"Crimea" (O151 : H10 and other H antigens)[b]
O25 : K1 (serotype 147, or 145–46, or Alkalescens-Dispar O2)	
O28a28c : K73(B18) (serotype 792)	O25 (serotype 301)[c]
O32 (serotype A-12)	
O112a112c : K66(B11)	
O115 (serotype 185)[d]	
O129	
O135	
O136 : K78(B22)	
O143 : Kx1(B)	
O144 : Kx2(B)	
O152[a]	

[a] Sakazaki et al. (1974*a*, *b*).
[b] Novgorodskaya et al. (1968*b*). Golubeva and Kiseleva (1974) identified these organisms as O151 : H10 and O151 : H11. Sakazaki et al. (1974*a*, *b*) described similar EEC O151 : H52.
[c] Adeeva et al. (1968).
[d] Stenzel (1975) proved it to belong to *E. coli* O152.

guinea pigs suggest that these organisms belong to the *Shigella* genus.* In 1962 the International Enterobacteriaceae Subcommittee** rejected the suggestion to regard biological tests on experimental animals as the principal criterion of *Enterobacteriaceae* classification and confirmed the generally accepted view that identification of *Enterobacteriaceae* should be based on biochemical and antigenic characteristics (Ewing, 1966; Edwards and Ewing, 1972).

Szturm-Rubinsten et al. (1964, 1967), Piéchaud et al. (1965) and Szturm-Rubinsten and Piéchaud (1971), pointing out that EEC O25 and O28a28c are similar to shigellae in their pathogenic properties but differ from them in a more pronounced saccharolytic activity, regard these organisms as intermediates between *Shigella* and *Escherichia* ("Alkalescens-Dispar" group) and even suggest to consider them as a special group "parashigella". The same view was held by Shmilovitz (1970) who included EEC O25 in a new group: "intermediate-Shigella-Coli-Alkalescens-Dispar". The same opinion was voiced by Aldova et al. (1968); these investigators regarded not only EEC O25 and O28a28c, but the whole series of agents associated with dysentery-like diseases, as intermediates.

* Manolov (1968) even suggested to name EEC O124 *Shigella intermedia* or *Escherichia shigelloides* while Stenzel (1962*a*, 1965) considered EEC O124 and O143 to be escherichiae but not shigellae and proposed to rank them into a new "dysentery-coli group".

** *Intern. J. Bacter. Nomencl. Taxonomy*, **14**, 16 (1964).

Experimental studies to be dealt with later (Chapters 20–22) have shown that the pathogenicity of organisms of the shigella-like EEC-II group is due to bacterial intraepithelial parasitism.

At the same time, the pathogenicity of other serotypes of *E. coli* that also induce dysentery-like diseases (though the mildest forms) in adults and children is not clear. The fact is that they do not bear any antigenic relationship to shigellae and are unable to elicit keratoconjunctivitis in guinea pigs. Two serotypes can be referred to this group of EEC-II: *E. coli* "Crimea" first isolated during an epidemic in the Crimea (Novgorodskaya et al., 1968*b*), and serotypes 301 of *E. coli* O25 serogroup isolated during an outbreak in Leningrad (Avdeeva et al., 1968). EEC "Crimea" was demonstrated to bear antigenic relationship not to shigellae but to salmonellae of the O47 serogroup; it had flagellar antigen H10 of *E. coli* and no K antigen (Novgorodskaya et al., 1968*b*). Golubeva and Kiseleva (1974) have recently identified EEC "Crimea" organisms as O151:H10 and O151:H11. Sakazaki et al. (1974*a*, *b*) have isolated and tested three similar strains EEC O151:H52 from adult patients with diarrhoea.

The aetiological role of EEC "Crimea" and serotype 301 in the diarrhoeal diseases was proved by clinical and microbiological investigations, epidemiological analysis, immunological examinations and pathogen circulation studies. In experiments these organisms multiplied in monolayer tissue cultures in contrast to NEEC and similarly to all enteropathogens (Arbuzova, 1970*a*, 1972). In addition, following multiple passages, serotype 301 multiplied in the lung tissue of intranasally challenged mice; it did not, however, penetrate the bronchial epithelium and caused almost no deaths in mice (Dragunskaya, 1970*a*; Smirnova, 1970; Smirnova and Dragunskaya, 1973*a*, *b*). Nevertheless, the above data failed to reveal the essence of the pathogenic properties of the organisms.

CHOLERA-LIKE ESCHERICHIOSIS OF ADULTS AND CHILDREN

It is well known that cholera vibrios cannot always be isolated from patients with typical "cholera syndrome", i.e. from gastroenteritis characterized by severe fluid loss due to profuse diarrhoea with "rice water" stools (McIntyre et al., 1965; Carpenter et al., 1965, etc.). A group of American and Indian cholera experts working in Calcutta have recently revealed that certain strains of *E. coli* multiply in the small intestine of some patients with cholera syndrome. They were able to prove this by perfusion of the small intestine (Glew et al., 1969; Gorbach, 1970; Gorbach et al., 1971; Sack et al. 1971). The strains isolated by them were defined as EEC O6:H16, O15:H11, O25:H42, O78:H12, O126:K71(B16):H12, O126:K?:H12 and several untypable ones (Gorbach et al., 1971). Like cholera enterotoxin, broth culture filtrates of these strains caused fluid accumulation and dilatation of isolated loops of rabbit small intestine. However, unlike in the former, the "vascular permeability factor" was absent in these enterotoxins of *E. coli* (as shown by intradermal injection to rabbits) and they

also differed from choleragen in their heat stability (Etkin and Gorbach, 1971).

Judging from biopsy specimens of small intestinal mucosa, such EEC producing enterotoxins, similarly to cholera vibrios, neither impaired the epithelium nor penetrated the mucosa (Gorbach, 1970; Banwell et al., 1971). As shown by clinical and experimental observations, the symptoms developed when the organisms multiplied in the upper portion of the small intestine, where their enterotoxins caused the abundant secretion of an isotonic fluid of the same chemical composition as found in cholera (Gorbach, 1970; Gorbach and Levitan, 1970; Banwell et al., 1971; Etkin and Gorbach, 1971).

It has been shown in recent experiments that enterotoxins of the above *E. coli* strains stimulate adenyl cyclase in the intestinal mucosa which results in fluid hypersecretion by epithelial cells of the small intestine (Al-Awqati et al., 1972; D. J. Evans jr. et al., 1972; Levitan et al., 1972; Pierce and Wallace, 1972; Guerrant et al., 1973; Sherr et al., 1973; Kantor et al., 1974*a*). These enterotoxins of *E. coli* were proved to contain both heat-stable and heat-labile components (D. G. Evans et al., 1973; D. J. Evans jr. et al., 1973; Nalin et al., 1974). They even possessed the "permeability factor" in concentrated preparations (D. J. Evans jr. et al., 1973). Strong immunologic cross-reactions of enterotoxins from *E. coli* and *Vibrio cholerae* have been revealed only for heat-labile *E. coli* enterotoxins (D. G. Evans et al., 1973; Pierce, 1973; N. W. Smith and Sack, 1973; Gyles, 1974*a*, *b*; Likhoded et al., 1974; Nalin et al., 1974; Sack et al., 1974). At the same time heat-stable *E. coli* enterotoxin differed from choleragen both immunologically and pathophysiologically, because the former had an earlier, weaker and shorter effect and it impaired glucose and glycine absorption (Pierce, 1973; Sherr et al., 1973; Nalin et al., 1974).

The so-called "travellers' diarrhoea", frequently observed in persons arriving in tropical countries, bears some resemblance to cholera (Gorbach, 1970, etc.). Rowe et al. (1970) described a new EEC strain (O148:H28) during an epidemic among British soldiers in Aden. In a case of unintentional laboratory infection with this EEC, one of the technical assistants developed profuse watery diarrhoea with symptoms of intoxication but without a rise of temperature.

Formal et al. (1971*a*) and DuPont et al. (1971) isolated O148:H28 and another agent, O6:H16, from American soldiers suffering from a similar disease in South Vietnam, and obtained cholera-like enterotoxins from these strains. Living cultures of these organisms as well as enterotoxins isolated from them (in filtrates and lysates) caused fluid accumulation resulting in the dilatation of ligated loops of the small intestine of rabbits. After oral challenge of starved guinea pigs, monkeys and adult volunteers with enterotoxigenic EEC, mild intestinal disorders developed with signs of impairment of small as well as large intestinal function. Unlike EEC-II O124 etc. and shigellae, enterotoxigenic EEC O6 and O148 neither induced keratoconjunctivitis in guinea pigs nor penetrated the epithelial cells in other experimental models. Strains of EEC O148:H28 and O148:H7, isolated during a gastroenteritis outbreak in Leningrad, showed the same

properties (Avdeeva et al., 1975; Kleganov et al., 1975; Chakhutinskaya et al., 1975).

In addition to the above pathogens of cholera-like diseases, a somewhat different group of enterotoxigenic EEC has been described. Some strains of *E. coli* O112a112b, O6, O1 and other serogroups were repeatedly isolated in sporadic cases and small-scale food-borne outbreaks of "gastroenteritis" or "undifferentiated diarrhoea" of adults and children in Leningrad (Arbuzova, 1970*a*, 1972; Avdeeva et al., 1973*a*, *b*, *c*; Vasser and Semenova, 1973). Epidemic outbreaks of mild cholera-like gastroenteritis caused by the same strains O112a112b and O1 were also observed (Arbuzova and Krivonosova, 1973; Balabanova et al., 1973; Karyagina and Raskina, 1973; Safonova 1973). These organisms did not induce keratoconjunctivitis in guinea pigs though *E. coli* O112a112b and *Shigella boydii* 15 were found to possess identical O antigens, while *E. coli* O1 was referred to the Alkalescens-Dispar group in which Sakazaki et al. (1967) had found shigella-like EEC. At the same time these organisms showed unusual toxicity in experiments with intranasal challenge of mice. They caused the death of almost all the mice during the first hours after challenge (Avdeeva et al., 1970*a*, 1973*a*, *b*, *c*). As shown by morphological studies (Chapter 21), they produced severe serous-haemorrhagic lung oedema and did not penetrate the epithelial cells (Dragunskaya et al., 1972, 1973). It has been demonstrated in experiments on ligated loops of rabbit small intestine (Chapter 22) that the ultrasonic lysates of some strains mentioned have an enterotoxigenic effect which disappears after heat treatment.

The lung model permitted to reveal certain members of the group of enterotoxigenic EEC, pathogens of acute diarrhoea of adults and children (Avdeeva et al., 1973*a*). One would assume that enterotoxigenic *E. coli* strains isolated by Gorbach et al. (1971), DuPont et al. (1971) and other investigators would also cause death of mice. However, T. A. Avdeeva and co-workers have not observed death of the animals in lung model experiments with living cultures, filtrates and lysates of enterotoxigenic *E. coli* strains obtained from S. L. Gorbach and H. L. DuPont and of their own isolates of O148:H28 and O148:H7 (Kleganov et al., 1975). These EEC, unlike the strains O1, O6, O112a112b and others, did not induce fatal serous-haemorrhagic lung oedema. The difference in the properties of the two varieties of enterotoxigenic *E. coli* (apparently associated with their different effect on the blood vessels) should be studied in special comparative investigations.

Most recently new enterotoxigenic EEC have been described, namely new members of serogroups O6 and O78 (Sakazaki et al., 1974*a*, *b*), O78:H11, O85:H7, O16:H39, O128:H21 (N. W. Smith and Sack, 1973; Sack et al., 1974), O27:H20 (Shore et al., 1974; Sakazaki et al., 1974*a*, *b*). Gorbach and Khurana (1972) reported on weak enterotoxigenic strains of *E. coli* O23, O75, O119 encountered in infants and children with diarrhoea in Chicago. Information on some of the new enterotoxigenic strains is frequently incomplete; epidemiological data are often absent, although detection of enterotoxigenicity in animal experiments in itself cannot be regarded as evidence of pathogenicity (Sakazaki et al., 1974*b*).

There are some descriptions of quite new *E. coli* O group representatives, namely O154:K94:H4, O155:H9, O156:H47, O160:H34, O161:H54, isolated from adults with "travellers' diarrhoea" in the Middle East (I. Ørskov et al., 1973, 1975*a*). However, the results of their enterotoxigenicity tests are not yet known. The list of EEC causing cholera-like diseases in adults and children is given in Table 19-III.

In animals further serological groups and types of *E. coli* play an important part in the normal development of the host and in the aetiology of various autoinfectious diseases. It is especially important that special pathogenic EEC serotypes are responsible for a whole group of infectious diseases designated with the common term "colibacillosis".

COLIBACILLOSIS OF ANIMALS

According to Barnum et al. (1967) the term colibacillosis may be misleading. Autoinfectious diseases such as mastitis or urinary tract infection induced by non-enteropathogenic serotypes (NEEC) of *E. coli* (or other organisms, *Enterobacter* and others) are not included here. Colibacillosis as a term encompasses syndromes occurring in newborn and older animals (e.g. weaned swine) which, as currently available evidence indicates, are caused by certain enteropathogenic *E. coli* serotypes. These diseases are extremely widespread. They affect domestic and laboratory animals, poultry, monkeys etc., causing thereby considerable economical losses. Their agents are EEC serotypes non-pathogenic for man and mostly specific for each animal species (Taylor, 1961; Ewing et al., 1963; Gay, 1965; Sojka, 1965; Barnum et al., 1967; H. W. Smith and Halls, 1967*a*; Glantz, 1971).

Colibacillosis is manifested by a variety of syndromes studied best in calves and in young swine (Gay, 1965, 1971; Sojka, 1965; Barnum et al., 1967; Barnum, 1971).

In calves colibacillosis mostly develops during the first two weeks of life especially when feeding with colostrum has not been started on the first day after birth or only with a delay. Three forms of colibacillosis are distinguished, namely septicaemic, enteric-toxaemic and enteric.

Colisepticaemia occurs during the first few days after birth; it usually has a fulminant course with fever and increased respiratory and heart rates. The affected calves die quickly. Less frequently there may be a more prolonged course with localized purulent or fibrino-purulent lesions (polyarthritis, leptomeningitis, omphalophlebitis, nephritis). Diarrhoea may not be observed in colisepticaemia. EEC do not multiply in the bowel; the pathogen is isolated in pure culture from the blood and viscera.

The "enteric-toxaemic" form of colibacillosis is manifested as a sudden and usually fatal collapse. Diarrhoea is usually absent, presumably because the time is too short for diarrhoea to develop (Barnum et al., 1967). Abundant EEC multiplication in the small intestine is evident, but no bacteraemia is observed.

Enteric colibacillosis, or *E. coli* enteritis of calves, is characterized by prolonged emaciating diarrhoea and dehydration. This form of colibacillo-

TABLE 19-III

Enteropathogenic E. coli—causative agents of cholera-like diseases of adults and children

Enterotoxin producing	Ability to produce enterotoxins has not yet been studied
O6 : H16 and other H antigens [1, 2, 3, 4, 5, 6]	O112a112b : K68(B13) : H18 [6]
O15 : H11 [1, 4, 5]	O1 [6]
O16 : H39 [4, 5]	O154 : K94 : H4 [10]
O25 : H42 [1, 4, 5]	O155 : H9 [10]
O27 : H20, H18 [3, 7]	O156 : H47 [10]
O78 : H12, H11 and other H antigens [1, 3, 4, 5]	O160 : H34 [11]
O85 : H7 [4, 5]	O161 : H54 [11]
O126 : K? : H12 [1, 4, 5]	
O126 : K71(B16) : H12 [1]	
O128 : H21 [4, 5]	
O148 : H28, H53, H30, H7 [2, 3, 8, 9]	

1. Gorbach et al. (1971)
2. DuPont et al. (1971)
3. Sakazaki et al. (1974*a*, *b*)
4. N. W. Smith and Sack (1973)
5. Sack et al. (1974)
6, Avdeeva et al. (1973*a*)
7. Shore et al. (1974)
8. Rowe (1974)
9. Avdeeva et al. (1975)
10. I. Ørskov et al. (1973)
11. I. Ørskov et al. (1975*a*)

sis is the most common one, and frequently occurs as a herd problem in enclosed premises. Occasionally almost all the calves are affected and die, but if the diagnosis is made in due time and the therapy and care are appropriate, most of the animals may recover. In the small intestine multiplication of EEC is revealed but invasion beyond the intestinal tract is not a consistent feature (Barnum et al., 1967).

At autopsy of calves that have died of colisepticaemia the intestinal tract is found normal in most of the cases; in some instances there may be a catarrhal enteritis (Barnum et al., 1967). In enteric forms the intestinal tract is distended with fluid; as a result, the intestinal walls are thin, atonic and occasionally congested. In addition, signs of extreme dehydration may be observed. Very little is known about the histological appearance of the affected intestine (most studies on colibacillosis of calves deal with the bacteriology, epidemiology and immunology of the disease). In most cases the bowel is not altered but in some calves foci of a catarrhal, mucopurulent or haemorrhagic inflammation of the small intestinal mucosa can be found (H. W. Smith, 1963; Barnum et al., 1967).

In histological examination of calves sacrificed in an advanced stage of colibacillosis (the enteric form, probably) T. Smith and Orcutt (1925) revealed that the bacilli form layers or films attached to the top plates of the epithelial cells. At this time morphologic changes in the cells are not yet recognizable.* Using fluorescent antibody technique in experimental

* These authors also suggested that the action of *E. coli* in bringing about the local hyperaemia and flux and the general intoxication is probably a result of the absorption of toxin produced and released during multiplication. This was assumed as early as 1925 and has now been confirmed.

E. coli enteritis of calves, Gilka and Salajka (1970*a*, *b*) proved that accumulations of multiplying organisms on the surface of small intestinal epithelium consist of EEC. Challenging newborn calves with EEC (strain O55:B5:H7 was, however, isolated from a case of human infantile gastroenteritis) Staley et al. (1970*a*) observed that the organisms were attached to the epithelial cells, penetrated them and were transported further to the lamina propria and mesenteric lymph nodes.

A whole series of serotypes of serogroups 78, 137, 35, 8, 9, 15, 2a, 101, 103, 115, 86, 26, 55 and others are referred to as agents of colibacillosis of calves (Fey, 1957; Rees, 1958; Gay, 1965; Sojka, 1965; Barnum et al., 1967; Glantz, 1971; Sidorov and Polyakova, 1974). The first three serogroups are believed to be more frequently related to colisepticaemia of calves deprived of colostrum, the rest are associated with *E. coli* enteritis though occasionally they also cause bacteraemia (Rees, 1958; Gay, 1965). Many strains isolated from diarrhoeal calves are referred to as yet untypable *E. coli* serogroups (Myers, 1975).

In pigs EEC induce even a greater variety of colibacillosis syndromes (H. W. Smith and Jones, 1963; Sojka, 1965; Barnum et al., 1967). Coliform enteritis frequently occurs in newborn piglets during the first week of life; the animals infrequently develop septicaemia. These diseases of newborn piglets are rather similar to calf colibacillosis but are induced by other EEC serotypes of O groups 8, 6, 141, 147, 116, 138, 20, 21, 23, 45, 149, 150, 157 and others (H. W. Smith and Jones, 1963; Sojka, 1965; Barnum, 1971; Glantz, 1971; Gyles et al., 1971; Söderlind, 1971; Furowicz and Ørskov, 1972).

Young swine (8 to 12 weeks of age) may develop oedema disease or coliform enteritis during weaning, after vaccination, shipping or if there has been a change in their diet. Oedema disease principally occurs in rapidly growing pigs (while others remain healthy) and is manifested by partial paralysis, muscular incoordination, blindness and extended oedema of the subcutaneous tissue (particularly the eyelids); most animals die within 24 hours.

Coliform enteritis of weanlings (8 to 12 weeks of age) develops under conditions similar to oedema disease but spreads epidemically to almost all piglets of the same age group in the herd. The usual clinical manifestations of this disease are fever, profuse protracted diarrhoea, dehydration, emaciation and toxicosis (occasionally with neurological symptoms or erythema and haemorrhagic rash). The majority of affected weanlings recover.

Bacterial counts used in the enteric forms of colibacillosis and oedema disease of pigs demonstrate great numbers of EEC in the small gut especially in its upper portions; no organisms are found in the blood (H. W. Smith and Jones, 1963; Moon et al., 1966*b*; Barnum et al., 1967). EEC serogroups 138, 139, 141, 147, 149, 8 and others are usually associated with diseases of weanlings (Rees, 1959; H. W. Smith and Jones, 1963; Gay, 1965; Sojka, 1965; Barnum, 1971; Glantz, 1971; Söderlind, 1971, etc.).

At necropsy of newborn piglets that died of *E. coli* enteritis, distension of the intestinal tract with fluid is apparent usually without any signs of congestion and inflammation. In most cases histological studies fail to

reveal any intestinal alterations (H. W. Smith and Jones, 1963; Moon et al., 1966*b*; Barnum et al., 1967). However, occasionally there is catarrhal enteritis, and in cases with a protracted course atrophy of the jejunal villi is observed (Moon et al., 1966*b*, 1970*a*; Barnum et al., 1967; Moon, 1969).

By using fluorescent antibody technique and bacterial staining in investigating the intestinal sections of affected piglets, Arbuckle (1970, 1971) revealed that EEC multiplied on the epithelial lining of the small bowel, adhering to the cells and destroying the mucopolysaccharide layer which covers them. Alterations of the intestine are usually absent in piglets succumbing to colisepticaemia as well as in calves (Barnum et al., 1967).

At necropsy of weanlings that have died of oedema disease, extensive, pronounced serous or haemorrhagic oedema of the gastric and intestinal wall, mesentery, lungs, subcutaneous tissue, brain and spinal cord is found. Occasionally, there are accumulations of serous fluid in the pleural, pericardial and abdominal cavities. Histological examination of the oedematous tissue does not reveal any cellular infiltration (Barnum et al., 1967, etc.).

According to the same authors, at autopsy of weanlings that have died of coliform enteritis the small intestine is not only atonic and distended with fluid but it is also congested. On the surface of the congested mucosa an extensive mucous film is usually seen. There may be petechial haemorrhages on the serosal surface. The mesenteric lymph nodes are enlarged and congested. Histologically, catarrhal, frequently haemorrhagic, enteritis is established. Inflammatory cells accumulate in the lamina propria; on the mucosal surface of the small intestine there are aggregations of sloughed epithelial cells, leukocytes, mucus and fibrin. Occasionally there may be atrophy of intestinal villi. Arbuckle (1970, 1971) found surface multiplication of EEC when examining stained intestinal sections histologically. In experiments on germ-free piglets not only surface multiplication but also penetration of EEC through the epithelial cells into the lamina propria, lymphatic vessels and blood has been observed (Christie, 1969; Drees and Waxler, 1970; Waxler et al., 1971). This finding has not been confirmed by Kenworthy (1970).

Antibodies of maternal milk (chiefly colostrum) are of even greater importance for the protection of newborn animals against EEC than they are for human infants (T. Smith and Little, 1922; Gay, 1965; Barnum et al., 1967). The mechanisms of passive and active immunity of newborn animals against EEC are currently being investigated (Gay, 1965, 1971; Kohler and Bohl, 1966; Fey, 1967, 1971; Kohler, 1967, 1974; Porter et al., 1970, 1974; H. W. Smith and Linggood, 1971, 1972; Rutter and Anderson, 1972; Rutter and Jones, 1973; Cardella et al., 1974; Hill and Porter, 1974). In this respect peculiarities of the permeability of the intestinal epithelial barrier in newborn animals are especially important (T. Smith, 1925; Möllendorff, 1925; Comline et al., 1951; Clark, 1959; Kraehenbuhl et al., 1966, 1967, 1971; Mattisson and Karlsson, 1967; Graney, 1968; Staley et al., 1968, 1969*c*, 1972*a*; Kraehenbuhl and Campiche, 1969; Veress and Baintner, 1970; Clarke and Hardy, 1971; Fey, 1971; Moon, 1972; Rundell and Lecce, 1972; Jeffcott, 1974; Jones, 1974).

Agents of animal colibacillosis have been studied by enteral challenge of newborn (or germ-free) animals and on ligated loops of small intestine of various animal species (H. W. Smith and Halls, 1967*a*; Moon and Whipp, 1971; Waxler et al., 1971; Myers et al., 1975, etc). The experiments have proved that these organisms produce enterotoxins very similar to cholera enterotoxin in the mechanism of their action (H. W. Smith and Halls, 1967*b*; Kohler, 1968, 1971*a*, *b*; Gyles and Barnum, 1969; Moon et al., 1970*a*, *b*, 1971; H. W. Smith and Gyles, 1970*a*; Gyles, 1971; Larivière et al., 1973; Jacks et al., 1973, etc.). These agents produce either only non-antigenic heat-stable (ST) toxins or both ST and antigenic heat-labile (LT) toxins. Both components, ST and LT, represent two forms of the same toxin, the production of which is determined by an episomal factor, the transmissible plasmid *Ent*, which is apparently the same in all agents of swine colibacillosis (H. W. Smith and Gyles, 1970*a*; Gyles, 1971, 1974*a*, *b*; Larivière et al., 1973; Gyles et al., 1974). The ST component is produced by all pathogens of swine colibacillosis, but the LT component only by those strains which produce ST and possess antigen K88 at the same time. This antigen, also determined by a transmissible plasmid, defines the ability of EEC strains to attach to the epithelium and to multiply in the small intestine of piglets. Derivatives of these strains lose their pathogenicity after the removal of this antigen or after its neutralization with antiserum (H. W. Smith and Linggood, 1971; Jones and Rutter, 1972,1974; Rutter and Jones, 1973). A similar protein antigen, K99, also determined by transmissible plasmid, is revealed in enteric colibacillosis pathogens of calves and lambs (H. W. Smith and Linggood, 1972; I. Ørskov et al., 1975*c*).

Pathogens of animal colibacillosis and human cholera-like disease have similar characteristics (H. W. Smith and Gyles, 1970*b*; Punyashthiti and Finkelstein, 1971; Dean et al., 1972; H. W. Smith and Linggood, 1972) (cf. Chapter 22). Also, there is a certain similarity between their enterotoxins and cholera enterotoxin, including even some common antigenic determinants (Moon et al., 1970*b*, 1971; Grady and Keusch, 1971; Holmgren et al., 1973; Pearce, 1973; Gyles, 1974*a*, *b*; Nalin et al., 1974).

Colibacillosis of animals, mainly its enteric forms, is frequently compared to infantile *E. coli* enteritis (Gay, 1965; Barnum et al., 1967; Gordon, 1971, etc.). At the same time the similarity of enteric colibacillosis of swine to human cholera has first been pointed out by T. Smith and Little (1927) and later by Gyles and Barnum (1969), by Moon et al. (1970*a*, *b*, 1971) and by a number of other investigators. Fey (1971) found an analogy between calf colisepticaemia and human generalized salmonelloses of typhoid type. Oedema disease of young pigs and the enteric-toxaemic form of calf colibacillosis seem to have no analogy among human diseases.

Summarizing all available data on the role played by different EEC in human and animal pathology, it should be stressed that these diseases (different escherichioses) are quite heterogeneous. Apparently, the reason for this lies in the considerable differences existing between the biological properties of their pathogens. With the list of EEC serotypes ever increasing, there arose a justified need to classify them into categories and groups.

Any such classification of EEC can, for the time being, be provisional only, and will certainly be revised as required by new findings. The pathogenesis of the diseases caused in man and animals by different EEC has not been studied extensively. Valuable data have been obtained recently by experimental studies to be discussed in the following Chapters.

The similarity of EEC-II such as O124 etc. to shigellae and their difference from EEC-I (like O111 etc.) have first been established in experiments with conjunctival and intravesical challenge of guinea pigs (see Chapter 20). Meanwhile, the lung model has proved to be more suitable for studying the dynamics of the interaction between different EEC and the host (see Chapter 21). The pathogenesis of infectious processes developing in the intestine when using the enteral mode of challenge with EEC will be discussed in Chapter 22. The earliest stages of the intestinal infectious process can be studied in experiments on ligated intestinal loops. Chapter 22 will also deal with the results of parallel experiments with shigellae and salmonellae, making use of the comparative analysis to judge the pathogenic properties of different EEC.

CHAPTER 20

CONJUNCTIVAL AND INTRAVESICAL CHALLENGE OF GUINEA PIGS WITH ENTEROPATHOGENIC *ESCHERICHIA COLI*. EXPERIMENTS WITH TISSUE CULTURES

by

YU. E. POLOTSKY

Many investigators have attempted to distinguish in animal experiments the agents of infantile enteritis (EEC-I) and other *E. coli* strains isolated from healthy humans or patients with non-intestinal diseases (NEEC). At first, conventional methods, such as intravenous and intraperitoneal challenge of mice, were used and the results were assessed in terms of animal mortality or bacterial counts. In this manner no essential difference was found between various EEC-I and NEEC. The effects of strains of even the same serotype of *E. coli* were found to differ in terms of animal mortality; NEEC strains were sometimes even more virulent for mice (Giles and Sangster, 1948; Taylor et al., 1949; Taylor, 1951; Braun et al., 1953; Rowley, 1954, 1955; Minck and Lavillaureix, 1955; Lindberg and Young, 1956; Demina and Chagodaeva, 1959; Shtriter and Barats, 1962*a*; Erlandson et al., 1964; Bergner et al., 1965; Filotti, 1965; Yakobson, 1965; Medearis and Kenny, 1968). Similar inconclusive results were obtained by intracerebral challenge of mice (Minck and Lavillaureix, 1955; Turgeon et al., 1960; Shtriter and Barats, 1962*a*; Erlandson et al., 1964) and in experiments on chick embryos (Demina and Chagodaeva, 1959; Turgeon et al., 1960; Shtriter and Barats, 1962*b*; Filotti, 1965; Yakobson, 1965).

Berman and Slavskaya (1959, 1961, 1962, 1966) noted that incomplete phagocytosis was usually evident in imprints of peritoneal exudate of mice challenged intraperitoneally with the majority of EEC-I O111 strains. However, about one-third of the NEEC strains were also resistant to leukocytes. They concluded that the outcome of their experiments did not depend upon the pathogenicity of the strain for infants, but upon the amount of endotoxin present in the bacteria.

By using these techniques no information can be obtained on agents of dysentery-like (EEC-II O124, etc.) and cholera-like (enterotoxigenic EEC) diseases of adults and children.

EXPERIMENTS WITH MONOLAYER CELL CULTURES

Lindberg and Young (1956) were the first to find a possibility of distinguishing EEC-I from NEEC in experiments with established monolayer cell cultures. They observed that the multiplication of EEC-I O111 in HeLa cells led to the death of these cells, whereas NEEC exerted no inju-

rious effect on cell cultures. These observations have been confirmed by several investigators using various primary and established cell cultures. It has been shown that even very small doses of EEC-I, more pathogenic for infants, destroy cell cultures, whereas with less pathogenic strains a positive test is not always obtained even when using large doses (Sharapova and Gavrilyuk, 1963; Baltrashevich and Ostrovskaya, 1964; Emelyanova, 1964; Safronov and Filippovich, 1966; Arbuzova, 1968*a*, 1970*a*, 1972; Sharapova, 1968). When stored under laboratory conditions EEC-I agents rapidly lose their ability to destroy the cellular monolayer (Filotti, 1965; Tallmeyster and Raudsik, 1965, etc.).

Morphological examinations made by Sharapova (1968) showed that bacteria of more pathogenic EEC-I strains were able to penetrate cultivated cells earlier and in higher numbers, multiplied in them more intensively and destroyed the cellular monolayer more rapidly. NEEC did not penetrate the cells at all or entered them in very small numbers and were incapable of multiplying in the cytoplasm. On the other hand, all *E. coli* strains investigated grew at the same rate outside the cells in the culture fluid. Arbuzova (1970*a*) came to similar conclusions. Ogawa et al. (1968*b*), however, have noted neither penetration into HeLa cells nor cellular monolayer destruction by EEC-I (salmonella-like EEC).

The ability to multiply in various cell cultures was also found in shigelia-like EEC-II (Arbuzova, 1968*a*, 1970*a*, 1972; Ogawa et al., 1968*b*; DuPont et al., 1971; Formal et al., 1971*a*; Osada et al., 1972*b*). According to the observations of Ogawa et al. (1968*b*) these bacteria, penetrating the cells behave like shigellae in similar experiments (see Chapter 5). Arbuzova (1970*a*) described that penetration and multiplication of EEC-II in the cytoplasm of cells of monolayer cultures took place later than that of shigellae.

Enterotoxin-producing *E. coli* strains (agents of cholera-like diseases) also destroy cell cultures, but do not penetrate the cells (Arbuzova, 1970*a*, 1972; DuPont et al., 1971; Formal et al., 1971*a*). According to the observations of DuPont et al. (1971) and Formal et al. (1971*a*), some of these organisms (e.g. *E. coli* O6) adhere to cultivated cells. Recently new methods have been developed for the evaluation of *E. coli* enterotoxin effect in tissue cultures, using the estimation of adenyl-cyclase activation in fat cells (Hewlett et al., 1974) or in intestinal epithelial cells (Kántor et al., 1974*b*) and the degree of stimulation of steroidogenesis in adrenal cells (Donta et al., 1974; Donta and Smith, 1974; Kwan and Wishnow, 1974).

CONJUNCTIVAL CHALLENGE

As pointed out in Chapter 19, of all the *E. coli* strains tested only shigella-like EEC-II organisms were capable of inducing the same experimental keratoconjunctivitis in guinea pigs as that obtained after challenge with dysentery agents.

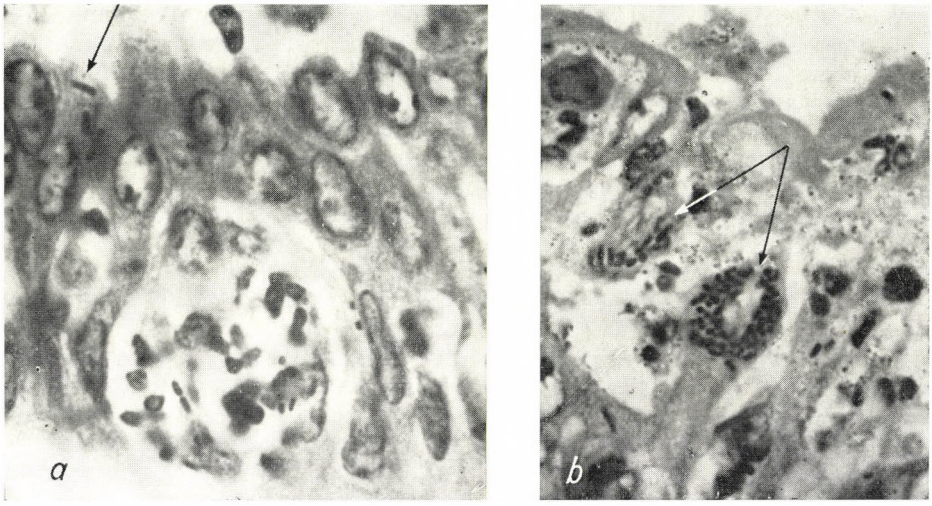


Fig. 20-1. *E. coli* O124 in the cytoplasm of conjunctival epithelial cells (arrows) 3 h (*a*) and 9 h (*b*) after challenge. Focal accumulation of leukocytes (microabscess) engulfing the organisms (*a*). Eosin-azure, ×1600

Our investigations* have shown a morphological similarity between the features of EEC-II and shigella keratoconjunctivitis, though inflammation, in the cornea in particular, arises somewhat later, is less pronounced and recovery occurs earlier. Similarly to shigellae, these EEC-II penetrate the conjunctival epithelial cells (Fig. 20-1*a*), multiply there (Fig. 20-1*b*) and, destroying the epithelial lining of the conjunctiva at certain sites during the first day after challenge, elicit focal purulent-ulcerative conjunctivitis. At this time EEC-II organisms appear in the cornea and multiply in the most superficial cells (Fig. 20-2*a*). Later, after the destruction or shedding of these cells, they penetrate deeper, down to the basal layer (Fig. 20-2*b*). The destruction of the corneal epithelium (starting from the periphery) and development of pronounced purulent-ulcerative keratitis usually occurred after 48 h and not after 24 h as in experiments with shigellae (Fig. 20-2*c*). Thereafter the inflammatory process subsided in the conjunctiva, while in the cornea it persisted resulting in the destruction of the whole epithelial lining. Here the inflammation also subsided earlier than in experiments with shigellae and, after 10–15 days, complete regeneration of the epithelial lining was usually observed.

Experiments with EEC-II strains losing their virulence were more successful on small guinea pigs weighing 120–180 g. However, the conjunctival inflammatory process was frequently less severe and of focal character even in these animals. Sometimes no inflammation of the cornea developed

* Polotsky and Vasser (1966*a*), Polotsky et al. (1966, 1971), Vasser and Polotsky (1970*b*).

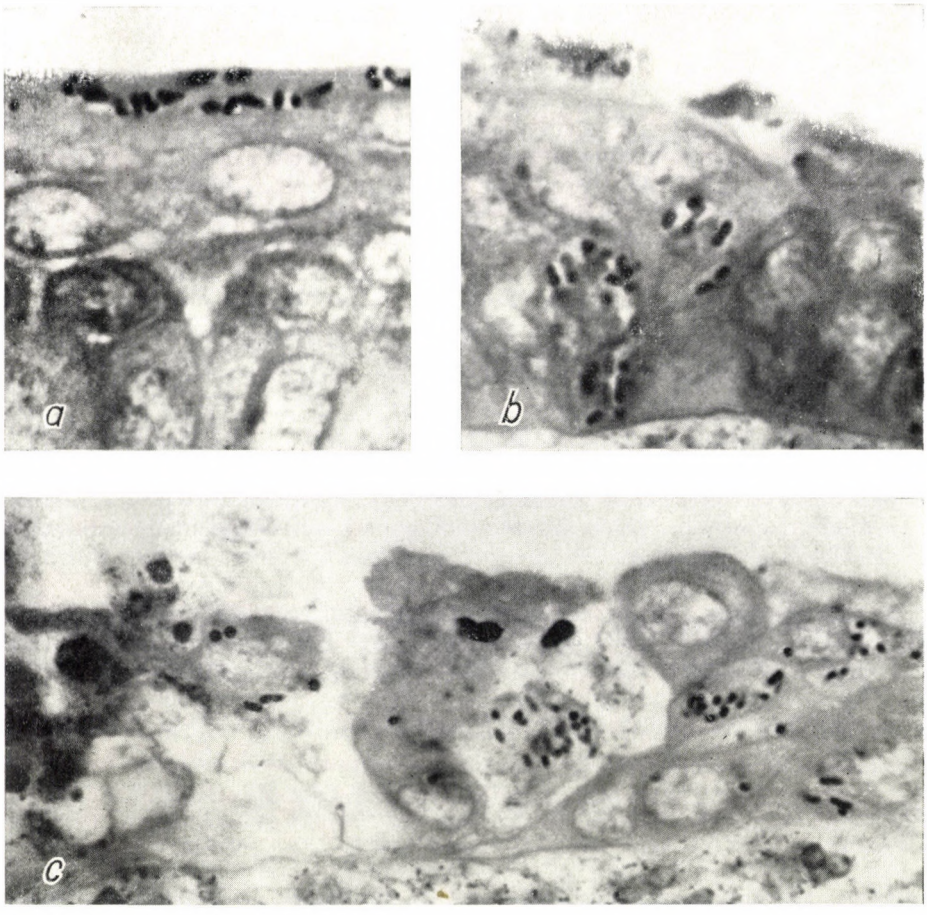


Fig. 20-2*a* and *b*. Shigella-like EEC-II in the cytoplasm of corneal epithelial cells 24 h after challenge (*a E. coli* O136, ×1700; *b E. coli* O144, ×1800) and *c* 48 h after challenge (*E. coli* O124, ×1540). Eosin-azure. Note the ulceration of corneal surface in *c*

at all, though at sites distinct multiplication of bacilli was revealed in the cytoplasm of single epithelial cells (Polotsky et al., 1971).

In experimental keratoconjunctivitis Ogawa et al. (1968*b*) also observed the growth of EEC-II (O136, O143, O144) in epithelial cells of the conjunctiva and cornea. It is noteworthy that in such experiments antibiotic treatment with drugs not penetrating the epithelial cells (kanamycin and streptomycin) did not prevent intracellular bacterial growth, while the administration of antibiotics penetrating the epithelial cells (rifampicin and tetracycline) resulted in rapid disappearance of the bacilli and in the recovery of the eye (Osada et al., 1972*a*).

INTRAVESICAL CHALLENGE

There are very few data available on the intravesical challenge of guinea pigs with enteropathogenic *E. coli*.* Braun et al. (1953) were the first to use this test for various cultures of enteropathogenic *E. coli* isolated from infants with *E. coli* enteritis. Using ordinary histological examinations, they observed the development of a moderate cystitis. Similar results were also obtained with NEEC cultures isolated from the urine of patients with pyelonephritis. On the other hand, agents of dysentery-like diseases (shigella-like EEC-II) were described to have induced purulent cystitis with signs of severe intoxication, similar to that observed with shigellae (Stenzel, 1962*b*, *c*, 1965). Challenging guinea pigs intravesically with shigella-like *E. coli*, Osada et al. (1971) even obtained severe damages of the vesical mucosa accompanied by extensive necrosis and thrombosis. However, these findings might be attributed to the administration of cortisone suppressing leukocyte response and to the fact that the organisms were inoculated in mucin preventing their elimination and phagocytosis. According to Osada et al. (1971) the pathogens, like shigellae (see Chapter 8), were predominantly revealed in epithelial cells. In control experiments, avirulent laboratory EEC-II strains caused no bladder infection and did not penetrate epithelial cells as NEEC strains isolated from patients with bacteriuria.

In experiments performed at the National Institute of Public Health (Budapest) by K. Tenner, freshly isolated strains of *E. coli* O111:K58(B4), the most typical representative of EEC-I, and of *E. coli* O124:K72(B17), the most frequently occurring shigella-like EEC-II, were used. Challenge with great numbers of EEC-I failed to cause death or severe disease in guinea pigs. At autopsy of animals sacrificed at different intervals after challenge, only a moderate oedema of the vesical wall and surrounding tissue was detected. Peculiar histological changes could be observed but none of these were marked. Six and 12 h after challenge, accumulations of bacteria occasionally forming whole films were revealed on the surface of the vesical mucosa. The organisms were seen not only to closely apply to the epithelial lining surface of the bladder, but also to densely adhere to its certain epithelial cells which had apparently been destroyed and shed into the vesical lumen (Fig. 20-3*a*, *b*). This was accompanied by a local moderate leukocyte response in the mucosa, which subsided 48–72 h after challenge, when the number of organisms on the mucosal surface also significantly decreased. There was no evidence of any defect of the vesical epithelial lining.

In contrast to EEC-I, *E. coli* O124 induced marked purulent cystitis, similar to that caused by shigellae, though the disease was milder and not fatal. EEC-II, as shigellae, penetrated into the cytoplasm of some epithelial

* Non-enteropathogenic *E. coli* of various serogroups (O4, O6 etc.) known as the most frequent agents of human pyelitis and cystitis were studied with intravesical challenge of various animals (Akopyan, 1965; Prát et al., 1970). However, these studies did not deal with the direct interaction between *E. coli* and the urinary tract epithelium, their aim being mostly the elucidation of the factors responsible for the development and spread of inflammation in the urinary bladder and renal pelves.

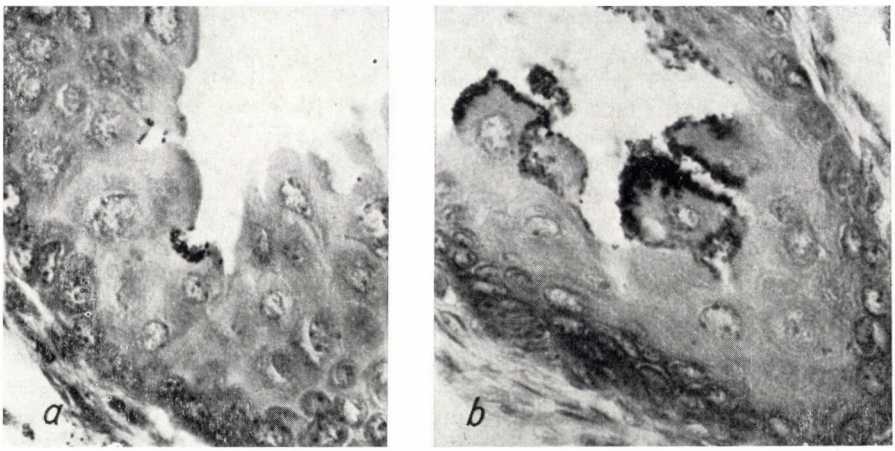


Fig. 20-3. EEC-I O111 multiplication on the surface of vesical epithelial cells 6 h (*a*) and 12 h (*b*) after challenge. Methyl green-pyronine, ×480

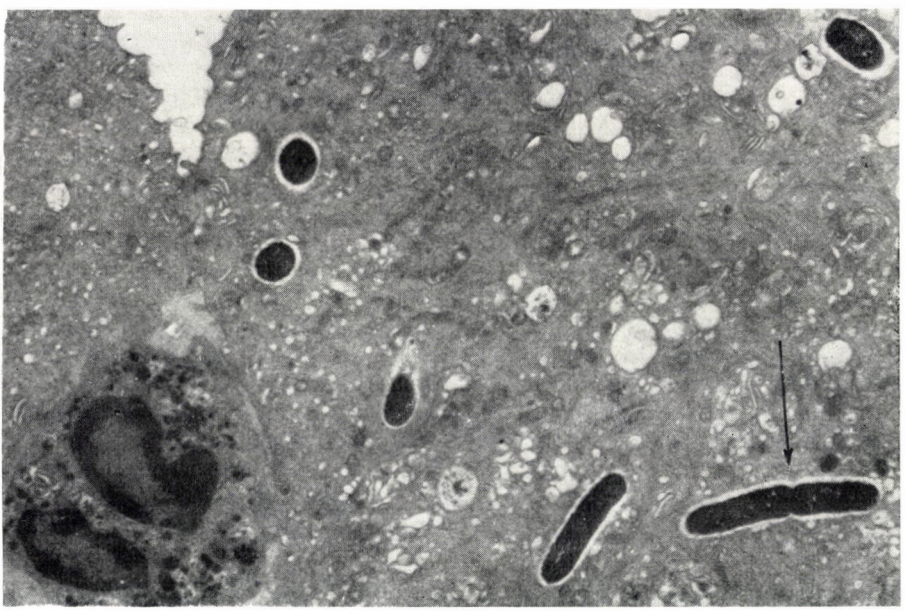


Fig. 20-4. *E. coli* O124 bacteria in the cytoplasm of vesical epithelial cells, 6 h after challenge. Membranes around bacteria are absent. Arrow indicates a dividing organism, ×5700

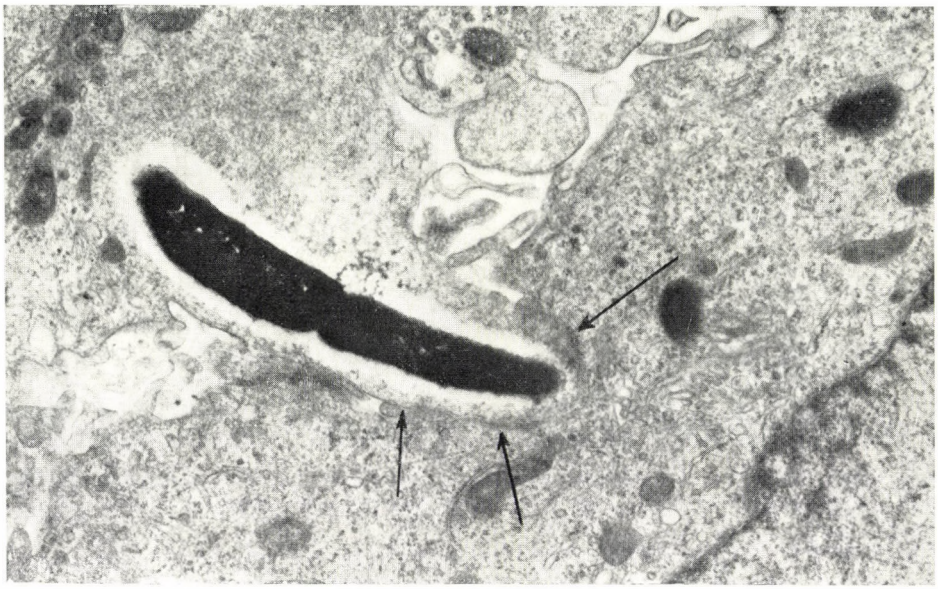


Fig. 20-5. Dividing *E. coli* O124 organism passing into neighbouring epithelial cell, pushing ahead and indenting the membranes of both cells (arrows) 6 h after challenge. × 15 400

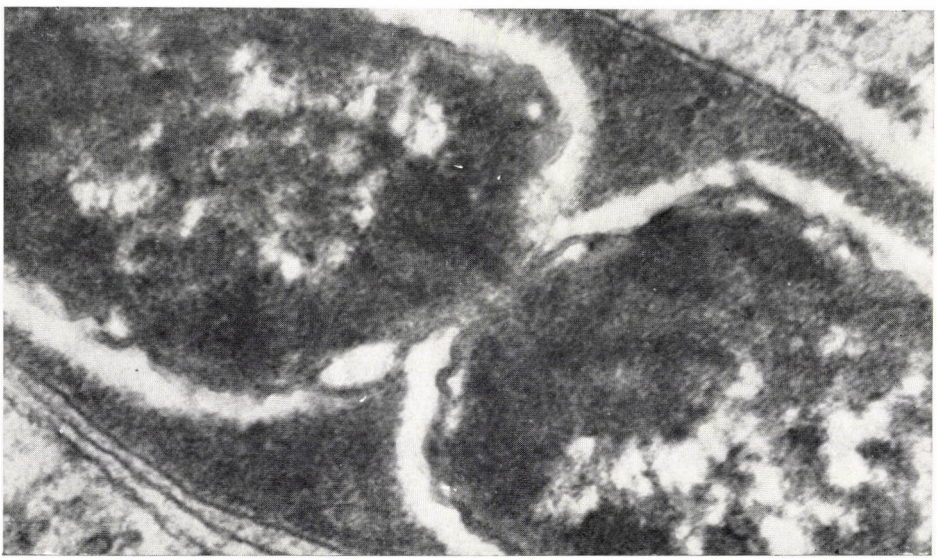


Fig. 20-6. Dividing *E. coli* O124 organism in the cytoplasm of a vesical epithelial cell enclosed into membrane-bound vacuole with electron dense osmiophilic material; 6 h after challenge. × 78 000

cells 3 h after challenge. Later they infected more cells and multiplied there, filling their cytoplasm. Following the destruction of these cells, superficial erosions and small ulcers filled by leukocytes appeared.

As with shigellae, electron-microscopic investigations of epithelial cells in most cases showed *E. coli* O124 bacteria to be unbounded by membranes in the cytoplasm (Fig. 20-4), where they multiplied and passed into neighbouring cells (Fig. 20-5). However, unlike with shigellae, a narrow electron transparent rim was detected on the surface of these organisms (Figs 20-4, 20-5). As no signs of artificial shrivelling are evident and some structures are discernible in this rim at higher magnifications, it seemed to represent a superficial layer of the bacterial wall (possibly a microcapsule or K-antigen which is absent in shigellae as a rule). In addition, unlike shigellae, *E. coli* O124 organisms were sometimes enclosed in membrane-bound vacuoles, inside which an electron dense osmiophilic material, resembling lysosome content, was distinguishable, though the bacteria showed no signs of alteration and even divided (Fig. 20-6).

Although the vesical epithelial lining was destroyed to a considerable extent during the first day after challenge, the inflammation decreased considerably after 48 h and subsided within 3–4 days after challenge giving place to epithelial regeneration.

Thus, intravenous, intraperitoneal and intracerebral routes of challenge are unsuitable for the study of different pathogenic properties of EEC. Tests on chick embryos do not reveal distinctive peculiarities either. In cell cultures representatives of different groups of EEC (except enterotoxigenic *E. coli*) penetrate the cells, multiply and destroy the cellular monolayer. The test permits to distinguish enteropathogens from NEEC, but does not allow a reliable differentiation between EEC-I and EEC-II. The most suitable method for this purpose is Serény's conjunctival challenge of guinea pigs. Keratoconjunctivitis induced by shigella-like EEC-II differs from shigella keratoconjunctivitis only in its less severe course. Morphological investigations showed that EEC-II, like shigellae, were able to parasitize epithelial cells. Intravesical challenge of guinea pigs with EEC permits the study of pathological processes induced not only by shigella-like EEC-II, but also by pathogens of infantile *E. coli* enteritis; the latter are able to multiply on the surface of the vesical mucosa.

CHAPTER 21

INTRANASAL CHALLENGE OF MICE WITH DIFFERENT *ESCHERICHIA COLI*

by

YU. E. POLOTSKY

The intranasal route of challenging animals with pathogens of enteric infections is being widely used at the Department of Pathological Anatomy, Institute of Experimental Medicine of the Academy of Medical Sciences of the USSR, and in the Department of Enteric Infections of the Pasteur Institute in Leningrad. The results of experiments with shigellae (see Chapter 9) and salmonellae (see Chapter 15) served as a ground for carrying out similar investigations with *E. coli*. Such studies are all the more justified because various *E. coli* strains are rather frequent agents of infantile pneumonia (MacGregor, 1946; Kravets et al., 1947; Tur, 1947; Lavnikova and Tsinzerling, 1958; Tsinzerling and Tsinzerling, 1963; Serzhanina, 1966; Zadvornyak et al., 1968; Tsinzerling, 1970). *E. coli* strains (usually non-enteropathogenic) are occasionally also detected in pure culture or associated with other organisms in bronchitis and pneumonias of adults (Wichert, 1959; Vygodchikov, 1960; Turck et al., 1962; Molchanov, 1965; Tillotson and Lerner, 1966, 1967*a*, *b*; Margolin et al., 1966; Vasilyeva and Polyak, 1967; Lerner and Tillotson, 1968; Lerner, 1969; Lerner and Federman, 1971; Solodova, 1970; Luneva, 1971). In pneumonias frequently complicating infantile *E. coli* enteritis, enteropathogenic *E. coli* (EEC) belonging to the same serotype as that isolated from the intestine of the patient is frequently encountered (Braun, 1956, 1971; Neter, 1959; Shastina, 1966; Tsinzerling et al., 1968; Drucker et al., 1970).

Joint morphological and bacteriological investigations were therefore made for studying the biological properties of different EEC serotypes and the infectious processes induced by them on the lung model. The method has been described in Chapter 9. Mainly freshly isolated strains were used since EEC organisms lose their virulence very rapidly not only when stored on artificial nutrient media but even when lyophilized.

The virulence of shigella-like EEC strains was preliminarily determined by the keratoconjunctival test. Immediately after challenge with 0.05 ml undiluted broth culture containing an average of 3×10^7–4×10^7 organisms, 0.7 ± 0.1 of the inoculated bacteria was, as a rule, recovered from the lungs (Ariel et al., 1970). In a number of experiments lower doses (from 10^6 to 10^7 organisms) were used for challenge.

EXPERIMENTS WITH NON-ENTEROPATHOGENIC *E. COLI* AND AGENTS OF INFANTILE *E. COLI* ENTERITIS*

After intranasal administration of 10^7–10^8 organisms of NEEC or EEC-I (O111:K58, O55:K59, O26:K60, O128:K67, O119:K69, O20:K84, O33:K (B), etc.) the general state of the animals apparently did not change and only single mice died 2–3 days after challenge. Bacteriological counts showed the number of organisms cultured from the lungs 3 h after challenge to be 2–25 times higher as compared with the inoculum, but 24 h after challenge it was 6–38 times lower as compared with the maximum level at 3 h after challenge; subsequently it kept decreasing considerably (Fig. 21-1). In mice sacrificed after identical intervals during the experiment the number of organisms in the lungs varied, especially from 9–12 to 18–24 h after challenge (in a few mice it even increased). However, the number of organisms decreased markedly in the lungs of all animals within 2–3 days even in dying mice.

According to morphological studies, the organisms introduced into the lungs were arranged singly or in small groups on the walls of the alveoli, alveolar ducts and the bronchi, predominantly in the hili and in the upper posterior parts of the lungs immediately after challenge. A certain number of organisms were immediately engulfed by alveolar macrophages. Three h after challenge the number of organisms observed in the same areas of

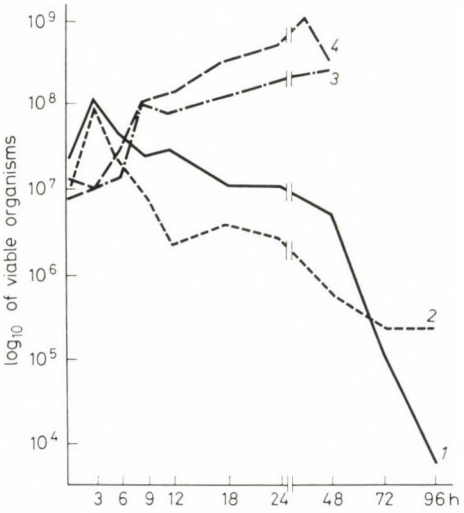


Fig. 21-1. The dynamics of microbial growth in the lungs of mice after intranasal challenge with various *E. coli* strains. Abscissa, time after challenge (in h); ordinate, bacterial counts in the lungs (absolute values presented on a $\log_{10}$ scale). *1* NEEC, *2* EEC-I O111:K58, *3* EEC-II O124:K72, *4* EEC-II O28a28c:K73

* Polotsky and Arbuzova (1966, 1967); Polotsky et al. (1968).

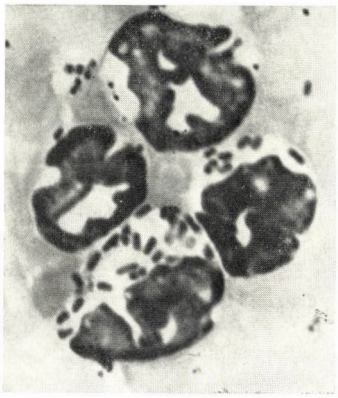


Fig. 21-2. Phagocytosis of organisms by leukocytes 3 h after challenge with NEEC. Imprint from lung tissue. Giemsa stain, ×1600

the lungs was much higher and signs of an inflammatory response were already apparent. Polymorphonuclears with phagocytized organisms appeared in the alveoli. In imprints some *E. coli* were seen to have been engulfed by leukocytes and to have lost their distinct outlines (Fig. 21-2), inside macrophages, however, most bacteria remained unchanged and even divided (Fig. 21-3*a*).

Subsequently the inflammatory process became more intensive; after 6–12 h the separate groups of alveoli filled with serous-leukocytic exudate became confluent and the pulmonary tissue in the hili and upper posterior parts of the lung acquired a kind of meshy or lacy appearance because of the presence of alternating air-containing and airless areas. Free organisms were scanty in the exudate and those which were present in polymorphonuclears bore signs of destruction. The organisms obviously multiplied in

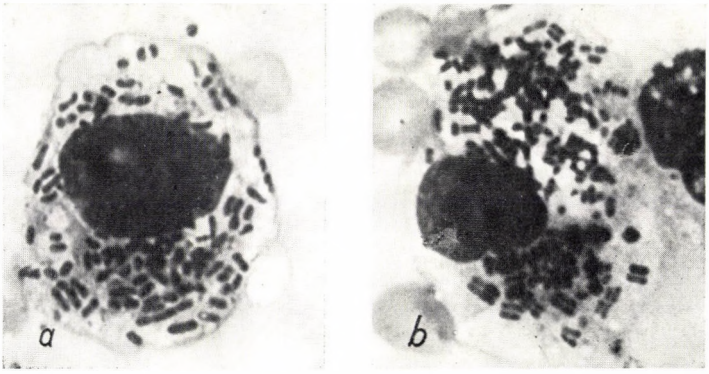


Fig. 21-3. Organisms in the cytoplasm of alveolar macrophages. NEEC, 3 h (*a*) and 12 h (*b*) after challenge. Imprints from lung tissue. Giemsa stain, ×1600

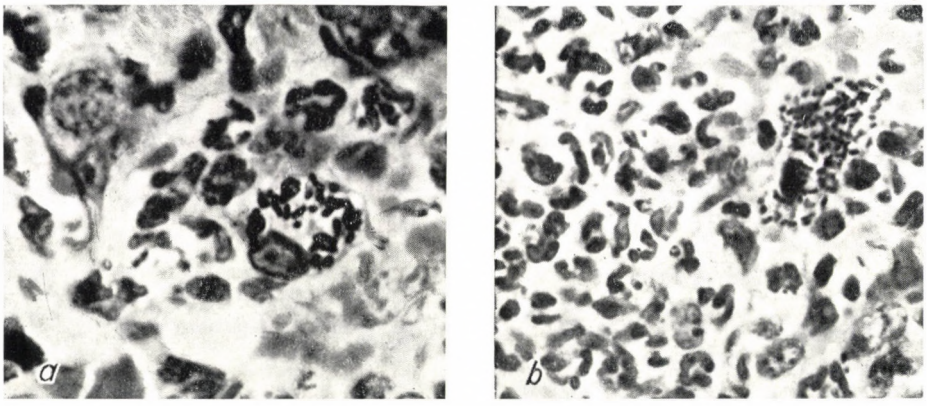


Fig. 21-4. Multiplication of the organisms in macrophages. *a* NEEC, 6 h after challenge. Eosin-azure, ×1600; *b* microcolony of organisms in the disintegrating macrophage with picnotic nucleus. 12 h after challenge with EEC-I O111. Eosin-azure, ×1070

macrophages; filling the cytoplasm of these cells (Fig. 21-3*b*), they finally caused their disintegration. Soon (6 h) after challenge with both NEEC and EEC-I, some well-preserved macrophages filled with bacteria (Fig. 21-4*a*) were detected in the alveoli among leukocytes, infrequently containing breakdown products of engulfed organisms. Later, after 9–12 h, these macrophages disintegrated (Fig. 21-4*b*), leaving behind clusters of organisms reproducing the outlines of the cell; the only remnants of the cell itself were a deformed picnotic nucleus or only chromatin blocklets.

By the end of the first day after challenge, there was a marked leukocyte response (though not to the same extent in all animals), while the number of organisms in the pulmonary tissue diminished. The density of leukocytes filling the alveoli varied. When leukocytes were less numerous in the alveoli and the serous exudate was more abundant, organisms were present in leukocytes in higher numbers; macrophages filled with *E. coli* (Fig. 21-5*a*) and microcolonies could be detected in place of disintegrated cells though less frequently than previously. In other cases, however, numerous leukocytes seemed to carry out their functions successfully and even in macrophages rather few disintegrating bacteria or their remnants were revealed (Fig. 21-5*b*, *c*).

Forty-eight h after challenge, organisms were rarely seen in sections and in imprints from the lungs; the inflammation subsided and the exudate and leukocyte breakdown products gradually disappeared from the alveoli.

Thus, intranasal challenge of mice showed that NEEC and EEC-I induce a similar infectious process which only develops during the first day after challenge and then subsides. If investigations had been carried out only with daily intervals and the fate of the organisms had not been studied, one might have believed the findings reflected merely a clearing response to the organisms rather than an infectious process developing in the lungs.

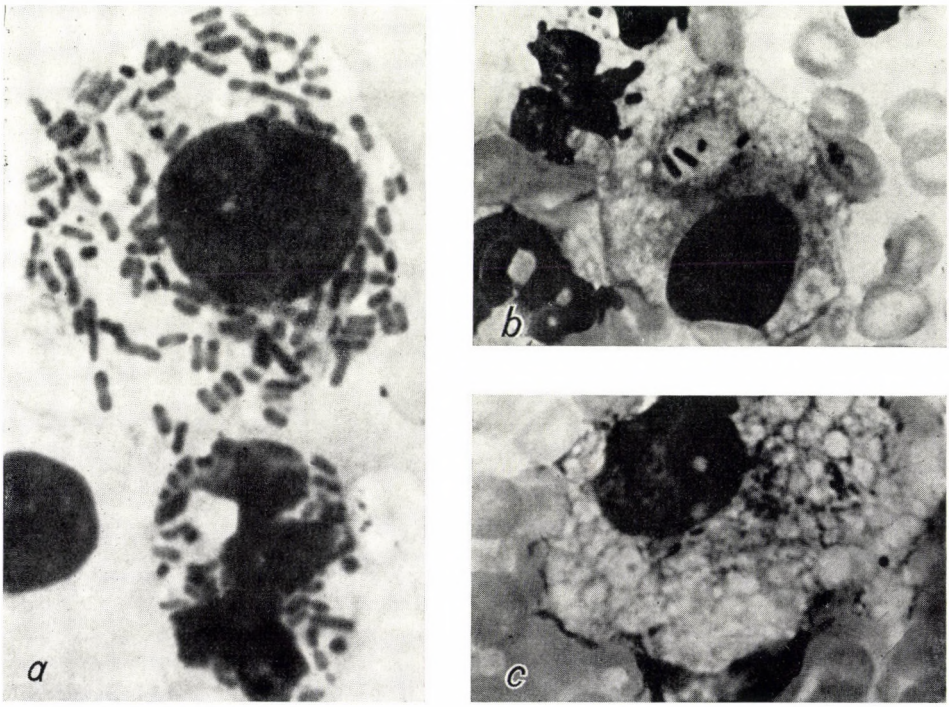


Fig. 21-5. Multiplication (*a*) and destruction (*b* and *c*) of organisms in macrophages. *a* NEEC, 18 h after challenge, ×2250; *b* EEC-I O111, 24 h after challenge, ×1600; *c* NEEC, 24 h after challenge, ×1600. Imprints from lung tissue. Giemsa stain

In similar experiments with NEEC and EEC-I Shastina and Mikhaylova (1967) used cultures grown on the surface of agar plates for intranasal challenge. In these experiments the number of organisms inoculated was much greater (about 4.5×10^9 were used for challenge and an average of 6×10^8 organisms entered the lungs). Shastina and Mikhaylova failed to follow escherichia multiplication in pulmonary macrophages. They obviously thought that the filling of these cells with organisms was merely the sequence of phagocytosis of numerous bacteria present in the alveoli. The serous-haemorrhagic character of the exudate in the pulmonary alveoli in later periods of observation might have been explained by the severity of infection (and consequently by the abundance of toxic breakdown products of organisms destroyed).

In our experiments there were few free organisms in the alveoli from the very start, and by the 9–12th h they had been taken up by phagocytes almost completely. Therefore, further increase in the number of bacteria of similar appearance (many of them dividing) in the cytoplasm of macrophages must have been due to intracellular multiplication. This property regularly observed in all experiments with NEEC and EEC-I resembles the

behaviour of salmonellae to some extent. At the same time, in intranasal challenge, salmonellae cause a long-term, progressing infectious process with constant cyclic microbial multiplication in macrophages (see Chapter 15). In experiments with NEEC and EEC-I, cyclic multiplication stops approximately by the end of the first day after challenge by which time macrophages have acquired the ability to destroy these organisms. Opsonization of the organisms liberated from the macrophages (e.g. with normal antibodies) may be possible in this case. Moreover, the enhancement of macrophage activity by breakdown products of leukocytes and *E. coli* organisms may also be involved.

EXPERIMENTS WITH AGENTS OF DYSENTERY-LIKE DISEASES OF ADULTS AND CHILDREN*

The outcome of challenging mice with shigella-like EEC-II (O28a28c:K73, O124:K72, O143:Kx1, O144:Kx2) was entirely different. Following intranasal administration of about 10^7 organisms, most animals died after 24–48 h. Cultures showed a 10-fold and even 100-fold increase in the number of organisms in the lungs. When mice were challenged with a lower number of EEC-II (about 10^6), part of them survived.

Comparison of the course of growth of the organisms in the lungs after challenge with shigella-like EEC-II and NEEC or EEC-I (about 10^7 organisms) showed a striking difference (see Fig. 21-1). The number of EEC-II increased markedly not after 3 but after 9–12 h. Even subsequently their number kept increasing. Challenge with smaller numbers of EEC-II (especially in experiments with strains losing virulence) yielded a similar course of growth up to 9–12 h; later the bacterial count increased in dying mice while in surviving animals it decreased, especially after 48–72 h.

Morphological studies showed that EEC-II gave rise to pneumonia, but the leukocyte response was less pronounced than in infections with EEC-I and NEEC strains; the exudate contained a considerable amount of serous fluid and erythrocytes. Leukocytes and macrophages phagocytized the organisms. Bacteria were destroyed in leukocytes though in some polymorphonuclears they remained unaltered and even divided. In macrophages the bacteria were obviously multiplying, filling the whole cytoplasm after 9–12 hours. By this time the organisms in the alveoli were mostly seen as microcolonies in macrophages. The infected macrophages disintegrated, then leukocytes engulfed and destroyed the organisms.

Thus, at first the infectious process generally resembled the experiments with NEEC and EEC-I. But already at the very beginning essential differences could be seen in the bronchi where EEC-II bacteria were observed in the cytoplasm of single epithelial cells; after 9–12 h the organisms filled the cells to abundance, destroyed them and infected the adjacent cells (Fig. 21-6*a*). This process was especially clearly visible in imprints, where

* Polotsky and Arbuzova (1965, 1966, 1967); Polotsky et al. (1966, 1968); Ariel et al. (1968); Novgorodskaya et al. (1968*a*); Avdeeva et al. (1970*b*).

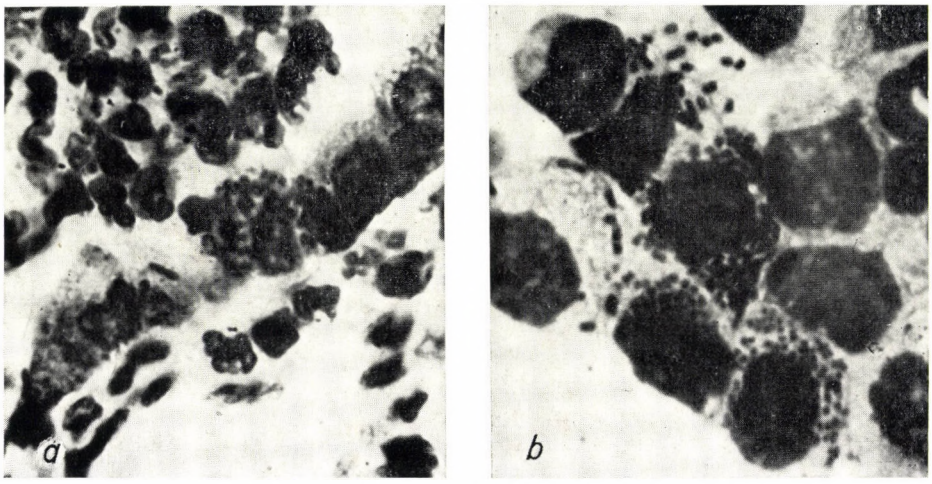


Fig. 21-6. Multiplication of shigella-like EEC-II in the cytoplasm of bronchial epithelial cells. *a E. coli* O124, 12 h after challenge, thionine, ×1600; *b E. coli* O124, 18 h after challenge. Imprint from lung tissue. Giemsa stain, ×1600

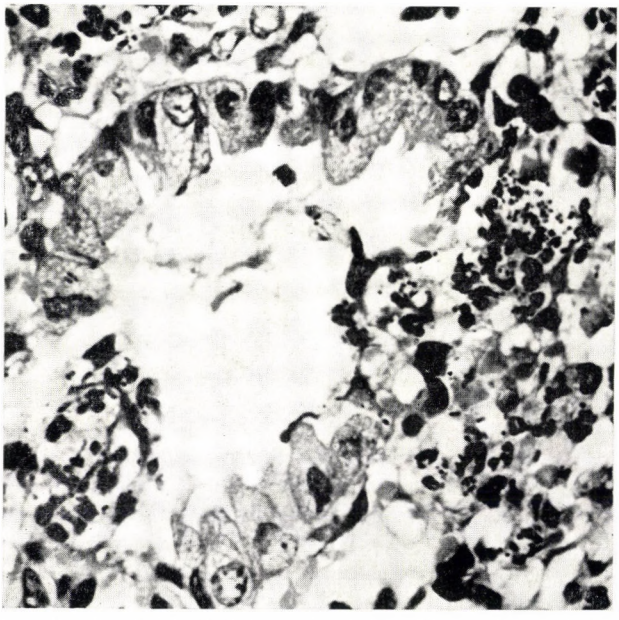


Fig. 21-7. Defects in the epithelial lining 'n a bronchus. *E. coli* O28a28c, 24 h after challenge. Thionine, ×850

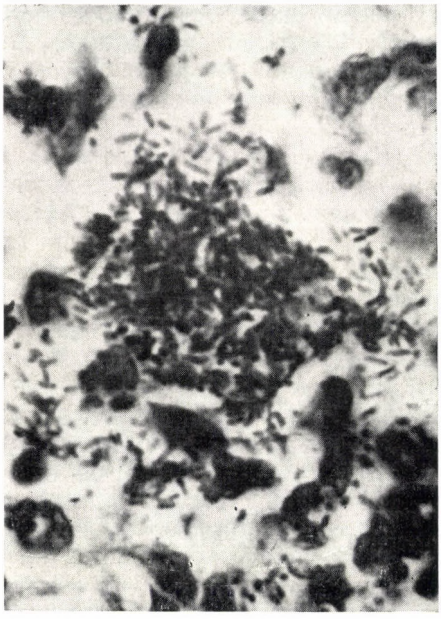


Fig. 21-8. Extracellular multiplication of *E. coli* O124, 24 h after challenge. Thionine, $\times 1600$

the epithelial cells are usually stretched out and are frequently seen as whole clusters (Fig. 21-6*b*). The destruction of infected epithelial cells *in situ* or their sloughing into the lumen resulted in defects in the epithelial lining of the bronchi (Fig. 21-7) becoming filled with leukocytic exudate.

At the end of the first day (18–24 h) after challenge with 10^6–10^7 organisms the infectious process in the lungs of most mice acquired a different character. Polymorphonuclears were obviously unable to destroy all bacteria released from the infected epithelial cells and macrophages. Accumulations of unaltered and even dividing rods in polymorphonuclears were frequently present and the leukocytes themselves displayed signs of death. The number of free organisms increased in the alveoli. At the periphery of pneumonic foci where there were few leukocytes and serous-haemorrhagic exudate was prevalent, the multiplying organisms formed large, loose accumulations (Fig. 21-8). A kind of "paralysis" of leukocyte response was apparent: serous-haemorrhagic oedema with unimpeded microbial multiplication in the exudate increased. Almost all animals died.

In a small number of animals that had been challenged with 10^6 EEC-II and survived, the infectious process subsided at the end of the first day; this finding was especially characteristic of experiments with strains losing virulence. The leukocyte response did not decline and free organisms could hardly be detected in the pulmonary alveoli of convalescent mice by the 18th to 24th h. Organisms in the epithelial cells and macrophages

were less frequently seen; in macrophages they disintegrated rather than multiplied (as observed with NEEC and EEC-I). In mice surviving 2–3 days, bacterial multiplication in the epithelium ceased, bronchial defects were covered with regenerating epithelium and the exudate cleared from the lungs.

Thus, in intranasal challenge shigella-like EEC-II, in contrast to NEEC and EEC-I, caused a dramatically progressing infectious process leading to the death of most animals. In this case EEC-II, similarly to shigellae, penetrate the cytoplasm of bronchial epithelial cells and multiply therein. However, as has been shown in Chapter 9, virulent shigellae are rarely engulfed by macrophages and are much more resistant to leukocytes. Moreover, in experiments with EEC-II, free organisms in the alveoli were almost completely destroyed by leukocytes in the early (up to 9–12 h) post-challenge period, and microbial multiplication in the bronchial epithelium was particularly evident. In the alveolar exudate EEC-II organisms at first even resembled NEEC and EEC-I in their growth in macrophages and in the absence of their extracellular multiplication. But after the first day, if the infectious process ran an unfavourable course, most animals exhibited an extracellular microbial multiplication associated with a "paralysis" of leukocyte response, which is exactly the case in experimental shigella pneumonia (see Chapter 9). In the latter, however, the organisms are much more abundant but they neither multiply nor appear in macrophages. It is noteworthy that in experiments with EEC-II O124, doses considerably larger than those used in experiments with shigellae are necessary to produce an infectious process fatal to all animals. Accordingly, in spite of the similarity between the pathogenic properties of this group of EEC-II and shigellae, there are differences between these organisms which are well discernible on the lung model.

EXPERIMENTS WITH ENTEROTOXIGENIC *E. COLI* ASSOCIATED WITH CHOLERA-LIKE DISEASES

As mentioned in Chapter 19, EEC-II serogroups O112a112b, O6, O1 and others have been isolated from adults and children in sporadic cases and epidemic outbreaks of gastroenteritis resembling mild forms of cholera. Immediately after isolation these organisms were tested with conjunctival challenge of guinea pigs and intranasal challenge of mice. In contrast to *E. coli* O124 and other shigella-like EEC-II strains, they did not induce keratoconjunctivitis. It was the lung model that revealed an essential difference between them and all other kinds of *Escherichia* strains. With intranasal administration of 10^6–10^7 organisms almost all the mice died during the first hours, displaying signs of asphyxia accompanied by convulsions and an abundant flow of bloody foam from the nose and mouth (Arbuzova, 1970*a*, 1972; Avdeeva et al., 1970*a*, 1973*b*; Arbuzova and Krivonosova, 1973; Smirnova, 1974). Bacterial counts from the lungs usually showed no essential increase during the first hours but in a few mice surviving for a longer period marked multiplication was observed after 9–12 h and later.

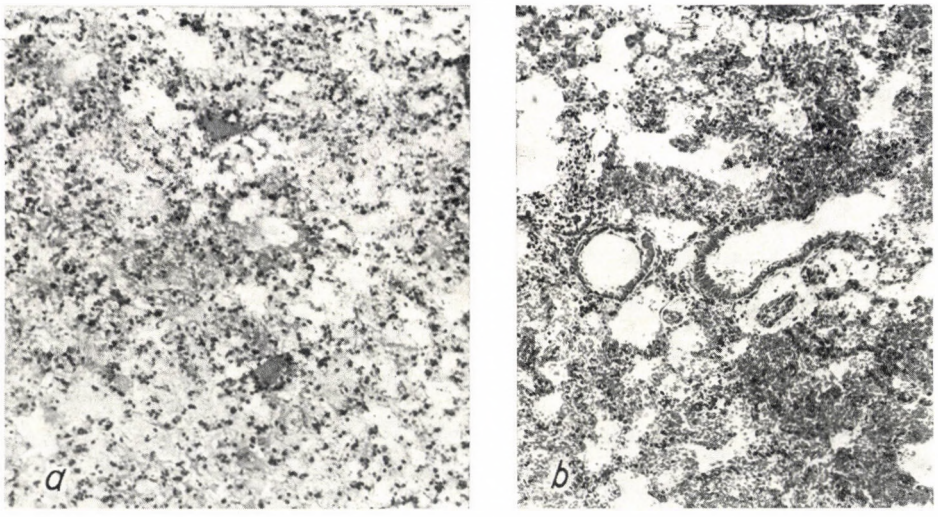


Fig. 21-9. Serous-haemorrhagic oedema (*a*) and haemorrhages (*b*) in the lungs after challenge with *E. coli* strains producing enterotoxin-like substances. *a* 2 h after exposure to *E. coli* O112a112b, eosin-azure, ×110; *b* 6 h after administration of *E. coli* O6 strain (No. 228). Dominici's stain, ×90 (Courtesy of E. M. Dragunskaya)

Morphological studies carried out by E. M. Dragunskaya at the Department of Pathological Anatomy, Institute of Experimental Medicine of the Academy of Medical Sciences of the USSR (Dragunskaya, 1970*b*, 1972; Dragunskaya and Smirnova, 1970; Dragunskaya et al., 1972, 1973) revealed a gradual increase in the number of organisms in the pulmonary alveoli during the very first hours of the experiment. The development of leukocyte response was appreciably inhibited. The alveoli were filled with abundant serous-haemorrhagic fluid (Fig. 21-9*a*); focal and extensive haemorrhages were frequently seen (Fig. 21-9*b*). The organisms multiplied in the alveoli extracellularly and were almost absent in the macrophages. In these cells (as well as in others) dystrophic alterations were noted. Small defects in the epithelial lining appeared in the bronchi though organisms were not detected in the cytoplasm of epithelial cells.

The results of morphological studies of the lungs suggested unusually severe and early toxic lesions. Since no considerable destruction of organisms was evident at such early observations, the alterations could not be attributed to endotoxin. Similar early fatal serous-haemorrhagic pulmonary oedema could be produced with intranasal administration of lethal doses of staphylococcal exotoxin (Bibinova et al., 1962*b*; Pigarevsky and Ilyin, 1967), a finding stimulating a search for exotoxin-like substances in these EEC. The similarity between diseases caused by these organisms and mild forms of cholera also might point to the possible involvement of enterotoxins.

As shown in Chapter 19, Gorbach et al. (1971), Sack et al. (1971), DuPont et al. (1971), Formal et al. (1971*a*) and a number of researchers after them

have demonstrated that *E. coli* strains associated with cholera-like diseases produced enterotoxins. Experiments on ligated rabbit small intestine loops to be dealt with in Chapter 22 are considered to be the generally accepted model for establishing microbial enteropathogenicity and particularly enterotoxigenicity. Experiments described in the above chapter have shown that ultrasonic lysates of EEC causing early death of mice after intranasal challenge (as well as their living cultures) elicit fluid accumulation and dilatation of intestinal loops (Avdeeva et al., 1973*a*, *c*). Heated lysates have been found to lose their activity. Thus, these organisms have been proved to be also capable of producing a heat-labile enterotoxin similar to that of cholera vibrios.

Intranasal administration of the enterotoxin (lysate) to mice caused the death of all animals as did the challenge with living organisms, but less rapidly (Avdeeva et al., 1973*c*). Morphological studies revealed the same serous-haemorrhagic lung oedema, but leukocyte response was less evident and less inhibited (Dragunskaya et al., 1973). The heated lysate was entirely harmless; it produced only leukocyte response with rapid clearance. At the same time, filtrates of the supernatants of the above cultures did not cause any damage in mice after intranasal exposure, though they occasionally produced a positive response in ligated rabbit small intestinal loops (Avdeeva et al., 1973*c*).

As noted in Chapter 19, the readily accessible and convenient lung model seemed to be the method of choice for the demonstration of all heat-labile enterotoxin-producing *E. coli* strains (Avdeeva et al., 1973*a*, *c*). However, intranasal inoculation of living cultures, filtrates and lysates of EEC O6:H16, O15:H11, O148:H28 and others, obtained from S. L. Gorbach and H. L. DuPont, and of EEC O148:H28 and O148:H7, isolated in Leningrad (Avdeeva et al., 1975), gave negative results (Kleganov et al., 1975). These strains, unlike *E. coli* O112a1,2b, O6, O1 and others described above, did not induce fatal lung oedema.

It is noteworthy that strains of animal colibacillosis had previously been tested on the same model (Thal and Dinter, 1953). The living culture of strain "455" and its haemolysin obtained by filtration were administered to mice. In both experiments early death of the animals due to haemorrhagic lung oedema was evident (morphological examination was not performed). These observations suggest that the lung model may be useful in studying agents of animal colibacillosis.

In testing *E. coli* type strains of the Copenhagen Escherichia Centre, Smirnova (1973, 1974) found that 40 strains out of 115 cultures belonging to 98 O-serogroups caused fatal infection in mice after intranasal challenge. Twenty-three strains killed practically all mice in the first three hours. Many of the cultures tested had originally been isolated from animal colibacillosis. Other strains, including many pathogens of infantile *E. coli* enteritis, caused death of mice later and killed only part of the animals. Thus, further studies are needed to define the nature of toxins which cause blood vessel lesions and fatal serous-haemorrhagic lung oedema, inhibit leukocyte response and cause local cell damage involving the bronchial epithelium (in contrast to the intestinal epithelium in experiments on ligated

rabbit small gut loops as will be shown in Chapter 22). It would be desirable to study these toxins in comparison with other enterotoxins of *E. coli* isolated from cholera-like diseases, with enterotoxins of animal colibacillosis agents, as well as with cholera enterotoxin and "vascular permeability factor". The problem of enterotoxin-like substances in EEC-I should also be elucidated. For all these aims the lung model seems to be the best method along with the isolated gut loop technique, enteral challenge and intracutaneous inoculation of toxin preparations.

Thus, intranasal challenge of mice with different *E. coli* strains has shown that the lung model is suitable for distinguishing the pathogenic properties of these organisms. Both NEEC and EEC-I (agents of infantile *E. coli* enteritis) only cause short-term infectious processes in the lungs, demonstrable by repeated bacteriological and morphological examinations in the course of the first day. These organisms multiply in alveolar macrophages.

Shigella-like EEC-II (O124 etc.) agents of dysentery-like diseases of adults and children multiply in the epithelial cells as observed in conjunctival challenge. In contrast to shigellae, these organisms can grow inside alveolar macrophages. Experimental lung infection induced by these EEC-II has a severe course with marked microbial multiplication in the pulmonary tissue, leading to the death of the majority of the animals.

E. coli O11,2a,12b, O6, O1 and others, isolated from adults and children with gastroenteritis resembling mild forms of cholera, inhibit leukocyte response, damage alveolar and bronchial cells locally and impair vascular permeability, resulting in the animals' death from serous-haemorrhagic lung oedema in the first hours after challenge. With intranasal administration of heat-labile toxins present in the lysates of these organisms similar results were obtained, and their enterotoxigenic effect could be proved in experiments on isolated intestinal loops (see Chapter 22). At the same time intranasal challenge with other agents of cholera-like diseases, such as *E. coli* O6:H16, O1,5:H11 and O148:H28, producing even stronger heat-labile, and particularly heat-stable, enterotoxins (see Chapter 22), does not cause the death of mice. Further studies are required to define the nature of both kinds of toxins and to compare them with choleragen (known to contain both enterotoxin and "vascular permeability factor").

CHAPTER 22

ENTERAL CHALLENGE OF ANIMALS WITH ENTEROPATHOGENIC *ESCHERICHIA COLI*. EXPERIMENTS ON ISOLATED INTESTINAL LOOPS

by

YU. E. POLOTSKY

ENTERAL CHALLENGE

EXPERIMENTS WITH AGENTS OF INFANTILE *ESCHERICHIA COLI* ENTERITIS (EEC-I)

The first attempts to challenge ordinary laboratory animals orally with *E. coli* O111, O55, O26, O127 and some other agents of infantile *E. coli* enteritis were unsuccessful (Giles and Sangster, 1948; Taylor, 1951; Braun et al., 1953; Novgorodskaya, 1953*a*, *b*, 1958; Rantasalo et al., 1955; and others). Several investigators reported on the reproduction of enteritis by feeding EEC-I cultures to kittens and puppies (Krepler and Zischka, 1952; Zaleski and Ceprynska-Ciekawa, 1956; Elshina and Molchenko, 1959; Illutovich et al., 1962; Emelyanova and Shvetsov, 1963; Ermolov, 1967; Staley et al., 1969*a*). EEC-I penetration into the mesenteric lymph nodes, liver, spleen or blood was occasionally noted, especially in experiments on newborn animals (Staley et al., 1969*a*; and others). A mild form of enteritis was also observed after oral challenge with large doses of EEC-I in young monkeys (Stasilevich, 1958*a*; Yakhnina et al., 1960*b*; Dzhikidze et al., 1965). The diseases induced have not been studied in detail.

The resistance of laboratory animals to infection with pathogens of human diseases is attributed to the antagonism of their intestinal microflora (see Chapter 3). When the mouse intestinal flora is inhibited with antibiotics, EEC-I strains resistant to them multiply and colonize the entire intestinal tract without producing pathological changes in it (Rauss and Kétyi, 1960; Aschburner and Mushin, 1962). The same may be observed in adult rabbits (Ravin and Fine, 1962) and in suckling mice challenged with non-pathogenic microorganisms during the first week of their life (Kenny et al., 1970). In these experiments we are not dealing with the so-called benign infectious process but with an implantation of organisms replacing the normal intestinal flora. These methods are used for studying immunity against EEC-I and for developing vaccination techniques in mice (Linde et al., 1969; Linde and Koch, 1970; and others) and even in monkeys (Felsenfeld et al., 1972).

The importance of the age of animals or of the antagonism of their intestinal flora in challenge with EEC-I is not quite clear. When challenging suckling mice orally with agents of infantile *E. coli* enteritis, Mushin and Dubos (1965) and Mushin et al. (1970) observed fatal enteric infection accompanied by intensive growth of the pathogen in the intestine and penetration into the blood and viscera. These were, however, animals of a

specific NCS colony with insignificant normal *E. coli* content in the gut. Such mice, unlike ordinary ones, displayed peculiar susceptibility to challenge with different viruses and bacteria, i.e. possessed a low level of non-specific immunity. In ordinary, non-inbred white suckling mice, EEC-I induced similar diseases but these were milder and of shorter duration. Administration of NEEC cultures (O81 and O117) to NCS suckling mice also resulted in bacterial multiplication and colonization of the gut but the animals did not succumb to the disease (Mushin et al., 1970).

Friedman and Halbert (1960) and Kétyi (1964, 1966*a*) used for infecting newborn ordinary mice *E. coli* O101:K30(A):H– isolated from calves that died of colibacillosis (Moll and Ingalsbe, 1955) instead of EEC-I. When challenged during the first three days after birth, the mice died rapidly of sepsis (resembling *E. coli* sepsis in newborn and premature infants), while in mice aged 4–6 days enteritis developed and the animals died of dehydration and toxicosis (like infants having *E. coli* enteritis). The resistance to EEC O101 infection which appeared after 7–8 days was not attributed to the appearance of an antagonistic intestinal flora (which was noted earlier), but to biological maturing of the intestinal epithelium (Kétyi, 1964, 1966*a*). However, morphological examinations were not performed.

The pathogenic properties of EEC-I have been studied by challenging germ-free newborn piglets. After feeding large numbers (10^9) of EEC O55, Miller et al. (1964), Rejnek et al. (1968) and Travniček et al. (1968) observed the development of fatal infection with signs of bacterial multiplication in the gut and sepsis within 24–48 h. Oral administration of a NEEC strain used both in these experiments and in the former works of Šterzl et al. (1960) proved to be harmless for piglets though the organisms were recovered from the viscera.

Staley et al. (1969*b*, *d*, 1970*b*) conducted histological and electron-microscopic studies on this model to clear up the fate of EEC-I O55:B5:H7, as well as its interaction with the intestinal mucosa. A mild form of enteritis was observed in these experiments during the first two days followed by mild or even marked colitis (Staley et al., 1970*b*). Shortly after challenge with *E. coli* O55 the organisms became attached at sites to the epithelial surface of small intestinal villi, and seemed to multiply there. By the end of the first day they adhered to the mucosa in large numbers and formed films covering the villi in 48 h (Fig. 22-1). At the sites of bacterial attachment microvilli were absent, and the cell membrane appeared to be monolayer, swollen and occasionally formed invaginations (Fig. 22-2*a*, *b*). Shortening, swelling and branching of adjacent microvilli to varying degrees were detected. Organisms were frequently found in the cytoplasm of some epithelial cells (Fig. 22-1, 22-2*a*), where they were, as a rule, separated from the cytoplasm by membranes and did not produce any conspicuous damage. Part of the organisms also penetrated the lamina propria where they were taken up by leukocytes and macrophages. Later similar events occurred in the large bowel mucosa. Staley and his co-workers (1970*b*) pointed out that, in spite of an extensive microbial invasion, they did not find essential lesions up to the 6th day of the experiment, when thrombosis of capillaries, oedema and haemorrhages in the lamina propria and in the

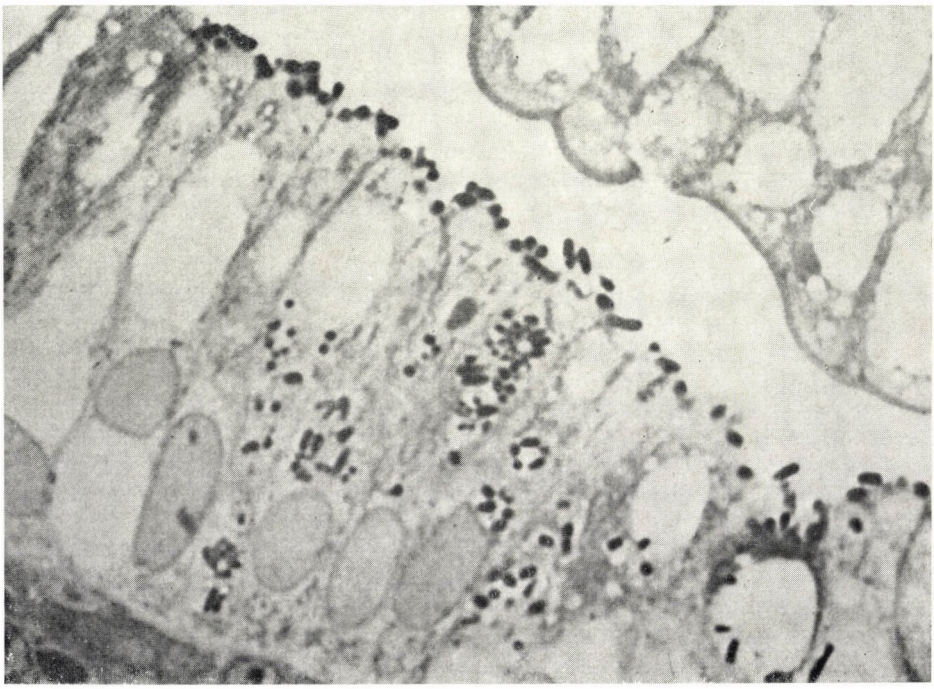


Fig. 22-1. *E. coli* O55:B5:H7 on the surface of the epithelium of ileal villi and inside cells 48 h after challenge of newborn germ-free piglets. Toluidine blue, ×1900 (Staley et al., 1969*b*)

submucosa appeared, but without damaging the integrity of the epithelial lining.

Similar signs, closely resembling pathological findings in infants who died of *E. coli* enteritis (see Chapter 19), were also observed by Staley et al. (1970*a*) in newborn calves challenged with *E. coli* O55:B5:H7. It is possible that in all of these cases multiplication of EEC-I in the gut, their growth on the surface of mucosal epithelium and their penetration through it is promoted not only by the absence of an antagonistic intestinal flora but also by the immaturity of the epithelial lining (Staley et al., 1972*b*, etc.).*

It is noteworthy that in animal experiments agents of the so-called colibacillosis of young pigs and calves also had the ability to grow on the

* After a certain interval following birth (varying in length with the species) intestinal epithelial cells are capable of absorbing and transporting protein macromolecules (immunoglobulins of maternal colostrum or milk, etc.) and corpuscular elements (Comline et al., 1951; Kraehenbuhl et al., 1966, 1967, 1971; Mattisson and Karlsson, 1967; El-Nagen, 1967; Graney, 1968; Staley et al., 1968, 1969*c*, 1970*a*, 1972*a*, *b*; Kraehenbuhl and Campiche, 1969; Veress and Baintner, 1970; Clarke and Hardy, 1971; Fey, 1971; Moon, 1972; Rundell and Lecce, 1972; Jeffcott, 1974; Jones, 1974).

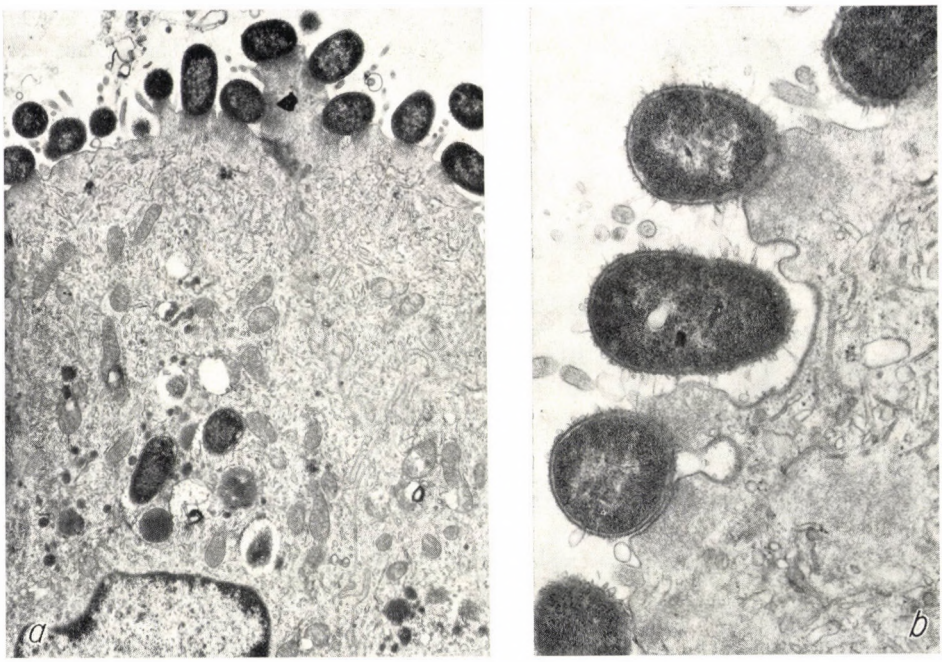


Fig. 22-2*a*, *b*. *E. coli* O55:B5:H7 on the surface of the intestinal epithelium and their partial penetration into the cytoplasm (*a*) 20 h after challenge of newborn germ-free piglets. *a* ×23 500; *b* ×50 000 (Staley et al., 1969*d*)*

mucosal epithelial surface (T. Smith and Orcutt, 1925; Arbuckle, 1970, 1971; Gilka and Salajka, 1970*a*, *b*; Drees and Waxler, 1970; Waxler et al., 1971; Bertschinger et al., 1972). It is a controversial problem whether they penetrate the mucosa and cause an inflammatory response (H. W. Smith, 1963; H. W. Smith and Jones, 1963; Kohler and Bohl, 1966; Moon et al., 1966*b*, 1970*a*; Kohler, 1967; Christie, 1969; Jones and Smith, 1969; Drees and Waxler, 1970; Kenworthy, 1970; Waxler et al., 1971; Bertschinger et al., 1972). Investigations carried out on isolated loops of small intestine (see later) have shown that the agents of animal colibacillosis produce enterotoxins. Oral administration of these preparations (sterile lysates or filtrates) to newborn animals causes a characteristic diarrhoea which is not accompanied by intestinal inflammatory alterations (Kohler, 1968, 1971*a*, *b*, 1974; Kohler and Cross, 1969, 1971; H. W. Smith and Gyles, 1970*a*; Gyles, 1971; H. W. Smith and Linggood, 1971, 1972). At the same time it has been stated that protein transmissive antigen K88 plays an important part in the pathogenicity of enterotoxigenic agents of swine colibacillosis. This antigen is responsible for the ability of bacteria to attach to the

* We express our thanks to dr. Theodore E. Staley for kindly sending us the originals of some of his electron micrographs.

intestinal epithelium; its removal or neutralization with antiserum results in the loss of pathogenicity (H. W. Smith and Linggood, 1971; Jones and Rutter, 1972, 1974; Rutter and Jones, 1973). A similar antigen, K99, has been revealed in enterotoxigenic agents of calf and lamb enteric colibacillosis (H. W. Smith and Linggood, 1972; I. Ørskov et al., 1975*c*).

EXPERIMENTS WITH PATHOGENS OF DYSENTERY-LIKE DISEASES OF ADULTS AND CHILDREN (SHIGELLA-LIKE EEC-II)

Administration of 1.3–4.4 $\times 10^9$ virulent *E. coli* O124:K72(B17) organisms into the stomach of guinea pigs pretreated according to the method of Formal et al. (1958) (see Chapter 10) resulted in severe disease (Polotsky and Vasser, 1966*b*, 1970*a*, *b*). About one-third of the animals died within 1–3 days. No diarrhoea was seen though stools were frequently loose, containing a considerable amount of mucus. At autopsy, loops of the small intestine and the caecum of animals sacrificed at different time intervals were seen to be filled with reddish-yellow or brown fluid containing

Fig. 22-3. Ulcer in the caecum 48 h after challenge with *E. coli* O124:K72(B17) of starved guinea pigs. Thionine, $\times 85$

gas bubbles; congestion and oedema of the intestinal walls were noted. On their mucosa flake-like films were occasionally seen. Cultures made from the liquid showed multiplication of the organisms, which was especially marked in severely affected animals.

Histological investigation showed inflammatory foci in the small intestinal and caecal mucosa; inflammation was most intensive by the 48th h, when in severely affected and dying guinea pigs the mucosal surface of the caecum was covered with a purulent exudate. In such areas the crypts were filled with leukocytes, and at sites their epithelium showed signs of destruction. Fairly large ulcers occurred in the caecum in some cases (Fig. 22-3). In convalescent animals the inflammatory process subsided gradually.

Within the first hours after challenge single organisms were revealed in the cytoplasm of some epithelial cells of the jejunum. After 24 h and especially after 48 h there was an increase in the number of infected cells (not in the jejunum but in the ileum and even more in the caecum) as well as in the number of bacilli in the cytoplasm of affected cells in severely ill or dying animals. Such cells were more frequently seen on the periphery of destroyed parts of the epithelial lining. Sloughed epithelial cells filled with organisms were frequently observed in the purulent exudate filling the gut lumen and in the crypts. In the cytoplasm of polymorphonuclears, few semi-destroyed bacteria were sometimes encountered. At damaged sites of the mucosa the organisms were sometimes also found in the cytoplasm of macrophages of the lamina propria, where they appeared to be unaltered and were even dividing.

Except for the ability of organisms to survive in macrophages, experimental enterocolitis of guinea pigs caused by *E. coli* O124 and by *Shigella* (see Chapter 10) are closely similar. However, much larger doses were required for challenge with EEC-II, while the infection produced was milder. The epithelial lesions caused by these organisms were less extensive, whereas the leukocyte response was more intensive and leukocytes destroyed the organisms much more quickly.

Formal et al. (1971*a*) and DuPont et al. (1971), conducting similar experiments on guinea pigs, also observed the penetration of *E. coli* O124, O143 and O144 into the epithelial cells and the lamina propria of the intestinal mucosa. Foci of inflammatory infiltration were detected in the ileal and caecal mucosa with corresponding defects of the mucosal epithelium (these studies were carried out 24 h post challenge).

The same authors observed a mild diarrhoea after oral challenge of monkeys with shigella-like EEC-II, and by feeding large doses of these organism to volunteers they succeeded in reproducing a typical dysentery-like disease with bloody diarrhoea, fever and marked signs of intoxication. At sigmoidoscopy the appearance of the colonic mucosa of the patients was similar to that in dysentery; it was red, friable with multiple punctate bleedings. The dose required to produce a dysentery-like disease in volunteers was 10,000 times higher for EEC-II than for shigellae.

EXPERIMENTS WITH ENTEROTOXIGENIC *ESCHERICHIA COLI*

Enterotoxigenic *E. coli* O6:H16 and O148:H28 isolated from patients (see Chapter 19) proved to be almost completely harmless for guinea pigs (Formal et al., 1971*a*; DuPont et al., 1971). Dean et al. (1972) suggested to introduce such bacterial cultures directly into the stomach of newborn mice by puncturing the anterior abdominal wall. The results of the experiments were assessed in terms of the amount of fluid accumulating in the intestinal lumen (determined by weighing the intestine). The data obtained generally conformed to the results of similar experiments on isolated intestinal loops (see later).

Challenging monkeys with *E. coli* O6 and O148, DuPont et al. (1971) observed a transient diarrhoea with watery stools in some animals (8 out of 36 monkeys). The same was noted in humans infected orally with very high doses of enterotoxigenic EEC. Bacteriological studies showed the presence of organisms mostly in the upper part of the small intestine. At sigmoidoscopy the colonic mucosa of infected volunteers was red but without haemorrhages. Congestion was the only histological change to be observed. The authors came to the conclusion that these organisms possessing no invasive properties but producing enterotoxins cause human diseases which are similar to cholera but not to dysentery.

Relying upon the results of oral challenge of newborn rabbits, Gorbach and Khurana (1972) and Kantor et al. (1974*a*) considered the agents of infantile *E. coli* enteritis (EEC-I), or at least some of them, as well as some members of other *E. coli* serogroups, to be capable of producing enterotoxins. These authors also maintained that the mechanism of action of enterotoxins is probably similar to that of pathogens of cholera-like disease of adults, but the former are weaker in effect.

EXPERIMENTS ON ISOLATED INTESTINAL LOOPS

Experiments on isolated segments of the intestine bear close resemblance to enteral challenge of animals. It is the principal advantage of the method that it excludes the mechanical elimination of organisms by peristalsis. Any dose of organisms or toxins, antisera, etc. can be introduced into the isolated intestinal loops, and the initial stages of interaction between organisms and intestinal epithelium can be studied, which is rarely possible in enteral challenge.

Artificial infection of ligated loops of the rabbit's small intestine was first suggested by Violle and Crendiropoulo (1915) and later by De and Chatterje (1953) for the experimental study of cholera; it has then been widely used for this purpose (Formal et al., 1961*b*; Fresh et al., 1964; Burrows and Musteikis, 1966; Patnaik and Ghosh, 1966; Finkelstein, 1969; Freter, 1969; Sinha et al., 1969; and many others). The experiments showed cholera agents to induce marked dilatation of infected loops due to accumulation of fluid resembling cholera patients' stools. Cholera vibrios do not penetrate the epithelial cells but multiply on their surface without injuring the

epithelial lining or causing an inflammatory response (in either humans or orally challenged animals) (Fresh et al., 1964; Patnaik and Ghosh, 1966; Freter, 1969). However, Formal et al. (1961*b*) observed a transient insignificant leukocyte response in the intestinal loops at early periods of the experiments. By the end of the observation period Formal and his co-workers (1961*b*), as well as De and Chatterje (1953) and Fresh et al. (1964), could observe necrotic changes of the epithelium, which were probably due to disturbance of circulation (Formal et al., 1961) or to compression of the mucosa by the great amount of fluid (Fresh et al., 1964).

Studies made with this model proved that the pathogenic effect of cholera vibrios is due to their exotoxin, i.e. a choleragen or a heat-labile enterotoxin present in broth culture filtrates and ultrasonic (or sonic) vibrio lysates (De, 1959; De et al., 1960; Burrows and Musteikis, 1966; Kasai and Burrows, 1966; Burrows, 1968; Finkelstein, 1969; Burrows et al., 1971; Kao et al., 1972; etc.). Recent studies have shown that the effluent in cholera, i.e. the serous isotonic fluid accumulating in the intestinal loops exposed to cholera enterotoxin and containing high levels of some electrolytes (large quantities of sodium and bicarbonate with lesser amounts of potassium and chloride) but poor in protein, is the product of epithelial hypersecretion rather than a transudate (Norris and Majno, 1967, 1968; Norris, 1968; Norris et al., 1969; Carpenter, 1971, 1972; Pierce et al., 1971). This has been confirmed by biochemical investigations showing that cholera enterotoxin stimulated the activity of adenyl cyclase, which is a cellular membrane-bound enzyme, increasing the fluid and electrolyte secretion of epithelial cells (Field, 1971; Kimberg et al., 1971; Sharp and Hynie, 1971; Guerrant et al., 1972; Beckman et al., 1974).

Experiments with cholera vibrios and enterotoxin are most frequently made on the rabbit's small gut. After fasting the animal for 1–3 days the peritoneal cavity is opened under anaesthesia and 10–15 cm long loops of small intestine are ligated, alternating with intact segments. With this method different enterotoxigenic organisms and their enterotoxins can be tested. Since the intestinal tract is obstructed, the period of observation is usually limited to 24 h. The more complicated Thiry-Vella technique is less frequently used (on rabbits or dogs); according to this method a small intestinal loop (20–25 cm long) is severed *in toto* by suturing its end to the abdominal wall. At the site of resection anastomosis is made to keep the alimentary tract passable; thus long-term experiments become possible. To detect the enterotoxigenicity of *E. coli* Punyashthiti and Finkelstein (1971) employed ligated loops of the mouse small gut, simultaneously assessing the "adhesiveness" of the organisms, i.e. their ability to become attached to epithelial cells using the erythrocyte slide haemagglutination test. Agents of animal colibacillosis are usually tested on the ligated gut of animals of the same species (H. W. Smith and Halls, 1967*a*; Myers et al., 1975).

EXPERIMENTS WITH ENTEROTOXIGENIC *ESCHERICHIA COLI* ASSOCIATED WITH CHOLERA-LIKE DISEASES

De et al. (1956) were the first to note that in India adult patients with acute enteritis with a typical cholera syndrome frequently discharged great numbers of *E. coli* organisms in the absence of cholera vibrios. Having tested 20 serologically unidentified isolates in ligated loops of the rabbit small gut, they found that within 24 h, 13 cultures caused dilatation of the loops and necrotic non-inflammatory changes of the epithelium. De and Chatterje (1953) considered the same findings to be typical for cholera vibrios.

As mentioned in Chapter 19, cholera-like diseases were shown to be associated with *E. coli* O6, O15, O78, O148 and other serogroups (see Table 19-III) which elaborate enterotoxins detected in broth culture filtrates and in whole microbial cell lysates (Gorbach et al., 1971; Sack et al., 1971, 1974; Formal et al., 1971*a*; DuPont et al., 1971; Sakazaki et al., 1974*a*, *b*; Shore et al., 1974; etc.). In contrast to NEEC filtrates and lysates and, similarly to cholera enterotoxin, enterotoxins of these organisms caused serous fluid accumulation and dilatation of isolated loops of the small gut in rabbits (and dogs). The enterotoxins of *E. coli* similarly to choleragen stimulated adenyl cyclase and elicited hypersecretion of the epithelial cells, although compared with the latter their action was weaker, started earlier and lasted shorter (Al-Awqati et al., 1972; Evans et al., 1972; Levitan et al., 1972; Pierce and Wallace, 1972; Guerrant et al., 1973; Sherr et al., 1973; Kantor et al., 1974*a*). They even had some antigenic relationship to cholera enterotoxin (N. W. Smith and Sack, 1973; Pierce, 1973; Gyles, 1974*a*, *b*; Likhoded et al., 1974; Sack et al., 1974), but differed from it by containing a heat-stable component (Etkin and Gorbach, 1971; D. J. Evans jr. et al., 1973; D. G. Evans et al., 1973; Nalin et al., 1974) as well as by impairing the absorption of glucose and glycine, suggesting a somewhat different pathophysiological response; this is of importance from the point of view of therapy in clinical practice (Sherr et al., 1973). The lack of inactivation of *E. coli* enterotoxins by natural cholera toxoid and ganglioside suggested a difference in mucosal receptors (Pierce, 1973). Meanwhile, separate experimental studies with heat-stable and heat-labile *E. coli* enterotoxins have shown that the effect of the former is earlier, weaker and lasts shorter than that of the latter, which is neutralized by antiserum to choleragen (Nalin et al., 1974).

DuPont et al. (1971) and Formal et al. (1971*a*) have shown that enterotoxigenic *E. coli* O6:H16 and O148:H28 failed to penetrate the intestinal epithelium and did not induce inflammation.

In our first experiments some other agents of diarrhoeal diseases of adults and children resembling mild forms of cholera were used (*E. coli* serogroups O6, O112a112b and others producing early death of intranasally challenged mice) (Avdeeva et al., 1973*a*, *c*; — see Chapters 19 and 21). The doses ranged from 10×10^9 to 75×10^9 (in 1–5 ml broth culture grown with shaking). Accumulation of great amounts of serous-haemorrhagic fluid started in almost half of the infected loops 6 h after challenge and reached its peak by the

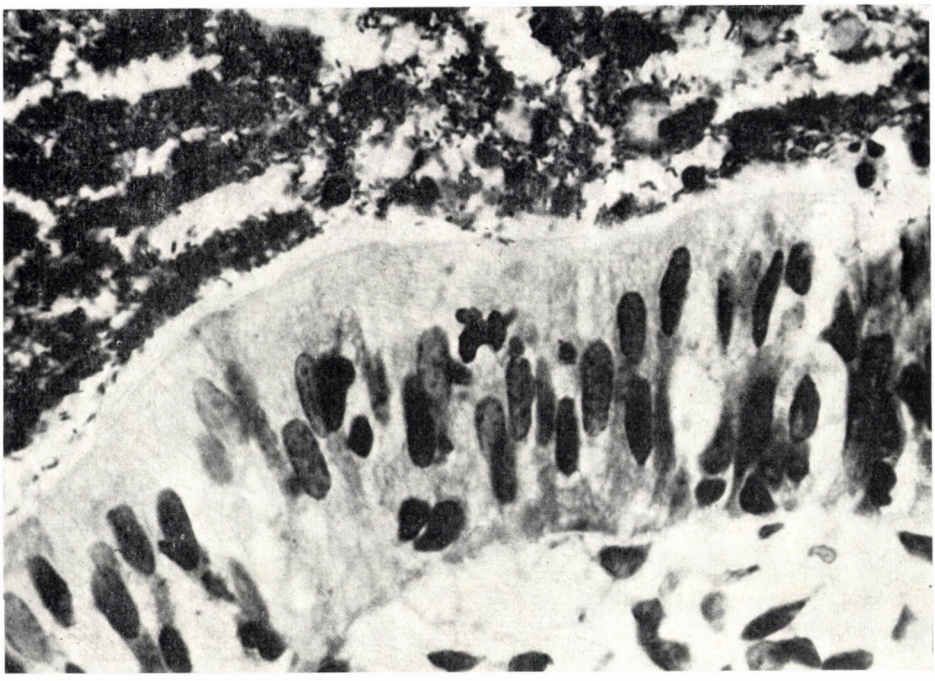


Fig. 22-4. Abundant accumulations of enterotoxigenic *E. coli* strain No. 3976 on the surface of the epithelium of the rabbit jejunal ligated loop 12 h after challenge. Thionine, ×1140

10th–14th h (Avdeeva et al., 1973*a*, *c*).* Sterile ultrasonic lysates of the two strains tested gave positive results somewhat more frequently than living cultures, however, sterile filtrates of supernatant culture fluids did so much more rarely. The activity of lysates was completely lost after heating them to 60 °C for 30 min, but filtrates were not inactivated even at higher temperatures (90 °C) (Avdeeva et al., 1973*c*).

Histological examinations showed that neither the ligation of intestinal loops nor the administration of sterile broth or NEEC cultures induced any conspicuous morphological alterations. Marked oedema and congestion were noted in the mucosa and submucosa of dilated gut loops after injection of enterotoxigenic *E. coli* strains or their enterotoxins. Haemorrhages were occasionally seen; leukocytes were few in number and were almost absent in the villi. In the first hours after challenge large accumulations

* Our experiments made with enterotoxigenic *E. coli* on ligated loops of rabbit small intestine were carried out in collaboration with T. A. Avdeeva, E. M. Dragunskaya and L. A. Smirnova. Studies on EEC-I were performed together with N. R. Vasser and E. M. Dragunskaya, those on shigella-like EEC-II (O124 etc.), *Shigella* and *Salmonella* with E. M. Dragunskaya and N. R. Vasser. E. S. Snigirevskaya contributed to the electron-microscopic studies.

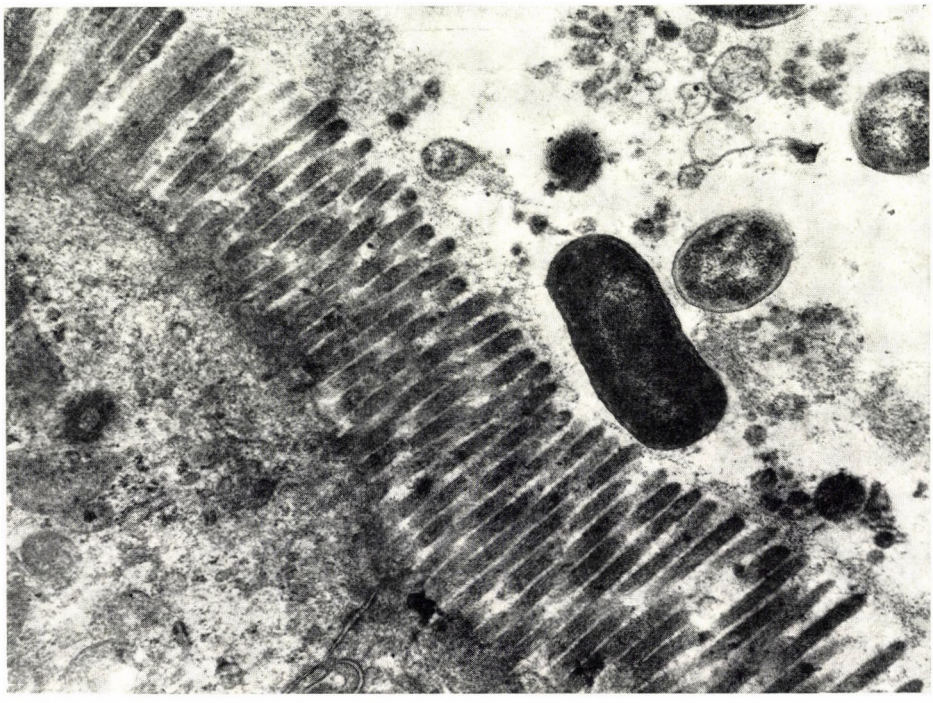


Fig. 22-5. Enterotoxigenic *E. coli* strain No. 3976 in the glycocalyx layer covering the microvilli of the epithelial cells of the rabbit small intestinal loop 12 h after challenge. ×23 000

of organisms were seen, often forming thick films, frequently with the admixture of mucus (discharged from goblet cells), eosinophilic fluid, erythrocytes and sloughed epithelial cells on the surface of the unaltered epithelial lining. At some sites the organisms seemed to be fixed to the brush border but they did not cause alterations in it or in the cytoplasm of the epithelial cells. After 9–12 h and later, bacilli were seen to have become attached to the epithelial surface in the infected loops overflowing with fluid less frequently. They seemed to have been washed out by the fluid effluent into the loop lumen where their amount increased. However, at some sites of the epithelial lining of the villi, accumulations of multiplying enterotoxigenic *E. coli* were still observed, and occasionally their number was considerable (Fig. 22-4).

Electron microscopy (Polotsky et al., 1974*a*, 1976) showed the organisms to be in contact only with the glycocalyx* covering the microvilli (Fig.

* The glycocalyx was detected by means of fixation in glutaraldehyde with addition of thiophosphamide followed by that of osmium tetroxide [technique of Komissarchik and Snigirevskaya (1973)]; instead of thiophosphamide, occasionally alcian blue, ruthenium red or lanthanum were used.

22-5), without penetrating deeper. An increased number of large mitochondria with distinct crystae, strands of rough endoplasmic reticulum, ribosomes and many secretory granules were apparent in many epithelial cells; these findings obviously reflected an increase in epithelial function. In addition, in many cells there were structures called autophagosomes, membrane-bound vacuoles containing cytoplasmic particles, organellae and secretory granules. Autophagosomes were especially numerous in desquamated epithelial cells (Fig. 22-6). They were also revealed outside the cells in the lumen together with a great number of free secretory granules, mucus and serous fluid.

Thus, our morphological studies have shown that enterotoxigenic EEC multiply on the epithelial surface and in the lumen of infected gut loops,

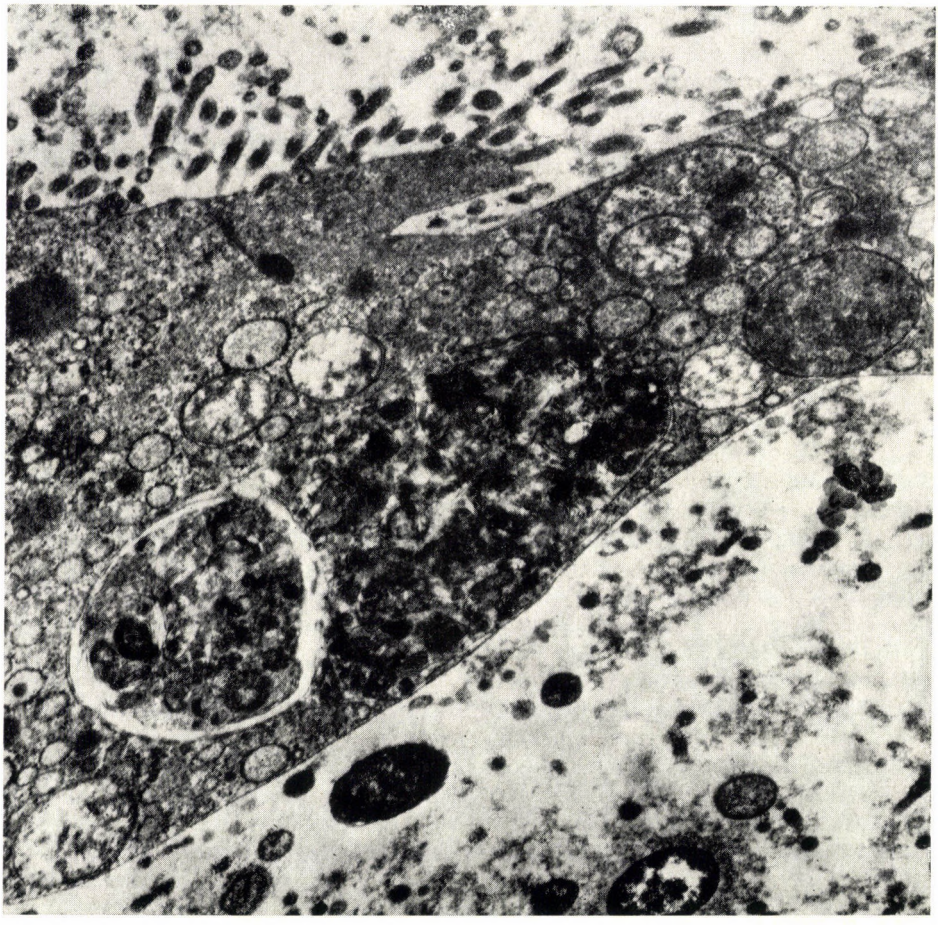


Fig. 22-6. Autophagosomes in a desquamated epithelial cell 12 h after challenging a ligated rabbit small gut loop with enterotoxigenic *E. coli* strain No. 3796. ×20 000

similarly to cholera vibrios. They do not penetrate the epithelial cells and cause no damage to their microvilli, interacting only with the glycocalyx. Their pathogenicity is due to enterotoxins causing marked hypersecretion of epithelial cells in the absence of inflammation. At the same time, the appearance of numerous autophagosomes and desquamation of epithelial cells indicate lesions which do not affect the continuity of the epithelial lining. The presence of secretory granules in the autophagosomes suggests that these changes are probably due to hypersecretion. It should be pointed out that in intranasal challenge of mice these enterotoxigenic EEC, as a rule, destroy the bronchial epithelial cells and focal defects of the epithelial lining appear (see Chapter 21). The role played by impaired vascular permeability determining the outcome of the process in the lung model (see Chapter 21) is not quite clear in intestinal loop experiments.

Similar results were seen in experiments with enterotoxigenic *E. coli* O6:H16, O15:H11 and O148:H28 obtained from S. L. Gorbach and H. L. DuPont and with EEC O148:H28 and O148:H7 isolated by T. A. Avdeeva and co-workers (1975) in Leningrad. However, living cultures of all these organisms and their enterotoxins—especially their heat-stable supernatant filtrates—evoked more frequent and pronounced overfilling of the gut loops with fluid without admixture of blood (Chakhutinskaya et al., 1975; Polotsky et al., 1976). As noted in Chapters 19 and 21, experiments with the lung model showed a conspicuous difference in toxic properties between *E. coli* O6, O15 and O148, which caused no early death of mice, and *E. coli* O6, O112a1,2b, O1 etc., which produced an early fatal serous-haemorrhagic lung oedema. The difference seems to be related to the different effect of these two types of enterotoxigenic *E. coli* on the blood vessels.

Agents of animal colibacillosis also produce enterotoxins causing similar dilatation of isolated intestinal loops without development of inflammation as living microbial cultures do (H. W. Smith and Halls, 1967*a*, *b*; Gyles and Barnum, 1969; H. W. Smith and Gyles, 1970*a*; Moon at al., 1966*a*, 1970*b*, 1971; Gyles, 1971; Whipp and Moon, 1972, etc.). Bertschinger et al. (1972) showed that agents of swine colibacillosis were attached to the supramembraneous coat of the brush border of epithelial cells and multiplied there, becoming spread along the whole length of the intestinal villi. Like in the case of oral challenge of piglets they did not penetrate the epithelium. EEC O6 and O148 associated with human cholera-like diseases behaved similarly in their experiments. It should be stressed that the pathogenicity of animal colibacillosis agents strongly depends on the ability to attach to the intestinal epithelial surface which, in turn, is associated with the presence of surface antigens (K88 in swine disease agents and K99 in agents of colibacillosis of calves and lambs). As mentioned above, enterotoxigenic *E. coli* strains deprived of the antigens or treated by specific antisera raised against them, cannot induce animal diseases. Gyles et al. (1974) have recently supposed that enterotoxigenic agents of human cholera-like diseases possess similar substances.

EXPERIMENTS WITH *ESCHERICHIA COLI* ASSOCIATED WITH INFANTILE ENTERITIS (EEC-I)

De et al. (1956) also tested EEC-I cultures (O26, O55 and O111) and observed the dilatation of infected rabbit intestinal loops due to fluid accumulation without inflammation but with necrosis of the epithelial lining. However, in subsequent investigations EEC-I induced mucosal inflammation, the degree of which depended on the virulence of the organism (McNaught and Roberts, 1958; Taylor et al., 1958, 1961; Trabulsi, 1964; Filotti, 1965; Carrillo et al., 1966; Drucker et al., 1967; Yahagi et al., 1967). Virulence probably also determines the extent of loop dilatation with the fluid exudate. The mode of interaction of EEC-I with the epithelial cells of the infected gut loop mucosa is not quite clear. Taylor et al. (1958) found organisms only in the intestinal lumen when the inflammatory response was slight, and mostly on the surface of the columnar epithelium of the villi when the response was more marked. The organisms did not penetrate deeper but infiltrated and replaced occasional dying cells. In a similar experiment Drucker et al. (1967) noted microbial adherence to the epithelial lining which was accompanied by the destruction of the brush border and damage to the apical cytoplasm of cells; the organisms could be seen in the cytoplasm of these cells. Drucker and his co-workers (using the most virulent EEC-I strains) found organisms in the lamina propria and vascular lumen, while at culturing they were recovered from the blood of mesenteric veins. Yahagi (1967) also demonstrated EEC-I in the cytoplasm of epithelial cells and in the lamina propria, in contrast to NEEC, which never penetrated into the epithelium.

Staley et al. (1970*c*), introduced EEC-I (O55:B5) into ligated loops of the small gut of newborn foals; after 2 h they observed the same attachment of the organisms to the epithelial cell surface with the destruction of the brush border and partial bacterial penetration into the cytoplasm, as found in experiments with orally challenged newborn germ-free piglets described above.

In our experiments using EEC-I and the generally accepted technique (Vasser and Polotsky, 1970*a*, 1972) we failed to evoke a distinct dilatation of exposed loops of the rabbit small gut. A little slightly turbid mucous fluid appeared in the lumen only occasionally. EEC-I organisms injected into the intestinal loops multiplied intensively in the lumen and were recovered from the mucosa washed off several times prior to homogenization and cultivation.

In histological studies mucus discharge from goblet cells, and moderate congestion and oedema of the mucosa were observed with the appearance of a small number of leukocytes. A more significant focal leukocyte response was also noted at sites. The epithelial lining was preserved throughout but organisms were seen to adhere to its surface at sites (Fig. 22-7). The brush border of the epithelial cells was frequently absent at these sites and the organisms adhered closely to the apical cytoplasm; occasionally bacteria (singly or even in clusters) were found inside the cytoplasm of the columnar absorptive and emptied goblet cells.

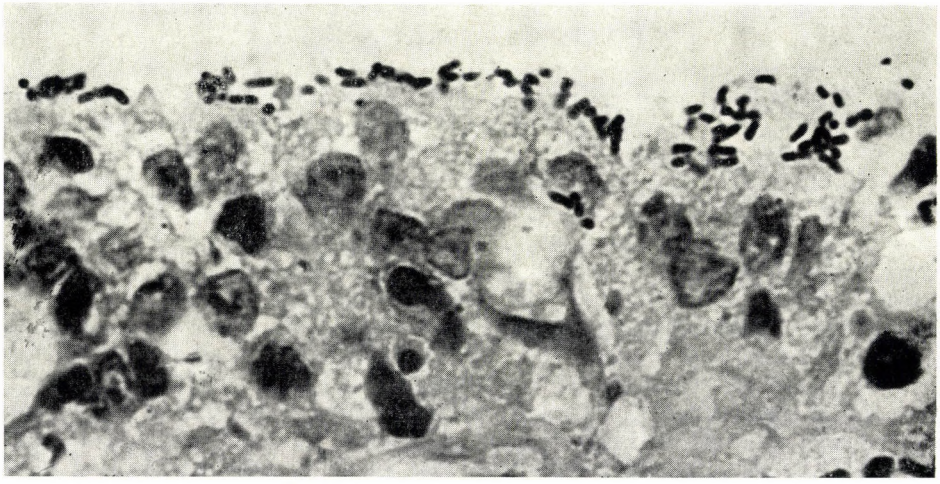


Fig. 22-7. *E. coli* O111:K58(B4) on the surface of the epithelium in a rabbit small intestinal loop, and partial penetration of the organisms into the cytoplasm of epithelial cells 24 h after challenge. Thionine, ×1500

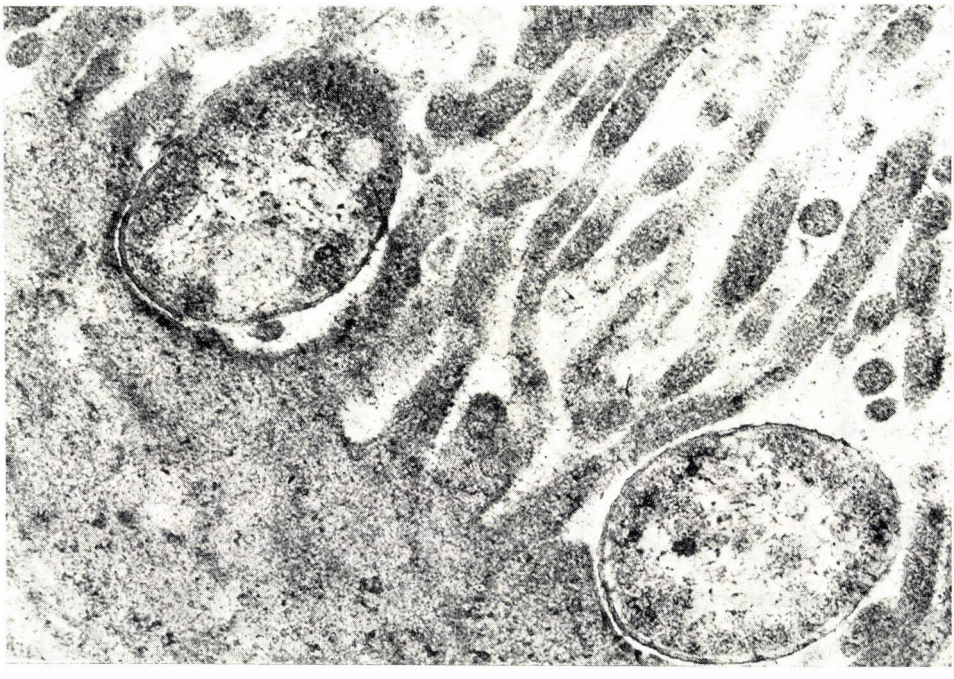


Fig. 22-8. *E. coli* O111:K58(B4) organisms push aside the microvilli of epithelial cells and become attached to the outer membrane of the cells 9 h after challenging a ligated rabbit small gut loop. ×53,000

Electron-microscopic investigations (Polotsky et al., 1976) showed that the organisms—many of them dividing—had pushed aside the microvilli and adhered to the outer membrane of epithelial cells (Fig. 22-8). Single bacilli were also revealed in the cytoplasm of epithelial cells where they were enclosed in membrane-bound vacuoles containing electron-dense osmiophilic material. Signs of damage to the organisms or to the cells were not seen.

In comparison with other authors' findings, our experiments yielded similar but less marked alterations. This might be attributed to the decrease noted recently in the virulence of *E. coli* strains associated with infantile enteritis. The lack of extensive detachment of the microvilli of the epithelial cells described by Staley and co-workers might have been due to the use of adult rabbits in our experiments instead of newborns.

Several investigators have reported on the isolation of enterotoxin-like substances in some EEC-I (Taylor and Bettelheim, 1966; Bettelheim and Taylor, 1970, 1971; H. W. Smith and Gyles, 1970*b*; Gorbach et al., 1971; Gorbach and Khurana, 1972; Kantor et al., 1974*a*; Sakazaki et al., 1974*b*). However, additional studies are needed in this field.

EXPERIMENTS WITH *ESCHERICHIA COLI* ASSOCIATED WITH DYSENTERY-LIKE DISEASE OF ADULTS AND CHILDREN (SHIGELLA-LIKE EEC-II)

Ogawa et al. (1968*b*) introduced EEC-I cultures ("salmonella-like" EEC) and shigella-like EEC cultures (EEC-II O28a28c, O124, O136, O143, O144) into ligated loops of the most terminal ileum and proximal colon of the rabbit. In experiments with EEC-I they observed macroscopic alterations very rarely: there was only histological evidence of moderate oedema. The organisms did not penetrate the mucosa. At the same time shigella-like EEC-II appeared in the cytoplasm of epithelial cells in the first hours after challenge. Subsequently their number increased, the epithelial cells were destroyed and a characteristic purulent-ulcerative inflammation with accummulation of an inflammatory exudate in the lumen of the infected loops developed. In experiments on ligated loops of the rabbit small gut Formal et al. (1971*a*) and DuPont et al. (1971) also observed penetration of EEC O124, O143, O144 and *Sh. flexneri* into the epithelial cells and lamina propria with development of an intensive inflammation and destruction of the epithelial lining. Broth culture filtrates of these organisms and their lysates caused no such changes suggesting that they failed to produce enterotoxin.*

In our experiments with shigella-like EEC-II (O28a28c, O124, O136, O143, O144), 2–4 $\times 10^8$ organisms in 1 ml of 18-h peptone water culture

* Sakazaki et al. (1974*b*) showed that filtrates of some cultures of shigella-like EEC-II and even 'normal' NEEC had occasionally an enterotoxigenic effect.

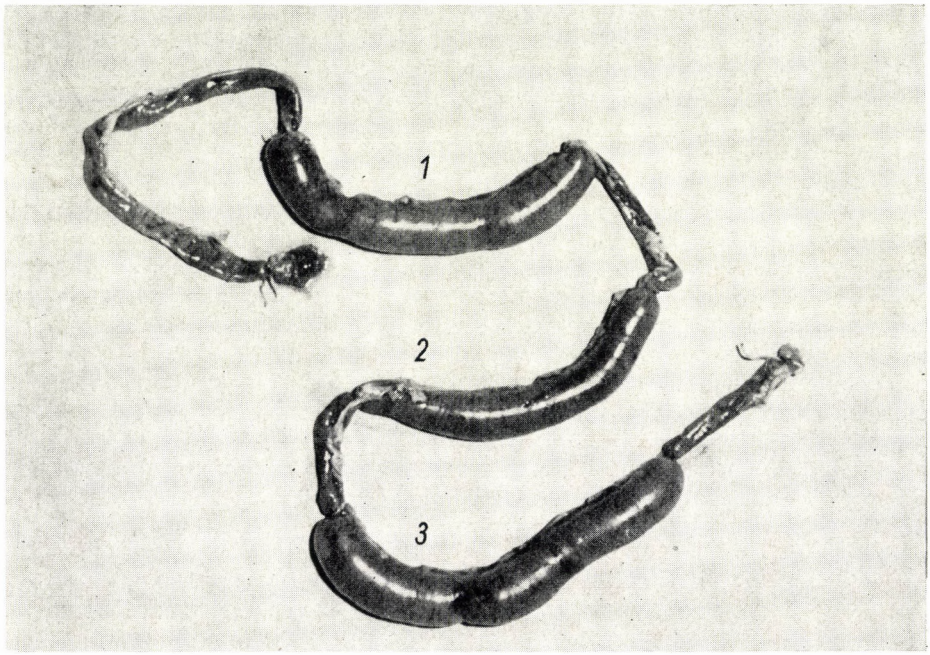
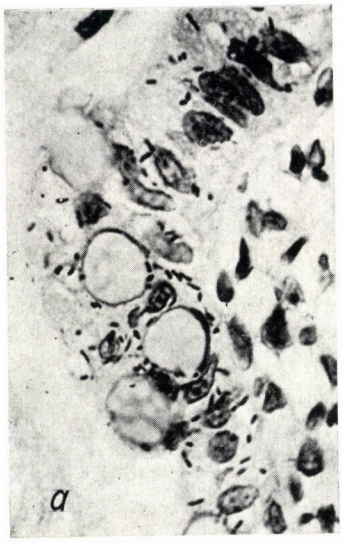


Fig. 22-9. Ligated rabbit ileal loops 9 h after challenge with *Sh. sonnei* (*1*), *E. coli* O124:K72(B17) (*2*) and *S. typhimurium* (*3*)

induced the dilatation of exposed loops of intestine approximately in one half of the cases after 24 h (Vasser and Polotsky, 1970*a*, 1972). Using larger doses ($3–42 \times 10^9$ organisms in 1–5 ml of 6-h broth culture grown with shaking), EEC-II O124 almost always caused accumulation of great amounts of purulent-haemorrhagic exudate as soon as after 9–12 h (Fig. 22-9).

Histological and electron-microscopic studies (Polotsky et al., 1976) showed that within the first hours after challenge shigella-like EEC-II organisms penetrated into the cytoplasm of unchanged epithelial cells of the mucosa and multiplied there, mostly without being separated by a membrane from the cytoplasm (Fig. 22-10*a*, *b*). The multiplying bacteria destroyed the epithelial lining of the villi and crypts (Fig. 22-10*c*). A purulent-ulcerative inflammation developed and the lumen of the challenged loops was filled with abundant purulent exudate. A small number of organisms were occasionally detected in the lamina propria before the appearance of the defects of the epithelial lining. Here they were engulfed by leukocytes destroying the organisms and by macrophages which left the bacteria unharmed so that even signs of division could be observed. Nine to 12 h after challenge erosions and small ulcerations appeared followed by accumulation of large numbers of free organisms in addition to those engulfed by leukocytes and macrophages. These findings were similar to



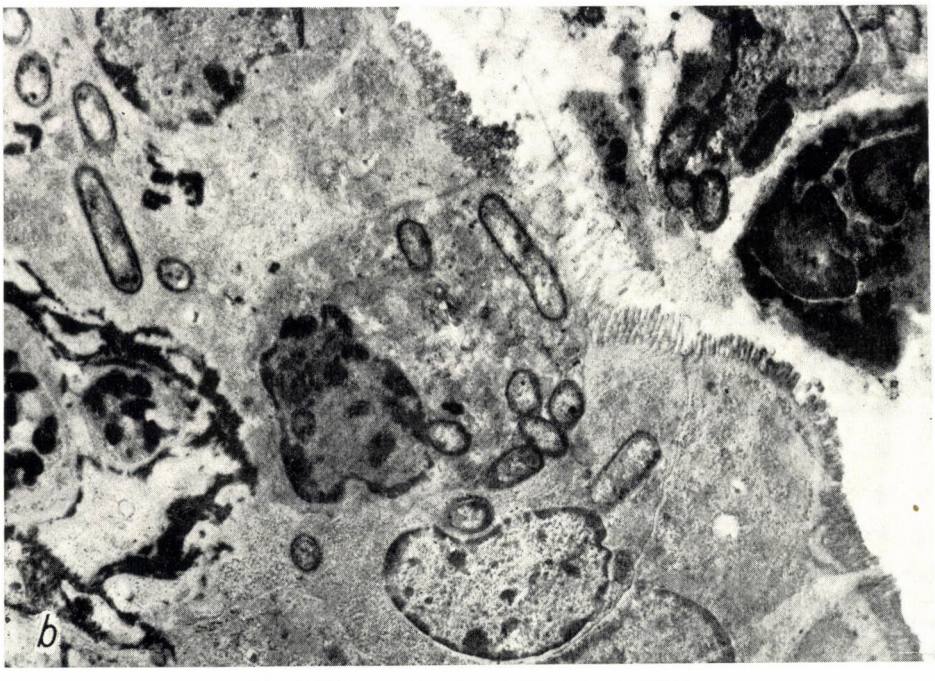


Fig. 22-10*a* and *b* (for caption see p. 314)

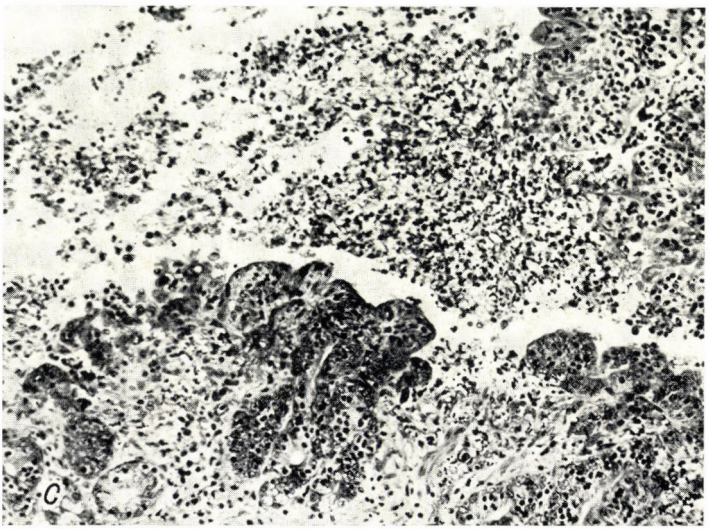


Fig. 22-10*a*, *b*. *E. coli* O124:K72(B17) in the cytoplasm of epithelial cells of an intestinal loop 9 h after challenge. *a* thionine, × 1000; *b* × 5300. *c* Development of purulent-ulcerative inflammation in the mucosa 12 h after challenge. Eosin-azure, × 180

those observed with shigellae in other models (see Chapter 7, 8 and 10), including passage of the organisms into adjacent cells (see Fig. 22-10*b*). In experiments with *E. coli* O124 we also found intraepithelial bacteria enclosed in membrane-bound vacuoles containing even electron-dense osmiophilic material. The vacuoles were very similar to phagolysosomes although no degenerative alterations of the organisms were seen in them. They also occurred in experiments using the urinary bladder model and *E. coli* O124 (see Chapter 20), rather than in the experiments with shigellae.

Thus, similarly to shigellae, agents of dysentery-like diseases (EEC-II O124 and others) exhibited intraepithelial parasitism in both this and other models. To correlate the pathological processes induced by these organisms, we extended our studies to shigellae.

EXPERIMENTS WITH SHIGELLAE

Taylor and Wilkins (1961) were the first to report that some *Shigella* and *Salmonella* strains (obviously, more freshly isolated ones) caused the dilatation of ligated loops of the rabbit's small intestine with fluid accumulation. In experiments with shigellae Arm et al. (1965) revealed leukocyte infiltration and mucosal ulceration along with marked oedema and haemorrhages. Killed shigellae and their endotoxins, filtrates and lysates induced no such changes. Yahagi et al. (1967) observed approximately the same alterations in the experiments with virulent shigellae. However, having found the organ-

isms in the epithelial cells and the lamina propria, Yahagi (1967) rejected the existence of a direct relationship between this fact and the development of an inflammatory process. Using ligated loops of the rabbit's terminal ileum and proximal colon Ogawa and Nakamura (1969*a*) established that the purulent-ulcerative inflammation of the mucosa was the result of shigella invasion into the epithelial cells.

In our experiments freshly isolated *Sh. sonnei*, *Sh. flexneri* and *Sh. boydii* induced the accumulation of a turbid, serous or haemorrhagic exudate in doses of $9–20 \times 10^7$ organisms in the majority of the cases (Vasser and Polotsky, 1970*a*, 1972) and in doses of $1–3 \times 10^9$ in almost all cases, as a rule, after 9–12 h (see Fig. 22-9).

Histological examinations revealed a marked inflammatory process in the small intestinal loops. Polymorphonuclears started accumulating in the mucosa as soon as after 1 h, and infiltrated the mucosa within 9–12 h. The epithelial lining, densely covered with a purulent exudate, was destroyed at many sites. Soon after the challenge (from 30 min to 2 h) the organisms could be seen attached to the epithelial cell surface, the brush border being absent at such places. Organisms (including dividing ones) were frequently found in the apical, paranuclear, and even basal cytoplasmic areas of the epithelial cells. The number of epithelial cells infected by shigellae as well as that of the organisms they harboured increased subsequently.

Thus, the pathological processes caused by shigellae and shigella-like EEC-II in the mucosa of isolated rabbit gut loops were fairly similar, though shigellae caused more severe lesions of the epithelial lining.* In addition, shigellae survived longer in the leukocytes.

Experiments with shigellae on the gut loop model also permitted the study of the early stages of interaction between the organisms and the gut epithelial cells. Electron microscopy (Polotsky et al., 1974*b*, 1976) showed that some of the shigellae were arranged on the surface of the glycocalyx layer** covering the microvilli of the epithelial cells, 30 min after challenge (Fig. 22-11). After 2 h the organisms were much more frequently found on the surface of epithelial cells; the glycocalyx was absent where organisms were located, while the microvilli in contact with shigellae were swollen and transformed into large bubbles protruding into the lumen (Fig. 22-12*a–d*). Such bubbles having a homogeneous finely ground content were also seen in the gut lumen. At some sites of shigella attachment, the microvilli were absent and the organisms were lying directly on the cell membrane giving rise to invaginations (Fig. 22-12*a-d*). Disintegration of the membrane was not observed, though during the same period shigellae were also found inside the epithelial cells (Fig. 22-12*a, c, e, f*). Some of the organisms in the most apical parts of the epithelial cells were completely or partly

* Working with freshly isolated strains of *Sh. dysenteriae* 1, Gemski et al. (1972) observed especially severe lesions in isolated loops of the rabbit small gut.

** As mentioned above, the glycocalyx was revealed by means of special fixation in glutaraldehyde with addition of thiophosphamide followed by osmium tetroxide according to the method of Komissarchik and Snigirevskaya (1973); alcian blue, ruthenium red or lanthanum were occasionally used instead of thiophosphamide.

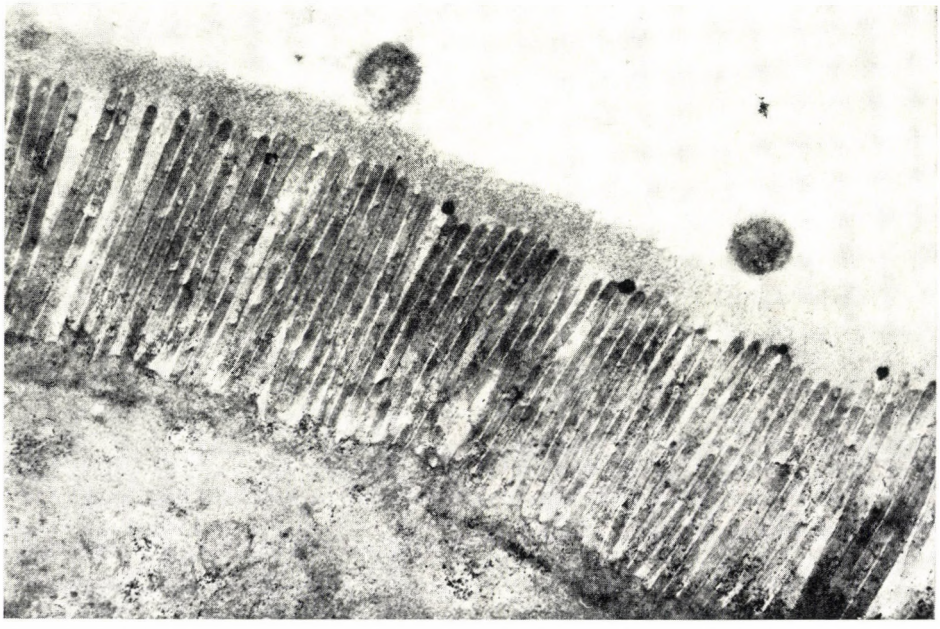


Fig. 22-11. *Sh. sonnei* on the surface of the glycocalyx layer covering the microvilli of the epithelial cells 30 min after challenging a ligated rabbit small gut loop. ×18,000

covered with a three-layer membrane (indistinguishable from the outer cell membrane) (Fig. 22-12*e*, *f*). In deeper portions of the cytoplasm the vigorously multiplying organisms were found to lie freely without being surrounded by a membrane. Meanwhile, in the cytoplasm of numerous leukocytes that accumulated in the lumen of the infected intestinal loops, and in the lamina propria, shigellae were seen in membrane-bound vacuoles also containing some electron-dense material (obviously, a product of lysosomes); many organisms in these phagolysosomes were apparently disintegrated.

Thus, shigellae are first attached to the glycocalyx of unaltered epithelial cells, then cause characteristic alterations, destroy the microvilli, and subsequently penetrate into the cytoplasm of epithelial cells. Here the organisms are first surrounded by a three-layer membrane which obviously represents a detached invagination of the outer cell membrane; this is then rapidly destroyed and the organisms multiply freely in the cytoplasm.

It has long been known that *Sh. dysenteriae* 1 ("Shiga bacillus") differs from all other shigellae in its ability to produce an exotoxin called "neurotoxin". It has recently been shown that this substance is a heat-labile enterotoxin, causing the dilatation of ligated loops of the rabbit's small gut; the liquid accumulating, however, contains higher amounts of protein, potassium and chlorides than after the administration of *Vibrio cholerae* or *E. coli* enterotoxin (Keusch et al., 1972*a*). Dysentery enterotoxin differs from that of cholera in the lack of the "vascular perme-

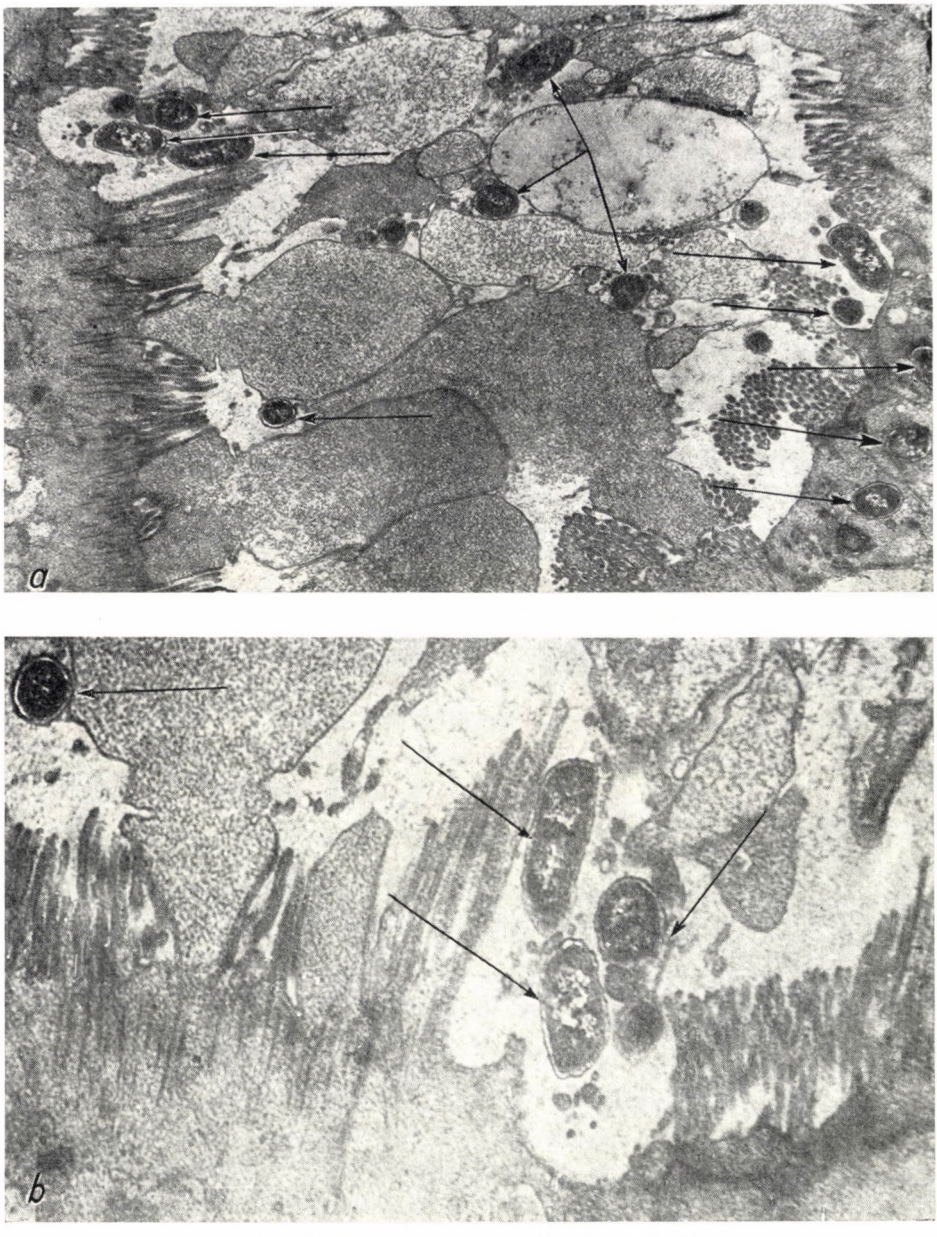


Fig. 22-12*a* and *b* (for caption see p. 319)

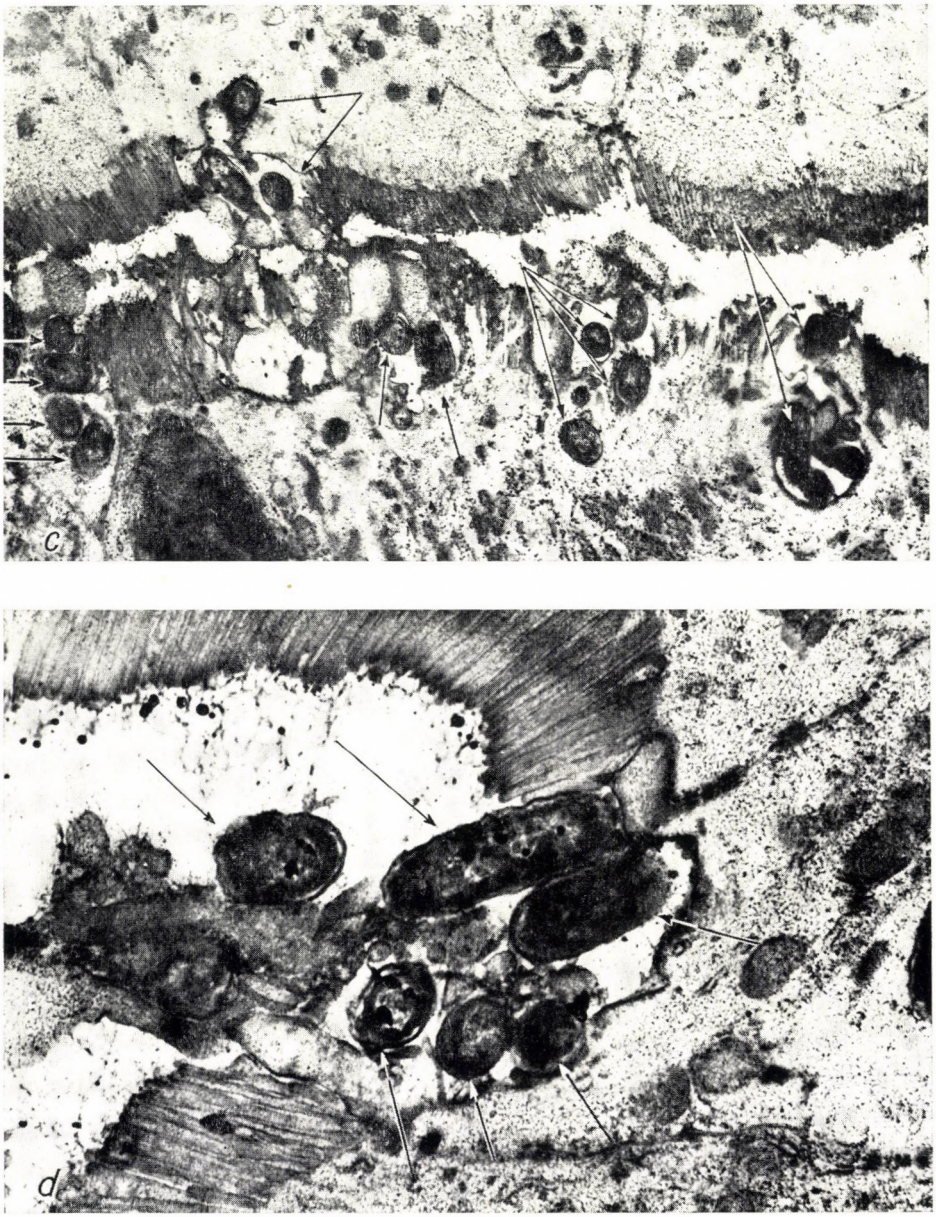


Fig. 22-12*c* and *d* (for caption see p. 319)

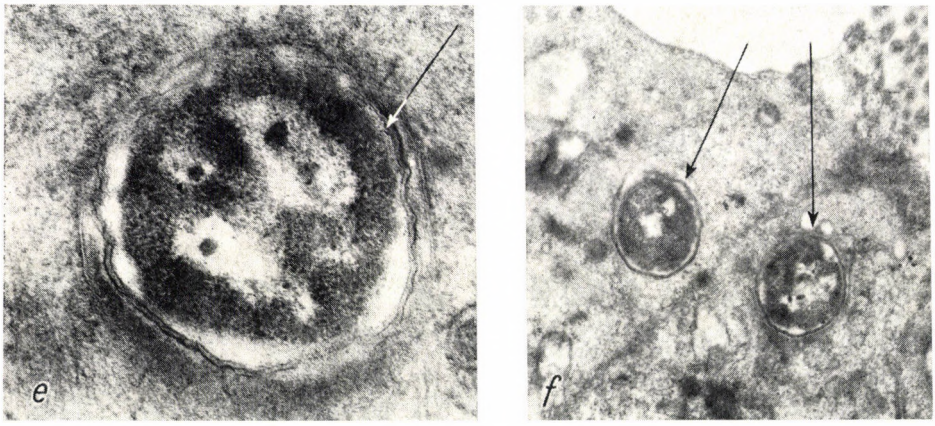


Fig. 22-12. *Sh. sonnei* organisms (arrows) on the surface (*a–d*) and in the cytoplasm (*a*, *c*, *e*, *f*) of epithelial cells of the small gut loop and alterations of the microvilli (*a–d*) 2 h after challenge. *a* ×4500, *b* ×9000, *c* ×5600, *d* ×14,400, *e* ×70,000, *f* ×20,000

ability factor" (demonstrated with intracutaneous test in rabbits) and also in *in vivo* and *in vitro* cytotoxicity (Grady and Keusch, 1971; Keusch et al., 1972*a*, *b*, *c*). When injected into the gut loops, dysentery enterotoxin induces lesions of the epithelial cells and slight inflammatory alterations (Keusch et al., 1972*b*). In HeLa cell cultures, it destroys the cellular monolayer (Keusch et al., 1972*c*). Biochemical studies have not revealed any effect of the dysentery enterotoxin on adenyl cyclase activity (Greenough and Keusch, cited by Carpenter, 1972). Consequently, it differs from the enterotoxins of the agents of cholera and cholera-like diseases.

EXPERIMENTS WITH SALMONELLAE

Administration of salmonellae into isolated small intestinal loops is of special interest as in natural infection these organisms usually attack the small intestine. The first experiments with salmonellae were carried out by Florey (1933), who challenged guinea pigs by injecting dense milk suspension of "*Bacillus aertrycke*" *(Salmonella typhimurium)* into the lumen of an intestinal loop isolated according to Thiry-Vella's technique. In contrast to coal particles and non-pathogenic organisms which became covered with mucus and moved away from the epithelial cell surface (single non-pathogenic organisms coming into contact with the brush border of the epithelial cells were destroyed), salmonella organisms penetrated through unaltered epithelial cells. In these cells Florey distinguished accumulations of small, poorly staining vacuole-bound organisms in the apical cytoplasm, paranuclear and basal areas. They seemed to enter the lamina propria therefrom. Microbial invasion produced an inflammatory

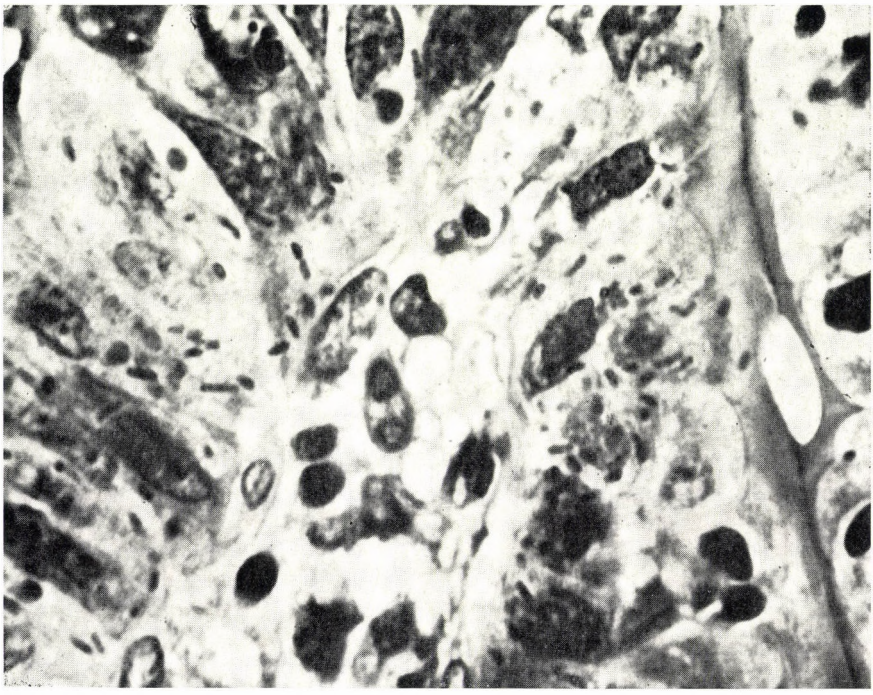


Fig. 22-13. *S. typhimurium* organisms in the cytoplasm of epithelial cells of a small gut loop 9 h after challenge. Eosin-azure, ×1600

process with infiltration of the mucosa with many leukocytes and few macrophages. Both kinds of phagocytes also emigrated into the lumen of the loops. The epithelial cells containing the organisms remained viable, whereas, in Florey's opinion, the organisms were partly destroyed in them. The epithelial lining preserved its continuity though an epithelial cell desquamation was noted. In recent studies Takeuchi (1967, 1971) and other authors confirmed the invasive properties of salmonellae and their ability to pass through the epithelium (see Chapter 17). It has, however, been found that they are not destroyed in the epithelial cells (they are, in fact, only stained less intensively) and that they are probably not transported through the epithelial lining by phagocytes which have engulfed them.

Our experiments with a number of *Salmonella* strains freshly isolated from patients (chiefly *Salmonella typhimurium*) were performed on ligated loops of rabbit small gut simultaneously and using the same method as with the other enteropathogens described above. Administration of $2–4 \times 10^8$ organisms resulted in dilatation of the loops with a serous or serous-haemorrhagic exudate in approximately 40 per cent of the cases; however, dilatation was, as a rule, noted with doses of $4–87 \times 10^9$ bacteria (see Fig.

22-9). Judging from the data of bacterial counts, salmonellae multiplied in the lumen much less intensively than *E. coli* or shigellae; in some cases there was no increase in their number at all (Vasser and Polotsky, 1970*a*, 1972).

Histological examinations showed that in contrast to shigellae, various *Salmonella* strains induced a much less marked inflammatory response which did not affect the integrity of the epithelial lining. The leukocyte response was somewhat increased in experiments with smaller doses. Polymorphonuclears were usually seen scattered in the lamina propria of the villi and deep mucosa, occasionally accumulating at the mucosal-submucosal border. They also emigrated into the gut lumen and crypts.

From 30 min to 2 h after challenge few organisms were attached to the surface of villous epithelial cells, and single bacilli were occasionally revealed in the brush border of the enterocytes. Later salmonellae were more frequently encountered inside epithelial cells (Fig. 22-13); they also penetrated into the lamina propria where some of them were free, others occurred in the cytoplasm of leukocytes and macrophages. Unaltered rods as well as their semi destroyed fragments could be seen in the polymorphonuclears while the macrophages contained intact bacteria; in the epithelial cells there were usually few salmonellae.

Electron-microscopic studies, in general, confirmed the observations of Takeuchi obtained in experiments with enteral challenge of guinea pigs (see Chapter 17). We also observed that, unlike shigellae, salmonellae were mainly transported via the epithelial cells without causing essential damage to them. They were, as a rule, enclosed in membrane-bound vacuoles. When thiophosphamide or other substances fixing the glycocalyx were added to the usual glutaraldehyde-osmium tetroxide fixation, an early attachment of salmonellae to this supramembraneous coat of enterocyte microvilli was noted (Polotsky et al., 1976). Takeuchi (1967, 1971), whose electron micrographs obviously did not preserve the glycocalyx, failed to observe this phenomenon. He noted the beginning of interaction between salmonellae and the epithelium as soon as the former approached the microvilli by less than 35 nm, after which they penetrated into the cytoplasm of enterocytes pushing the microvilli aside.

Tannock et al. (1975), describing histological examinations following intragastric challenge of mice with salmonellae, stressed that the organisms first became attached to the surface of small intestinal epithelium and then penetrated into the mesenteric lymph nodes and the spleen.

Challenging ligated rabbit small gut loops with *S. typhimurium*, Giannella et al. (1973*a*) also found intraepithelial localization of salmonellae and the development of focal inflammation without damage to the epithelial lining. They drew attention to the fact that the amount of fluid accumulation often did not correspond to the degree of mucosal inflammation, and made an attempt to find enterotoxin-like substances in their cultures. However, injection of salmonella filtrates and lysates into the loops gave a negative result. Nevertheless, Sakazaki et al. (1974*c*), using a new culture medium for growing *Salmonella* claimed to have shown enterotoxigenicity of filtrates of all eleven cultures isolated by them from patients. Living cul-

tures, however, caused the distention of intestinal loops less frequently. In our experiments living salmonella cultures (used approximately in the same doses as by Sakazaki et al.) almost always gave positive results. This discrepancy may be due to the exclusive use of strains freshly isolated from the most severe cases in our experiments. Koupal and Deibel (1975) described protein enterotoxin produced by *S. enteritidis* and localized in the cell wall or outer membrane. It gave positive results when applied to rabbit small gut loops, but was much easier to demonstrate in newborn mice using the modified technique of Dean et al. (1972).

Thus, the results of animal experiments described in this Chapter have shown the diverse nature of pathogenicity of enteropathogenic *E. coli*. They allow to assess the pathogenic properties of EEC of different categories and groups established in Chapter 19 with greater precision and to compare them with one another and with those of other enteropathogens.

EEC-I, agents of infantile *E. coli* enteritis, exhibited an ability to attach to and multiply on the surface of the intestinal epithelium in enteral challenge of piglets and in experiments on isolated intestinal loops, similarly to EEC-I behaviour, as is the case in affected infants (see Chapter 19). Some of the organisms also penetrate the epithelial cells, but do not multiply therein. They cause a focal inflammatory response related, evidently, to the degree of the agent's virulence.

There are no pathological data on intestinal lesions in diseases of adults and children caused by shigella-like and enterotoxigenic EEC. Clinical observations suggest a close similarity of the former to mild forms of dysentery while the latter greatly resemble mild forms of cholera (see Chapter 19). These analogies were confirmed experimentally.

Similarly to shigellae, shigella-like EEC-II (O124, etc.) penetrate into unaltered epithelial cells in all models; they multiply and destroy the cells causing a purulent-ulcerative inflammation of the mucosa. Like shigellae, these organisms are usually not separated from the cytoplasm of epithelial cells by membranes. On the other hand, shigella-like EEC-II are less virulent than shigellae, attack a smaller number of epithelial cells, are occasionally enclosed in phagosome-like vacuoles in the cytoplasm of these cells, and are more rapidly destroyed by leukocytes. Experimental observations suggest that in affected persons these organisms induce similar, though less severe, intestinal lesions as shigellae.

Enterotoxigenic EEC associated with diarrhoeal disease of adults and children resembling mild forms of cholera exhibit properties that are similar to those of cholera vibrios tested by enteral challenge of animals and in experiments on isolated gut loops. Their pathogenicity is based on the production of cholera-like enterotoxins bringing about abundant hypersecretion. Multiplying on the surface of the mucosa and in the small gut lumen, they do not induce inflammation or conspicuous lesions. However, electron microscopic studies have shown that numerous autophagosomes are formed in epithelial cells, along with signs of markedly increased secretion, pointing to damage of the cytoplasm and cell organelles. In spite of the desquamation of many of these cells the integrity of the intestinal epithelial lining is preserved.

The early stages of the interaction between different EEC, shigellae, salmonellae and the intestinal epithelium were studied and compared on intestinal loops. In spite of recent progress in our knowledge of the pathogenic properties of different enterobacteria, a number of unsolved problems remain, e.g. that of the ability of EEC-I, salmonellae and other *Enterobacteriaceae* to produce enterotoxin-like substances, of the similarities and differences between these substances, their importance in the pathogenesis of infectious processes, and of the nature of pathogenic factors associated with the ability of microorganisms to interact with the intestinal epithelium in so different ways.

CHAPTER 23

GENERAL CONCLUSIONS

by

M. V. VOINO-YASENETSKY

Although infectious processes induced by pathogenic organisms and viruses affect the whole body of the patient, usually in each contagious disease there is a special selectivity and not infrequently a characteristic impairment of separate organs and systems. Direct interaction between parasites and the host is best displayed in these pathologic processes. Findings described in this volume prove that the specific features of enteric infections are due to special biological properties of their agents.

Different pathogenic enterobacteria have some common properties, which are, however, of different importance in each group of organisms. Almost all of them are able to penetrate into epithelial cells; however, their settling and growth in the intestinal epithelium are the main pathogenic factors only with shigellae and shigella-like *E. coli*. Salmonellae only pass through the epithelium and then settle in mucosal macrophages. *E. coli* may also survive and even grow in macrophages but this ability (also characteristic of non-pathogenic *E. coli*) does not play an important part in the development of the infection they induce.

If engulfed by macrophages, shigellae are usually destroyed, although in lung model experiments Dragunskaya and Smirnova (1973) encountered a typical *Sh. sonnei* strain which multiplied not only in the bronchial epithelium but also in alveolar phagocytes. Some of our *Sh. flexneri* strains behaved similarly, while in properties they resembled common shigellae.

E. coli strains isolated recently from cholera-like diseases of man show an outstanding behaviour. Similarly to cholera vibrios, they produce an enterotoxin responsible for characteristic manifestations of the disease but do not possess the ability of intracellular parasitism. Thus, the most important factor of their pathogenicity is the production of toxic substances. The effect of similar exotoxins may apparently be involved in the development of acute salmonella gastroenteritis as well (Chapter 18).

Enterotoxin-type substances have been suggested to play an important part in the pathogenesis of dysentery (Keusch et al., 1970), though up to the present they have only been detected in *Sh. dysenteriae* 1. Advances in microbial genetics helped to disprove this hypothesis.

Certain pathogenic properties of enterobacteria are known to be due to the presence of the appropriate genes (Formal et al., 1971*b*; Timakov and Petrovskaya, 1972; Petrovskaya, 1974). It has become possible to obtain mutants and hybrids of these organisms, including attenuated ones suitable

TABLE 23-I

Studies in animal models with strain 3817 (T form) of Shigella dysenteriae 1 and its mutant derivatives
(Gemski et al., 1972)

Strain	Invasion	Toxin production	Conjunctival test	Rabbit ileal loop	Invasion of guinea pig intestine	Clinical disease in monkey
3818-T	+	+	4/4*	6/6	4/4	9/15
3818-O	−	+	0/4	4/4	0/4	0/15
725–78	+	−	4/4	7/8	4/4	3/6
735–19	−	−	0/4	0/4	0/4	not done

* Number of positive/total tested.

for immunization or special studies. Using fresh strains of *Sh. dysenteriae* 1 isolated during the recent epidemic in Guatemala, Gemski et al. (1972) selected four variants of these organisms:

1. Organisms which have preserved their biological properties, i.e. are capable of invading epithelial cells and producing enterotoxin;
2. Variants producing this toxin but unable to penetrate the epithelium;
3. Variants penetrating the epithelium but devoid of the toxin;
4. Variants devoid of both properties.

The testing of these strains on various experimental models (Table 23-I) confirmed that the capacity to parasitize epithelial cells is the main factor of shigella pathogenicity. Without this property, the organisms are not dangerous even if producing enterotoxin. While the toxin causes fluid accumulation in the isolated intestinal loop, infection fails to develop, as the organisms do not penetrate the epithelium. In contrast, a non-toxic but invasive strain is markedly pathogenic.

Having established which properties of intestinal organisms play a decisive role in causing diseases, one cannot neglect other biological peculiarities. Probably the severity of intestinal lesions in dysentery caused by *Sh. dysenteriae* 1 is related to the action of their toxin which enhances the injury resulting from intracellular parasitism.

In experiments on tissue cultures, *Sh. dysenteriae* 1 exotoxin exhibited distinct cytopathogenicity (Vigari et al., 1960; Keusch et al., 1972*c*). Compared with a penetrating non-toxigenic culture, a toxigenic penetrating *Sh. dysenteria* 1 strain was found by Gemski et al. (1972) to induce a more marked intestinal inflammation both by enteral challenge in guinea pigs and by being injected into an isolated intestinal loop of rabbit. It is still unclear why the toxin of these organisms, differing from other enterotoxins in a number of features (see Chapter 22), does not affect the blood vessels, although the impairment of the permeability of vascular walls resulting in haemorrhages with fibrin deposition in the affected intestinal areas, is a characteristic feature of Shiga dysentery. However, these vascular disturb-

ances may result from a non-specific secondary infection owing to the extensive destruction of the colonic epithelial lining (see Chapter 12).

Properties of organisms not obligatory for the primary induction of a disease but still influencing its course and issue may be called secondary pathogenicity factors. In addition to exotoxins of *Sh. dysenteriae* 1, endotoxins released on disintegration of all Gram-negative organisms may be included among these factors. They certainly play a less important role in the pathogenesis of intestinal infections than formerly believed, but obviously have some part in the development of local lesions and cellular (especially macrophage) response as well as in that of general signs like fever, non-specific resistance, etc.

Relative resistance to phagocytosis by polymorphonuclears should be recognized as the most important secondary factor of pathogenicity. In different experiments (especially in the lung model) it was observed that polymorphonuclears actively destroyed parasites when they were outside the epithelial cells. However, leukocytes were frequently unable to kill the organisms engulfed by them and perished themselves. This usually occurred with highly virulent *Shigella*, *Salmonella* and *Escherichia* strains. The same "incomplete phagocytosis" has also been noted in experiments with leukocytes *in vitro* (Berman and Slavskaya, 1962; Mikhailov et al., 1968, etc.). Further, leukocytes of different animals do not cope equally well with pathogenic intestinal organisms (with salmonellae, in particular) having, obviously, an effect on the course of experimental infections and on their reproducibility.

It is quite probable that besides exotoxin production, which currently attracts attention of most workers, the ability of different enterobacteria to parasitize either epithelial or macrophage-type cells is due to the activity of certain enzymes or substances the nature of which is not yet known. For the elucidation of such factors combined bacteriological and morphological studies would be needed that might throw light upon the behaviour of the organisms in the host. Many important biological properties of pathogenic organisms are only exhibited during their immediate interaction with live tissues and cells. Probably some change in their biochemical structure only occurs when they settle in the body "sensitive" to them. This assumption is supported by the fact that the same organisms may considerably differ even in their appearance when growing on dead tissues or in living cells.

INTRACELLULAR PARASITISM OF DIFFERENT *ENTEROBACTERIACEAE*

The way in which *Salmonella* and *Escherichia* enter macrophages is known well enough. These phagocytic cells engulf any extraneous particles, including microorganisms, they encounter in the internal medium of the body. Far less convincing is the suggestion (Szturm-Rubinsten and Piéchaud, 1963; Wessel and Rácz, 1967) that the intestinal epithelium takes up shigellae since these bacteria are devoid of motile organs and cannot,

therefore, invade the epithelial cells by themselves. There is no need to discuss the extremely controversial problem of the phagocytic capacity of the epithelium. Even taking this improbable possibility for granted, it remains to explain why the epithelial cells select from the abundant intestinal flora only organisms dangerous to them. It is, therefore, generally accepted that invasion of the epithelium is a genetically determined property of the organisms. Data have recently become available on the modes of penetration into epithelial cells by shigellae, salmonellae and pathogenic *E. coli*. It is significant that these were not at all identical for different organisms.

To get inside the cells of the intestinal epithelium, the organisms must, first of all, overcome resistance offered by the brush border. Electron-microscopic studies described in Chapters 17 and 22 have suggested that pathogenic organisms are able to destroy the microvilli forming this border. However, salmonellae and agents of *E. coli* enteritis only impair relatively few microvilli. In contrast, shigellae damage the brush border more extensively (see Fig. 22-12).

Having destroyed the brush border, pathogenic organisms still have no direct access to epithelial cells: the cytoplasm of these cells is covered with an external membrane preserving its continuity even after the destruction of microvilli. Shigellae seem to indent this membrane (see Fig. 22-12*b–d*); the resulting pouch then becomes detached, and an isolated vacuole containing the organisms might be found in the cytoplasm. Similar observations have been made in experiments with salmonellae (Takeuchi, 1967) and *E. coli* enteritis agents (Stales et al., 1969*b*).

Certainly, the electron micrographs may be explained in a different manner. It may be assumed that the cell itself plays the active part in engulfing the organisms which come near its surface similarly to droplets of liquid or fat under normal conditions. But this phenomenon, defined as pinocytosis, normally occurs without any lesions of the microvilli (Holter, 1961). Pinocytotic vesicles are much smaller than the vacuoles containing the organisms. In case of salmonella and shigella penetration, the vacuoles may contain several organisms, remnants of microvilli and, as observed by Takeuchi (1967, 1971), cytoplasmic components of the epithelial cell (endoplasmic reticulum, mitochondria and ribosomes). Thus they bear more resemblance to autophagosomes (membrane-bound structures occurring as a result of local damage to the cytoplasm). It seems likely that when penetrating, pathogenic organisms make, to some extent, use of physiological mechanisms of the epithelial cell; this, however does not justify the presumption of pinocytosis or some peculiar form of phagocytosis of parasites.

After penetrating the intestinal epithelium, salmonellae remain membrane-bound (or bound by some electron-dense substance), migrate to the base of the cell and enter the lamina propria (see Chapters 17 and 22). They do not pass into neighbouring epithelial cells.* Even in the epithelium of the

* At the same time, when penetrating the mucosa from the intestinal lumen, salmonellae are able to pass not only through the brush border but also between the adjacent epithelial cells, penetrating their lateral surface (Takeuchi, 1967).

urinary bladder, where they are capable of multiplying, these organisms do not pass beyond the border of single cells (see Chapter 16). Agents of *E. coli* enteritis are similar to salmonellae in this respect, although they are more frequently found on the surface of the epithelium than inside the cells (see Chapter 22).

Shigellae and shigella-like *E. coli* (EEC-II) exhibit a quite different behaviour in the cytoplasm of epithelial cells. They soon dispose of the cell membrane fragment surrounding them at the moment of penetration, and grow directly in the cytoplasm. They frequently pass from one cell to another, causing extensive lesions of the epithelial lining. The manner in which shigellae pass into adjacent epithelial cells is of interest. Figure 7-5 convincingly proves that they push ahead, indenting the external membranes of both the host cell and of an adjacent cell. Such a way of penetration is confirmed by the presence of double membranes occasionally seen to surround the organisms (see Fig. 7-6).

The powers enabling pathogenic organisms to penetrate epithelial cells are not known. There is no ground for the assumption that shigellae acquire some kind of "organ of locomotion" like flagellae, during their growth in live tissues. Fimbria occurring in some pathogenic enterobacteria may, apparently, contribute to the attachment of organisms to the cell surface (see Fig. 22-2*b*), but inside the cell they would only be impeded by such a fringe. Figure 7-5 shows an interesting phenomenon: at the anterior end of a shigella passing from one epithelial cell into another, a peculiar cap is apparent. This might be interpreted as a sign of secretion of some substance altering the state of the cell membrane.

Obviously, while lodging in the cells, microorganisms do not secrete toxins. Frequently the host cell is not impaired even if the organisms grow directly in its cytoplasm* (Takeuchi et al., 1965; Tenner et al., 1970). The bacteria are not digested or destroyed in epithelial cells; consequently, endotoxins are not active under these conditions. They are, however, released during successful ingestion of the organisms by leukocytes and macrophages which results in bacterial disintegration. The endotoxins may damage not only the phagocyte itself but the adjacent tissue elements as well. It is not quite clear whether the effect of endotoxins contributes to further invasion of the cells by organisms (shigellae in particular). At any rate, the cells involved must not die because shigellae do not grow in dead cells (Watkins, 1960).

MODEL EXPERIMENTS AND THE NATURAL COURSE OF INFECTION

Discussion of the results of various model experiments with enteric agents (see Chapters 12 and 18) has confirmed the methodological premises considered in Chapter 3. In these experiments local specific infectious processes, but not any specific disease (dysentery or typhoid) are reproduced.

* Toxic lesion of the cytoplasm of tissue culture cells during intracellular growth of shigellae, which has been described by Nosova et al. (1972), seems to be exaggerated by an artifact due to the embedding of samples in methacrylate.

There are a number of models available for experimental and other purposes (e.g. determination of bacterial virulence, testing of drugs, etc.) and care should be taken to select the most appropriate one. The widespread and simple intraperitoneal test e.g. proved unsuitable for experiments with *Shigella* and *E. coli*. In various studies made with this model the results were usually only assessed in terms of mortality rate, as there was no evidence why the animals died.

Unfortunately the pathological lesions in typhoid infection (non-reproducible experimentally) and animal salmonelloses were found to be basically different in spite of the many similar features. Thus the pathogenesis of human typhoid and the so-called toxinfections could not sufficiently be cleared up. In contrast, a striking similarity and even identity has been found between dysenteric intestinal lesions in humans and in monkeys (see Chapter 12), therefore, the results of studying dysentery in monkeys are useful for understanding the human pathology of the disease.

Without wanting to repeat the facts dealt with in Chapter 12, an important feature of the morphological changes occurring in the intestines in dysentery must be emphasized, namely that they are not uniform. Two different pathological processes may be observed: the first is specific for dysentery and consists of destruction of the mucosa to varying degrees which is directly due to shigellae and of a local inflammatory response with the active participation of polymorphonuclears. The inflammation is always focal; even at the sites of most severe and seemingly continuous lesions, areas of mucosa which are damaged and abundantly covered by leukocyte aggregations, alternate with slightly changed ones. Common catarrh is the second constant component of dysenteric colitis. Its only sign is an increased secretory activity of the congested mucosa, while organisms and leukocytes are absent. Catarrh may involve almost the whole colonic mucosa (and occasionally that of the small intestine) except for the areas of true inflammation.

On the basis of observations made on infected monkeys (see Chapter 11), one may conclude that in the catarrhal form of colitis, foci of true inflammation, due to shigella parasitism in the epithelium, are seen with varying frequency, depending on the severity of the disease and on the acuteness of its course. In the presence of mild intestinal lesions characteristic of modern dysentery, catarrhal phenomena prevail.

In such cases the search for specific small, usually microscopic, foci may prove difficult. It is even less probable to find them in the tiny tissue sample taken at biopsy. In contrast to the opinion of Tsinzerling (1973*a*, *b*) and Pokrovsky and Shalygina (1973), the failure of such attempts casts no doubt on the decisive role of intracellular shigella parasitism in dysentery.

The cause of common catarrh developing together with the specific inflammation in the intestine in dysentery, is not clear. Swelling and a pink colour of the mucosa, which are characteristic of catarrh, are more pronounced *in vivo* than at autopsy. This finding suggests a functional character of the congestion of the intestinal wall. Increased secretory activity of the epithelium of colonic mucosa must obviously also be considered a function-

al disorder. As mentioned, there is no associated bacterial tissue invasion. It is no use searching for intracellular shigellae in the areas of common catarrh.

Catarrhal manifestations may be due to the direct effect of certain noxious or irritative substances on the mucosa. If so, it may be that the colonic mucosa responds locally to toxins of shigellae abundantly multiplying in the intestinal contents. However, in such cases cultivation of the patients' faeces shows small numbers of shigellae and frequently even negative results are obtained. Thus, the extensive catarrhal response must be attributed to other causes. Foci of specific inflammation may cause and maintain it in an indirect (reflex) way even when they are small in size and in number. This assumption helps to explain the protracted or atypical forms of dysentery, in which functional disorders prevail and morphological lesions of the intestine are less marked. There is, however, no evidence available to this point.

*

The major findings which have completely changed the early theories on the pathogenesis of intestinal infections became known more than 10 years ago. Similarly to many other discoveries, the facts were described almost at the same time by several groups of investigators working independently in different countries. However, several years had to pass until the different teams came to unanimous understanding and the data were correctly estimated. Further investigations confirmed and complemented the early findings.

Certainly, our knowledge of the pathological processes characteristic of dysentery, salmonelloses and diseases caused by enteropathogenic *E. coli* is still incomplete and further research is needed in order to answer all the questions. However, there is no doubt that the understanding of the pathogenesis of intestinal infections is impossible without studying the peculiarities of the interactions between their agents and the host.

REFERENCES

Abrams, G. D., Bauer, H., Sprinz, H.: Influence of the normal flora on mucosal morphology and cellular renewal in the ileum. *Lab. Invest.*, **12**, 355–364 (1963).
Abrams, G. D., Bishop, J. E.: Normal flora and leukocyte mobilization. *Acrh. Path.*, **79**, 213–217 (1965).
Abrams, G. D., Bishop, J. E.: Effect of the normal microbial flora on the resistance of the small intestine to infection. *J. Bact.*, **92**, 1604–1608 (1966).
(Abrikosov, A. I.) Абрикосов, А. И.: Воспаление кишечника. В кн.: *Многотомное руководство по патологической анатомии.* т. 4, кн. 2, стр. 78–168, Медгиз, Москва (1957).
Adam, A.: Über die Biologie der Dyspepsiecoli und ihre Beziehungen zur Pathogenese der Dyspepsie und Intoxikationen. *Jb. Kinderheilk*, **51**, 295–314 (1923).
Adam, A.: Dyspepsiecoli. Zur Frage der bakteriellen Ätiologie der sogennanten alimentären Intoxikation. *Jb. Kinderheilk.*, **66**, 8–40 (1927).
Adam, A.: Pathogenese, Klinik und Therapie. In: *Säuglings-Enteritis.* Ed. by A. Adam. Thieme, Stuttgart. pp. 389–489 (1956).
Adam, A., Froboese, C.: Untersuchungen zur Pathologie der Durchfallserkrankungen des Säuglings. *Z. Kinderheilk.*, **39**, 267–313 (1925).
Adams, J. W.: Intracellular bacilli in intestinal and mesenteric lesions of typhoid fever. *Amer. J. Path.*, **15**, 560–566 (1939).
(Ado, A. D.) Адо, А. Д.: О механизмах патогенного действия эндотоксинов микробов кишечной группы. *Успехи совр. биол.*, **55**, 239–254 (1963).
(Afanasyeva, V. M.) Афанасьева, В. М.: О патоморфологической картине колибациллярной диспепсии и заболеваний, обусловленных патогенными штаммами кишечной палочки. *Вопр. охр. мат. дет.* **2**, 26–32 (1959).
(Afanasyeva, V. M.) Афанасьева, В. М.: Патологоанатомические данные при колибациллярных заболеваниях у детей. В кн.: *Колиэнтериты.* Под ред. О. В. Барояна. Медгиз, Москва. 183–198 (1962).
(Afanasyeva, V. M.) Афанасьева, В. М.: Колибактериальная инфекция. В кн.: *Многотомное руководство по патологической анатомии.* Медицина, Москва, т. **9**, 457—476 (1964).
(Akatova, N. S.) Акатова, Н. С.: К вопросу о природе феномена цветного свечения бактериальных культур при косом освещении. *Ж. микробиол. (Москва)*, **3**, 51–56 (1964).
(Akatova, N. S.) Акатова, Н. С.: Причины различного свечения колоний микробов при косом освещении. *Ж. микробиол. (Москва)*, **3**, 25–28 (1965).
Akazaki K. et al.: Über die Natur der Epithelioidzellen und der Typhuszellen. *Beitr. path. Anat.*, **116**, 200–237 (1956).
(Akopyan, A. B.) Акопян, А. Б.: Материалы к изучению патогенеза пиелонефрита (экспериментальное исследование). Автореферат дисс. канд., Ленинград, (1965).
(Aksenova, A. S.) Аксенова, А. С.: Паратифозное бациллоносительство у обезьян. В кн.: *Вопросы инфекционной патологии в эксперименте на обезьянах.* Под ред. Б. А. Лапина. Институт экспериментальной патологии и терапии АМН СССР, Сухуми. 159–165, (1958).
Al-Awqati, Q., Wallace, C. K., Greenough, W. B.: Stimulation of intestinal secretion *in vitro* by culture filtrates of *Escherichia coli*. *J. infect. Dis.*, **125**, 300–303 (1972).
Aldová, E., Lázničková, K.: Isolation of an *Escherichia coli* serotype provoking keratoconjunctivitis in guinea pigs. *J. Hyg. Epidem. (Praha)*, **2**, 439–442 (1967).

Aldová, E., Sourek, J., Lázničková, K.: On the taxonomy of *Escherichia coli* serotypes causing experimental keratoconjunctivitis shigellosa in guinea pigs. *Arch. Immunol. Ther. Exp. (Warsz.)*, **16**, 228–242 (1968).

(Alferyeva, M. A., Zakharova, O. A.) Алферьева, М. А., Захарова, О. А.: Материалы к вопросу о морфологической и бактериологической характеристике колибациллярной инфекции. В кн.: *Перинатальная патология и колиэнтерит*. Беларусь, Минск 285–290 (1964).

Alivisatos, G. P., Yovanovic, M.: Experimenteller Beitrag zur peroralen Immunisierung des Kaninchens gegen Ruhr. *Zbl. Bakt., I Abt. Orig.*, **98**, 311–325 (1926).

(Alov, A. I., Braude, A. I., Aspiz, M. E.) Алов, А. И., Брауде, А. И., Аспиз, М. Е.: *Основы функциональной морфологии клетки*. Медицина, Москва (1966), 2-ое изд., Медицина, Москва (1969).

Alp, M. H., Hislop, I. G.: The effect of *Giardia lamblia* infestation on the gastrointestinal tract. *Aust. Ann. Med.*, **18**, 232–237 (1969).

(Ambarnikov, I. M.)Амбарников, И. М.: Изучение действия токсинов кишечной группы на некоторые энтеровирусы. *Вопр. вирусол.*, **1**, 61–65 (1967).

Ament, M. E., Rubin, C. E.: Relation of giardiasis to abnormal intestinal structure and function in gastrointestinal immunodeficiency syndromes. *Gastroenterology*, **62**, 216–226 (1972).

(Andreeva, Z. M.) Андреева, З. М.: Циркуляция и выделение микробов и антигенов при экспериментальной дизентерии. *Ж. микробиол. (Москва)*, **6**, 22–26 (1962).

(Andreeva, Z. M.) Андреева, З. М.: Некоторые особенности патогенеза и иммунитета экспериментальной дизентерийной инфекции. Автореф. дисс. докт. мед. наук (Москва) (1965).

(Andreeva, Z. M., Bakhutashvili, V. I.) Андреева, З. М., Бахуташвили, В. И.: Сравнительное изучение патогенности шигелл в кератоконъюнктивальной пробе и в культуре ткани. *Лабор. дело*, **2**, 95–97 (1968).

(Andreeva, Z. M., Eklinova, E. A., Akatova, N. S.) Андреева, З. М., Еклинова, Е. А., Акатова, Н. С.: Селекция вирулентных вариантов шигелл. *Ж. микробиол. (Москва)*, **11**, 129–130 (1965).

Anitschkow, N. N.: Über die experimentelle autogene Infektion. *Zbl. allg. Path. path. Anat.*, **101**, 151–159 (1960).

Arbuckle, J. B. R.: The location of *Escherichia coli* in the pig intestine. *J. Med. Microbiol.*, **3**, 333–336 (1970).

Arbuckle, J. B. R.: Enteropathogenic *Escherichia coli* on the intestinal mucopolysaccharide layer of pigs. *J. Path.*, **104**, 93–98 (1971).

(Arbuzova, V. A.) Арбузова, В. А.: Материалы к биологической характеристике бреславльской палочки и некоторые эпидемиологические особенности современного бреславльского сальмонеллеза. *Ж. микробиол. (Москва)*, **8**, 101–105 (1957).

(Arbuzova, V. A.) Арбузова, В. А.: Течение экспериментальной инфекции, вызываемой у белых мышей культурами бреславльской палочки. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **21**, 148–163 (1960).

(Arbuzova, V. A.) Арбузова, В. А.: Изучение некоторых биологических свойств сальмонелл с помощью культуры перитонеальных макрофагов. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **27**, 71–91 (1964*a*).

(Arbuzova, V. A). Арбузова, В. А.: Развитие и исход сальмонеллезной инфекции при интраназальном заражении белых мышей. I. Микробиологические исследования. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **27**, 92–105 (1964*b*).

(Arbuzova, V. A.) Арбузова, В. А.: Изучение роста на культурах тканей микробных возбудителей острых кишечных заболеваний. 1. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **33**, 30–43 (1968*a*).

(Arbuzova, V. A.) Абрузова, В. А.: Некоторые вопросы эпидемиологии сальмонеллезов. *Тр. Ин-та эпидемиол. микробиол им. Пастера (Ленинград)*, **33**, 353–357 (1968*b*).

(Arbuzova, V. A.) Арбузова, В. А.: Изучение роста на культурах тканей микробных возбудителей острых кишечных инфекций. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **36**, 133–141 (1970*a*).

(Arbuzova, V. A.) Арбузова, В. А.: К биологической характеристике и систематике бактерий Аризона. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **36**, 323–331 (1970*b*).

(Arbuzova, V. A.) Арбузова, В. А.: К вопросу о специфичности и чувствительности культуры ткани как теста для выявления энтеропатогенных свойств кишечных бактерий. *Ж. микробиол. (Москва)*, **9,** 72–77 (1972).

(Arbuzova, V. A.) Арбузова, В. А.: Сравнительное изучение характера взаимодействия шигелл и сальмонелл с клетками брюшной полости (на модели сальника белых мышей). *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **40,** 197–203 (1973).

(Arbuzova, V. A., Krivonosova, K. I.) Арбузова, В. А., Кривоносова, К. И.: Материалы к биологической характеристике энтеропатогенной кишечной палочки серологической группы O112a112b: K68 (B13). *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **40,** 93–101 (1973).

(Arbuzova, V. A., Krivonosova, K. I., Drobyshevskaya, E. I.) Арбузова, В. А., Кривоносова, К. И., Дробышевская, Э. И.: К биологической характеристике энтеропатогенных кишечных палочек серологической группы 044, выделенных при групповых острых кишечных заболеваниях новорожденных. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **36,** 287–294 (1970).

(Arbuzova, V. A., Kuzmenko, V. V.) Арбузова, В. А., Кузьменко, В. В.: К этиологии острых кишечных заболеваний новорожденных. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **33,** 200–209 (1968).

(Ariel, B. M., Bernovskaya, A. A.) Ариэль, Б. М., Берновская, А. А.: Электронномикроскопические наблюдения за взаимоотношениями сальмонелл с клетками организма (на легочной модели). *Арх. пат. (Москва)*, **11,** 17–21 (1970).

(Ariel, B. M., Dragunskaya, E. M., Arbuzova, V. A., Polotsky, Yu. E.) Ариэль, Б. М., Драгунская, Е. М., Арбузова, В. А., Полоцкий, Ю. Е.: Статистическая оценка интраназального заражения белых мышей («легочная» модель). I. Характеристика дозы заражения в опытах с *Escherichia coli*. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **36,** 217–220 (1970).

(Ariel, B. M., Polotsky, Yu. E., Dragunskaya, E. M.) Ариэль, Б. М., Полоцкий, Ю. Е,. Драгунская, Е. М.: Изучение биологических свойств энтеропатогенной палочки O28a28c в эксперименте на животных. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **33,** 104–110 (1968).

Arm, H. G., Floyd, T. M., Faber, J. R., Hayes, J. R.: Use of ligated segments of rabbit small intestine in experimental shigellosis. *J. Bact.*, **89,** 803–809 (1965).

(Arons, R. M.) Аронс, Р. М.: О специфической профилактике дизентерии в эксперименте с использованием полного антигена. *Ж. микробиол. (Москва)*, **2,** 140 (1966).

(Aruin, L. I.) Аруин, Л., И.: Морфологическое исследование материала биопсий тонкого кишечника. *Арх. пат. (Москва)*, **9,** 3–12 (1967).

(Aruin, L. I., Modestova, E. V., Sharov, V. G.) Аруин, Л. И., Модестова, Е. В., Шаров, В. Г.: Гистохимия и ультраструктура слизистой оболочки тонкой кишки при болезни Уиппля. *Арх. пат. (Москва)*, **11,** 67–73 (1971).

Aschoff, L.: *Pathologische Anatomie*. 7th ed., G. Fischer. Vol. 2. Jena (1928).

Ashburner, F. M., Mushin, R.: Experimental intestinal coliform infections in mice. *J. Hyg.*, **60,** 175–181 (1962).

Auzins I., Rowley D.: Factors involved in the adherence of *S. typhimurium* C5 and mouse peritoneal macrophages. *Aust. J. exp. Biol. med. Sci.*, **41,** 539–546 (1963).

(Avdeeva, T. A.) Авдеева, Т. А.: Материалы к иммунологической и количественной микробиологической характеристике острого дизентерийного процесса. *Тр. ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **24,** 124–140 (1963).

(Avdeeva, T. A.) Авдеева, Т. А.: *Количественное микробиологическое исследование при дизентерии*. Автореф. дисс. докт. мед. н., Ленинград, (1964).

(Avdeeva, T. A.) Авдеева, Т. А.: Результаты дальнейшего изучения культур *E. coli* серологической группы 028. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **33,** 93–103 (1968).

(Avdeeva, T. A., Arbuzova, V. A., Smirnova, L. A., Dragunskaya, E. M., Krol, Ya. Ya., Ozeryanova, A. G.) Авдеева, Т. А., Арбузова, В. А., Смирнова, Л. А., Драгунская, Е. М., Кроль, Я. Я., Озерянова, А. Г.: К биологической характеристике энтеропатогенной кишечной палочки 0144:K?(B). *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **36,** 187–193 (1970b).

(Avdeeva, T. A., Arbuzova, V. A., Smirnova, L. A., Ozeryanova, A. G.) Авдеева, Т. А., Арбузова, В. А., Смирнова, Л. А., Озерянова, А. Г.: Экспериментальные модели как возможный путь выявления возбудителей острых кишечных заболеваний неуста-

новленной этиологии. *Авторефераты Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, 37–39 (1970*a*).

(Avdeeva, T. A., Ivanova, V. V., Turuleva, V. T., Pik-Levontin, E. M.) Авдеева, Т. А., Иванова, В. В., Турулева, В. Т., Пик-Левонтин, Э. М.: К изучению новых разновидностей *E. coli* — возбудителей дизентериеподобных заболеваний у взрослых и детей. *Авторефераты Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, 28–32 (1966).

(Avdeeva, T. A., Kleganov, V. K., Karyagina, E. I., Toluzakova, N. V.) Авдеева, Т. А., Клеганов, В. К., Карягина, Е. И., Толузакова, Н. В.: О групповых острых кишечных заболеваниях, вызванных энтеротоксигенными кишечными палочками серологической группы O148. В кн.: *Эпидемиол., диагностика и профилактика кишечных, респираторных и природноочаговых инфекций*. Ин-т эпидемиол. микробиол. им. Пастера (Ленинград), 59–60 (1975).

(Avdeeva, T. A., Polotsky, Yu. E., Smirnova, L. A., Dragunskaya, E. M., Poyasova, E. V., Chalenko, V. G.) Авдеева, Т. А., Полоцкий, Ю. Е., Смирнова, Л. А., Драгунская, Е. М., Поясова, Э. В., Чаленко, В. Г.: Характеристика новой группы энтеропатогенных кишечных палочек, продуцирующих энтеротоксин. *Ж. микробиол. (Москва)*, **11,** 9–12 (1973*a*).

(Avdeeva, T. A., Smirnova, L. A., Arbuzova, V. A., Dragunskaya, E. M., Ozeryanova, A. G.) Авдеева, Т. А., Смирнова, Л. А., Арбузова, В. А., Драгунская, Е. М., Озерянова, А. Г.: К характеристике инфекционного процесса у белых мышей, интраназально зараженных рядом представителей рода эшерихиа, выделяемых от больных острыми кишечными расстройствами. *Ж. микробиол. (Москва)*, **2,** 20–25 (1973*b*).

(Avdeeva, T. A., Smirnova, L. A., Polotsky, Yu. E., Dragunskaya, E. M., Poyasova, E. V., Chalenko, V. G.) Авдеева, Т. А., Смирнова, Л. А., Полоцкий, Ю. Е., Драгунская, Е. М., Поясова, Э. В., Чаленко, В. Г.: Интраназальное заражение белых мышей как метод выявления энтеротоксигенных кишечных палочек. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **40,** 78–85 (1973*c*).

(Avdeeva, T. A., Smirnova, L. A., Safonova, N. V., Efron, N. N.) Авдеева, Т. А., Смирнова, Л. А., Сафонова, Н. В., Эфрон, Н. Н.: К этиологической роли представителей эшерихиа коли О группы 25 (серотип 301) при групповых дизентериеподобных заболеваниях у взрослых. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **33,** 111–116 (1968).

(Avdeeva, T. A., Turuleva V. T.) Авдеева, Т. А., Турулева, В. Т.: Опыт использования дополнительных методов микробиологической диагностики дизентерии. I. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **33,** 305–310 (1968).

(Avdeeva, T. A., Turuleva, V. T.) Авдеева, Т. А., Турулева, В. Т.: Дальнейшее изучение специфических серологических сдвигов при дизентериеподобных заболеваниях, вызванных энтеропатогенной кишечной палочкой серотипа 301. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **36,** 221–228 (1970).

(Avetikyan, B. G.) Аветикян Б. Г.: цит. по Бибиновой и др. (1960).

(Avtsyn, A. P., Berezina, E. K.) Авцын, А. П., Березина, Е. К.: Изменения патологоанатомической картины при инфекционных болезнях в связи с применением антибиотиков. *Антибиотики (Москва)*, **5,** 67–71 (1958).

(Avtsyn, A. P., Berezina, E. K.) Авцын, А. П., Березина, Е. К.: Патоморфологический метод в экспериментальной химиотерапии острых бактериальных инфекций. В кн.: *Методы экспериментальной химиотерапии*. Изд. 2-е. Под ред. Г. Н. Першина, Медицина, Москва, 152–166 (1971).

(Avtsyn, A. P., Shakhlamov, V. A., Sageeva, O. F., Lobanov, V. N., Petrova, L. S., Kenig, E. E.) Авцын, А. П., Шахламов, В. А., Сагеева, О. Ф., Лобанов, В. Н., Петрова, Л. С., Кениг, Э. Э.: Интраэпителиальная инвазия холерных вибрионов при экспериментальной холере. *Вестник Акад. Мед. Наук СССР*, **7,** 50–55 (1971).

Awataguchi, S., Kawano, Y., Kojima, A., Sakuma, S.: An attempt to establish experimental dysenteric bacilli cystitis. *Jap. J. Microbiol.*, **13,** 325–333 (1969).

Babes, V.: Untersuchungen über Koch's Kommabacillus. *Virchows Arch. path. Anat.*, **99,** 148–163 (1885).

Bacon, G. A., Burrows, T. W., Yates, M.: The effects of biochemical mutation on the virulence of Bacterium typhosum: the virulence of mutants. *Brit. J. exp. Path.*, **31,** 714–724 (1950).

Bacon, G. A., Burrows, T. W., Yates, M.: The effects of biochemical mutation on the virulence of Bacterium typhosum, the loss of virulence of certain mutants. *Brit. J. exp. Path.*, **32**, 85–96 (1951).

Bakács, T., Rácz, P., Rédei, A., Tenner, K.: Contributions to cellular immunity with special regard to intracellular parasitism. *Egészségtudomány*, **14**, 94–103 (1970).

(Bakhutashvili, V. I.) Бахуташвили, В. И.: Методы выявления патогенности шигелл при взаимодействии с тканевыми культурами. *Лабор. дело*, **11**, 683–686 (1967).

(Bakhutashvili, V. I.) Бахуташвили, В. И.: Значение вирулентности шигелл и вирусной инфекции в патогенезе дизентерии. Автореф. дисс. докт. мед. наук, Москва, (1968).

(Bakhutashvili, V. I.) Бахуташвили, В. И.: Внутриклеточное паразитирование шигелл и смешанная инфекция культур тканей шигеллами и вирусами. *Сов. мед.*, **6**, 21–25 (1969).

(Bakhutashvili, V. I., Gulevich, N. E.) Бахуташвили, В. И., Гулевич, Н. Е.: Особенности течения микробновирусной инфекции в клетках лейкемии, восприимчивых и резистентных к вирусу. *Ж. микробиол.*, **10**, 150–153 (1968).

(Balabonova, L. S., Lyamtseva, G. A., Bartysheva, V. A., Bogolapova, N. M.) Балабонова, Л. С., Лямцева, Г. А., Бартышева, В. А., Боголапова, Н. М.: Материалы к эпидемиологии сальмонеллезов в Ленинграде. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **36**, 309–317 (1970).

(Balabonova, L. S., Lyamtseva, G. A., Bogolapova, N. I., Poplavskaya, Zh. V., Smirnova, N. M., Turyanskaya, S. B., Goldenberg, R. A., Raskina, T. M., Kalinova, E. A.) Балабонова, Л. С., Лямцева, Г. А., Боголапова, Н. И., Поплавская, Ж. В., Смирнова, Н. М., Турьянская, С. Б., Гольденберг, Р. А., Раскина, Т. М., Калинова, Е. А.: О групповых заболеваниях, вызванных энтеропатогенной кишечной палочкой O112a112b. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **40**, 102–104 (1973).

Balogh, E.: Unter dem Bilde der toxischen Formen von Paratyphus verlaufende Typhusinfectionen. *Wien. klin. Wochenschr.*, **35**, 844–845 (1922).

Bals, M., Leonesco, M.: Étude sur l'application du test de pathogénité de Serény dans l'isolement des Shigella. *Arch. roum. Path. exp.* **20**, 365–373 (1961).

(Baltrashevich, A. K., Ostrovskaya, M. B.) Балтрашевич, А. К., Островская, М. Б.: Цитопатогенное действие разных серологических типов культур энтеропатогенных кишечных палочек группы 0111. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **27**, 307–310 (1964).

Banwell, J. G., Gorbach, S. L., Mitra, R. C., Pierce, N. F., Mondal, A.: Acute undifferentiated diarrhea in the tropics. II. Alterations in intestinal fluid and electrolyte movement. *J. clin. Invest.*, **50**, 890–900 (1971).

Barbieri, D., De Brito, T., Hoshino, S.: Giardiasis in childhood. *Arch. Dis. Childh.*, **45**, 466–472 (1970).

Barnum, D. A.: The control of neonatal colibacillosis of swine. *Ann. N. Y. Acad. Sci.*, **176**, 385–400 (1971).

Barnum, D. A., Glantz, P. J., Moon, H. W.: *CIBA Vet. Monogr. Ser., Colibacillosis.* Summit, New Jersey. (1967).

Baron, L. S., Spilman, W. M., Carey, W. F.: Diploid heterozygous hybrids from matings between *Escherichia coli* and *Salmonella typhosa*. *J. exp. Med.*, **112**, 361 (1960).

(Barsky, I. Ya., Khavkin, T. N.) Барский, И. Я., Хавкин, Т. Н.: Фотографический способ микрофлуориметрии при иммуно-люминесцентных исследованиях. *Цитология*, **10**, 1358–1362 (1969).

Baskerville, A., Dow, C., Curran, W. L., Hanna, J.: Ultrastructure of phagocytosis of *Salmonella cholerae suis* by pulmonary macrophages *in vivo*. *Brit. J. exp. Path.*, **53**, 641–647 (1972).

Baskerville, A., Dow, C., Curran, W. L., Hanna, J.: Further studies on experimental bacterial pneumonia. *Brit. J. exp. Path.*, **54**, 90–98 (1973).

Batson, H. C., Landy, M., Brown, M.: Determination of differences in virulence of strains of *Salmonella typhosa*. *J. exp. Med.*, **91**, 219–229 (1950).

Bauer, H.: Defence mechanisms in germfree animals. Part II. Cellular defence mechanisms. In: *The Germfree Animals in Research*. Ed. by M. E. Coates, H. A. Gordon, B. S. Wostmann. London—New York. pp. 210–226 (1968).

(Bayramova, E.) Байрамова, Э.: К методике воспроизведения дизентерийного кератоконъюнктивита у морских свинок. Тез. докл. итоговой научн. конф. АШИЭиГ Ашхабад, 20–21 (1962).

(Bayramova, E.) Байрамова, Э.: Экспериментальное воспроизведение дизентерийного и бреславльского кератоконъюнктивита. *Здравоохр. Туркменистана*, **7**, 3–5 (1963).

(Bayramova, E., Gleiberman, S. E.) Байрамова, Э., Глейберман, С. Е.: Сравнительная клинико-морфологическая характеристика экспериментального шигеллезного и бреславльского кератоконъюнктивита. *Здравоохр. Туркменистана*, **8**, 30–35 (1963).

Beckman, B., Flores, J., Witkum, P. A., Sharp, G. W. G.: Studies on the mode of action of cholera toxin. Effects on solubilized adenylate cyclase. *J. clin. Invest.*, **53**, 1202–1205 (1974).

(Belaya, Yu. A.) Белая, Ю. А.: К вопросу об использовании кошек в качестве экспериментальной модели при дизентерии. *Ж. микробиол. (Москва)*, **4**, 77–80 (1958).

(Belaya, Yu. A.) Белая, Ю. А.: Материалы к изучению биологических свойств дизентерийных штаммов при испытании на куриных эмбрионах и на модели экспериментального дизентерийного кератоконъюнктивита. *Ж. микробиол. (Москва)*, **12**, 44–47 (1959).

(Belaya, Yu. A.) Белая, Ю. А.: Сохранение вирулентности дизентерийных бактерий методом пассирования в конъюнктивальном мешке морских свинок. *Ж. микробиол. (Москва)*, **1**, 18–21 (1962*a*).

(Belaya, Yu. A.) Белая, Ю. А.: О закономерностях размножения свежевыделенных и лабораторных дизентерийных штаммов на куриных эмбрионах. *Ж. микробиол. (Москва)*, **3**, 65–68 (1962*b*).

(Belaya, Yu. A.) Белая, Ю. А.: Изучение лабильных антигенов дизентерийных бактерий методом диффузии в агаре. *Ж. микробиол. (Москва)*, **8**, 59–64 (1963).

(Belaya, Yu. A.) Белая, Ю. А.: Инфекционный и постинфекционный иммунитет при экспериментальном кератоконъюнктивите. *Ж. микробиол. (Москва)*, **1**, 44–48 (1964).

(Belaya, Yu. A.) Белая, Ю. А.: Иммуноэлектрофоретический анализ структуры антигенов дизентерийных бактерий Флекснера и Бойда. *Ж. микробиол. (Москва)*, **7**, 6–12 (1965).

(Belaya, Yu. A.) Белая, Ю. А.: К вопросу о специфической профилактике дизентерии. 1. *Ж. микробиол. (Москва)*, **4**, 14–19 (1968).

(Belaya, Yu. A.) Белая, Ю. А.: Биология и антигенная структура шигелл в связи с патогенезом дизентерии. Автореф. дисс. докт. мед. наук (Москва), (1970*a*).

Belaya, Yu. A.: Antigen structure and virulence of *Salmonella typhi*. *J. Hyg. Epidemiol. (Praha)*, **14**, 263–273 (1970*b*).

(Belaya, Yu. A., Braude, A. I., Shcherbakova, E. G., Solovyev, N. N.) Белая, Ю. А., Брауде, А. И., Щербакова, Э. Г., Соловьев, Н. Н.: Фагоцитоз вирулентных и авирулентных шигелл в культуре мышиных перитонеальных макрофагов. *Ж. микробиол. (Москва)*, **7**, 26–31 (1968).

(Belaya, Yu. A., Kavtaradze, K. N., Dzhikidze, E. K.) Белая, Ю. А., Кавтарадзе, К. Н., Джикидзе, Э. К.: К вопросу о специфической профилактике дизентерии. VI. *Ж. микробиол. (Москва)*, **2**, 22–27 (1970).

(Belyanin, V. L.) Белянин, Б. Л.: Поражения кишечника при экспериментальной сальмонеллезной инфекции. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **33**, 364–371 (1968).

(Belyanin, V. L.) Белянин, В. Л.: Взаимодействие между микробами и организмом при экспериментальной сальмонеллезной инфекции. Автореф. дисс. канд. мед. наук (Ленинград), (1969).

Bennett, B.: A method for performing differential counts on peritoneal cell populations from mice. *Transplantation*, **3**, 254–257 (1965).

Bennett, B.: Comparative morphology of macrophages in tissue culture. *Adv. exp. med. biol.*, **1**, 74–84 (1967*a*).

Bennett, B.: Isolation and cultivation *in vitro* of macrophages from various sources in the mouse. *Amer. J. Path.*, **48**, 165–181 (1967*b*).

Berczi, J.: Stability of *Escherichia coli* endotoxin in the rat gastrointestinal tract. *J. Path. Bact.*, **96**, 487–491 (1968).

Berczi, J., Bertók, L., Baintner, K., Veress, B.: Failure of oral *Escherichia coli* endotoxin to induce either specific tolerance or toxin symptoms in rats. *J. Path. Bact.*, **96**, 481–486 (1968).

Berczi, J., Bertók, L., Baintner, K., Veress, B.: Experiments to induce endotoxin tolerance and toxin effects by orally administered *E. coli* endotoxin in rats. *Ann. immunol. hung.* **12,** 117–121 (1968/1969).

Bergman, S., Weibul, C.: Effects of endotoxin on tissue culture cells. *Acta path. microbiol. scand.*, **77,** 698–706 (1969).

Bergner, E., Stern, A., Vancea, D.: Patogenitatea tulpinor hemolitice de *E. coli*. *Microbiol. (Buc.)*, **10,** 41–48 (1965).

(Berman, V. M.) Берман, В. М.: О некоторых чертах возрастной ретивности в инфекционных процессах. В кн.: *Вопросы патогенеза и патологической анатомии инфекционных болезней*. Медгиз, Ленинград. 13–26 (1957).

(Berman, V. M.) Берман, В. М.: Микробный фактор и реактивность организма в этиологии, патогенезе, профилактике и терапии колиэнтеритов. *Вопр. охр. мат. дет.*, **9,** 17–23 (1961).

(Berman, V. M., Panov, N. P., Slavskaya, E. M.) Берман, В. М., Панов, Н. П., Славская, Е. М.: Методика экспериментального изучения тифозной инфекции и иммунитета на белых мышах путем создания местных инфекционных очагов в лимфатических узлах. *Арх. биол. наук (Ленинград)*, **55,** 88–103 (1939).

(Berman, V. M., Slavskaya, E. M.) Берман, В. М., Славская, Е. М.: Завершенный фагоцитоз. I. Новый методический принцип изучения завершенной фагоцитарной реакции. *Ж. микробиол. (Москва)*, **3,** 8–13 (1958).

(Berman, V. M., Slavskaya, E. M.) Берман, В. М., Славская, Е. М.: Реакция завершенного фагоцитоза. В кн.: *Современные проблемы иммунологии. ИЭМ АМН СССР, Ленинград* 33–46 (1959).

(Berman, V. M., Slavskaya, E. M.) Берман, В. М., Славская, Е. М.: К сравнительной характеристике инфекционного процесса у взрослых и развивающихся животных при их заражении кишечными палочками. *Тр. Педиатрического мед. ин-та (Ленинград)*, **29,** 171–180 (1961).

(Berman, V. M., Slavskaya, E. M.) Берман, В. М., Славская, Е. М.: Сравнительная характеристика патогенных свойств кишечных палочек в опыте внутрибрюшинного заражения белых мышей. *Тр. Педиатрического мед. ин-та (Ленинград)*, **29,** 157–169 (1962).

(Berman, V. M., Slavskaya, E. M.) Берман, В. М., Славская, Е. М.: О природе и свойствах факторов, стимулирующих состояние неспецифического иммунитета. *IX. Междунар. конгресс по микробиологии. Тезисы докладов.* (Москва), 646 (1966).

Bernstein, J. D., Ovary, Z.: Absorption of antigens from the gastrointestinal tract. *Int. Arch. Allergy*, **33,** 525–527 (1968).

Berry, L. J., Mitchell, R. B.: The effect of malonate on *Salmonella typhimurium* infection in mice. *Science*, **118,** 140–141 (1953*a*).

Berry, L. J., Mitchell, R. B.: The relation of the bicarboxylic acid cycle to bacterial infection. I. The effect of malonate on *Salmonella typhimurium* infection in mice. *J. infect. Dis.*, **93,** 75–92 (1953*b*).

Bertschinger, H. U., Moon, H. W., Whipp, S. C.: Association of *Escherichia coli* with the small intestinal epithelium. *Infect. Immunity*, **5,** 595–611 (1972).

Besredka, A.: Du mécanisme de l'infection dysentérique, de la vaccination contre la dysenterie par la voie buccale. *Ann. Inst. Pasteur*, **33,** 301–317 (1919).

(Besredka, A. M.) Безредка, А. М.: *Местная иммунизация*. "France-Russie", Paris (1925).

Bessau, G.: Zur Pathogenese der Intoxikation. *Wschr. Kinderheilk.*, **38,** 138—144 (1928).

Bessau, G., Bossert, O.: Zur Pathogenese der akuten Ernährungsstörungen. *Jb. Kinderheilk.*, **39,** 213–269 (1919).

Bettelheim, K. A., Breadon, A., Faiers, M. S., O'Farrell, S. M., Shooter, R. A.: The origin of O-serotypes of *Escherichia coli* in babies after normal delivery. *J. Hyg. (Lond.)*, **72,** 67–70 (1974).

Bettelheim, K. A., Taylor, J.: Soluble antigens of enteropathogenic *Escherichia coli*. *J. Med. Microbiol.*, **3,** 655–667 (1970), *Ann. N. Y. Acad. Sci.*, **176,** 301–313 (1971).

(Bibinova, L. S.) Бибинова, Л. С.: К вопросу об экспериментальной дизентерии. *Арх. биол. наук*, **32,** 271–283 (1932*a*).

Bibinova, L. S.: Zur Frage der experimentellen Dysenterie. *Z. exp. Med.*, **85,** 674–689 (1932*b*).

(Bibinova, L. S.) Бибинова, Л. С.: Экспериментальный паратифозный энтерит у кролика. *Арх. биол. наук*, **55,** 176–183 (1939).
(Bibinova, L. S.) Бибинова, Л. С.: Морфологическая характеристика дизентерии у детей в разных ее стадиях и формах. Автореф. дисс. докт. мед. наук (Ленинград), (1954).
(Bibinova, L. S.) Бибинова, Л. С.: О катаральных поражениях кишечника при дизентерии у детей. В кн.: *Вопросы патогенеза и патологической анатомии инфекционных болезней.* Медгиз, Ленинград 251–263 (1967).
Bibinova, L. S., Khavkin, T. N., Nikulnikova, N. S., Kruglikhina, Z. M.: Experimental dysentery in enterally infected guinea pigs. Experiments using fluorescent antibodies. *J. Hyg. Epidemiol. (Praha)*, **12,** 449–455 (1968*a*).
(Bibinova, L. S., Khavkin, T. N., Nikulnikova, N. S., Kruglikhina, Z. M.) Бибинова, Л. С., Хавкин, Т. Н., Никульникова, Н. С., Круглихина, З. М.: Экспериментальный шигеллезный энтероколит у морских свинок. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **33,** 329–337 (1968*b*).
(Bibinova, L. S., Kopytovskaya, L. P., Bukina, A. P.) Бибинова, Л. С., Копытовская, Л. П., Букина, А. П.: Морфологические проявления общей реакции организма при дизентерийной интоксикации у мышей, лишенных надпочечников. В кн.: *Вопросы инфекционной патологии. ИЭМ АМН СССР,* Ленинград. 97–106 (1962*a*).
(Bibinova, L. S., Kopytovskaya L. P., Bukina A. P.) Бибинова, Л. С., Копытовская, Л. П., Букина, А. П.: Морфологические изменения легких адреналэктомированных мышей при интраназальном введении стафилококков и стафилококкового токсина. В кн.: *Вопросы инфекционной патологии. ИЭМ АМН СССР,* Ленинград. 107–115 (1962*b*).
Bibinova, L. S., Kruglikhina Z. M., Nikulnikova N. S.: Morphological changes induced in the guinea pig intestinal tract by enterally administered dysentery endotoxin. *J. Hyg. Epidem. (Praha)*, **15,** 212–219 (1971).
(Bibinova, L. S., Osipova, P. V.) Бибинова, Л. С., Осипова, П. В.: Местное действие бактериальных токсинов. *Ежегодник Ин-та экспер. мед. АМН СССР за 1958 г.* Ленинград. 301–306 (1959).
(Bibinova, L. S., Panova, M. F.) Бибинова, Л. С., Панова, М. Ф.: О морфологии некоторых общих реакций при бактериальных интоксикациях. *Ежегодник Ин-та экспер. мед. АМН СССР за 1957. г.* Ленинград. 362–366 (1958).
(Bibinova, L. S., Voino-Yasenetskaya, M. K.) Бибинова, Л. С., Войно-Ясенецкая, М. К.: Экспериментальные исследования дизентерии. В кн.: *Конференция по итогам научно-исследовательской работы за 1953 г. Ин-т экспер. мед. АМН СССР.* Ленинград. **2,** 43–44 (1954).
(Bibinova, L. S., Voino-Yasenetskaya, M. K., Uglova, T. V., Nikulnikova, N. S.) Бибинова, Л. С., Войно-Ясенецкая, М. К., Углова, Т. В., Никульникова, Н. С.: Об экспериментальном воспроизведении дизентерийной инфекции. *Вестн. АМН СССР* **2,** 34–46 (1960).
(Bilibin, A. F.) Билибин, А. Ф.: Брюшной тиф, паратиф А и паратиф В. Пищевые токсикоинфекции. В кн.: *Руководство по инфекционным болезням.* Под ред. А. Ф. Билибина, Г. П. Руднева, Медгиз, Москва. 127–176 (1962).
(Bilibin, A. F., Timakov, V. D., Bondarenko, V. M., Ivanova, L. V.) Билибин, А. Ф., Тимаков, В. Д., Бондаренко, В. М., Иванова, Л. В.: К патогенезу тифо-паратифозного бактерионосительства. *Ж. микробиол. (Москва)*, **1,** 12–18 (1970.)
Bingel, K. F.: Eine tierexperimentelle Methode zum Studium der Infektion mit gramnegativen Darmbakterien, insbesondere der Ruhr. *Z. Hyg. Infekt.-Kr.*, **125,** 110–122 (1943*a*).
Bingel, K. F.: Phasen und Pathogenität der Kruse-Sonne-Ruhr im Tierexperiment. *Zbl. Bakt. I., Abt. Orig.*, **150,** 225 (1943*b*).
Bingel, K. F.: Prophylaxe der Ruhr im Tierexperiment. *Z. Hyg. Infekt.-Kr.*, **125,** 574 (1944*a*).
Bingel, K. F.: Tierexperimentelle Beiträge zur Pathogenese der Ruhr. *Z. Infekt-Kr., Hyg.*, **125,** 610–650 (1944*b*).
Bizzozero, G.: Über das konstante Vorkommen von Bakterien in den Lymphofolikeln des Kaninchendarmes. *Zbl. Med. klin. Wissensch.*, **45,** 801–804 (1885).
Blanden, R. V., Lefford, M. J., Mackaness, G. B.: The host response to Calmette–Guérin bacillus infection in mice. *J. exp. Med.*, **129,** 1079–1106 (1969).
Blanden, R. V., Mackaness, G. B., Collins, F. M.: Mechanisms of acquired resistance in mouse typhoid. *J. exp. Med.*, **124,** 585–600 (1966).

(Blank, G. F.) Бланк, Г. Ф.: Изучение поствакцинального иммунитета на модели легочного шигеллеза у белых мышей. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **33,** 275–277 (1968).

(Blank, G. F.) Бланк, Г. Ф.: Опыт изучения противодизентерийного иммунитета на модели шигеллезной пневмонии у белых мышей. Автореф. дисс. канд. мед. наук (Ленинград) (1969).

(Blank, G. F., Avetikyan, B. G.) Бланк, Г. Ф., Аветикян, В. Г.: К характеристике модели экспериментальной шигеллезной пневмонии у белых мышей. *Ж. микробиол. (Москва)*, **1,** 115–119 (1972).

(Blinkin, I. N.) Блинкин, И. Н.: *Методы ускоренной бактериологической диагностики кишечных инфекций.* Медгиз, Москва. 199–219 (1963).

(Blinova, N. I.) Блинова, Н. И.: Использование «легочной модели» для определения вирулентности гибридов шигелл с различной генетической характеристикой. *Ж. микробиол. (Москва)*, **9,** 32–36 (1970).

(Blokhov, V. P.) Блохов, В. П.: Случай нестерильного иммунитета у морских свинок с экспериментальным дизентерийным кератоконъюнктивитом. *Ж. микробиол. (Москва)*, **4,** 147 (1964).

(Bluger, A. F., Novitsky, I. N., Terebkova, V. F.), Блюгер, А. Ф., Новицкий, И. Н., Теребкова, В. Ф.: *Сальмонеллез.* Зинатне, Рига, (1975).

(Bluger, A. F., Sinelnikova, M. P., Veksler, Kh. M., Terentyeva, L. A., Novitsky, I. N.) Бглюгер, А. Ф., Синельникова, М. П., Векслер, Х. М., Терентьева, Л. А., Новицкий, И. Н.: Изменения слизистой оболочки тонкой кишки при дизентерии. *Арх. пат. (Москва)*, **2,** 38–43 (1973*a*).

(Bluger, A. F., Veksler, Kh. M., Novitsky, I. N., Sinelnikova, M. P., Terentyeva, L. A.) Блюгер, А. Ф., Векслер, Х. М., Новицкий, И. Н., Синельникова, М. П., Терентьева, Л. А.: *Биопсия тонкой кишки при острых кишечных инфекциях.* Зинатне, Рига, (1973*b*).

Board, R. G., Fuller, R.: Non-specific antimicrobial defences of the avian egg, embryo and neonate. *Biol. Rev.*, **1,** 15–49 (1974).

(Bogdanova, V. V.) Богданова, В. В.: Сравнительное изучение некоторых свойств дизентерийных бактерий, патогенных для глаз морских свинок, и полученных из них непатогенных вариантов. *Ж. микробиол. (Москва)*, **3,** 140 (1965).

Boehme, D. H.: Resistance to salmonella infections in inbred mouse strains. *Bull. N. Y. Acad. Med.*, **46,** 495–508 (1970).

Boehme, D. H., Schneider, H. A., Lee, J. M.: Some physiopathological parameters of natural resistance to infection in murine salmonellosis. *J. exp. Med.*, **110,** 9–26 (1959).

(Bondarenko, V. M.) Бондаренко, В. М.: Цитопатическое действие *S. typhi* в культуре ткани. *Ж. микробиол. (Москва)*, **1,** 65–67 (1967).

(Bondarenko, V. M., Maslova, T. N., Petrovskaya, V. G.) Бондаренко, В. М., Маслова, Т. Н., Петровская, В. Г.: Цитотоксическое действие шигелл Флекснера различных серотипов в культуре перитонеальных макрофагов морской свинки. *Вест. АМН СССР*, **7,** 52–57 (1973*a*).

(Bondarenko, V. M., Petrovskaya, V. G., Korn, M. Ya., Gadoshevich, V. N.) Бондаренко, В. М., Петровская, В. Г., Корн, М. Я., Гадошевич, В. Н.: Изучение в культуре Нер-2 взаимодействия с клеткой вирулентных шигелл Флекснера и гибридов, утративших способность вызывать кератоконъюнктивит. *Ж. микробиол. (Москва)*, **11,** 87–95 (1973*b*).

(Bondarenko, V. M., Pinegin, B. V., Ivanova, L. V.) Бондаренко, В. М., Пинегин, Б. В., Иванова, Л. В.: Изучение взаимодействия брюшнотифозных бактерий с лимфоцитами в культуре лейкоцитов периферической крови неиммунных людей и хронических брюшнотифозных бактерионосителей. *Вест. АМН СССР*, **8,** 78–84 (1970).

Bonhoff, M., Drake, B. L., Miller, C. P.: Effect of streptomycin on susceptibility of intestinal tract to experimental salmonella infections. *Proc. Soc. exp. Biol. (N. Y.)*, **86,** 132–137 (1954).

Bonhoff, M., Miller, C. P., Martin, W.: Resistance of the mouse intestinal tract to experimental salmonella infection. *J. exp. Med.*, **120,** 805–828 (1964).

Bonventre, P. F., Hayes, R., Imhoft, J.: Autoradiographic evidence for the impermeability of mouse peritoneal macrophages to tritiated streptomycin. *J. Bact.*, **93,** 445–450 (1967).

Borián, A., Csizmazia, F., Karvaly, E., Mihalffy, F., Rédey, B.: Enterocolitis epidemic in Veszprém caused by water infected by Coli O124. *Orv. Hetil.*, **100**, 1072–1074 (1959).

Borman, E. K., Stuart, C. A., Wheeler, K. M.: Taxonomy of the family *Enterobacteriaceae*. *J. Bact.*, **48**, 351–367 (1944).

Boros, D. L., Gerichter, C. B.: The effect of reticuloendothelial blockade and passively transferred peritoneal cells on experimental infection of mice with *Salmonella typhi*, strain Ty_2. *Ann. Inst. Pasteur*, **114**, 367–377 (1968).

(Borovaya, A. Ya., Verholomov, E. E., Arbuzova, V. A.) Боровая, А. Я., Верхоломов, Е. Е., Арбузова, В. А.: Биологические особенности шигелл Бойд-10, выделенных при групповой заболевании дизентерией. *Ж. микробиол. (Москва)*, **9**, 147 (1967).

Borowski, J., Dzieržanowska, D., Zaremba, M.: The diagnostic and epidemiologic significance of nephropathogenic *Escherichia coli* serotypes. *Arch. Immunol. Therap. Exper. (Wrocław)*, **18**, 332–345 (1970).

Brandborg, L. L.: Structure and function of the small intestine in some parasitic diseases. *Amer. J. clin. Nutr.*, **24**, 124–132 (1971).

Brandborg, L. L., Goldberg, S. B., Breidenbach, W. C.: Human coccidiosis — a possible cause of malabsorption. The life cycle in small bowel mucosal biopsies as a diagnostic feature. *New Engl. J. Med.*, **283**, 1306–1313 (1970).

Brandborg, L. L., Tankersley, C., Gottlieb, S., Barancik, M., Sartor, V. E.: Histological demonstration of mucosal invasion by *Giardia lamblia* in man. *Gastroenterology*, **52**, 143–150 (1967).

Branham, S. E., Robey, L., Day, L. A.: A poison produced by *Bacterium enteritidis* and *Bacterium aertricke* which is active in mice when given by mouth. *J. infect. Dis.*, **43**, 507–515 (1928).

Branham, S. W., Habel, K., Lillie, R. D.: Studies with *Shigella dysenteriae* (Shiga). III. *J. infect. Dis.*, **85**, 295–303 (1949).

Brasher, P. H., Landor, J. H., Rigler, S. P., Dragstedt, L. R.: Gastric, duodenal and colonic ulceration induced by histamine. *Arch. Surg.*, **71**, 299–303 (1955).

Brauer-Eppendorf, L.: *Die Ruhr, ihr Wesen und ihre Behandlung*. Kornfeld, Berlin (1922).

Braun, O. H.: Das Problem der Pathogenität von *Escherichia coli* in Säuglingsalter. *Ergebn. inner. Med. Kinderheilk.*, **4**, 78–194 (1953).

Braun, O. H.: Die Epidemiologie der Säuglings-Enteritis. In: *Säuglings-Enteritis*. Ed. by A. Adam. G. Thieme, Stuttgart. 81–169 (1956).

Braun, O. H.: Darmflora des enteritisch erkrankten Säuglings. *Mod. Probl. Pädiatr.* **2**, 24–38 (1957).

Braun, O. H.: Die Colienteritis der Säuglinge. In: *Infektionskrankheiten*. Ed. by O. Gsell, W. Mohr. Springer, Berlin—Heidelberg—New York. Part 2, 643–658. (1968).

Braun, O. H.: *E. coli* enteritis in Germany: epidemiology and recent research. *Ann. N. Y. Acad. Sci.*, **176**, 126–134 (1971).

Braun, O. H., Henckel, H.: Über epidemische Säuglingsenteritis. *Z. Kinderheilk.*, **70**, 33–59 (1951).

Braun, O. H., Henckel, H.: Säuglingsenteritis durch pathogene Colitypen, insbesondere *E. coli* 55:B5. *Z. Kinderheilk.*, **71**, 273–285 (1952).

Braun, O. H., Resemann, G.: Über das Vorkommen von *E. coli* O26:B6 und O86 bei der Säuglingsenteritis. *Klin. Wschr.*, **30**, 853–854 (1952).

Braun, O. H., Resemann, G., Stöckle, Ch.: Untersuchungen über die Tierpathogenität der Dyspepsie-colibacterien. *Z. Hyg. Infekt.-Kr.*, **137**, 581–610 (1953).

Braun, O. H., Sprecht, H., Lüderitz, O., Westphal, O.: Untersuchungen über die Endotoxine aus Dyspepsiecoli-Bakterien. *Z. Hyg. Infekt.-Kr.*, **139**, 565–572 (1954).

Braun, W., Kessel, W. J.: Cytotoxity of endotoxin in relation to their effects on host resistance. In: *Bacterial Endotoxins*. Ed. by M. Landy, W. Braun. New Brunswick, New Jersey. pp. 397–409 (1964).

Brede, H. D., Coldrey, N. A., Coates, J. K., Finlayson, M. H.: *Escherichia coli* serotypes associated with urinary tract infections in the Western Cape. *S. Afr. Med. J.*, **48**, 261–262 (1974).

Brooks, S. E. H., Audretsch, J., Miller G. G.: Electron microscopy of *Giardia lamblia* in human jejunal biopsies. *J. Med. Microbiol.*, **3**, 196–199 (1970).

Brown, C. A., Draper, P., Hart, P. D.: Mycobacteria and lysosomes; a paradox. *Nature* **221**, 658–660 (1969).

Brown, J. H., Brenn, L.: A method for the differential staining of Gram-positive and Gram-negative bacteria in tissue sections. *Johns Hopk. Hosp. Bull.* **4**, 69–73 (1931).

Brown, R. C., Richter, C. B., Bloomer, M. D.: Ultrastructural pathology of an acute fatal enteritis of captive cottomal rabbits. *Amer. J. Path.*, **57**, 93–126 (1969).

(Buchin, P. I., Zinin-Bermes, N. N., Protsenko, O. A., Komarova, M. A.) Бучин, П. И., Зинин-Бермес, Н. Н., Проценко, О. А., Комарова, М. А.: Материалы о носительстве дизентерийных и тифо-паратифозных бактерий при пероральном заражении в эксперименте. *Ж. микробиол. (Москва)*, **6**, 136–137 (1961).

(Budilina, V. V., Illutovich, A. Yu., Petrova, Z. S., Golubeva, E. E., Titrova, A. I., Chetvernina, R. S.) Будилина, В. В., Иллютович, А. Ю., Петрова, З. С., Голубева, Е. Е., Титрова, А. И., Четвернина, Р. С.: Экспериментальная модель бактериальной дизентерии. *Бюлл. экспер. биол. мед.*, **2**, 70–75 (1957).

Burrows, W.: Endotoxins. *Ann. Rev. Microbiol.*, **5**, 181–196 (1951).

Burrows, W.: Cholera toxins. *Ann. Rev. Microbiol.*, **22**, 245–268 (1968).

Burrows, W., Kaur, J., Cercavski, L.: The cholera enterotoxin and local immunity. *Ann. N. Y. Acad. Sci.*, **176**, 324–329 (1971).

Burrows, W., Musteikis, G. M.: Cholera infection and toxin in the rabbit ileal loop. *J. infect. Dis.*, **116**, 183–190 (1966).

Calabi, O.: *In vitro* interaction of *Shigella flexneri* with leukocytes and HeLa cells. *J. Bact.* **122**, 1–9 (1970).

Calmette, A.: *Infection bacillaire et la tuberculose*. Acad. Med. Paris (1928).

Cardella, M. A., Huhn, R. G., Wilson, M. R.: Immunity to neonatal colibacillosis: field studies. *J. Amer. vet. med. Ass.*, **164**, 299–304 (1974).

Carpenter, C. C. J.: Cholera enterotoxin — recent investigations yield insights into transport processes. *Amer. J. Med.*, **50**, 1–7 (1971).

Carpenter, C. C. J.: Cholera and other enterotoxin-related diarrheal diseases. *J. infect. Dis.*, **126**, 551–564 (1972).

Carpenter, C. C. J., Barua, D., Wallace, C. K., Sack, R. B, Mitra, P. P., Werner, A. S., Duffy, T. P., Oleinick, A., Khanra, S. R., Lewis, G. W.: Clinical and physiological observations during an epidemic outbreak of non-vibrio cholera-like disease in Calcutta. *Bull. Wld. Hlth. Org.*, **33**, 665–671 (1965).

Carpenter, K. P.: The relationship of the Enterobacterium A-12 (Sacks) to *Shigella boydii* 14. *J. gen. Microbiol.*, **26**, 535–542 (1961).

Carpenter, K. P.: Report of the Subcommittee on Taxonomy of the *Enterobacteriaceae*. *Int. Bull. bact. Nomencl.*, **13**, 69 (1963*a*).

Carpenter, K. P.: Minutes of Enterobacteriaceae Subcommittee meeting, Montreal, August, 1962. *Int. Bull. bact. Nomencl.* **13**, 139–143 (1963*b*).

Carpenter, K. P.: The catalase activity of *Shigella dysenteriae*, *Shigella boydii*, *Shigella flexneri* and other Enterobacteria. *Ann. Immunol. Hung.*, **9**, 29–34 (1966).

Carrillo, J., Hashimoto, B., Kumate, J.: Content of heterogenetic antigen in *Escherichia coli* and its relationship to diarrhea in newborn infants. *J. infect. Dis.*, **116**, 285–296 (1966).

Cefalu, M., Brancato, P., Saeli, M.: La moltiplicatione intracellulare di *Shigella flexneri* in colture di macrofagi di cavie in rapporto allo virulenzia. *Riv. Ist. sieroter. ital.*, **38**, 197–219 (1963).

Cefalu, M., Puglisi, C.: La virulenza delle Shigella. Potere moltiplicativo intracellulare in colture in cico di macrofagi e attività patogena cheratocongiuntivale nella cavia. *Riv. Ist., sieroter. ital.*, **42**, 34–50 (1967*a*).

Cefalu, M., Puglisi, C.: Attività pathogena cheratocongiuntivale in cavia di Shigella dotate e prive di fimbrie. *Riv. Ist. sieroter. ital.*, **42**, 248–257 (1967*b*).

(Chakhutinskaya, M. G.) Чахутинская, М. Г.: Материалы к биологической характеристике относительно редких представителей сальмонелл. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **21**, 175–183 (1960).

(Chakhutinskaya, M. G.) Чахутинская, М. Г.: Сравнительная клинико-микробиологическая характеристика групповых и спорадических заболеваний, вызываемых сальмонеллой тифи муриум. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **33**, 358–362 (1968).

(Chakhutinskaya, M. G., Avdeeva, T. A., Kleganov, V. K.) Чахутинская, М. Г., Авдеева, Т. А., Клеганов, В. К.: К обоснованию энтеротоксигенности культур кишечной палочки серологической группы 0148, выделенных от больных острыми кишечными заболеваниями в 1974 году в Ленинграде. В кн.: *Эпидемиол., диагностика и профилактика кишечных, респираторных и природноочаговых инфекций.* Институт эпидемиол. микробиол. им. Пастера, Ленинград. 63–65 (1975).

(Chilingaryan, A. V.) Чилингарян, А. В.: Результаты параллельного применения легочной модели, реакции непрямой гемагглютинации и реакции агглютинации для выявления противодизентерийных антител в крови здоровых. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград),* **36,** 93–101 (1970).

(Chilingaryan, A. V., Avdeeva, T. A.) Чилингарян, А. В., Авдеева, Т. А.: К иммунологической характеристике населения г. Еревана в отношении дизентерии. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград),* **33,** 259–267 (1968).

(Chistovich, F. Ya.) Чистович, Ф. Я.: Особенности строения воспалительных новообразований, развивающихся на месте введения составных веществ туберкулезных палочек. *Харьков. мед. ж.,* **2,** 169–187 (1906).

Christeller, E.: Der Typhus abdominalis. In: *Handb. der spez. path. Anatomie und Histologie.* Ed. by F. Henke, O. Lubarsch. **4/2,** 500–609 (1928).

Christiansen, M.: Bakterien der Typhus-Coli-Gruppe im Darm von gesunden Spankälbern und bei deren Darminfektionen. *Zbl. Bakt. I. Abt. Orig.,* **79,** 196–248 (1917).

Christie, B. R.: The pathogenesis of colibacillary diarrhea of the newborn. An electron-microscopic and histochemical study of the disease in neonatal gnotobiotic pigs. Ph. D. Thesis. East Lansing, Michigan (1969).

Ciufeco, C.: Recherches concernant l'immunité croisée entre différents sérotypes de bacilles dysentériques et la souche 47552 kératoconjonctivité, lac dépourvue d'agglutinogènes de Shigella. *Arch. roum. Path. exp.,* **28,** 389–394 (1969).

Ciufeco, C.: Le test de la kérato-conjonctivité. *Arch. roum. Path. exp.,* **29,** 7–27 (1970).

Ciufeco, C., Ciplea, A. G., Ionescu, M. D., Voiculescu, R.: La kératite expérimentale du cobaye. *Arch. roum. Path. exp.,* **29,** 355—383 (1970).

Ciufeco, C., Grigoresco E., Istrati G.: La vaccination antidysentérique. *Arch. roum. Path. exp.* **24,** 669–676 (1965).

Ciufeco, C., Istrati, G.: La vaccination antidysentérique. XV. *Arch. roum. Path. exp.,* **25,** 875–880 (1966).

Ciufeco, C., Jonesco, D.: Étude de quelques Entérobactéries kératoconjonctivité-positive dépourvues d'agglutinogènes de Shigella. *Arch. roum. Path. exp.,* **26,** 781–786 (1967).

Clark, S. L.: The ingestion of proteins and colloidal materials by columnar absorptive cells of the small intestine in the suckling rats and mice. *J. biophys. biochem. Cytol.,* **5,** 41–50 (1959).

Clarke, R. M., Hardy, R. N.: Histological changes in the small intestine of the young pig and their relation to macromolecular uptake. *J. Anat. (Lond.),* **108,** 63–77 (1971).

Coates, M. E.: Nutrition and metabolism. In: *The Germfree Animals in Research.* Ed. by M. E. Coates, H. A. Gordon, B. S. Wostmann. London–New York. pp. 161–179 (1968).

Cohrs, P., Jaffe, R., Meessen, H.: *Pathologie der Laboratoriumstiere.* Springer, Berlin–Göttingen–Heidelberg (1958).

Collins, F. M.: The effect of the growth rate on the composition of *S. enteritidis* cell walls. *Austr. J. exp. biol.,* **42,** 255–262 (1964).

Collins, F. M.: Effect of specific immune mouse serum on the growth of *Salmonella enteritidis* in non-vaccinated mice challenged by various routes. *J. Bact.* **97,** 667–675 (1969).

Collins, F. M.: Mechanisms of antimicrobial immunity. *J. Reticuloendothel. Soc.,* **10,** 58–99 (1971).

Comline, R. S., Roberts, H. E., Titchen, D. A.: Histological changes in the epithelium of the small intestine during protein absorption in the newborn animal. *Nature (Lond.),* **168,** 84–85 (1951).

Conradi, H.: Über lösliche, durch aseptische Autolyse erhaltene Giftstoffe von Ruhr- und Typhusbazillen. *Dtsch. med. Wschr.,* **2,** 26–28 (1903).

Coons, A. H.: Fluorescent antibody methods. In: *General Cytochemical Methods.* Ed. by J. F. Danielli. Acad. Press. New York. pp. 399–422 (1958).
Cooper, G. N.: Experimental shigellosis in mice. *Austr. J. exp. Biol. med. Sci.*, **37,** 193–200 (1959).
Cooper, M. L., Keller, H. M.: Immunization of mice with dysentery antigen administered by gavage and by voluntary drinking. *Proc. Soc. exp. Biol. (N. Y.)*, **64,**422–423 (1947).
Cooper, M. L., Keller, H. M.: Invasion by *Shigella sonnei* of tissues of mice following gavage with viable shigella. *Proc. Soc. exp. Biol. (N. Y.).*, **68,** 87–90 (1948).
Cooper, M. L., Keller, H. M., Walters, E. W.: Microscopic characteristics of colonies of *Shigella flexneri* 2a and 2b and their relation to antigenic composition, mouse virulence and immunogenicity. *J. Immunol.*, **78,** 160–171 (1957).
Costin, I. D.: Serotipuri enteropathogene de *Escherichia coli*, izolate in Banot. *Microbiologia (Buc.)*, **8,** 333–339 (1963).
Costin, I. D.: Diarrhoeal disease in children and adults associated with *Escherichia coli* strains not belonging to enteropathogenic serotypes. *Path. et Microbiol. (Basel)*, **29,** 214–217 (1966).
Costin, I. D., Olinici, N.: Studiul unor tulpini de *Escherichia coli* serotip 124 : 72(B17) izolate de la copii şi adulti in enterite. *Microbiologia (Buc.)*, **10,** 49–58 (1965).
Costin, I. D., Voiculescu, D., Gorcea, V.: An outbreak of food poisoning in adults associated with *E. coli* serotype 86 : B7 : H34. *Path. et Microbiol. (Basel)*, **27,** 68–78 (1964).
Cotchin, E., Roe, F. J. C.: *Pathology of laboratory rats and mice.* Blackwell, Oxford–Edinburgh (1967).
Crane, R. K.: Digestive-absorptive surface of the small bowel mucosa. *Ann. Rev. Med.*, **19,** 57–68 (1968).
Cross, W. R., Nakamura, M.: Analysis of the virulence of *Shigella flexneri* by experimental infection of the rabbit eye. *J. infect. Dis.*, **122,** 394–400 (1970).
Cruickshank, R., Ghosh, G. K., Letif, M. A.: Pathogenesis of experimental cholera. In: *9th Internat. Congr. Microbiol., Symposia, Moscow.* 169–172 (1966).
Dack, G. M.: *Food Poisoning.* 3rd ed., Univ. Press., Chicago. (1956).
Dack, G. M.: Salmonella food infections. In: *Diseases Transmitted from Animals to Man.* Ed. by T. G. Hill. 5th ed. Thomas, Springfield. 210–234 (1963).
Darlow, H. M., Bale, W. R., Carter, G. B.: Infection of mice by the respiratory route with *Salmonella typhimurium. J. Hyg. (Lond.)*, **59,** 303–308 (1961).
David, H.: *Elektronenmikroskopische Organpathologie.* Springer, Berlin (1967).
Davis, C. P., Savage, D. C.: Habitat, succession, attachment, and morphology of segmented, filamentous microbes indigenous to the murine gastrointestinal tract. *Infect. Immun.*, **10,** 948–956 (1974).
(Davydovsky, I. V.) Давыдовский, И. В.: *Патологическая анатомия и патогенез болезней человека.* Медгиз, Москва **1,** 146–178 (1956).
(Davydovsky, I. V.) Давыдовский, И. В.: *Общая патология человека.* Медгиз, Москва (1961).
De, S. N.: Enterotoxicity of bacteria-free culture-filtrate of *Vibrio cholerae. Nature (Lond.)*, **183,** 1533–1534 (1959).
De, S. N., Bhattacharya, K., Sarkar, J. K.: A study of the pathogenicity of strains of *Bacterium coli* from acute and chronic enteritis. *J. Path. Bact.*, **71,** 201–209 (1956).
De, S. N., Chatterje, D. N.: An experimental study of the mechanism of action of *Vibrio cholerae* on the intestinal mucous membrane. *J. Path. Bact.*, **66,** 559–562 (1953).
De, S. N., Ghose, M. L., Sen, A.: Activities of bacteria-free preparations from *Vibrio cholerae. J. Path. Bact.*, **79,** 373–380 (1960).
Dean, A. G., Ching, Y. C., Williams, R. G., Harden, L. B.: Test for *Escherichia coli* enterotoxin using infant mice. *J. infect. Dis.*, **125,** 407–411 (1972).
Deane, H. W.: Some electron-microscopic observations on the lamina propria of the gut. *Anat. Rec.*, **149,** 453–474 (1964).
Deák, Z.: Phage-typing of *Escherichia coli* O124 : K72(B17) by the examination of lysogenity. *Acta microbiol. Acad. Sci. hung.*, **12,** 261–267 (1965).
De Assis, A.: Estudos sobre "*Shigella alkalescens* Andrewes"; compartamento fermentativo. *Hospital (Rio de J.)*, **15,** 447–456 (1939*a*).

De Assis, A.: Estudos sobre "*Shigella alkalescens* Andrewes"; compartamento sorológico. *Hospital (Rio de J.)* **15,** 655–666 (1939*b*).
De Assis, A.: *Shigella guanabara*, tipo sorologico destacado do grupo do B. ceylonensis-dispar. *Hospital (Rio de J.)*, **33,** 508–518 (1948*a*).
De Assis, A.: *Shigella tieté*, novo tipo sorologico de bacilo disentérico. *Hospital (Rio de J.)*, **32,** 667–671 (1948*b*).
Delaunay, A.: La diapédèse leucocytaire, ses facteurs et son mécanisme. *Rev. canad. Biol.*, **12,** 340–346 (1953).
Delaunay, A., Lebrun J., Kerneis S. P., Delaunay M.: Le mode d'action des endotoxines bactériennes. *Rev. Immunol. (Paris)*, **12,** 23–42 (1948).
(Demina, A. A., Chagodaeva, E. A.) Демина, А. А., Чагодаева, Е. А.: Определение вирулентности патогенных серологических типов кишечной палочки на куриных эмбрионах. *Тр. Ин-та вакцин и сывороток им. Мечникова (Москва)*, **14,** 133–141 (1959).
(Dergachev, I. S.) Дергачев, И. С.: Некоторые вопросы патогенеза дизентерии детей грудного возраста. *Педиатрия*, **4,** 14–20 (1954).
Doycke: Zur Aetiologie der Dysenterie. *Dtsch. med. Wschr.*, **27,** 10–12 (1901).
Dobbins, W. O., Ruffin, J. M.: A light- and electron-microscopic study of bacterial invasion in Whipple's disease. *Amer. J. Path.*, **51,** 225–242 (1967).
Doerr, R.: Das Dysenterietoxin. *Wien. klin. Wschr.*, **19,** 1218–1220 (1906).
Doerr, R.: *Das Dysenterietoxin*. Fischer, Jena (1907).
Donaldson, R. M.: Microbial population of the human intestine. *Gastroenterology*, **53,** 1003–1005 (1967*a*).
Donaldson, R. M.: Role of enteric microorganisms in malabsorption. *Fed. Proc.*, **26,** 1426–1431 (1967*b*).
Donaldson, R. M.: Role of indigenous enteric bacteria in intestinal function and disease. In: *Handbook of Physiology*. American Physiological Society, Washington. Section 6, Vol. 5, pp. 2807–2837. (1968).
Donellan, W. L.: The structure of the colonic mucosa. *Gastroenterology*, **49,** 496–514 (1965).
Donta, S. T., Sack, D. A., Walace, R. B., DuPont, H. L., Sack, R. B.: Tissue-culture assay of antibodies to heat-labile *Escherichia coli* enterotoxins. *New Engl. J. Med.*, **291,** 117–120 (1974).
Donta, S. T., Smith, D. M.: Stimulation of steroidogenesis in tissue culture by enterotoxigenic *Escherichia coli* and its neutralization by specific antiserum. *Infect. Immunity*, **9,** 500–505 (1974).
Dopter, C., Repaci, G.: La dysentérie bacillaire expérimentale par ingestion. *C. R. Soc. Biol. (Paris)*, **68,** 52–54 (1910).
(Dosser, E. M.) Доссер, Е. М.: Типоспецифичность иммунитета при дизентерии Флекснера. *Ж. микробиол. (Москва)*, **7,** 74–76 (1953).
Drach, G. W., Cox, C. E.: Immunofluorescence studies of the bladder defence mechanism. I. Localization of *Escherichia coli* in the human bladder. *J. Amer. Geriat. Soc.*, **15,** 1114–1124 (1967).
(Dragunskaya, E. M.) Драгунская, Е. М.: К биологической характеристике энтеропатогенной кишечной палочки серотипа 301 серологической группы 025. II. *Тр. Ин-та эпидемиол. микробиол. им. Пастера, (Ленинград)*, **36,** 211–216 (1970*a*).
(Dragunskaya, E. M.) Драгунская, Е. М.: Изучение токсических штаммов кишечных палочек в опытах внутрилегочного заражения белых мышей. *Материалы конференции I Ленингр. мед. ин-та и Ин-та экспер. мед. АМН СССР*, 24–26 (1970*b*).
(Dragunskaya, E. M.) Драгунская, Е. М.: Экспериментальное изучение патогенных свойств *Escherichia coli* — возбудителей острых кишечных заболеваний взрослых и детей. Дисс. канд. Ленинград (1972).
(Dragunskaya, E. M., Avdeeva, T. A., Smirnova, L. A.) Драгунская, Е. М., Авдеева, Т. А., Смирнова, Л. А.: Морфологические изменения, вызываемые в легких интраназально зараженных белых мышей энтеротоксигенными кишечными палочками и полученными из них клеточными лизатами. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **40,** 86–92 (1973).
(Dragunskaya, E. M., Smirnova, L. A.) Драгунская, Е. М., Смирнова, Л. А.: К количественной микробиологической и патоморфологической характеристике процесса, вызываемого некоторыми представителями рода эшерихия у интраназально зара-

женных белых мышей. *Авторефераты Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, 39–41 (1970).
(Dragunskaya, E. M., Smirnova, L. A.) Драгунская, Е. М., Смирнова, Л. А.: Особенности инфекционного процесса в легких белых мышей после интраназального введения одного из штаммов шигеллы Зонне. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **40,** 70–72 (1973).
(Dragunskaya, E. M., Smirnova, L. A., Arbuzova, V. A., Avdeeva, T. A.) Драгунская, Е. М., Смирнова, Л. А., Арбузова, В. А., Авдеева, Т. А.: Особенности инфекционных процессов в легких мышей после интраназального заражения некоторыми штаммами кишечных палочек. *Тр. Ленинградского общества патологоанатомов*, **13,** 210–213 (1972).
Drees, D. T., Waxler, G. L.: Enteric colibacillosis in gnotobiotic swine. *Amer. J. vet. Res.*, **31,** 1147–1171 (1970).
(Drobyshevskaya, E. I.) Дробышевская, Э. И.: Материалы к биологической характеристике энтеропатогенных кишечных палочек серологических групп 0126, 0127 и 0128. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **36,** 295–303 (1970).
(Drozdova, M. V., Petrova, P. A.) Дроздова, М. В., Петрова, П. А.: Палочки дизентерии в патологии глаза. *Русс. офтальмол. ж.* **2,** 204–208 (1929).
Drucker, M. M., Polliack, A., Yeivin, R., Sacks, T. G.: Immunofluorescent demonstration of enteropathogenic *Escherichia coli* in tissues of infants dying with enteritis. *Pediatrics*, **46,** 855–864 (1970).
Drucker, M. M., Yeivin, R., Sacks, T. G.: Pathogenesis of *Escherichia coli* enteritis in the ligated rabbit gut. *Israel J. med. Sci.*, **3,** 445–452 (1967).
Dubos, R. J.: *Biochemical Determinants of Microbiol Diseases*. Univ. Press, Cambridge, Massachusetts (1954).
Dubos, R. J.: Metabolic interrelationships between host and parasite. In: *Host-Parasite Relationships in Living Cells*. Ed. by H. M. Felton, Thomas, Springfield. pp. 172–190 (1957).
Dubos, R. J.: The microbiota of the gastrointestinal tract. *Gastroenterology*, **51,** 868–874 (1966).
Dubos, R. J., Savage, D.C., Schaedler, R. W.: The indigenous flora of the gastrointestinal tract. *Diseases Colon and Rectum*, **10,** 23–34 (1967).
Dubos, R. J., Schaedler, R. W., Costello, R., Hol, P.: Indigenous, normal and autochtonous flora of the gastrointestinal tract. *J. exp. Med.*, **122,** 67–76 (1965).
Duguid, J. P.: The function of bacterial fimbriae. *Arch. Immunol. Ther. exp.*, **16,** 173–188 (1968).
Duguid, J. P., Gillies, R. R.: Fimbriae and adhesive properties in dysentery bacilli. *J. Path. Bact.*, **74,** 397–411 (1957).
Duke-Elder, S.: Diseases of the conjunctiva and associated disease of the corneal epithelium. In: *System of Ophthalmology*. Ed. by S. Duke-Elder, London. Vol. 8, Part 1, pp. 1–598 (1965).
Dumas, Y. A., Combiesco, D.: L'intoxication dysentérique du lapin et l'intoxication cholérique du cobaye par ingestion de toxins dysentérique et cholérique solubles. *C. R. Acad. Sci. (Paris)*, **175,** 652–654 (1922).
Dunareanu, H., Brindus, Gh.: Recherches sur la valeur du test de la kérato-conjonctivité dans l'isolement des Shigella. *Arch. roum. Path. exp.*, **20,** 503–509 (1961).
DuPont, H. L., Formal, S. B., Hornick, R. B., Snyder, M. J., Libonati, J. P., Sheahan, D. G., LaBrec, E. H., Kalas, J. P.: Pathogenesis of *Escherichia coli* diarrhea. *New Engl. J. Med.*, **285,** 1–9 (1971).
DuPont, H. L., Hornick, R. B., Dawkins, A. T., Snyder, M. J., Formal, S. B.: The response of man to virulent *Shigella flexneri* 2a. *J. infect. Dis.* **119,** 296–299 (1969).
Dutt, A. R.: Distribution of *Vibrio cholerae* in the intestine. *Indian J. med. Res.*, **55,** 314–317 (1967).
(Dvizhkov, P. P.) Движков, П. П.: Дизентерия. В кн.: *Многотомное руководство по патологической анатомии*. Медицина, Москва. **9**, 289–300 (1964).
(Dzhikidze, E. K., Stasilevich, Z. K., Pekerman, S. M., Kavtaradze, K. N.) Джикидзе, Э. К., Стасилевич, З. К., Пекерман, С. М., Кавтарадзе, К. Н.: Моделирование кишечных инфекций человека в эксперименте на животных. *Вестн. АМН СССР*, **11,** 38–49 (1965).

Eberth, C. J.: Die Organismen in den Organen bei Typhus abdominalis. *Virchows Arch. path., Anat.*, **81**, 56–74 (1880).
Eberth, C. J.: Neue Untersuchungen über den Bacillus des Abdominaltyphus. *Virchows Arch. path. Anat.*, **83**, 486–501 (1881).
Edsall, G., Gaines, S., Landy, M., Tigertt, W. D., Sprinz, H., Trapani, R. J., Mandel, A. D., Benenson, A. S.: Studies on infection and immunity in experimental typhoid fever, *J. exp. Med.*, **112**, 143–166 (1960).
Edwards, P. R., Ewing, W. H.: *Identification of Enterobacteriaceae*. Burgess Publ. Co., Minneapolis, 2nd ed., (1962); 3rd ed., (1972).
(Elchinova, E. A.) Ельчинова, Е. А.: Культивирование микробов Флекснера в куриных эмбрионах. *Ж. микробиол. (Москва)*, **3**, 119–122 (1960).
(Eliseev, V. G., Afanasyev, Yu. I., Kopaev, Yu. N., Yurina, N. A.) Елисеев, В. Г., Афанасьев, Ю. И., Копаев, Ю. Н., Юрина, Н. А.: *Гистология*. 2-е изд., Медицина, Москва (1972).
Elliot, H. L., Carpenter, C. C. J., Sack, R. B., Yardley, J. H.: Small bowel morphology in experimental canine cholera. *Lab. Invest.*, **22**, 112–120 (1970).
El-Nagen, M. M.: Période de perméabilité de l'intestin du veau nouveau-né aux gamma-globulines du colostrum. *Ann. Méd. vét.*, **6**, 370–379 (1967).
(Elshina, M. A., Molchenko, E. F.) Елшина, М. А., Мольченко, Е. Ф.: Некоторые данные об этиологической роли кишечной палочки серотипа 0111: B4 при острых кишечных заболеваниях у детей. *Ж. микробиол. (Москва)*, **4**, 44–49 (1959).
(Emelyanova, O. I.) Емельянова, О. И.: Изучение биологических свойств кишечных палочек патогенных серологических типов. Автореф. дисс. канд. Харьков (1964).
(Emelyanova, O. I., Shvetsov, V. S.) Емельянова, О. И., Швецов, В. С.: Котята как модель для изучения колиэнтерита. *Ж. микробиол. (Москва)*, **4**, 93–96 (1963).
Erlandsen, S. L., Chase, D. G.: Morphological alterations in the border of villous epithelial cells produced by intestinal microorganisms. *Amer. J. clin. Nutr.*, **27**, 1277–1286 (1974).
Erlandsen, S. L., Parsons, J. A., Taylor, T. D.: Ultrastructural immunocytochemical localization of lysozyme in the Paneth cells of man. *J. Histochem. Cytochem.*, **22**, 401–413 (1974).
Erlandson, A. L., Lamley, E. L., Floid, T. M.: Experimental *Shigella flexneri* infections in chick embryos. *J. infect. Dis.*, **102**, 237–242 (1958).
Erlandson, A. L., Nemer, M. A., Pearson, I. A.: Characteristics of various experimental human adult virulent *Escherichia coli* infections in mice. *J. infect. Dis.*, **114**, 163–168 (1964).
(Ermolyeva, Z. V., Avtsyn, A. P., Semich, A. J., Berezina, E. K.) Ермольева, З. В., Авцын, А. П., Семич, А. И., Березина, Е. К.: Изучение химиотерапевтических свойств биомицина при экспериментальной брюшнотифозной инфекции. *Ж. микробиол. (Москва)*, **6**, 3–7 (1954).
(Ermolov, V. I.) Ермолов, В. И.: Экспериментальная инфекция у щенков собак, вызванная *E. coli*. *Ж. микробиол. (Москва)*, **8**, 102–105 (1967).
Escherich, T.: Die Darmbacterien des Neugeborenen und Säuglings. *Fortschr. Med.*, **3**, 515–522, 547–554 (1885).
Escherich, T.: *Die Darmbacterien des Säuglings und ihre Beziehung zur Physiologie der Verdauung*. F. Enke, Stuttgart (1886).
Essbach, H.: *Paidopathologie. Kyemopathien. Neogonopathien. Thelamonopathien. Lehrbuch and Atlas*. G. Thieme, Leipzig (1961).
Etinger-Tulczynska, R.: Nasale Infection mit Mäusetyphus. *Z. ges. Hyg.*, **113**, 493–497 (1932).
Etkin, S., Gorbach S. L.: Studies on enterotoxin from *Escherichia coli* associated with acute diarrhea in man. *J. Lab. clin. Med.*, **78**, 81–87 (1971).
Evans, D. G., Evans, D. J. Jr., Pierce N. F.: Differences in the response of rabbit small intestine to heat-labile and heat-stable enterotoxins of *Escherichia coli*. *Infect. Immunity*, **7**, 873–880 (1973).
Evans, D. J. Jr., Chen, L. C., Curlin, C. T., Evans, D. G.: Stimulation of adenyl cyclase by *Escherichia coli* enterotoxin. *Nature New Biology*, **236**, 137–138 (1972).
Evans, D. J. Jr., Evans, D. G., Gorbach, S. L.: Production of vascular permeability factor by enterotoxigenic *Escherichia coli* isolated from man. *Infect. Immunity*, **8**, 725–730 (1973).

Ewing, W. H.: Serological relationships between *Shigella* and coliform cultures. *J. Bact.*, **66,** 333–340 (1953).
Ewing, W. H.: Enteropathogenic *Escherichia coli* serotypes. *Ann. N. Y. Acad. Sci.*, **66,** 61–70 (1956).
Ewing, W. H.: Sources of *Escherichia coli* cultures that belonged to O-antigen groups associated with infantile diarrheal disease. *J. infect. Dis.*, **110,** 114–120 (1962).
Ewing, W. H.: *Enterobacteriaceae taxonomy and nomenclature.* Commun. Dis. Center, Atlanta, Georgia (1966).
Ewing, W. H.: *Differentiation of Enterobacteriaceae by biochemical reactions.* Commun. Dis. Center, Atlanta, Georgia (1968).
Ewing, W. H.: Isolation and preliminary identification of enteropathogenic serotypes of *Escherichia coli. J. Conf. Publ. Health Lab. Directors*, **27,** 19–30 (1969).
Ewing, W. H., Davis, B. R.: *The O-antigen groups of Escherichia coli cultures from various sources.* Commun. Dis. Center, Atlanta, Georgia (1961).
Ewing, W. H., Davis, B. R., Martin, W. J.: *Biochemical characterization of Escherichia Coli.* Commun. Dis. Center, Atlanta, Georgia (1972).
Ewing, W. H., Davis, B. R., Montague, T. S.: *Studies on the occurrence of Escherichia coli serotypes associated with diarrheal disease.* Commun. Dis. Center, Atlanta, Georgia (1963).
Ewing, W. H., Edwards, P. R.: The principal divisions and groups of Enterobacteriaceae and their differentiation. *Int. Bull. bact. Nomencl.* **10,** 1–12 (1960).
Ewing, W. H., Gravatti, J. L.: *Shigella* types encountered in the Mediterranean area. *J. Bact.*, **53,** 191–195 (1947).
Ewing, W. H., Hucks, M. C.: Two intermediate members of *Enterobacteriaceae. J. Bact.*, **60,** 367–368 (1950).
Ewing, W. H., Hucks, M. C., Taylor, M. W.: Interrelationship of certain *Shigella* and *Escherichia* cultures. *J. Bact.*, **63,** 319–325 (1952).
Ewing, W. H., Kauffmann, F.: A new coli O-antigen group. *U. S. Publ. Health Repts.*, **65,** 1341–1343 (1950).
Ewing, W. H., Reavis, R. W., Davis, B. R.: Provisional *Shigella* serotypes. *Canad. J. Microbiol.*, **4,** 89–107 (1958).
Ewing, W. H., Tanner, K. E.: The B-antigens of *Shigella dysenteriae* 2 and *Escherichia coli* O-group 112a112c. *J. Bact.*, **69,** 89–91 (1955).
Ewing, W. H., Taylor, M. W., Hucks, M. C.: The Alkalescens-Dispar group. *U. S. Publ. Health Repts.*, **65,** 1474–1480 (1950).
Faber, H.: Die typhösen Knötchen in Leber, Milz und Knochenmark. *Beitr. path. Anat.*, **68,** 458–485 (1921).
Falkow, S., Schneider, H., Baron, L. S., Formal, S. B.: Virulence of *Escherichia-Shigella* genetic hybrids for the guinea pig. *J. Bact.* **86,** 1251–1258 (1963).
Fell, B. F., Boyne, R., Cuthbertson, D. P.: Intestine lesions following histamine liberation in the rat. *J. Path.*, **82,** 445–452 (1961).
Felsen, J.: Acute and chronic bacillary dysentery. *Amer. J. Path.*, **12,** 395–404 (1936).
Felsen, J.: *Bacillary Dysentery, Colitis and Enteritis.* Saunders, Philadelphia–London. (1945).
Felsenfeld, O., Wolf, R. H., Greer, W. E., Brannon, R. B.: Oral immunization with polyvalent streptomycin-dependent *Escherichia coli* vaccine. *Appl. Microbiol.*, **23,** 444–448 (1972).
Felton, H. M. (ed.): *Host-parasite relationships in living cells.* Symposium. Springfield, (1957).
Ferguson, W. W.: Experimental diarrheal disease of human volunteers due to *Escherichia coli. Ann. N. Y. Acad. Sci.*, **66,** 71–77 (1956).
Ferguson, W. W., June, R. C.: Experiments on feeding adult volunteers with *Escherichia coli* 111 : B4, a coliform organism associated with infant diarrhea. *Amer. J. Hyg.*, **55,** 155–169 (1952).
Fernandes, M. R. F., Trabulsi, L. R.: A new *Escherichia coli* serotype causing experimental keratoconjunctivitis in guinea-pig (culture 412-DEC-66). *Rev. Inst. Med. trop. S. Paulo*, **9,** 62 (1967).
Fernandes, M. R. F., Trabulsi, L. R.: Enteropatogénicida do e comportamento bioguimico do colibacilo O136 : K78. *Rev. Inst. Med. trop. S. Paulo*, **11,** 101–103 (1969).

Fey, H.: On coliform bovine mastitis with special reference to infantile enteritis. In: *Proc. 6th Internat. Congr. Microbiol.* Roma. vol. **4**, pp. 263–264 (1953).

Fey, H.: *Serologische, biochemische und biologische Untersuchungen an Stämmen aus boviner Colimastitis mit spezieller Berücksichtigung der Colisäuglingsenteritis.* Thesis, Zürich (1955).

Fey, H.: Bakteriologie und Serologie der Colisepsis des Kalbes. I. Serologische und biochimische Untersuchungen. II. Bedeutung des Colityps 78 : B80 für die Kälberruhr. *Z. Veterinaermed.*, **4**, 309–318, 447–458 (1957).

Fey, H.: Die Bedeutung des Gammaglobulins für das neugeborene Kalb. In: *Internat. Tierärztl. Arbeitsgemeinschaft. Tierernährung. Symposium.* Salzburg. pp. 17–18 (1967).

Fey, H.: Immunology of the newborn calf: its relationship to colisepticemia. *Ann. N. Y. Acad. Sci.*, **176**, 49–63 (1971).

Field, M.: Intestinal secretion: effect of cyclic AMP and its role in cholera. *New Engl. J. Med.*, **284**, 1137–1144 (1971).

(Filichkin, S. E.) Филичкин, С. Е.: Антагонизм между колициногенными дизентерийными бактериями и кишечными палочками, выделенными от больных дизентерией. *Ж. микробиол. (Москва)*, **4**, 64–69 (1969).

Filotti, A.: Sur le pouvoir pathogène expérimental de certaines souches du genre Escherichia. *Arch. roum. Path. exp.*, **24**, 843–850 (1965).

Fine, J., Frank, E. D., Ravin, H. A., Rutenberg, S. H., Schweinburg, F. B.: The bacterial factor in traumatic shock. *New Engl. J. Med.*, **260**, 214–220 (1959).

Finkelstein, R. A.: The role of choleragen in the pathogenesis and immunology of cholera. *Tex. Rep. Biol. Med.*, **27**, Suppl. 1, 181–201 (1969).

Fischer, W.: Ruhr und asiatische Cholera. In: *Handb. d. spez. pathol. Anatomie und Histologie.*, Ed. by F. Henke and O. Lubarsch. Berlin. vol. **4/3**, pp. 417–441. (1929).

Flexner, S., Sweet, J. E.: The pathogenesis of experimental colitis, and the relation of colitis in animals and man. *J. exp. Med.*, **8**, 514–535 (1906).

Florey, H. W.: Observations on the functions of mucus and the early stages of bacterial invasion of the intestinal mucosa. *J. Path. Bact.*, **37**, 283–289 (1933).

Florey, H. W.: Electron microscopic observations on goblet cells of the rat's colon. *Quart. J. exp. Physiol.*, **45**, 329–336 (1960).

Florey, H. W.: *General pathology.* 3rd ed., Lloyd-Luke, London (1962). 4th ed. (1970).

Floyd, T. M., Clarke, R. B.: The effect of some Krebs' cycle inhibitors and intermediates on Shigella infections in mice. *Bact. Proc.*, **90**, (1957).

Floyd, T. M., Hoogstral, H.: The susceptibility of same desert rodents to experimental infection with Shigella and Brucella organisms. *J. Hyg.*, **52**, 516–524 (1954).

Foo, M. C., Lee, A., Cooper, G. N.: Natural antibodies and the intestinal flora of rodents. *Austr. J. exp. Biol. med. Sci.*, **52** (part 2), 321–330 (1974).

Formal, S. B., Abrams, G. D., Schneider, H., Sprinz, H.: Experimental Shigella infection. VI. Role of the small intestine in an experimental infection in guinea pigs. *J. Bact.* **85**, 119–125 (1963*a*).

Formal, S. B., Dammin, G. J., LaBrec, E. H., Schneider, H.: Experimental Shigella infections: characteristics of a fatal infection produced in guinea pigs. *J. Bact.* **75**, 604–610 (1958).

Formal, S. B., Dammin, C. J., Schneider, H., LaBrec, E. H.: Experimental Shigella infections. II. Characteristics of a fatal enteric infection in guinea pigs following the subcutaneous inoculation of carbon tetrachloride. *J. Bact.* **78**, 800–804 (1959).

Formal, S. B., Dammin, G., Sprinz, H., Kundel, D., Schneider, H., Horowitz, M., Forbes, M.: Experimental Shigella infections. V. Studies in germ free guinea pigs. *J. Bact.* **82**, 284–287 (1961*a*).

Formal, S. B., DuPont, H. L., Hornick, R., Snyder, M. J., Libonati, J., LaBrec, E. H.: Experimental models in the investigation of the virulence of dysentery bacilli and *Escherichia coli*. *Ann. N. Y. Acad. Sci.*, **176**, 190–196 (1971*a*).

Formal, S. B., Gemski, P., Baron, L., LaBrec, E.: A chromosomal locus which controls the ability of *Shigella flexneri* to evoke keratoconjunctivitis. *Infect., Immunity*, **3**, 73–79 (1971*b*).

Formal, S. B., Kent, T. H., Austin, S., LaBrec, E. H.: Fluorescent-antibody and histological study of vaccinated and control monkeys challenged with *Shigella flexneri*. *J. Bact.*, **91**, 2368–2376 (1966*a*).

Formal, S. B., Kent, T. H., May, H. C., Palmer, A., Falkow, S., LaBrec, E. H.: Protection of monkeys against experimental Shigellosis with a living attenuated oral polyvalent dysentery vaccine. *J. Bact.* **92**, 17–22 (1966*b*).

Formal, S. B., Kundel, D., Schneider, H., Kunev, N., Sprinz, H.: Studies with *Vibrio cholerae* in the ligated loop of the rabbit intestine. *Brit. J. exp. Path.*, **42**, 504–510 (1961*b*).

Formal, S. B., LaBrec, E. H., Kent, T. H., Falkow, S.: Abortive intestinal infection with an *Escherichia coli-Shigella flexneri* hybrid strain. *J. Bact.*, **89**, 1374–1382 (1965*a*).

Formal, S. B., LaBrec, E. H., Kent, T. H., May, H. C., Lowenthal J. P., Berman S.: Biological properties of freeze-dried attenuated Shigella vaccines. *Proc. Soc. exp. Biol.*, **124**, 284–289 (1967*a*).

Formal, S. B., LaBrec, E. H., Palmer, A., Falkow, S.: Protection of monkeys against experimental shigellosis with attenuated vaccines. *J. Bact.*, **90**, 63–68 (1965*c*).

Formal, S. B., LaBrec, E. H., Schneider, H.: Pathogenesis of bacillary dysentery in laboratory animals. *Fed. Proc.* **24**, 29–34 (1965*d*).

Formal, S. B., LaBrec, E. H., Schneider, H., Falkow, S.: Restoration of virulence to a strain of *Shigella flexneri* by mating with *Escherichia coli*. *J. Bact.*, **89**, 835–838 (1965*b*).

Formal, S. B., LaBrec, E. H., Schneider, H., Sprinz, H.: The pathogenesis of bacillary dysentery in laboratory animals. In: *Proceedings. — Symposium on the characterization and uses of human diploid cell strains*. Opatija, Yugoslavia. 641–650 (1963*b*).

Formal, S. B., Maenza, R. M., Austin, S., LaBrec, E. H.: Vaccines to protect monkeys against experimental shigellosis. *Proc. Soc. exp. Biol.*, **125**, 347–349 (1967*b*).

Formal, S. B., Noyes, H. E., Schneider, H.: Experimental Shigella infections. III. Sensitivity of normal, starved and carbon tetrachloride treated guinea pigs to endotoxin. *Proc. Soc. exp. Biol.*, **103**, 415–418 (1960).

(Forshter, Kh. K.) Форштер, Х. К.: Влияние кортизона на процесс фагоцитоза при брюшнотифозной инфекции у мышей. *Ж. микробиол. (Москва)*, **4**, 139–146 (1957).

Fraenkel, E., Simmonds, M.: *Die ätiologische Bedeutung des Typhus-Bacillus*. Hamburg–Leipzig. (1886).

Fraenkel, E.: Über Roseola typhosa. *Z. ges. Hyg.*, **34**, 483–495 (1900).

Franke, F. E.: Action of toxic doses of the polysaccharide from *Serratia marcescens* (Bacillus prodigiosus) on the dog and guinea pig. *J. nat. Cancer Inst.*, **5**, 185–193 (1944–1945).

(Freidlin, I. S.) Фрейдлин, И. С.: Изменение фагоцитарной активности и некоторых цитохимических реакций лейкоцитов перитонеального экссудата морских свинок в процессе иммунизации. *Бюлл. экспер. биол. мед.* **9**, 80–83 (1961).

(Freidlin, I. S.) Фрейдлин, И. С.: Фагоцитарная активность и некоторые цитохимические реакции лейкоцитов в перитонеальном экссудате иммунизированных тетравакциной морских свинок в условиях различного насыщения витамином С. *Ж. микробиол. (Москва)*, **10**, 101–105 (1962).

Frenkel, J. K.: Models for infectious diseases. *Fed. Proc.*, **28**, 179–190 (1969).

Fresh, J. W., Versage, P. M., Reyes, V.: Intestinal morphology in human and experimental cholera. *Arch. Pathol.*, **77**, 529–537 (1964).

Freter, R.: The fatal enteric cholera infection in the guinea pig, achieved by inhibition of normal enteric flora. *J. infect. Dis.*, **97**, 57–65 (1955).

Freter, R.: Experimental enteric Shigella and vibrio infections in mice and guinea pigs. *J. exp. Med.*, **104**, 411–418 (1956).

Freter, R.: *In vitro* and *in vivo* antagonism of intestinal bacteria against *Shigella flexneri*. *J. infect. Dis.*, **110**, 38–46 (1962).

Freter, R.: Studies on the mechanism of action of intestinal antibody in experimental cholera. *Tex. Rep. Biol. Med.*, **27**, Suppl. 1, 299–316 (1969).

Friedman, D. R., Halbert, S. R.: Mixed bacterial infections in relation to antibiotic activities. IV. *J. Immunol.*, **84**, 11–19 (1960).

(Frolova, M. A., Sergeev, V. V., Golubeva, O. S., Protasova, O. V., Elkina, S. I., Shuster, B. Yu.) Фролова, М. А. Сергеев, В. В., Голубева, О. С., Протасова, О. В., Елкина, С. И., Шустер, Б. Ю.: К механизму иммуногенеза при вакцинации стрептомицинзависимыми мутантами сальмонелл и шигелл. 1. *Ж. микробиол. (Москва)*, **4**, 96–100 (1970).

Fukumi, H., Kosakai, N.: Resident and disease-producing effect of the coliform organism. *Japan. Med. News* (Nippon Izi Sinpo), 914 (1953). — cited by Ferguson (1956).
Furness, G.: Interaction between *Salmonella typhimurium* and phagocytic cells in cell culture. *J. infect. Dis.*, **103**, 272–277 (1958).
Furness, G., Ferreira, J.: The role of macrophages in natural immunity to salmonellae. *J. infect. Dis.*, **104**, 203–206 (1959).
Furness, G., Rowley, D.: Transduction of virulence with the species *Salmonella typhimurium*. *J. gen. Microbiol.* **15**, 140–145 (1956).
Furowicz, A. J., Ørskov, F.: Two new *Escherichia coli* O antigens, O150 and O157, and one new K antigen, K 93, in strains isolated from veterinary diseases. *Acta path. microbiol. scand., Section B*, **80**, 441–444 (1972).
Furth, R., Cohn, Z. A.: The origin and kinetics of mononuclear phagocytes. *J. exp. Med.*, **128**, 415–435 (1968).
Gabliks, J., Solotorovsky, M.: Cell culture reactivity to diphtheria, staphylococcus, tetanus and *Escherichia coli* toxins, *J. Immunol.*, **88**, 505–512 (1962).
(Gabrichevsky, G.) Габричевский, Г.: *Bacterium coli commune* и его роль в патологии человека. *Мед. обозрение (Москва)*, **41**, 1087–1102 (1894).
Gaffky, G.: Zur Ätiologie des Abdominaltyphus. *Mitt. a. d. kaiserl. Gesundheitsamt*, **2**, 372–420 (1884).
Gaines, S., Sprinz, H., Tully, J. G., Tigertt, W. D.: Studies on infection and immunity in experimental typhoid fever. VII, VIII. *J. infect. Dis.*, **118**, 293–306 and 393–401 (1968).
Gangarosa, E. J., Perera, D. R., Mata, L. J.: Epidemic Shiga bacillus dysentery in Central America. II. *J. infect. Dis.*, **122**, 181–190 (1970).
Garber, E. D.: The host as a growth medium. *Ann. N. Y. Acad. Sci.*, **88**, Art. 5, 1187–1194 (1960).
(Gavrilyuk, B. K.) Гаврилюк, Б. К.: Определение степени патогенности сальмонелл, кишечных палочек и стафилококков на культуре ткани. *Уч. записки Хабаровского ин-та эпидемиол., микробиол.* **8**, 179–180 (1966).
Gay, C. C.: *Escherichia coli* and neonatal disease of calves. *Bact. Rev.*, **29**, 75–101 (1965).
Gay, C. C.: Problems of immunization in the control of *Escherichia coli* infection. *Ann. N. Y. Acad. Sci.*, **176**, 336–349 (1971).
Gear, E. V., Dobbins W. O.: Rectal biopsy. *Gastroenterology* **55**, 522–544 (1968).
(Gekker, V. D.) Геккер, В. Д.: О вирулентности дизентерийных бактерий (Обзор литературы). *Ж. микробиол. (Москва)*, **1**, 39–43 (1964).
(Gekker, V. D., Belaya, Yu. A.) Геккер, В. Д., Белая, Ю. А.: Иммунитет при экспериментальном дизентерийном кератоконъюнктивите. *Ж. микробиол. (Москва)*, **3**, 18–23 (1958).
(Gekker, V. D., Belaya, Yu. A., Melnik, E. G.) Геккер, В. Д., Белая, Ю. А., Мельник, Е. Г.: Экспериментальный дизентерийный кератоконъюнктивит. *Ж. микробиол. (Москва)*, **7**, 110–114 (1957*a*).
Gekker, V. D., Belaya, Yu. A., Melnik, E. G.: Experimental keratoconjunctivitis as a model for the study of dysentery. *J. Hyg. (Praha)*, I. 70–74 (1957*b*).
Gemski, P., Takeuchi, A., Washington, O., Formal, S. B.: Shigellosis due to *Shigella dysenteriae* 1: relative importance of mucosal invasion versus toxin production in pathogenesis. *J. infect. Dis.*, **126**, 523–530 (1972).
(Gennadyeva, T. Ya., Khazenson, L. B.) Геннадьева, Т. Я., Хазенсон, Л. Б.: Устойчивость антител к энтеропатогенным кишечным палочкам и шигеллам в женском молоке и сыворотке крови к температуре, pH, пищеварительным ферментам. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **40**, 165–170 (1973).
(Gennadyeva, T. Ya., Khazenson, L. B., Nikolaeva, T. A.) Геннадьева, Т. Я., Хазенсон, Л. Б., Николаева, Т. А.: Иммунохимическая природа антител к энтеропатогенным кишечным палочкам и шигеллам в женском молоке. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **40**, 159–164 (1973).
Gerber, D. F., Watkins, H. M. S.: Growth of Shigellae in monolayer tissue culture. *J. Bact.*, **82**, 815–822 (1961).
Gerichter, C. B.: The dissemination of *Salmonella typhi*, *S. paratyphi-A.*, and *S. paratyphi-B.*, through the organs of the white mouse by oral infection. *J. Hyg.*, **58**, 307–319 (1960).

Gershon, Z.: The effect of phagocytosis on the succinic dehydrogenase activity on mouse polymorphonuclears determined macroscopically and histochemically. *Path. et Microbiol. (Basel)*, **28**, 501–511 (1965).
Giannella, R. A., Broitman, S. A., Zamcheck, N.: Salmonella enteritis. *Amer. J. dig. Dis.*, **16**, 1000–1013 (1971).
Giannella, R. A., Formal, S. B., Dammin, G. J., Collins, H.: Pathogenesis of salmonellosis: studies of fluid secretion, mucosal invasion and morphologic reaction in the rabbit ileum. *J. clin. Invest.*, **52**, 441–453 (1973*a*).
Giannella, R. A., Washington, O., Gemski, P., Formal, S. B.: Invasion of HeLa cells by *Salmonella typhimurium:* a model for study of invasiveness of Salmonella. *J. infect. Dis.*, **128**, 69–75 (1973*b*).
Gilbert, A.: De la collibacillose. *Sem. méd. (Paris)*, **15**, 1–3 (1895).
Gilbert, R. P.: Mechanisms of the hemodynamic effect of endotoxin. *Physiol. Rev.*, **40**, 245–279 (1960).
Giles, C. M. B., Sangster, M. B.: An outbreak of infantile gastroenteritis in Aberdeen. *J. Hyg. (Cambridge)*, **46**, 1–9 (1948).
Gilka, F., Salajka, E.: Die Immunfluoreszenz bei der Detektion von pathogenen Serotypen der *E. coli* bei experimenteller Kolienteritis bei Kälbern. *Fortpfl. Haust.*, **6**, 287–289 (1970*a*).
Gilka, F., Salajka, E.: Die Immunofluoreszenz bei der experimentellen infektiösen akuten Gastroenteritis neugeborener Kälber und Ferkel. *Zbl. allg. Path., path. Anat.*, **113**, 466 (1970*b*).
(Gindes, E. A.) Гиндес, Е. А.: *Желудочно-кишечные заболевания у детей.* Азернешер, Баку. (1940).
Glantz, P. J.: Serotypes of *Escherichia coli* associated with colibacillosis in neonatal animals. *Ann. N. Y. Acad. Sci.*, **176**, 67–79 (1971).
(Gleiberman, S. E., Shashikhina, M. N., Bayramova, E., Stepanyan, E. G., Yurko, L. P.) Глейберман, С. Е., Шашихина, М. Н., Байрамова, Э., Степанян, Е. Г., Юрко, Л. П.: Конъюнктивальная биопроба как метод изучения дизентерийной и сальмонеллезной инфекций. *Тр. Ашхабад. Ин-та эпид. и гиг.*, **7**, 1–144 (1964).
Glew, R. H., Gorbach, S. L., Sack, R. B., Wallace, C. K.: Gut fluid loss produced by culture filtrates of *Escherichia coli* isolated from small bowel of patients with acute diarrhea. *Clin. Res.*, **17**, 368 (1969).
(Godun, V. M.) Годун, В. М.: Терапевтическая эффективность альбомицина и колимицина, взятых отдельно и в сочетании на модели шигеллезного кератоконъюнктивита. *Антибиотики (Москва)*, **10**, 920 (1962).
Gogolak, F. M.: A quantitative study of the infectivity of murine pneumonitis virus in mice infected in a cloud chamber of improved design. *J. infect. Dis.*, **92**, 240–247 (1953).
Göing, H., Keiser, P.: Aufbau und Wirkungsweise bakterieller Endotoxine. *Ergebn. Mikrobiol.*, **39**, 243–326 (1966).
Goldmann, J.: Zur Frage der Lipoidgranula in den Blutelementen der blutbildenden Organe und des peripherischen Blutes. *Z. mikr.-anat. Forsch.*, **18**, 143–157 (1929*a*).
Goldmann, J.: Über die Lipoidfärbung mit Sudan (Scharlach R)-α-Naphthol. *Zbl. allg. Path. path. Anat.* **46**, 289–290 (1929*b*).
Goldmann, J.: Ergänzender Beitrag zur Lipoidfärbung mit Sudan III (Scharlach R)-α-Naphthol. *Zbl. allg. Path. path. Anat.*, **58**, 275–276 (1933).
Goldschmidt, R.: Untersuchungen zur Ätiologie der Durchfallserkrankungen des Säuglings. *Jb. Kinderheilk.*, **89**, 318–358 (1933).
(Golubeva, I. V.) Голубева, И. В.: О токсинах кишечной палочки. В кн.: *Вопросы эпидемиологии, профилактики и клиники кишечных инфекций.* Под ред. В. А. Крестовниковой, Москва. 116–127 (1954).
(Golubeva, I. V.) Голубева, И. В.: Кишечная коли-инфекция. Этиология. Иммунология. Эпидемиология. Лабораторный бактериологический диагноз. Профилактика. В кн: *Многотомное руководство по микробиологии, клинике и эпидемиологии инфекционных болезней.* Медицина, Москва. **6**, 126–135, 143–147, 148–151 (1964).
(Golubeva, I. V., Kiseleva, B. S.) Голубева, И. В., Киселева, Б. С.: О расшифровке антигенной структуры энтеропатогенных эшерихий с условным обозначением «Крым». В. кн.: *Сб. тезисов докладов 8-й республиканской научной конференции «Кишечные*

и респираторные инфекции, физическое развитие и состояние здоровья молодежи Эстонской ССР»· Таллин. 19–21 (1974).
Gonzalez-Lizea, A., Landa, L., Segovia, E., Velasco, E.: Estudio de la amibiasis invasora del recto con microscopio de luz y electrónico. *Arch. invest. med.*, **4**, Supl. I, 57–66 (1973).
Goodpasture, E. W.: Histopathology of intestine in cholera. *Philipp. J. Sci.* (Soc. B), **22**, 413–420 (1923).
Goodpasture, E. W.: Concerning the pathogenesis of typhoid fever. *Amer. J. Path.*, **13**, 175–185 (1937).
Goodpasture, E. W., Anderson, K.: The problem of infection as presented by bacterial invasion of the chorioallantoic membrane of chick embryos. *Amer. J. Path.*, **13**, 149–174 (1937).
Gorbach, S. L.: Acute diarrhea — a "toxin" disease? *New Engl. J. Med.*, **283**, 44–45 (1970).
Gorbach, S. L.: Intestinal microflora. *Gastroenterology* **60**, 1110–1129 (1971).
Gorbach, S. L., Banwell, J. G., Chatterjee, B. D., Jacobs, B., Sack, R. B.: Acute undifferentiated human diarrhea in the tropics: I. Alterations in intestinal microflora. *J. clin. Invest.*, **50**, 881–889 (1971).
Gorbach, S. L., Khurana C. M.: Toxigenic *Escherichia coli*. A cause of infantile diarrhea in Chicago. *New Engl. J. Med.*, **287**, 791–795 (1972).
Gorbach, S. L., Levitan, R.: Intestinal flora in health and in gastrointestinal diseases. In: *Progress in Gastroenterology*. Ed. by G. B. J. Glass. Grune, Stratton. New York. vol. **2**, pp. 252–275 (1970).
Gordon, H. A.: The germ free animal. *Amer. J. dig. Dis.*, **5**, 841–867 (1960).
Gordon, J. E.: Diarrheal disease of early childhood — worldwide scope of the problem. *Ann. N. Y. Acad. Sci.*, **176**, 9–15 (1971).
Gordon, J. H., Dubos, R.: Observations on the normal gastrointestinal flora of the mouse. *Ann. N. Y. Acad. Sci.*, **176**, 30–39 (1971).
Gorea, A.: Recherches sur la kératoconjonctivité des cobayes, déterminée par les germes du groupe Shigella. *Arch. roum. Path. exp. Microbiol.*, **18**, 245–255 (1959).
(Goryunova, A. G.) Горюнова, А. Г.: Изучение вирулентности дизентерийных бактерий у облученных животных (на модели экспериментального кератоконъюнктивита). *Ж. микробиол. (Москва)*, **6**, 133-134 (1963).
Gowen, J. W.: Genetic effects in nonspecific resistance to infectious diseases. *Bact. Rev.*, **24**, 192–200 (1960).
Grabar, J., Le Minor, S.: Test de séroprotection antityphoïdique sur l'embryon de poulet. *Ann. Inst. Pasteur*, **81**, 528–540 (1951).
Grady, G. F., Keusch, G. T.: Pathogenesis of bacterial diarrheas. *New Engl. J. Med.*, **285**, 831–841, 891–900 (1971).
Gräff, S.: Pathologisch-anatomische Beiträge zur Pathogenese der Typhus abdominalis. *Dtsch. Arch. klin. Med.*, **125**, 352–414, **126**, 1–44 (1918).
Gräff, S.: Pathologische Anatomie des Typhus abdominalis (Eberth). *Med. Klin.*, **3**, 101–103 (1927).
Graney, D. O.: The uptake of ferritin by ileal absorptive cells in suckling rats. An electron microscope study. *Amer. J. Anat.*, **123**, 227–253 (1968).
(Grechko, V. V., Sedova, T. S.) Гречко, В. В., Седова, Т. С.: Судьба меченного J^{131} антигена дизентерийных бактерий после перорального введения животными. *Ж. микробиол. (Москва)*, **11**, 117–122 (1960).
Greenberger, N. J., DeLor, C. S., Fisher, S., Perkins, R. L., Murad, T., Kapral, F.: Whipple's disease. *Amer. J. dig. Dis.*, **16**, 1127–1136 (1971).
Grys, S.: The mode of passage of viable *E. coli* and its antigens across the intestinal barrier in sheep. II. *Bull. Inst. Weteryn. Pulawach*, **10**, 70–71 (1966).
(Grigoryev, A. V.) Григорьев, А. В.: К вопросу о микроорганизмах при дизентерии. *Военно-мед. ж.* **171**, июнь, 73–102 (1891).
Grinberg, L. D.) Гринберг, Л. Д.: Материалы к изучению энтеропатогенной кишечной палочки серологической группы 0129. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **33**, 87–92 (1968).
(Grinfeld, A. A., Nikolaeva, V. L., Makarovkina, V. I., Sotnichenko, L. A., Eydelman, M. R.) Гринфельд, А. А., Николаева, В. Л., Макаровкина, В. И., Сотниченко, Л. А., Эйдельман, М. Р.: Материалы о здоровом носительстве при дизентерии. *XIII Все-*

союзный съезд гигиенистов, эпидемиологов, микробиологов и инфекционистов, **2**, 552–554, Москва, (1959).
Gross, W.: Untersuchungen über Bazillenruhr. *Münch. med. Wschr.*, **24**, 644–649 (1919).
Gruber, G. B.: Über die durch Infektion mit Bakterien der Typhusgruppe in der Leber bedingten knötchenförmigen Nekroseherde (sogenannten "miliaren Lymphome"). *Zbl. Bakt., I. Abt., Orig.*, **77**, 301–308 (1916).
Gruber, G. B.: Spezielle Infektionsfolgen der Leber. In: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Ed. by F. Hende und O. Lubars, Berlin. vol. **5/1**, pp. 536–547 (1930).
Grünbaum, A. S.: Some experiments on enteric, scarlet fever, and measles in chimpanzee. *Brit. med. J.*, **1**, 817–819 (1904).
Guerrant, R. L., Chen, L. C., Charp, G. W. G.: Intestinal adenyl-cyclase activity in canine cholera: correlation with fluid accumulation. *J. infect. Dis.*, **125**, 377–381 (1972).
Guerrant, R. L., Ganguly, U., Casper, A. G. T., Moore, E. J., Pierce, N. F., Carpenter, C. C. J.: Effect of *Escherichia coli* on fluid transport across canine small bowel. Mechanism and time-course with enterotoxin and whole bacterial cells. *J. clin. Invest.*, **52**, 1707–1714 (1973).
Güldner, E.: Wirkung der Proteusendotoxine auf den tierischen Organismus. *Z. Immunitätsforsch.*, **89**, 189–198 (1936).
(Gulkevich, Yu. V.) Гулькевич, Ю. В.: *Перинатальные инфекции*. Беларусь, Минск (1966).
Gupta, J. D., Reed, C. E.: Distribution and degradation of subletal doses of J^{125} labeled endotoxin from *Salmonella enteritidis* in mice. *Proc. Soc. Exp. Biol. Med.*, **131**, 481–487 (1969).
(Gurevich, E. S.) Гуревич, Е. С.: *Паратифозные заболевания (сальмонеллезы) группы С*. Медгиз, Москва (1956).
(Gurevich, E. S.) Гуревич, Е. С.: Проблемы эволюции сальмонеллезов и очередные вопросы борьбы с ними. *Труды Ленинград. педиатрич. мед. ин-та*, **53**, 5–22 (1970).
Gyles, C. L.: Heat-labile and heat-stable forms of the enterotoxin from *Escherichia coli* strains enteropathogenic for pigs. *Ann. N. Y. Acad. Sci.*, **176**, 314–322 (1971).
Gyles, C. L.: Relationships among heat-labile enterotoxins of *Escherichia coli* and *Vibrio cholerae*. *J. infect. Dis.*, **129**, 277–283 (1974*a*).
Gyles, C. L.: Immunological study of the heat-labile enterotoxins of *Escherichia coli* and *Vibrio cholerae*. *Infect. Immunity*, **9**, 564–570 (1974*b*).
Gyles, C. L., Barnum, D. A.: A heat-labile enterotoxin from strains of *Escherichia coli* enteropathogenic for pigs. *J. infect. Dis.*, **120**, 419–426 (1969).
Gyles, C., So, M., Falkow, S.: The enterotoxin plasmids of *Escherichia coli*. *J. infect. Dis.*, **130**, 40–49 (1974).
Gyles, C. L., Stevens, J. B., Craven, J. A.: A study of *Escherichia coli* strains isolated from pigs with gastrointestinal disease. *Canad. J. comp. Med.*, **35**, 258–266 (1971).
Haglund, J. R., Ayres, J. C., Paton, A. M., Kraff, A. A., Quinn, L. J.: Detection of Salmonella in eggs and products with fluorescent antibody. *Appl. Microbiol.*, **12**, 447–450 (1964).
Halbert, S. P.: The relation of antagonistic coliform organisms to Shigella infections. *J. Immunol.*, **60**, 359–381 (1948).
Haltalin, K. C., Nelson, J.: Fatal Shigella infection induced by folic acid deficiency in young guinea pigs. *J. infect. Dis.*, **121**, 275–287 (1970).
Hampton, J. C., Rosario, B.: The attachment of microorganisms to epithelial cells in the distal ileum of the mouse. *Lab. Invest.*, **14**, 1464–1481 (1965).
Hanny, S., Horváth, I.: Étel által közvetített coli O124 okozta házijárvány a kecskeméti Országos Nevelőintézetben (Food-borne epidemic caused by *E. coli* O124 at a Kecskemét school). *Népegészségügy*, **44**, 313–314 (1963).
Hara, S., Matsumoto, H.: An outbreak of enteritis due to *Escherichia coli* O28a28c : B18 (Katwijk). *J. Japan. Ass. infect. Dis.*, **32**, 35–38 (1958).
Hara, S., Matsumoto, H.: An *Escherichia coli* strain (O143) possessing identical O antigen with that of *Shigella boydii* 8. *J. Japan. Ass. infect. Dis.*, **33**, 1–3 (1959).
Harada, K., Shimizu, K., Matsuyama, T., Komiyayama, S., Mitsuhashi, S.: Studies on enteropathogenic *Escherichia coli*. *Japan. J. Bacteriol.*, **14**, 206–210 (1959).

Hardy, A. V., DeCapito, T., Halbert, S. P.: Studies of the acute diarrheal diseases. XIX. Immunization in shigellosis. *Publ. Hlth. Rep. (Wash.)* **63,** 685–688 (1948).

Hardy, A. V., Shapiro, R. L., Chant, H. L., Siegel, M.: Studies of the acute diarrheal diseases. IX-A. *Shigella dysenteriae* infections among institutional inmates. *Publ. Hlth. Rep. (Wash.),* **57,** 1079–1094 (1942).

Harland, W. A., Lee, F. D.: Intestinal spirochaetosis. *Brit. med. J.,* **3,** 718–719 (1967).

Hartz, H., Pot, A. W., Felsenfeld, O.: Fatal fulminant colitis due to *Salmonella saintpaul. Amer. J. clin. Path.,* **20,** 184–187 (1950).

Heilman, D. H.: Cellular aspects of the action of endotoxin. In: *Bacterial endotoxin.* Ed. by M. Landy and W. Braun. New Brunswick, New Jersey. pp. 610–617. (1964).

Heilman, D. H.: Selective toxicity of endotoxin for phagocytic cells of the reticuloendothelial system. *Int. Arch. Allergy.,* **26,** 63–79 (1965).

Hentges, D. J.: Inhibition of *Shigella flexneri* by the normal intestinal flora. *J. Bact.,* **97,** 513–517 (1969).

Hentges, D. J.: Enteric pathogen-normal flora interactions. *Amer. J. clin. Nutr.,* **23,** 1451–1456 (1970).

Heubner, O.: Dysenterie. In: *Handbuch der speziellen Pathologie und Therapie.* Ed. by H. Ziemsson, Leipzig., 3rd ed., vol. 2/3, pp. 4–42 (1886).

Hewlett, E. L., Guerrant, R. L., Evans, D. J. Jr., Greenough, W. B.: Toxins of *V. cholerae* and *E. coli* stimulate adenyl cyclase in rat fat cells. *Nature* **249,** 371–373 (1974).

Heymann, G.: Experimentelle Untersuchungen über die Gifte der Ruhrbakterien. *Z. Hyg.,* **138,** 81–93 (1953).

Heyman, G.: Über die Spezifität der Ruhrgifte. *Zbl. Bakt., I. Abt. Orig.,* **164,** 35–38 (1955).

Higgins, A. R., Floyd, T. M.: Studies in shigellosis I. General considerations, local of studies and methods. *Am. J. trop. Med. Hyg.* **4,** 263–270 (1955*a*).

Higgins, A. R., Floyd, T. M., Kader, M. A.: Studies in shigellosis II. Observations on incidence and etiology of diarrheal disease in Egyptian village children. *Amer. J. trop. Med. Hyg.,* **4,** 281–288 (1955*b*).

Hill, I. R., Porter, P.: Studies of bactericidal activity to *E. coli* of porcine serum and colostral immunoglobulins and the role of lysozyme with secretory IgA. *Immunology,* **26,** 1239–1250 (1974).

Hill, M. J., Drasar, B. S.: The normal colonic bacterial flora. *Gut,* **16,** 318–323 (1975).

Hiroki, H.: On *Escherichia coli* strains isolated from infantile enteritis in Japan. In: *6th Internat. Congr. Microbiol.,* Roma. vol. **4,** p. 258 (1953).

Hobbs, B. S., Thomas, M. E. M., Taylor, J.: School outbreak of gastro-enteritis associated with a pathogenic paracolon bacillus. *Lancet* **257,** 530–532 (1949).

Hobson, D.: Chronic bacterial carriage in survivors of experimental mouse typhoid. *J. Path. Bact.,* **73,** 399–410 (1957).

Hoffman, H. H., Frank, M. E.: Microbial burden of mucosal squamous epithelial cells. *Acta cytol. (Philad.),* **10,** 272–285 (1966).

Holmgren, J., Söderlind, O., Wadström, T.: Cross-reactivity between heat labile enterotoxins of *Vibrio cholerae* and *Escherichia coli* in neutralization tests in rabbit ileum and skin. *Acta Path. microbiol. scand., Section B.,* **81,** 757–762 (1973).

Holter, H.: How things get into cells. *Sci. Amer.,* **205,** 167–180 (1961). (и в кн.: *Живая клетка,* пер. с англ., Мир, Москва. 130–145 (1962).

Horowitz, R. E.: Collular and humoral defence mechanisms in germfree animals. In: *9th Internat. Congr. Microbiol., Symposia.* Moscow. pp. 251–255 (1966).

Horowitz, R. E., Bauer, H., Paronetto, F., Abrams, G. D., Watkins, K. S., Popper, H.: The response of the lymphatic tissue to bacterial antigen. Studies in germfree mice. *Amer. J. Path.,* **44,** 747–761 (1964).

Hoskins, L. C., Winawer, S. J., Broitman, S. A.: Clinical giardiasis and intestinal malabsorption. *Gastroenterology* **53,** 265–279 (1967).

Howard, H. J.: Role of the epithelial cells in conjunctival and corneal infections. *Amer. J. Ophthal.* **7,** 909–936 (1924).

Howie, J. W., O'Hea, A. J. (Eds): Mechanisms of microbial pathogenicity. *5th Symposium of the Society for General Microbiology.* Cambridge University Press, Cambridge (1955).

Hsu, H. S., Radcliffe, A. S.: Interactions between macrophages of guinea pigs and salmonellae. *J. Bact.*, **96**, 191–197 (1968).

Hübner, G.: Ultrastructurelle Leberveränderungen bei direkter Einwirkung von Tetrachlorkohlenstoff *in vivo* and *in vitro*. *Virchows Arch. path. Anat.*, **339**, 187–197 (1965).

Huebschmann, P.: Die pathologische Anatomie und Pathogenese der gastro-intestinalen Paratyphuserkrankungen. *Beitr. pathol. Anat.*, **56**, 514–579 (1913).

Huebschmann, P.: Beiträge zur Bakteriologie und pathologischen Anatomie der Ruhr. *Virchows Arch. path. Anat.*, **254**, 811–829 (1925).

Hueppe, F.: Zur Aetiologie der Cholerine. *Berl. klin. Wschr.*, **24**, 591–592 (1887).

Hughes, W. H.: The structure and development of the induced long forms of bacteria. *6th Sympos. Soc. gen. Microbiol.* Univ. Press, Cambrige. pp. 341–360 (1956).

Hugon, J. S.: Absorption of horse-radish peroxidase by the mucosae cell of the duodenum of mouse. II. The newborn mouse. *Histochemie*, **26**, 19–27 (1971).

Ilgner, G.: Pathologische Anatomie der Säuglings-Enteritis. In: *Säuglings-Enteritis*. Ed. by A. Adam. G. Thieme, Stuttgart. pp. 172–231 (1956).

(Illutovich, A. Yu., Petrova, Z. S., Khoteeva, R. S., Makhlinovsky, L. I.,Golubeva, E. E., Raikis, B. N.) Иллютович, А. Ю., Петрова, З. С., Хотеева, Р. С., Махлиновский, Л. И., Голубева, Е. Е., Раикис, Б. Н.: Экспериментально-биологическая модель колиэнтерита и некоторые вопросы патогенеза этой инфекции. *Ж. микробиол. (Москва)* **1**, 83–89 (1962).

(Ilyin, G. I.) Ильин, Г. И.: Реактивные изменения в легких белых мышей при интраназальном заражении эолотистым стафилококком. *Ежегодник Ин-та экспер. мед. АМН СССР, за 1957. г.* , Ленинград 356–359 (1958).

(Ismailov, F. N.) Исмаилов, Ф. Н.: Биологическая характеристика штаммов сальмонелл, выделенных при заболеваниях с различной клинической картиной. *Тезисы докладов 2-го Всесоюзного симпозиума «Факторы патогенности микроорганизмов»*. Институт эпидемиол. микробиол. им. Н. Ф. Гамалеи АМН СССР, Москва. 30–31 (1974).

Istrati, G.: Recherches sur l'immunité active de l'homme dans la dysentérie bacillaire. *Arch. roum. Path. exp.*, **20**, 53–60 (1961).

Istrati, G., Ciufeco, C.: Méthode d'isolement des Shigella II. Décèlement des Shigella qui se trouvent en nombre réduit dans les matières fécales. *Arch. roum. Path., exp.* **21**, 539–543 (1962).

Istrati, G., Ciufeco, C., Cimpeanu, I.: Méthode d'isolement des Shigella et des Salmonella. I. Isolement des eaux polluées. *Arch. roum. Path. exp.*, **21**, 89–99 (1962*a*).

Istrati, G., Gorea, A., Maldaresco, I.: Vaccination antidysentérique. II. Expériences d'immunisation des souris vis-à-vis de l'infection homologue et hétérologue avec des souches *Sh. flexneri* de types différents. *Arch. roum. Path. exp.*, **17**, 369–382 (1958).

Istrati, G., Istrati, M.: La vaccination dysentérique. VII. Détermination de l'immunité locale et générale antidysentérique utilisant des doses infectantes conjonctivales (D1/100) réduites. *Arch. roum. Path. exp.*, **23**, 87–90 (1964*a*).

Istrati, G., Istrati, M.: VIII. Vaccination conjonctivale des cobayes avec des bacilles dysentériques vivants non-pathogènes. *Arch. roum. Path. exp.*, **23**, 91–93 (1964*b*).

Istrati, M., Istrati, G.: XIV. Action immunisante sur la conjonctive du cobaye de quelques extraits toxiques de *Shigella flexneri 2a* (TI) pathogène en comparaison avec la culture vivante de deux souches non-pathogènes de *Shigella flexneri 2a*. *Arch. roum. Path. exp.*, **25**, 55–64 (1966).

Istrati, G., Istrati, M., Meitert, T., Ciufeco, C.: Vaccination antydysentérique. Recherches experimentales sur l'homme et les animaux. *Arch. roum., Path. exp.*, **22**, 531–536 (1963*a*).

Istrati, G., Istrati, M., Meitert, T., Ciufeco, C.: Stabilité génétique de caractère non-pathogène et immunogène antiinfectueux de quelques souches de *Sh. flexneri* 2a. *Arch. roum. Path. exp.*, **24**, 867–874 (1965*a*).

Istrati, G., Meitert, T.: Lysotypie de la *Shigella flexneri*. *Arch. roum. Path. exp.*, **22**, 903–908 (1963).

Istrati, G., Meitert, T., Ciufeco, C.: Recherches sur l'immunité dans la dysentérie bacillaire. *Arch. roum. Path. exp.*, **24**, 677–686 (1965*b*).

Istrati, G., Meitert, T., Ciufeco, C.: Innocuité de la souche vivante et non-pathogène *Shigella flexneri* 2a TXXXII administrée par voie souscutanée et intramusculaire à des volontaires. *Arch. roum. Path. exp.*, **24**, 875–878 (1965*c*).

Istrati, G., Meitert, T., Ciufeco, C.: Recherches sur l'immunité active de l'homme dans la dysentérie bacillaire, III. Maintien du caractère non-pathogène de la souche. *Sh. flexneri* 2a TXXXII administrée "per os" à des volontaires. *Arch. roum. Path. exp.* **24**, 677–686 (1965*d*).

Istrati, G., Meitert, T., Ciufeco, C.: Transformation von Shigellen in Stämme, die für den Menschen und für das Meerschweinchenauge apathogen sind. *Zbl. Bakt., I. Abt. Orig.*, **203**, 295 (1967).

Istrati, G., Meitert, T., Ciufeco, C.: Treatment of dysentery bacilli carrier with a live nonpathogenic antidysenteric vaccine. *Arch. Immunol. Therap. Exper. (Wrocław)* **16**, 333–337 (1968).

Istrati, G., Meitert, T., Ciufeco, C., Filipesco, S.: Recherches sur l'immunité active de l'homme dans la dysentérie bacillaire. *Arch. roum. Path. exp.*, **23**, 141–148 (1964).

Istrati, G., Szégli, L., Ciufeco, C., Filipesco, S., Dobre, M.: Test de Serény produit par certaines Salmonella. *Arch. roum. Path. exp.*, **22**, 101–107 (1963*b*).

Istrati, T., Meitert, T., Ciufeco, C., Hentin, V. Deleanu, L.: Lysotypie des Shigella. *Arch. roum. Path. exp.*, **21**, 288—294 (1962*b*).

Ito, S.: The surface coating of enteric microvilli. *Anat. Rec.*, **148**, 294 (1964).

Ito, S.: Structure and function of the glycocalyx. *Fed. Proc.*, **28**, 12–25 (1969).

(Ivanenko, T. P.) Иваненко, Т. П.: Опыт использования культуры ткани для определения степени патогенности сальмонелл. *Тр. Владивостокского ин-та эпидемиол. микробиол., гигиены*, **2**, 232–235 (1962).

(Ivanov, L. K.) Иванов, Л. К.: Модель экспериментального кератоконъюнктивита при оценке качества антибиотиков. *Ж. микробиол. (Москва)* **3**, 62–66 (1960).

(Ivanova, V. V.) Иванова, В. В.: Применение легочной модели с целью иммунологической характеристики дизентерии у детей. *Ж. микробиол. (Москва)*, **11**, 53–57 (1968).

(Ivanova, V. V., Avdeeva, T. A.) Иванова, В. В., Авдеева, Т. А.: Применение иммунологической пробы на мышах («легочная модель») для определения специфических серологических сдвигов в крови детей, больных острой дизентерией. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)* **33**, 267–274 (1968).

(Ivashentsov, G. A.) Ивашенцов, Г. А.: Об осложнениях при возвратном тифе. *Терап. арх. (Москва)*, **1**, 104–116 (1923).

Ivashentsov, G. A.: N-Paratyphobazillose und Febris recurrens. *Arch. f. Schiffs- u. Tropenhyg.*, **30**, 1–17 (1926).

(Izrailimsky, A. S.) Израилимский, А. С.: Возбудители кишечных инфекций в трупах детей, погибших от дизентерии. *Педиатрия (Москва)*, **4**, 36–40 (1939).

Jacks, T. M., Wu, B. J., Braemer, A. C., Bidlack, D. E.: Properties of the enterotoxic component in *Escherichia coli* enteropathogenic for swine. *Infect. Immunity*, **7**, 178–189 (1973).

Jacoby, F.: Macrophages. In: *Cells and tissues in culture*. Ed. by E. N. Willmer, N. Y. vol. **2**, pp. 1–95 (1965).

Jaffé, H.: Zur Histogenese der typhösen Leberveränderungen. *Virchow's Arch. path. Anat.*, **228**, 366–383 (1920).

Jaffé, R. H., Laing, D. R.: Changes of the digestive tract in uremia. *Arch. int. Méd. exp.*, **53**, 851–864 (1934).

Jeffcott, L. B.: Studies on passive immunity in the foal. II. The absorption of ^{125}I-labelled PVP (Polyvinyl Pyrrolidone) by the neonatal intestine. *J. comp. Path.*, **84**, 279–290 (1974).

Jenkin, C. R., Rowley, D.: *Salmonella typhimurium*, a parasite of the reticulo-endothelial system. *Colloq. internat. centre nat. rech. scientifique. (Paris)*, **115**, 391–417 (1963).

Jenkin, C. R., Rowley, D., Auzins, J.: The basis for immunity to mouse typhoid I. The carrier state. *Austral. J. exp. Biol. med. Sci.*, **42**, 215–228 (1964).

Jensen, C. O.: Über die Kälberruhr und deren Ätiologie. *Monatsch. prakt. Tierheilk.*, **4**, 97–115 (1892).

Jensen, C. O.: Kälberruhr. In: *Handb. path. Mikroorganismen*. G. Fischer, Jena. vol. **6**, pp. 131–144 (1913).

Jervis, H. R., LaBrec, E. H.: Correlation of acid phosphatase activity with degree of bacterial infection in HeLa cells. *J. Histochem. Cytochem.*, **14**, 196–202 (1966).

Joest, E.: Vergleichende Untersuchungen über die durch Bacterien der Gärtnergruppe in der Leber des Kalbes und die Typhusbazillen in der Leber des Menschen bedingten Pseudotuberkel. *Verhandl. Dtsch. path. Ges.*, **17**, 238–260 (1914).

Johnson, E. M., Snellings, N. J., Life, Ch. A., Baron, L. S.: Intraperitoneal mouse virulence of *Salmonella typhimurium* hybrids expressing somatic antigen 9. *Infect. Immunity*, **10**, 669–671 (1974).

Jones, G. W., Rutter, J. M.: Role of the K88 antigen in the pathogenesis of neonatal diarrhea caused by *Escherichia coli*. *Infect. Immunity*, **6**, 918–927 (1972).

Jones, G. W., Rutter, J. M.: Contribution of the K88 antigen of *Escherichia coli* to enteropathogenicity: protection against disease by neutralizing of the adhesive properties of K88 antigen. *Amer. J. clin. Nutr.*, **27**, 1441–1449 (1974).

Jones, J. E. T., Smith, H. W.: Histological studies on weaned pigs suffering from diarrhoea and oedema disease produced by oral inoculation of *Escherichia coli*. *J. Path. Bact.*, **97**, 168–172 (1969).

Jones, R. E.: Studies *in vivo* and *in vitro* of the transfer of rat IgG and rat albumen across the intestinal walls of young rats. *Biol. Neonat.* (Basel), **24**, 220–229 (1974).

June, R. C., Ferguson, W. W., Worfel, M. T.: Experiments on feeding adult volunteers with *Escherichia coli* 55 : B5, a coliform organism associated with infant diarrhea. *Amer. J. Hyg.*, **57**, 222–236 (1953).

(Kabanova, E. N.) Кабанова, Е. Н.: Влияние факторов внешнего воздействия на резистентность животных при заражении дизентерией Флекснера. Автореф. дисс. канд. мед. наук, Москва (1952).

(Kagan, G. Ya., Levashev, V. S.) Каган, Г. Я., Левашев, В. С.: Об L-трансформирующем действии некоторых антибиотиков в экспериментальных условиях. *Антибиотики (Москва)*, **5**, 383–385 (1961).

(Kamenskaya, I. N.) Каменская, И. Н.: К вопросу о виде и типоспецифичности иммунитета при дизентерии. *Ж. микробиол. (Москва)*, **3**, 22 (1956).

(Kamzolkina, N. B., Zakharov, N. S., Lukashova, N. I.) Камзолкина, Н. Б., Захаров, Н. С., Лукашова, Н. И.: Размножение брюшнотифозного микроба в макрофагах морской свинки и мыши. *IX Междунар. конгр. по микробиол. Тезисы докл. (Москва)*, 603–604 (1966).

(Kandyurina, V. G.) Кандюрина, В. Г.: Биологическая характеристика шигелл Зонне разных ферментативных типов по данным двух экспериментальных моделей — кератоконъюнктивальной пробы Шереня и легочной модели Войно-Ясенецкой. I. II. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **36**, 32–45 (1970*a*).

(Kandyurina, V. G.) Кандюрина В. Г.: К биологической характеристике шигелл Зонне разных ферментативных типов. *Ж. микробиол. (Москва)*, **11**, 49–54 (1970*b*).

Kantor, H. S., Tao, P., Gorbach, S. L.: Stimulation of intestinal adenyl cyclase by *Escherichia coli* enterotoxin: comparison of strains from an infant and an adult with diarrhea. *J. infect. Dis.*, **129**, 1–9 (1974*a*).

Kantor, H. S., Tao, P., Wisdom, C.: Action of *Escherichia coli* enterotoxin: adenylate cyclase behavior of intestinal epithelial cells in culture. *Infect. Immunity*, **9**, 1003–1011 (1974*b*).

Kao, V. C. Y., Sprinz, H., Burrows, W.: Localization of cholera toxin in rabbit intestine. *Lab. Invest.*, **26**, 149–153 (1972).

(Karyagina, E. I., Raskina, T. M.) Карягина, Е. И., Раскина, Т. М.: Материалы к характеристике культур энтеропатогенной кишечной палочки серологической группы 0112a112b: K68(B13), выделенных в 1972 году. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **40**, 105–108 (1973).

(Kasatkina, Z. N., Bazarov, T. D., Bugel, N. E., Makarov, E. I., Anikina, A. Z.) Касаткина, З. Н., Базаров, Т. Д., Бугель, Н. Е., Макаров, Е. И., Аникина, А. З.: Некоторые клинические особенности заболеваний, вызываемых сальмонеллой панама. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **36**, 318–322 (1970).

Kashiba, S., Imanishi, T., Migama, A., Yamade, S.: The use of young mouse vagina as a model for experimental shigellosis. *Biken's J.*, **10**, 25–32 (1967).

Kasai, G. J., Burrows, W.: The titration of cholera toxin and antitoxin in the rabbit ileal loop. *J. infect. Dis.*, **116**, 606–614 (1966).

(Katsnelson, I. A.) Кацнельсон, И. А.: Опыт применения экспериментального кератоконъюнктивита для бактериологической диагностики дизентерии. *Лаборат. дело*, **4**, 39–41 (1961).

Kaufmann, E.: Die Sublimatintoxikation. Thesis. Breslau (1888).

Kauffmann, F.: Zur Serologie der Coli-Gruppe. *Acta path. microbiol. scand.*, **21**, 20–45 (1944).

Kauffmann, F.: The serology of the coligroup. *J. Immunol.*, **57**, 71–100 (1947).
Kauffmann, F.: Die Coli-Theorie der Appendicitis. *Schweiz. Z. Path.*, **11**, 553–569 (1948).
Kauffmann, F.: Schlussbemerkungen zu den Coli-Enteritis Vorträgen. In: *6th Internat. Congr. Microbiol.*, Roma. **4**, 280–281 (1953).
Kauffmann, F.: Zur biochemischen und serologischen Gruppen — und Typen — Einteilung der Enterobacteriaceae. *Zbl. Bakt. I. Abt. Orig.*, **165**, 344–354 (1956*b*).
Kauffmann, F.: A simplified biochemical table of *Enterobacteriaceae*. *Acta path. microbiol. scand.*, **39**, 103–106 (1965).
Kauffmann, F.: *The bacteriology of Enterobacteriaceae*. Munksgaard, Copenhagen. (1966).
Kauffmann, F., Ørskov, F.: Die Bakteriologie der *Escherichia coli* Enteritis. In: *Säuglings-Enteritis*. Ed. by A. Adam., G. Thieme, Stuttgart. pp. 1–41 (1956).
Kaye, G. J., Fenoglio, C. M., Pascal, R. R., Lane, N.: Comparative electron microscopic features of normal, hyperplastic and adenomatous colonic epithelium. *Gastroenterology*, **64**, 926–945 (1973).
(Kazarinov, G. N.) Казаринов, Г. Н.: Палочка Шига как возбудитель кровавого поноса. *Русс. врач*, **41**, 1417–1420 (1903).
Kazarinov, G. N.: Über die Rolle des Shiga-Bazillus als Erreger der Dysenterie. *Arch. Hyg.*, **50**, 66–76 (1904).
Keiserling, E., Rácz, P., Tenner, K., Lennert, K.: Vorkommen intracellulärer Bakterien in Makrophagen beim Typhus abdominalis des Menschen. *Virchow's Arch., Abt. B.*, **2**, 343–357 (1972).
Kennedy, D. H., Walker, G. H., Fallon, R. J., Boyd, J. F., Gross, R. J., Rowe, B.: An outbreak of infantile gastroenteritis due to *E. coli* O142. *J. clin. Path.*, **26**, 731–737 (1973).
Kenny, J. F., Woleslagle, D. L., Gray, J. A., Michaels, R. H., Pearson, M. A.: Enteric infection with *Escherichia coli* O127 in the mouse. I. Characteristics of infection and systemic and local immune responses in mice of different ages. *J. infect. Dis.*, **121**, 528–540 (1970).
Kent, T. H., Formal, S. B., LaBrec, E. H.: Acute enteritis due to *Salmonella typhimurium* in opium-treated guinea pigs. *Arch. Path.*, **81**, 501–508 (1966*a*).
Kent, T. H., Formal, S. B., LaBrec, E. H.: Salmonella gastroenteritis in rhesus monkeys. *Arch. Path.*, **82**, 272–279 (1966*b*).
Kenworthy, R.: Effect of *Escherichia coli* on germfree and gnotobiotic pigs. I. Light and electron microscopy of the small intestine. *J. comp. Path.*, **80**, 53–63 (1970).
Kerekes, L.: Colonical variants of *Shigella flexneri*. *Acta microbiol. Acad. Sci. hung.* **9**, 123–132 (1962).
Kerekes, L.: Salicin-fermenting variant of *Shigella flexneri* serotype 2a. *Acta microbiol. Acad. Sci. hung.* **12**, 7–13 (1965).
Kerekes, L.: Virulence of Shigella strains isolated from dysenteric patients and symptomless carriers. *Acta microbiol. Acad. Sci. hung.*, **15**, 361–367 (1968).
Kétyi, I.: Modellversuche an Säuglingsmäusen mit einem enteropathogenen Coli-Stamm. *Zbl. Bakt., I. Abt. Orig.*, **194**, 332–341 (1964).
Kétyi, I.: Über den interferenzähnlichen Inplantationsantagonismus. *Ernährungsforsch.*, **10**, 143–147 (1965).
Kétyi, I.: Model experiments with suckling mice with an enteropathogenic strain of *E. coli*. *Proc. 4th Congr. Hung. Assoc. Microbiol. (Budapest)*, **270**, (1966*a*).
Kétyi, I.: Virulence test of Shigellae by intravenous inoculation to chick embryo. *Ann. Immunol. Hung.*, **9**, 81–86 (1966*b*).
Kétyi, I., Kneffel, P., Domjan, J.: Über das Vorkommen einer Gastro-Enteritis-Epidemie, bedingt durch einen in die O-124-Gruppe der *Escherichia coli* gehörenden Stamm. *Zbl. Bakter. I. Abt. Orig.*, **170**, 423–427 (1958).
Keusch, G. T., Grady, G. F., Mata, L. J., McIver, J.: The pathogenesis of Shigella diarrhea. I. Enterotoxin production by *Shigella dysenteriae* 1. *J. clin. Invest.*, **51**, 1212–1218 (1972*a*).
Keusch, G. T., Grady, G. F., Takeuchi, A., Sprinz, H.: The pathogenesis of Shigella diarrhea. II. Enterotoxin-induced acute enteritis in the rabbit ileum. *J. infect. Dis.*, **126**, 92–95 (1972*b*).
Keusch, G. T., Jacewicz, M., Hirschman, S. Z.: Quantitative microassay of *Shigella dysenteriae* 1 enterotoxin in cell culture. *J. infect. Dis.*, **125**, 539–541 (1972*c*).

Keusch, G. T., Mata, L. J., Grady, G.: Shigella enterotoxin. *Clin. Res.*, **18,** 442 (1970).
(Khazenson, L. B.) Хазенсон, Л. Б.: Некоторые вопросы эпидемиологии колиэнтеритов. Характеристика очагов колиэнтерита, возникавших в ясельных условиях. Характеристика очагов колиэнтерита, возникавших в больницах. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **21,** 5–14, 15–23, 24–32 (1960).
(Khazenson, L. B.) Хазенсон, Л. Б.: О некоторых факторах, определяющих уровень заболеваемости колиэнтеритом. Закономерности циркуляции энтеропатогенных кишечных палочек в коллективе. Роль реконвалесцентов колиэнтерита как источников инфекции. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **27,** 200–212, 213–223, 224–240 (1964).
(Khazenson, L. B.) Хазенсон, Л. Б.: О некоторых механизмах различной восприимчивости детей раннего возраста к дизентерии и колиэнтериту. *Вопр. охр. мат. дет.*, **7,** 49–55 (1967).
(Khazenson, L. B.) Хазенсон, Л. Б.: О некоторых механизмах различной восприимчивости детей раннего возраста к дизентерии и колиэнтериту. К вопросу о факторах, определяющих уровни заболеваемости в очагах колиэнтерита. К сравнительной эпидемиологической значимости больных колиэнтеритом и носителей ЭПКП как источников инфекции. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **33,** 176–182, 183–192, 193–199 (1968).
(Khazenson, L. B.) Хазенсон, Л. Б.: Вопросы иммунологии, эпидемиологии и профилактики колиэнтерита детей раннего возраста. Автореферат дисс. докт. Ленинград. (1970).
(Khazenson, L. B., Gennadyeva, T. Ya.) Хазенсон, Л. В., Геннадьева, Т. Я.: Некоторые закономерности трансплацентарной передачи полных и неполных 0-антител к возбудителям колиэнтерита и дизентерии. *Ж. микробиол. (Москва)*, **11,** 115–121 (1969).
(Khazenson, L. B., Gennadyeva, T. Ya., Nikolaeva, T. A.) Хазенсон, Л. Б., Геннадьева, Т. Я., Николаева, Т. А.: Иммунохимическая природа копроантигел к возбудителям дизентерии и колиэнтерита у детей раннего возраста. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **40,** 171–176 (1973).
(Khazenson, L. B., Tseprinskaya, E. V., Bogolapova, N. I., Tseplyaeva, L. G.) Хазенсон, Л. Б., Цепринская, Е. В., Боголапова, Н. И., Цепляева, Л. Г.: Оценка эффективности мероприятий по предупреждению колиэнтерита. III. Анализ эффективности мероприятий, проведенных в Ленинграде (1958–1965 гг.). *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **33,** 233–239 (1968).
(Kheisin, E. M.) Хейсин, Е. М.: *Жизненные циклы кокцидий домашних животных.* Наука, Ленинград (1967).
(Khesin, Ya. E.) Хесин, Я. Е.: *Размеры ядер и функциональное состояние клеток.* Наука, Москва (1967).
(Khlopin, N. G.) Хлопин, Н. Г.: *Общебиологические и экспериментальные основы гистологии.* Медгиз, Москва (1946).
(Khlystova, Z. S., Zaitsev, T. I.) Хлыстова, З. С., Зайцев, Т. И.: Морфология органов безмикробныхъ животных б постнатальном онтогенезе. *Онтогенез*, **2,** 145–151 (1971).
(Khomik, S. P.) Хомик, С. П.: Кошка как экспериментальная модель при изучении дизентерии. *Ж. микробиол. (Москва)*, **40,** 62–65 (1957).
Kimberg, D. V., Field, M., Johnson, J., Henderson, A., Gershon, E.: Stimulation of intestinal mucosal adenyl cyclase by cholera enterotoxin and protoglandins. *J. clin. Invest.*, **50,** 1218–1230 (1971).
Kind, P. D., Claman, H. N., Rowlands, D. T.: Alteration of the mouse thymus by endotoxin. *Fed. Proc.*, **23,** 139 (1964).
(Kiparisov, L. N.) Кипарисов, Л. Н.: Патологическая анатомия пищевой токсикоинфекции, вызванной бреславльской палочкой. *Труды Всесоюз. конференции патологоанатомов в г. Л-де 1954.* Медгиз, Москва, 136–141 (1956).
(Kiparisov, L. N.) Кипарисов, Л. Н.: Сальмонеллезы. В кн.: *Многотомное руководство по патологической анатомии.* Медицина, Москва. **9,** 276–288 (1964).
Kirby, A. C., Hall, E. G., Coackly, W.: Neonatal diarrhoea and vomiting. *Lancet*, **259,** 201–207 (1950).
(Kleganov, V. K., Avdeeva, T. A., Toluzakova, N. V., Karyagina, E. I.) Клеганов, В. К., Авдеева, Т. А., Толузакова, Н. В., Карягина, Е. И.: Результат идентификации и изучения биологических свойств культур энтеротоксигенных кишеч-

ных палочек серологической группы 0148, выделенных в 1974 году в Ленинграде. В. кн.: *Эпидемиол., диагностика и профилактика кишечных, респираторных и природноочаговых инфекций.* Ин-т эпидемиол. микробиол. им. Пастера, Ленинград. 61–62 (1975).

(Klemparskaya, N. N.) Клемпарская, Н. Н.: К вопросу о проницаемости кишечной стенки белых мышей для брюшнотифозных бактерий. *Ж. микробиол. (Москва),* **10,** 41–44 (1952).

(Klodnitsky, N. N., Kats, L. Ya.) Клодницкий, Н. Н., Кац, Л. Я.: Роль крыс в эпидемиологии кишечных инфекций. *Ж. микробиол. (Москва),* **4,** 604–606 (1936).

(Klyachko, N. S.) Клячко, Н. С.: Течение инфекций Гейдельберг в эксперименте на белых мышах. *Ж. микробиол. (Москва),* **8,** 34–38 (1947).

Koch, A.: Intracellular symbiosis in insects. *Ann. Rev. Microbiol.*, **14**, 121–140 (1960).

Koch, R. (1883): *Gesammelte Werke.* vol. 2, T. I., pp. 2–17, Leipzig (1912).

Koch, R.: Conferenz zur Erörterung der Cholerafrage. *Berlin. klin. Wsch.*, **37a**, 1–24 (1885).

Kohler, E. M.: Studies of *Escherichia coli* in gnotobiotic pigs. IV. Comparison of enteropathogenic and nonenteropathogenic strains. *Canad. J. comp. Med.*, **31**, 277–282 (1967).

Kohler, E. M.: Enterotoxin activity of filtrates of *Escherichia coli* in young pigs. *Am. J. Vet. Sci.*, **29**, 2263–2274 (1968).

Kohler, E. M.: Enterotoxic activity of whole cell lysates of *Escherichia coli* in young pigs. *Amer. J. Vet. Sci.*, **32**, 731–737 (1971*a*).

Kohler, E. M.: Observations on enterotoxins produced by enteropathogenic *Escherichia coli*. *Ann. N. Y. Acad. Sci.*, **176**, 212–219 (1971*b*).

Kohler, E. M.: Protection of pigs against neonatal enteric colibacillosis with colostrum and milk from orally vaccinated sows. *Amer. J. vet. Res.*, **35**, 331–338 (1974).

Kohler, E. M., Bohl, E. H.: Studies of *Escherichia coli* in gnotobiotic pigs. I. Experimental reproduction of colibacillosis. *Canad. J. comp. Med.*, **30**, 199–203 (1966).

Kohler, E. M., Cross, R. F.: Studies of *Escherichia coli* in gnotobiotic pigs. VI. Effect of feeding bacteria-free filtrates of broth cultures. *Canad. J. comp. Med.*, **33**, 173–177 (1969).

Kohler, E. M., Cross, R. F.: Feeding bacteria-free whole cell lysates of *Escherichia coli* to gnotobiotic pigs and the effects of giving antiserums. *Amer. J. vet. Res.* **32**, 739–748 (1971).

Kolta, F.: *Escherichia coli* O124 törzsek által okozott coli-enteritisek jelentősége. (Importance of colienteritis caused by *E. coli* O124 strains). *Orv. Hetil.*, **106**, 1952–1954 (1965).

Kolta, F.: *E. coli* O124 : K72 (B) strains, their occurrence, diagnostic and epidemic importance. Thesis. Budapest (1967).

Kolta, F., Deák, Z.: *Escherichia coli* O124 : K72(B17) törzsek előfordulása Komárom megyében (Occurrence of *E. coli* O124 : K72(B17) strains in county Komárom) *Egészségtudomány*, **6**, 363–371 (1962).

(Komissarchik, Ya. Yu., Snigirevskaya, E. S.) Комиссарчик, Я. Ю., Снигиревская, Е. С.: Использование тиофосфамида для выявления примембранных слоев грегарин. В кн.: *Структура, функция и реактивность клеток.* Ин-т цитологии АН СССР, Ленинград. (1973).

(Komissarchik, Ya. Yu., Ugolev, A. M.) Комиссарчик, Я. Ю., Уголев, А. М.: Ультраструктура и возможное функциональное значение гликокаликса микроворсинок кишечных клеток. *Докл. Акад. Наук СССР,* **194,** 731–733 (1970).

Kotelko, K., Szydlowski, S., Zablocki, B.: Badania nad zmienoscia farowa *Shigella sonnei. Med. Doswiad. Microbiol.*, **12**, 53–59 (1960).

Koupal, L. R., Deibel, R. H.: Assay, characterization and localization of an enterotoxin produced by Salmonella. *Infect. Immunity.*, **11**, 14–22 (1975).

Kourany, M., Kendrick, P. L.: Interaction between a human monocytic cell line and *Salmonella typhosa. J. infect. Dis.*, **116**, 495–513 (1966).

(Kovalev, G. K.) Ковалев, Г. К.: Лечение экспериментальной дизентерийной инфекции, вызванной антибиотикоустойчивым штаммом дизентерийных бактерий Флекснера, комбинацией антибиотика и препарата, повышающего иммуногенез. *Антибиотики (Москва),* **2,** 148–151 (1965*a*).

(Kovalev, G. K.) Ковалев, Г. К.: Лечение экспериментальной дизентерийной инфекции комбинацией антибиотика и препарата, вызывающего иммуногенез. *Ж. микробиол. (Москва)*, **6**, 21–24 (1965*b*).

(Kovaleva, A. I., Mironova, M. B., Khatuntseva, N. B., Belova, S. U.), Ковалева, А. И., Миронова, М. Б., Хатунцева, Н. Б., Белова, С. У.: Об иммунохимических свойствах культур возбудителя паратифа В. *Ж. микробиол. (Москва)*, **9**, 54–58 (1967).

Koya, G., Kosakai, N., Fukusawa, G.: Supplementary studies on multiplication of *Escherichia coli* O111 : B4 in intestinal tract of adult volunteers and its relation to manifestations of coli-enteritis. *Japan. J. med. Sci. Biol.*, **7**, 655–661 (1954*a*).

Koya, G., Kosakai, N., Kono, M., Mori, M., Fukusawa, G.: Observations on the multiplication of *Escherichia coli* O111 : B4 in the intestinal tract of adult volunteers in feeding experiments. The intubation study with Miller-Abbot's double lumen tube. *Japan. J. med. Sci. Biol.*, **7**, 197–203 (1954*b*).

Kraehenbuhl, J. P., Campiche, M. A.: Early stages of intestinal absorption of specific antibodies in the newborn. *J. Cell Biol.*, **42**, 345–365 (1969).

Kraehenbuhl, J. P., De Grandi, P. B., Campiche, M. A.: Ultrastructural localization of intracellular antigen using enzyme-labeled antibody fragments. *J. Cell Biol.*, **50**, 432–445 (1971).

Kraehenbuhl, J. P., Gloor, E., Blanc, B.: Morphologie comparée de la muqueuse intestinale de deux espèces animales aux possibilités d'absorption protéique néonatale différentes. *Z. Zellfsch.*, **70**, 209–219 (1966).

Kraehenbuhl, J. P., Gloor, E., Blanc, B.: Résorption intestinale de la ferritine chez deux espèces animales aux possibilités d'absorption protéique néonatale differentes. *Z. Zellfsch.*, **76**, 170–186 (1967).

Kraft, S. C., Kirsner, J. B.: Immunological apparatus of the gut and inflammatory bowel disease. *Gastroenterology*, **60**, 922–951 (1971).

(Krasnitskaya, E. S., Stremlina, S. M., Bryzgalova, E. A.) Красницкая, Е. С., Стремлина, С. М., Брызгалова, Е. А.: К вопросу о сальмонеллезных токсикоинфекциях. *Ж. микробиол. (Москва)*, **1**, 35–38 (1970).

(Kravets, E. M., Sinyushina, M. N., Startseva, P. S.) Кравец, Э. М., Синюшина, М. Н., Старцева, П. С.: Эффективность комплексной терапии пневмонии у недоношенных детей. *Вопросы педиатрии*, **6**, 10–11 (1947).

Krepler, P., Zischka, W.: Zur Frage der ätiologischen Bedeutung des *B. coli* 111 : B4 bei Säuglingsenteritiden. *Österr. Z. Kinderheilk.*, **7**, 89–94 (1952).

Krishnan, N., Stenger, R.: Effects of starvation on the hepatotoxicity of carbon tetrachloride. *Amer. J. Path.*, **49**, 239–256 (1966).

Kroneberg, G.: Pharmakologische Untersuchungen über das Gift der Flexner-Ruhr-Bakterien. *Zbl. Bakt. I. Abt. Orig.*, **158**, 326–328 (1952).

Kroneberg, G., Sandritter W.: Kreislaufkollaps und morphologische Veränderungen bei der Vergiftung mit Flexner-Ruhr-Endotoxin. *Z. ges. exp. Med.*, **120**, 329–346 (1953).

(Krotkova, M. R.) Кроткова, М. Р.: Изучение фагоцитоза *S. typhi* в культуре ткани. *Ж. микробиол. (Москва)*, **7**, 22–26 (1971).

Kubinyi, L.: A veszprémi és parádsasvári ivóvíz által terjesztett enteritis járványok tanulsága (Consequences drawn from the water-borne enteritis epidemics at Veszprém and Parádsasvár). *Egészségtudomány*, **4**, 343–350 (1959).

Kubinyi, L.: Ivóvíz által terjesztett járványok Magyarországon az utolsó tíz évben (Water-borne epidemics in Hungary in the last ten years). *Egészségtudomány*, **4**, 348–354 (1960).

(Kucheryavy, F. Kh.) Кучерявый, Ф. Х.: К методике получения экспериментальной паратифозной инфекции у кроликов. *Бюлл. экспер. биол. мед.*, **11**, 48–51 (1956).

(Kucheryavy, F. Kh.) Кучерявый, Ф. Х.: Влияние искусственного повышения и понижения температуры тела на развитие, течение и исход экспериментальной паратифозной инфекции у кроликов. В кн.: *Физиологические механизмы лихорабочной реакции*. Под ред. П. Н. Веселкина, Медгиз, Ленинград. 270–274 (1957).

(Kudlay, D. G., Likhoded, V. G.) Кудлай, Д. Г., Лиходед, В. Г.: *Бактериоциногения*. Медицина, Ленинград, (1966).

Kulesha, G. S., Titova N. A.: Die pathologische Anatomie und Aetiologie der Komplikationen des Rückfallfiebers. *Virchow's Arch. path. Anat.*, **241**, 319–354 (1923*a*).

(Kulesha, G. S., Titova, N. A.) Кулеша, Г. С., Титова, Н. А.: Патологическая анатомия и этиология осложнений возвратного тифа. *Врач. газета,* **3—4,** 66–70, **5—6,** 131–134, **7—8,** 181–186 (1922).; **241,** 319–354 (1923).

Kwan, C. N., Wishnow, R. M.: *Escherichia coli* enterotoxin-induced steroidogenesis in cultured adrenal tumor cells. *Infect. Immunity,* **10,** 146–151 (1974).

LaBrec, E. H., Formal, S. B.: Experimental Shigella infections. IV. *J. Immunol.,* **87,** 562–572 (1961).

LaBrec, E. H., Schneider, H., Magnani, T. J., Formal, S. B.: Epithelial cell penetration as an essential step in the pathogenesis of bacillary dysentery *J. Bact.,* **88** 1503–1518 (1964).

LaBrec, E. H., Sprinz, H., Schneider, H., Formal, S. B.: Localization of vibrios in experimental cholera. In: *Proc. of the cholera research.* Symposium. Honolulu. pp. 272–276 (1965).

Lachowicz, K.: Escherichia. In: *Enterobacteriaceae-Infektionen.* 2nd ed., Ed. by J. Sedlák, H. Rische. G. Thieme, Leipzig. pp. 445–519 (1968).

Laehr, G.: *Über den Untergang des Staphylococcus pyogenes aureus in den durch ihr hervorgerufenen Entzündungsprozessen der Lunge.* Thesis, Bonn. (1887).

Landy, M., Weidanz, W. P.: Natural antibodies against Gram-negative bacteria. In: *Bacterial endotoxine.* Ed. by M. Landy, W. Braun, Quinn-Boden. Rahway, New Jersey. pp. 275–290 (1964).

Lange, B., Keschischian, K. H.: Über Versuche weisse Mäuse durch Einatmung von Krankheitserregern zu infizieren. *Z. Hyg.,* **103,** 569–589 (1924).

Lányi, B., Szita, J., Ringelhann, B., Kovách, K.: *Escherichia coli* 124 : 72 : 32 serotypus által okozott parádsasvári vízjárvány (Water-borne epidemic at Parádsasvár caused by *E. coli* 124 : 72 : 32 serotype). *Orv. Hetil.,* **100,** 1075–1077 (1959).

Lapin, B. A.: Disease in monkey within the period of acclimatization and during long-term stay in animal houses. In: *The problem of laboratory animal disease.* Academic Press, London–New York (1961).

(Lapin, B. A., Yakovleva, L. A.) Лапин, Б. А., Яковлева, Л. А.: *Очерки сравнительной патологии обезьян.* Медгиз, Москва (1960).

Lapin, B. A., Yakovleva, L. A.: *Comparative pathology in monkeys.* Springfield, Ill. (1963).

(Lapin, B. A., Yakovleva, L. A., Pekerman, S. M.) Лапин, Б. А., Яковлева, Л. А., Пекерман, С. М.: Воспроизведение паратифа В у обезъян. *Бюлл. экспер. биол. и. мед.,* **1,** 33–38 (1956).

Larivière, S., Gyles, C. L., Barfium, D. A.: Preliminary characterization of the heat-labile enterotoxin of *Escherichia coli* FII (P155). *J. infect. Dis.,* **128,** 312–320 (1973).

Lavergne, V. de: De la signification biologique des lésions intestinales de la fièvre typhoïde. *Ann. Méd.,* **14,** 149–361 (1923).

(Lavnikova, G. A., Tsinzerling, V. D.) Лавникова, Г. А., Цинзерлинг, В. Д.: О пневмониях у новорожденных. *Труды Санитарно-Гигиенического ин-та (Ленинград),* **41,** 134–150 (1958).

(Lazovsky, Yu. M., Kudryavtseva, E. H., Melnik, E. G.) Лазовский, Ю. М., Кудрявцева, Е. Н., Мельник, Е. Г.: К учению о пневтотифе. *Клин. мед.,* **1,** 35–45 (1947); *Арх. патол. (Москва),* **6,** 53–60 (1948).

(Lebedeva, E. A.) Лебедева, Е. А.: О роли атипичных вариантов флекснера в возникновении инфекционного процесса в эксперименте. *Ж микробиол. (Москва),* **4,** 97–102 (1959).

(Lebedeva, M. N., Goncharova, G. I., Lizko, N. N.) Лебедева, М. Н., Гончарова, Г. И., Лизько, Н. Н.: Современные аспекты нормы и патологии кишечной микрофлоры (обзор литературы). *Ж. микробиол. (Москва),* **9,** 36–41 (1974).

Lee, F. D., Krazewski, A., Gordon, J., Howie, J. G. R., McSeveney, D., Harlan, W. A.: Intestinal spirochaetosis. *Gut,* **12,** 126–133 (1971).

Lemann, J. J.: A case of bacillary dysentery with pseudomembrane in the vagina and oedema of the abdominal wall. *J. amer. med. Assoc.,* **75,** 1716–1717 (1920).

Lennert, K.: Lymphknoten-Diagnostik, Schnitt und Ausstrich. Bandteil A. Cytologie und Lymphadenitis. In: *Handbuch der speziellen pathologischen Anatomie und Histologie.* Ed. by O. Lubarsch and F. Henke. Vol. I., Part 3/A, pp. 428–435, Springer, Berlin–Göttingen–Heidelberg (1961).

Lentz, O., Prigge, R.: Dysenterie. In: *Handbuch der pathogenen Mikroorganismen.* Ed. by Kolle-Kraus-Uhlenhuth, Berlin. vol. **3,** pp. 1377–1584, (1931).
Leppänen, M. K.: Investigation on *Escherichia coli* in acute appendicitis and in normal appendices and faeces, with special reference to O-groups 1–25. *Ann. Med. exp. Fenn.* **36,** Suppl. 2 (1958).
Lerner, A. M.: Primary Gram-negative pneumonias. *Med. Tms.* (Lond.) **97,** 118–126 (1969).
Lerner, A. M., Federman, M. J.: Gram-negative bacillary pneumonia. *J. infect. Dis.,* **124,** 425–427 (1971).
Lerner, A. M., Tillotson, J. R.: Pneumonias caused by Gram-negative bacilli. *Mich. Med.,* **67,** 35–38 (1968).
Letterer, E.: Beiträge zur Pathogenese der Bacillenruhr. *Virchows Arch. path. Anat.,* **312,** 673–725 (1944).
Letterer, E.: Experimentelle und morphologische Untersuchungen über die Wirkungsweise reiner Ruhrgiftstoffe. *Virchows Arch. path. Anat.,* **317,** 34–55 (1949).
Letterer, E., Seybold, G.: Untersuchungen mit dem Bingelschen Harnblasenversuch über den Angriffspunkt der Ruhr- und Diphtheriegiftstoffe am Gewebe. *Z. Hyg.,* **129,** 466–487 (1949).
(Levenbuk, I. S., Andreeva, Z. M.) Левенбук, И. С., Андреева, З. М.: К патологической анатомии экспериментальной дизентерии. *Вестн. Акад. Мед. наук СССР,* **2,** 70–76 (1962).
(Levenbuk, I. S., Andreeva, Z. M.) Левенбук, И. С., Андреева, З. М.: Дизентерийный цистит в эксперименте. *Ж. микробиол.,* **6,** 37–39 (1965*a*).
(Levenbuk, I. S., Andreeva, Z. M.) Левенбук, И. С., Андреева, З. М.: О паразитировании шигелл в эпителиальных клетках. В кн.: *Материалы межинститутской научной конференции памяти Л. А. Тарасевича.* Москва. 200–201 (1965*b*).
(Levenbuk, I. S., Belaya, Yu. A., Chigirinsky, A. E.) Левенбук, И. С., Белая, Ю. А., Чигиринский, А. Е.: К вопросу о специфической профилактике дизентерии. II. *Ж. микробиол. (Москва),* **4,** 37–41 (1968).
(Levenbuk, I. S., Belaya, Yu. A., Kavtaradze, K. N., Dzhikidze, E. K., Chigirinsky, A. E., Gutkina, A. V.) Левенбук, И. С., Белая, Ю. А., Кавтарадзе, К. Н., Джикидзе, Э. К., Чигиринский, А. Е., Гуткина, А. В.: Морфологические изменения у обезьян при энтеральной иммунизации живой вакциной. *Ж. микробиол. (Москва),* **10,** 105–111 (1970).
Levenson, S. M.: Some effects of *Escherichia coli* and other microbes on mammalian metabolism and nutrition. *Ann. N. Y. Acad. Sci.,* **176,** 273–285 (1971).
Levine, M.: Some changing concepts regarding the coliform group: pathogenicity, index of pollution and protective properties. *Iowa State J. Sci.,* **38,** 51–59 (1963).
Levitan, R., Gorbach, S. L., Maddock, M., Coleman, R. D.: Response of the canine gut to the diarrhea toxin of *Escherichia coli. J. clin. Invest.,* **51,** 57a (1972).
Lewis, R., Holian, O., Gorbach, S.: Enterotoxin of *Escherichia coli* causing diarrhea in adults. *Clin. Res.,* **20,** 533 (1972).
(Li, G.) Ли, Г.: Всасывание меченого антигена дизентерийных бактерий Ньюкесла из кишечника в кровь в опыте на лабораторных животных. *Ж. микробиол. (Москва),* **12,** 30–33 (1959).
(Likhoded, V. G., Tabachnik, A. L., Temper, R. M.) Лиходед, В. Г., Табачник, А. Л., Темпер, Р. М.: Способ получения и характеристика антисыворотки, нейтрализирующей энтеротоксины кишечной палочки и холерного вибриона. *Ж. микробиол. (Москва),* **6,** 36–40 (1974).
Lillehei, R. C., MacLean, L. D.: The intestinal factor in irreversible endotoxin shock. *Ann. Surg.,* **148,** 513–525 (1958).
Lillehei, R. C., Washington, D. S.: The intestinal factor in irreversible hemorrhagic shock. *Surgery* **42,** 1043–1054 (1957).
Lin, F. C., Yang, T. L., Wu, P. F.: Experimental keratoconjunctivitis shigellosa in guinea pigs. II. Histological findings. *Chin. med. J.,* **83,** 463–469 (1964).
Lindberg, R. B., Young, V. M.: Observations on enteropathogenic *Escherichia coli. Ann. N. Y. Acad. Sci.,* **66,** 100–107 (1956).
Linde, K., Koch, H.: Untersuchungen zur oralen Immunisierung gegen Coli-Enteritis mit Streptomycin-dependenten Coli-Keimen. V. *Zbl. Bakt. I. Abt. Orig.,* **215,** 286–295 (1970).

Linde, K., Koch, H., Kittlick, M., Kapell, J.: Untersuchungen zur oralen Immunisierung gegen Coli-Enteritis mit Streptomycin-dependenten Keimen. I. *Zbl. Bakt. I. Abt. Orig.*, **211**, 453–465 (1969).
Lindner, K.: Über die Topographie der parasitären Bindehautkeime. *Graefes Arch. Ophthalmol.*, **105**, 727–777 (1921).
Lipkin, M.: Cell replication in the gastrointestinal tract of man. *Gastroenterology*, **48**, 616–624 (1965).
Lipkin, M., Bell, B.: Cell proliferation. In: *Handbook of Physiology*. Amer. Physiol. Soc. Washington. Section 6, vol. **5**, pp. 2861–2880 (1968).
Lobo, M. C., Mandell, G. L.: The effect of antibiotics on Escherichia coli ingested by macrophages. *Proc. Soc. exp. Biol.*, **142**, 1048–1050 (1973).
Löhlein, M.: Ruhr. In: *Handbuch der ärztlichen Erfahrungen im Weltkriege 1914/1918*. Leipzig. vol. **8**, 100–117 (1921).
Löhlein, M.: Bindehaut. In: *Handbuch der speziellen pathologischen Anatomie und Histologie*. Ed. by F. Henke und O. Lubarsch, Berlin. vol. 1, pp. 1–215 (1928).
Lojda, Z., Frič, P., Jodl, J., Chmelik, V.: Cytochemistry of the human jejunal mucosa in the norm and malabsorption syndrome. *Curr. Top. Path.*, **52**, 1–63 (1970).
Lorentzen, W.: Beitrag zur Pathogenese der Bazillenruhr. *Virchows Arch. path. Anat.*, **240**, 184–202 (1923).
Lovell, R.: Classification of *Bacterium coli* from diseased calves. *J. Path. Bact.*, **44**, 125–139 (1937).
(Luneva, O. K.) Лунева, О. К.: Значение палочки инфлюэнцы и пневмококка при хронической бронхиальной инфекции. *Ж. микробиол. (Москва)*, **11**, 65–69 (1971).
Lüderitz, O., Staub, A. M., Westphal, O.: Immunochemistry of O and R antigens of Salmonella and related Enterobacteriaceae. *Bact. Rev.*, **30**, 193–255 (1966).
Luria, S. E., Burrous, J. W.: Hybridization between *E. coli* and *Shigella*. *J. Bact.* **74**, 461–476 (1957).
Maaløe, O.: *On the relation between alexin and opsonin*. Copenhagen. (1946). Cit. by G. B. Mackaness (1964).
MacCallum, W. G.: *A textbook of pathology*. 7th ed. Saunders Co., Philadelphia–London. (1945).
McGeachie, J.: Serological grouping of urinary *Escherichia coli*. *J. clin. Path.*, **18**, 428–431 (1965).
MacGregor, A. R.: The pathology of still-birth and neonatal death. *Brit. Med. Bull.*, **4**, 174–178 (1946).
McGuire, C. D., Floyd, Th. M.: Studies on experimental shigellosis I. Shigella infections of normal mice. *J. exp. Med.*, **108**, 269–276 (1958).
McGuire, E. A., Young, V. R., Newberne, P. M., Pajne, B. J.: Effects of *Salmonella typhimurium* infection in rats fed varying protein intakes. *Arch. Path.*, **86**, 60–68 (1968).
McIntyre, O. R., Feeley, J. C., Greenough, W. B., Benenson, A. S., Hassan, S. J., Saad, A.: Diarrhea caused by non-cholera vibrios. *Amer. J. trop. Med.*, **14**, 412–418 (1965).
Mackaness, G. B.: The behaviour of microbial parasites in relation to phagocytic cells *in vitro* and *in vivo*. In: *Microbial behaviour "in vivo" and "in vitro"*. Ed. by H. Smith and J. Taylor. Cambridge. pp. 213–240 (1964).
Mackaness, G. B.: The mechanism of macrophage activation. In: *Infectious agents and host reactions*. Ed. by S. Mudd. Saunders, Philadelphia pp. 61–75 (1970).
Mackaness, G. B., Blanden, R. V.: Cellular immunity. *Progr. Allergy*, **2**, 89–140 (1967).
Mackaness, G. B., Blanden, R. V.: Cellular immunity. In: *Infect. agents and host reactions*. Ed. by S. Mudd., Saunders, Philad., pp. 22–60 (1970).
Mackel, D. C., Langley, L. F., Venice, L. A.: The use of the guinea-pig conjunctivae as an experimental model for the study of virulence of Shigella organisms. *Amer. J. Hyg.*, **73**, 219–223 (1961).
MacLean, L. D., Well, M. A.: Hypotension (shock) in dogs produced by *Escherichia coli* endotoxin. *Circulat. Res.*, **4**, 546–556 (1956).
McNaught, W., Roberts, G. B. S.: Enteropathogenic effects of strains of *Bacterium coli* isolated from cases of gastro-enteritis. *J. Path. Bact.*, **76**, 155–188 (1958).
McNaught, W., Stevenson, J. S.: "Coliform diarrhoea" in adult hospital patients. *Brit. med. J.*, **2**, 182–184 (1953).

Maenza, R. M., Powel, D. W., Plotkin, G. R., Formal, S. B., Jervis, H. R., Sprinz, H.: Experimental diarrhea: Salmonella enterocolitis in the rat. *J. infect Dis.*, **121**, 475–485 (1970).

(Maksimovich, N. A.) Максимович, Н. А.: *Патологическая анатомия дизентерии у детей раннего возраста.* Здоровя, Киев. (1960).

Mallory, F. B.: A histological study of typhoid fever. *J. exp. Med.*, **3**, 611–638 (1898).

Manolov, D. G.: A study of some questions of pathogenicity and immunity in dysentery using experimental models of Shigella keratoconjunctivitis in guinea pigs. *J. Hyg. Epidem. (Praha)*, **1**, 322–328 (1957*a*).

(Manolov, D. G.) Манолов Д. Г.: Изучение некоторых вопросов патогенеза и иммунитета на модели дизентерийного кератоконъюнктивита на морских свинках. *Ж. микробиол. (Москва)*, **10**, 94–98 (1957*b*).

(Manolov, D. G.) Манолов Д. Г.: Новый вид бактерий из рода шигелл. *Ж. микробиол. (Москва)*, **12**, 98–101 (1958).

Manolov, D. G.: A new type of the genus Shigella—"Shigella 13". *J. Hyg. Epidem. (Praha)*, **3**, 184–190 (1959).

Manolov, D. G.: The interference phenomenon in different species of Shigellae in animal experiments. *J. Hyg. Epidem. (Praha)*, **7**, 482–486 (1963).

Manolov, D. G.: Strains of *Escherichia coli* O124 : 72 with Shigella-like properties. *J. Hyg. Epidem. (Praha)*, **12**, 102–106 (1968).

(Manolov, D. G., Gesheva, R. L., Jr., Toshkov, A. S.) Манолов, В. Г., Гешева, Р. Л., Тошков, А. С.: Шигелла-кератоконъюнктивальная реакция у морских свинок как модель для изучения действия стрептомицина и хлортетрациклина. *Антибиотики (Москва)*, **5**, 72–75 (1958).

(Manolov, D. G., Kosturkov, G. B.) Манолов, Д. Г., Костурков, Г. Б. : Влияние дизентерийного бактериофага на течение экспериментального шигеллезного цистита у морских свинок. *Ж. микробиол. (Москва)*, **7**, 144–145 (1965); *Ж. гиг. эпид., (Прага)*, **9**, 408–412 (1965).

Manolov, D. G., Trifonova, A., Ghinchev, P.: A new lactose-fermenting species of the Shigella genus. *J. Hyg. Epidem. (Praha)*, **6**, 422–427 (1962).

(Margolin, V. N.) Марголин, В. Н.: О методах выявления лейкоцитов в тканях при помощи реакции на оксидазу. *Арх. пат. (Москва)*, **5**, 63–69 (1948).

(Margolin, A. M., Vasilyeva, N. G., Ryabinin, I. F.) Марголин, А. М., Васильева, Н. Г., Рябинин, И. Ф.: О воспалительных процессах в легких при наличии в мокроте больных кишечной палочки. В кн.: *Морфология, физиология и патология органов дыхания.* I ЛМИ, Ленинград. 90–93 (1966).

Marier, R., Wells, J. G., Swanson, R. C., Callahan, W., Mehlman, I. J.: An outbreak of enteropathogenic *Escherichia coli* food-borne disease traced to imported French cheese. *Lancet*, **2**, 1376–1378 (1973).

Markov, K. J., Veljanov, D., Kalojenov, J.: Vermehrungsvermögen und Steigerung der Virulenz bei *S. typhimurium*-Stämmen. *Z. Naturforsch.*, **21**b., 710–711 (1966).

(Maslov, M. S.) Маслов, М. С. К вопросу об изучении этиологии и патогенеза желудочно-кишечных расстройств, вызываемых различными серотипами кишечной палочки у детей раннего возраста. В кн.: *Колиэнтериты.* Под ред. О. В. Барояна. Медгиз, Москва. 5–41 (1962).

Mata, L., Urrutia, J. J.: Intestinal colonization of breast-fed children in rural area of low socioeconomic level. *Ann. N. Y. Acad. Sci.*, **176**, 93–109 (1971).

Matsumoto, H., Hara, S.: *Escherichia coli* O124 : K72(B17) : H12; a new serotype of *E. coli*. *Jap. J. med. Sci. Biol.*, **12**, 471–472 (1959).

Mattisson, A. G. M., Karlsson, B. W.: Electron microscopic and immunochemical studies on the small intestine of newborn piglets. *Arkiv Zool. (Stockholm)*, **18**, 575–589 (1967).

Maximow, A. A.: Gewebe der serösen Membranen. In: *Handb. der mikroskopischen Anatomie des Menschen.* Ed. by W. Z. Möllendorff, Berlin. vol. 2/I, pp. 289–314 (1927).

Medearis, D. N., Kenny, J. F.: Observation concerning the pathogenesis of *E. coli* infections in mice. *J. Immunol.*, **101**, 534–540 (1968).

(Medovikov, P. C.) Медовиков, П. С.: К вопросу о понижении бактерицидности в тонких кишках под ивлянием некоторых внутренних и внешних воздействий. *Русский врач*, **9**, 414–421, 453–455, 519–524 (1910).

(Medzhinov, B. F.) Меджинов, Б. Ф.: Предварительные итоги изучения эффективности конкретных мероприятий по профилактике и борьбе с дизентерией. *Ж. микробиол. (Москва)*, **11,** 27–28 (1953).

(Meshalova, A. N.) Мешалова, А. Н.: Состояние научно-производственной деятельности институтов вакцин и сывороток и задачи на ближайшие годы. *Ж. микробиол. (Москва)*, **7,** 3–7 (1955).

Mesrobeanu, L., Mesrobeanu, I., Mitricá, N.: Les endotoxines thermolabiles (neurotoxines) des germes gram-négatifs I. Les neurotoxines du bacille Typhi murium S et R. *Arch. roum. Path. exp.*, **20,** 399–423 (1961).

Mestitz, W.: Zur Frage der Leberveränderungen bei Typhus und Paratyphus. *Virchows Arch. path. Anat.*, **244,** 498–509 (1923).

Mechnikov, I.: *Immunity in Infectious Diseases.* Cambridge University Press, Cambridge. (1905).

Metchnikoff, E., Besredka, A.: Recherches sur la fièvre typhoïde expérimentale. *Ann. Inst. Pasteur*, **25,** 193–221 (1911).

(Mechnikov, I. I.) Мечников И. И. *Академ. собр. соч.* т. **9,** 257–282 (1955).

Michael, J. G., Ringelback, R., Hottenstein, S.: The antimicrobial activity of human colostral antibody in the newborn. *J. infect. Dis.*, **124,** 445–448 (1971).

Midtvedt, T., Trippestad, A.: Opsonizing and bactericidal effects of sera from gnotobiotic and conventionalized rats on P^{32}-labeled *Escherichia coli*. *Acta path. microbiol. scand. Section B*, **78,** 1–5 (1970).

Midtvedt, T., Trippestad, A.: Specificity of opsonic and bactericidal response of gnotobiotic rat sera. *Acta path. microbiol. scand. Section B*, **79,** 291–296 (1971).

(Mikhailov, I. F., Sergeev, V. V., Elkina, S. I.) Михайлов, И. Ф., Сергеев, В. В. Елкина, С. И.: Патогенные свойства стрептомицинзависимых мутантов *Sh. flexneri* и их ревертантов. *Ж. микробиол. (Москва)*, **11,** 10–14 (1968).

Miles, A. A.: Mechanisms of microbiol. pathogenicity. In: *Mechanisms of Microbiol. Pathogenicity.* Ed. by J. W. Howie and A. J. O'Hea. Cambridge (1955).

Miller, I., Šterzl, J., Kostka, J., Lanc, A.: Effect of antigens of the intestinal flora on the development of specific and nonspecific reactions in newborns of different species. In: *Bacterial Endotoxins.* Ed. by M. Landy and W. Braun. Rayway, New Jersey. pp. 291–305 (1964).

Minck, R., Lavillaureix, J.: Pouvoir pathogène expérimental pour la souris de certains *E. coli* de gastro-entérites infantiles. *Ann. Inst. Pasteur*, **89,** 582–585 (1955).

Miniats, O. P., Valli, V. E.: The gastrointestinal tract of gnotobiotic pigs. In: *Germfree Res. Biol. Effect Gnotobiol. Environ.* New York–London. 575–583 (1973).

(Minkevich, I. E.) Минкевич, И. Е.: *Кишечная палочка как патогенный фактор.* Медгиз, Ленинград. (1950).

Miraglia, G. J., Berry, L. J.: Enhancement of salmonellosis and emergence of secondary infection in mice exposed to cold. *J. Bact.*, **84,** 1173–1180 (1962).

(Mironchik, I. N.) Мирочник, И. Н.: Морфология и патогенез кишечных заболеваний, вызываемых кишечной палочкой у детей раннего возраста. *Арх. пат. (Москва)*, **2,** 3–10 (1961).

(Mirzoev, G. G.) Мирзоев, Г. Г.: Выживаемость и изменчивость дизентерийных бактерий Флекснера во внешней среде. *Ж. микробиол. (Москва)*, **7,** 88–91 (1962).

(Mirzoev, G. G., Abramova, N. T., Krushinsky, D. G., Potapova, A. T., Shchepina, A. K.) Мирзоев, Г. Г., Абрамова, Н. Т., Крушинский, Д. Г., Потапова, А. Т., Щепина, А. К.: Этиологическая структура дизентерии в одном из районов Приполярья. *Ж. микробиол. (Москва)*, **2,** 129–130 (1966).

Mitsuhashi, S., Kawakami, M., Goto, S., Yoshimura, T., Hashimoto, H.: Studies on the experimental typhoid. *Japan. J. exp. Med.*, **29,** 311–321 (1959).

Mitsuhashi, S., Sato, J., Tanaka, T.: Experimental salmonellosis: intracellular growth of *Salmonella enteritidis* ingested in mononuclear phagocytes of mice, and cellular basis of immunity. *J. Bact.*, **81,** 863–868 (1961).

Miyakawa, M., Gordon, H. A., Wostmann, B. S.: The gnotobiotic animal as a tool in the study of inflammation. *Sience*, **173,** 171–173 (1971).

Miyakawa, M., Uno, Y., Asai, J.: Studies on the local defence mechanism of the mesenchymal cells (including RES) of the intestinal tract, using germ free animals. In: *Reticuloendothelial System.* Japan Soc. Promot. Sci., Tokyo, 132–138 (1965).

Moeller, H. C., Kirsner, J. B.: The effect of drug-induced hypermotility on the gastrointestinal tract of dogs. *Gastroenterology*, **26**, 303–311 (1954).
(Molchanov, N. S.) Молчанов, Н. С.: *Острые пневмонии.* Медицина, Ленинград. (1965).
Moll, T., Ingalsbe, C. K.: The pathogenicity of certain strains of *Escherichia coli* for young mice and calves. *Am. J. Vet. Res.*, **16**, 337–341 (1955).
Möllendorff, W.: Beiträge zur Kenntnis der Stoffwanderungen bei wachsenden Organismen. IV. *Z. Zellforsch.*, **2**, 129–202 (1925).
Moller, V.: Activity determination of amino acid decarboxylase in Enterobacteriaceae. *Acta path. microbiol. scand.*, **34**, 102–114 (1954*a*).
Moller, V.: Diagnostic use of the Braun KCN test within the Enterobacteriaceae. *Acta path. microbiol. scand.*, **34**, 115–126 (1954*b*).
Moon, H. W.: Enteric colibacillosis in the newborn pig: problems of diagnosis and control. *J. Amer. vet. med. Ass.*, **155**, 1853–1859 (1969).
Moon, H. W.: Vacoulated villous epithelium of the small intestine of young pigs. *Vet. Path.*, **9**, 3–21 (1972).
Moon, H. W., Nielsen, N. O., Kramer, T. T.: Experimental enteric colibacillosis of the newborn pig: histopathology of the small intestine and changes in plasma electrolytes. *Amer. J. vet. Res.*, **31**, 103–112 (1970*a*).
Moon, H. W., Sorensen, D. K., Sautter, J. H.: *Escherichia coli* infection of the ligated intestinal loop of the newborn pig. *Amer. J. vet. Res.*, **27**, 1317–1325 (1966*a*).
Moon, H. W., Sorensen, D. K., Sautter, J. H., Higbee, J. M.: Association of *Escherichia coli* with diarrheal disease of the newborn pig. *Amer. J. vet. Res.*, **27**, 1007–1011 (1966*b*).
Moon, H. W., Whipp, S. C.: Systems for testing the enteropathogenicity of *Escherichia coli*. *Ann. N. Y. Acad. Sci.*, **176**, 197–211 (1971).
Moon, H. W., Whipp, S. C., Baetz, A. L.: Comparative effects of enterotoxins from *Escherichia coli* and *Vibrio cholerae* on rabbit and swine small intestine. *Lab. Invest.*, **25**, 133–140 (1971).
Moon, H. W., Whipp, S. C., Engstrom, G. W., Baetz, A. L.: Response of the rabbit ileal loop to cell-free products from *Escherichia coli* enteropathogenic for swine. *J. infect. Dis.*, **121**, 182–187 (1970*b*).
Moore, B.: Observations pointing to the conjunctiva as the portal of entry in Salmonella infection of guinea pigs. *J. Hyg. (Cambr.)*, **55**, 414–433 (1957).
Morecki, R., Parker, J. G.: Ultrastructural studies of the human *Giardia lamblia* and subjacent jejunal mucosa in a subject with steatorrhea. *Gastroenterology*, **52**, 151–164 (1967).
(Morgunov, I. N., Yagud, S. L., Barshtein, Yu. A.) Моргунов, И. Н., Ягуд, С. Л., Барштейн, Ю. А.: Экспериментальное воспроизведение брюшного тифа у морских свинок и кроликов. *Ж. микробиол. (Москва)*, **1**, 40–45 (1962).
(Morgunov, I. N., Yagud, S. L., Barshtein, Yu. A., Novikova, L. B.) Моргунов, И. Н., Ягуд, С. Л., Барштейн, Ю. А., Новикова, Л. Б.: Экспериментальные поиски модели дизентерийной инфекции. Сообщ. 5, *Ж. микробиол. (Москва)*, **4**, 72–78 (1963).
(Morgunov I. N., Yagud, S. L., Barshtein, Yu. A.) Моргунов, И. Н., Ягуд, С. Л., Барштейн, Ю. А.: Некоторые данные экспериментального изучения патогенеза и иммунитета при дизентерии. В кн.: *Дизентерия*, Киев. 20–23 (1965).
Moro, E.: Bemerkungen zur Lehre von der Säuglingsernährung. *Jb. Kinderheilk.*, **34**, 1–9 (1916).
Moulder, J. W.: *The biochemistry of intracellular parasitism.* Univ. Press, Chicago–London. (1962).
Mueller, J. C., Jones, A. L., Brandborg, L. L.: Scanning electron microscope observation in human giardiasis. In: *Scanning Electron Microscopy* (Part III). Ed. by O. Johari, Chicago. pp. 557–564 (1973).
Mullens, J. E., McKay, K. A., Jubb, K. V.: Experimental enteritis produced in dogs by *Escherichia coli* endotoxin. *Canad. J. Surg.*, **7**, 79–88 (1964).
Mushin, R., Dubos, R.: Colonization of the mouse intestine with *Escherichia coli*. *J. exp. Med.*, **122**, 745–757 (1965).
Mushin, R., Ford, F. C. P., Hughes, J. C.: Aspects of colonization of the mouse intestine by coliform bacteria. *J. Med. Microbiol.*, **3**, 573–583 (1970).
Myers, L. L.: Characterization of *Escherichia coli* obtained from newborn calves with diarrhea. *Infect. Immunity*, **11**, 493–496 (1975).

Myers, L. L., Newman, F. S., Warren, G. R., Catlin, J. E., Anderson, C. K.: Calf ligated intestinal segment to detect enterotoxigenic *Escherichia coli*. *Infect. Immunity* **11,** 588–591 (1975).

Myren, J.: Injury of liver tissue in mice after single injections of carbon tetrachloride. *Acta path., microbiol. scand.*, **116,** Suppl., 1–64 (1956).

(Naftulyeva, K. A., Gefter, L. G., Mekler, M. I., Lisenko, A. N.) Нафтульева, К. А., Гефтер, Л. Г., Меклер, М. И., Лисенко, А. Н.: Дальнейшее изучение циркуляции энтеропатогенной кишечной палочки 0124 : К72(В17). *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **36,** 183–186 (1970).

Nakamura, A.: Virulence of Shigella isolated from healthy carriers. *Jap. J. med. Sci. Biol.*, **20,** 213–223 (1967).

Nakamura, M., Jackson, K. E., Gross, R.: Correlation pathogenicity of Shigella and intraperitoneal survival in mice. *Infect. Immunity* **2,** 570–573 (1970).

Nakanishi, R., Suchi, H., Tajiri, I., Yamasato, M.: An enteropathogenic *Escherichia coli* isolated from a patient with Ekiri. *J. japan. Ass. infect. Dis.*, **31,** 169–174 (1956).

Nakanishi, R., Suchi, H., Tajiri, I., Yamasato, M.: Additional report on the enteropathogenic *Escherichia coli* (O28a28c : B18) isolated from case affected with dysentery. *J. japan. Ass. infect. Dis.*, **32,** 531–535 (1958).

Nalin, D. R., Bhattacharjee, A. K., Richardson, S. H.: Cholera-like toxic effect of culture filtrates of *Escherichia coli*. *J. infect. Dis.*, **130,** 595–601 (1974).

Nelson, D. P., Mata, L. J.: Bacterial flora associated with the human gastrointestinal mucosa. *Gastroenterology*, **58,** 56–61 (1970).

Neter, E.: Enteritis due to enteropathogenic *Escherichia coli*. *J. Pediat.*, **55,** 223–239 (1959).

Neter, E.: Enteritis due to enteropathogenic *Escherichia coli*. *Amer. J. dig. Dis.*, **10,** 883–886 (1965).

Neter, E.: Discussion. *Ann. N. Y. Acad. Sci.*, **176,** 136 (1971).

Neter, E., Shumway, C. N.: *Escherichia coli* serotype D 433: occurrence in intestinal and respiratory tracts, cultural characteristics, pathogenicity, sensitivity to antibiotics. *Proc. Soc. exp. Biol.*, **75,** 504–507 (1950).

Neufeld, F., Kuhn, H.: Versuche über Pathogenität verschiedener Stämme der Typhus-Paratyphusgruppe bei pulmonaler Einführung. *Z. Hyg.*, **118,** 653–655 (1936).

Newberne, P. M., Hunt, C. E., Young, V. P.: The role of diet and the reticuloendothelial system in the response of rats to *Salmonella typhimurium* infections. *Brit. J. exp. Med.*, **49,** 448–457 (1968).

(Nisevich, N. I.) Нисевич, Н. И.: Кишечная коли-инфекция. Клиника. Лечение. В кн.: *Многотомное руководство по микробиологии, клинике и эпидемиологии инфекционных болезней*. Медицина, Москва. **6,** 138–143, 147–148 (1964).

Noble, R. L., Collip, J. B.: A quantitative method for the production of experimental traumatic shock without haemorrhage in unanaesthetized animals. *Quart. J. exp. Physiol.*, **31,** 187–199 (1942).

Norris, H. T.: Back to parenchymal inflammation? Pathogenesis of fluid production by the ileum in experimental cholera: an unusual type of host response to bacterial agents. *Biochem. Pharmacol.* Suppl., 133–141 (1968).

Norris, H. T., Curran, P. F., Schultz, S. G.: Modification of intestinal secretion in experimental cholera. *J. infect. Dis.*, **119,** 117–125 (1969).

Norris, H. T., Majno, G.: The advance of cholera. *Lancet*, **1,** 1229–1230 (1967).

Norris, H. T., Majno, G.: On the role of the ileal epithelium in the pathogenesis of experimental cholera. *Amer. J. Path.*, **53,** 263–279 (1968).

(Noskov, F. S.) Носков, Ф. С.: К вопросу о значении конъюнктивального заражения морской свинки для идентификации дизентерийных бактерий. *Ж. микробиол. (Москва)*, **7,** 124–125 (1959).

(Nosova, L. S., Salnikova, L. E., Trofimova, M. N., Znamenskaya, T. M.) Носова, Л. С., Сальникова, Л. Е., Трофимова, М. Н., Знаменская, Т. М.: Цитологическое изучение взаимодействия дизентерийных бактерий с клеточными культурами. В кн.: *Сравнительная физиология микроорганизмов*. Горький. 86–94 (1970).

(Nosova L. S., Trofimova, M. N., Salnikova, L. S., Zhdanov, E. E.) Носова, Л. С., Трофимова, М. Н., Сальникова, Л. С., Жданов, Е. Е.: Электронномикроскопическое

изучение взаимодействия шигелл с клеточными культурами. *Цитология*, **14,** 783–787 (1972).
(Novgorodskaya, E. M.) Новгородская, Э. М.: О так называемых токсико-септических заболеваниях новорожденных. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **11,** 169–202 (1948).
(Novgorodskaya, E. M.) Новгородская, Э. М.: Материалы к биологии культур кишечных палочек, выделенных от новорожденных. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **15,** 176–254 (1953*a*).
(Novgorodskaya-Linetskaya, E. M.) Новгородская-Линецкая, Э. М.: Микробиологические и эпидемиологические наблюдения при острых кишечных заболеваниях новорожденных. II. Заболевания новорожденных, вызываемые представителями рода Escherichia. Дисс. докт. Ленинград (1953*b*).
(Novgorodskaya, E. M.) Новгородская, Э. М.: Острые кишечные инфекции новорожденных. В кн.: *Эпидемиология*. Медгиз, Москва. 194–209 (1957).
(Novgorodskaya, E. M.) Новгородская, Э. М.: О колиэнтеритах детей раннего возраста. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **18,** 290–316 (1958).
Novgorodskaya-Linetskaya, E. M.: Acute intestinal infections of nondysenteric etiology. *Bull. Wld Hlth Org.*, **21,** 299–307 (1959).
(Novgorodskaya, E. M.) Новгородская, Э. М.: Сальмонеллезы человека и борьба с ними. *Тр. Ин-та эпидемиол. микродиол. им. Пастера (Ленинград)*, **21,** 105–129 (1960).
(Novgorodskaya, E. M.) Новгородская, Э. М.: Некоторые вопросы этиологии и эпидемиологии острых кишечных инфекций. *Ann. Immunol. hung.* **9,** 151–157 (1966*a*).
(Novgorodskaya, E. M.) Новгородская, Э. М.: Об изучении острых кишечных инфекций неустановленной этиологии. *Авторефераты Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, 5–9 (1966*b*).
(Novgorodskaya, E. M.) Новгородская, Э. М.: Некоторые новые аспекты изучения этиологии и диагностики острых кишечных заболеваний у взрослых и детей. *Материалы II Всероссийского съезда эпидемиологов, микробиологов и инфекционистов.* Москва. 168–169 (1966*c*).
(Novgorodskaya, E. M.) Новгородская, Э. М.: О некоторых новых аспектах изучения острых кишечных инфекций. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **33,** 7–16 (1968).
(Novgorodskaya, E. M.) Новгородская, Э. М.: О дизентерииподобных заболеваниях взрослых и детей, вызываемых энтеропатогенными кишечными палочками. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **36,** 117–132 (1970).
(Novgorodskaya, E. M.) Новгородская, Э. М.: О неоднородности эшерихиозов второй категории. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **40,** 73–77 (1973).
(Novgorodskaya E. M., Avdeeva, T. A., Arbuzova, V. A.) Новгородская, Э. М., Авдеева, Т. А., Арбузова, В. А.: Актуальные вопросы эпидемиологии острых кишечных инфекций в свете задачи их снижения и ликвидации. *IX Междунар. конгр. микробиол. Тезисы докл.* Москва, 718–719 (1966*a*).
(Novgorodskaya, E. M., Khazenson, L. B., Krivonosova, K. I.) Новгородская, Э. М., Хазенсон, Л. В., Кривоносова, К. И.: О колиэнтерите новорожденных, вызванном кишечными палочками редкого серологического типа O111 : B4 : H12. *Ж. микробиол. (Москва)*, **9,** 116–119 (1963).
(Novgorodskaya, E. M., Khazenson, L. B., Loseva, A. G., Krivonosova, K. L.) Новгородская, Э. М., Хазенсон, Л. Б., Лосева, А. Г., Кривоносова, К. И.: О групповых заболеваниях колиэнтеритом O44 среди новорожденных детей. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **36,** 279–286 (1970*a*).
(Novgorodskaya, E. M., Khazenson, L. B., Tseprinskaya, E. V.) Новгородская, Э. М., Хазенсон, Л. Б., Цепринская, Е. В.: Вопросы эпидемиологии и профилактики колиэнтерита детей раннего возраста. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **27,** 183–199 (1964*b*).
(Novgorodskaya, E. M., Krivonosova, K. I., Arbuzova, V. A., Polotsky, Yu. E.) Новгородская, Э. М., Кривоносова, К. И., Арбузова, В. А., Полоцкий, Ю. Е.: К биологической характеристике энтеропатогенной кишечной палочки O143 — возбудителя острого энтероколита взрослых и детей. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **33,** 76–86 (1968*a*).

(Novgorodskaya, E. M., Krivonosova, K. I., Karyagina, E. I., Semenova, O. A., Bartysheva, V. A., Balabonova, L. S.) Новгородская, Э. М., Кривоносова, К. И., Карягина, Е. И., Семенова, О. А., Бартышева, В. А., Балабонова, Л. С.: Материалы к изучению острого энтероколита у взрослых и детей, вызываемого энтеропатогенной кишечной палочкой O124 : K72 (B17). *Ж. микробиол. (Москва)*, **8,** 21–25 (1966*b*).

(Novgorodskaya, E. M., Krivonosova, K. I., Karyagina, E. I., Semenova, O. A., Bartysheva, V. A., Balabonova, L. S., Khazenson, L. B., Blokhina, T. F.) Новгородская, Э. М., Кривоносова, К. И., Карягина, Е. И., Семенова, О. А., Бартышева, В. А., Балабонова, Л. С., Хазенсон, Л. Б., Блохина, Т. Ф.: Об острых кишечных заболеваниях взрослых и детей, вызванных энтеропатогенной кишечной палочкой O124 : K72 (B17). *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **27,** 372–381 (1964*a*).

(Novgorodskaya, E. M., Loseva, A. G., Krivonosova, K. I.) Новгородская, Э. М., Лосева, А. Г., Кривоносова, К. И.: О колиэнтеритах детей раннего возраста, вызываемых энтеропатогенной кишечной палочкой серологического типа «9». *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **21,** 40–53 (1960).

(Novgorodskaya, E. M., Polotsky, Yu. E.) Новгородская, Э. М., Полоцкий, Ю. Е.: Биологическое значение кишечных палочек для нормального организма и их роль в патологии человека и животных. *Труды Ленинградского научного общества патологоанатомов за 1971* г. **13,** 31–38 (1972).

(Novgorodskaya, E. M., Semenova, O. A., Arbuzova, V. A.) Новгородская, Э. М., Семенова, О. А., Арбузова, В. А.: Новые данные к этиологии дизентериеподобных заболеваний взрослых и детей (Об энтеропатогенной кишечной палочке "Escherichia Крым»). *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **33,** 117–132 (1968*b*).

Oakberg, E. F.: Constitution of liver and spleen as a physical basis for genetic resistance to mouse typhoid. *J. infect. Dis.*, **78,** 79–98 (1946).

(Obukhova, O. V.) Обухова, О. В.: Распространение дизентерийных бактерий Sonne в организме мышей при энтеральном заражении. В кн.: *Кишечные инфекции*. Медгиз, Ленинград. 155–161 (1956).

Ocklitz, H. W.: Die Prophylaxe. In: *Säuglings-Enteritis*. Ed. by A. Adam. G. Thieme, Stuttgart. pp. 491–523 (1956).

Ogawa, H.: Experimental approach in studies on pathogenesis of bacillary dysentery — with special references to the invasion of bacilli into intestinal mucosa. *Acta path. Jap.*, **20,** 261–277 (1970).

Ogawa, H., Honjo, S., Takasaka, M., Fujiwara, T., Imaizumi, K.: Shigellosis in cynomolgus monkeys (Macaca iris). IV. Bacteriological and histopathological observations on the earlier stage of experimental infection with *Shigella flexneri* 2a. *Jap. J. med. Sci. Biol.*, **19,** 23–32 (1966*a*).

Ogawa, H., Nakamura, A.: Effect of active or passive immunization on the response of the ligated rabbit intestine to *Shigella flexneri* infection. *Jap. J. med. Sci. Biol.*, **22,** 351–362 (1969*a*).

Ogawa, H., Nakamura, A.: Experimental bacillary dysentery in rabbits. *Jap. J. med. Sci. Biol.*, **22,** 383–387 (1969*b*).

Ogawa, H., Nakamura, A., Nakaya, R.: Immunity to experimental keratoconjunctivitis shigellosa in guinea pigs. *Jap. J. med. Sci. Biol.*, **19,** 209–214 (1966*b*).

Ogawa, H., Nakamura, A., Nakaya, R.: Cinemicrographic study of tissue cell cultures infected with *Shigella flexneri*. *Jap. J. med. Sci. Biol.*, **21,** 259–273 (1968*a*).

Ogawa, H., Nakamura, A., Nakaya, R., Mise, K., Honjo, S., Takasaka, M., Fujiwara, T., Imaizumi, K.: Virulence and epithelial cell invasiveness of dysentery bacilli. *Jap. J. med. Sci. Biol.*, **20,** 315–328 (1967*a*).

Ogawa, H., Nakamura, A., Sakazaki, R.: Pathogenic properties of enteropathogenic *Escherichia coli* from diarrheal children and adults. *Jap. J. med. Sci. Biol.*, **21,** 333–349 (1968*b*).

Ogawa, H., Takahashi, R., Honjo, S., Takasaka, M., Fujiwara, T., Ando, K., Nakagawa, M., Muto, T., Imaizumi, K.: Shigellosis in cynomolgus monkeys (Macaca iris). III. Histopathological studies of natural and experimental shigellosis. *Jap. J. med. Sci. Biol.*, **17,** 321–332 (1964).

Ogawa, H., Yoshikura, H., Nakamura, A., Nakaya, R.: Susceptibility of cell cultures from various mammalian tissues to the infection by Shigella. *Jap. J. med. Sci. Biol.*, **20,** 329–339 (1967*b*).

Okada, S., Sakai, T., Hoshino, T.: A strain of *Escherichia coli* identical in antigenic structure with *Shigella boydii* 8. *Jap. J. Microbiol.*, **2**, 13–16 (1958).
Olitsky, P. K., Kligler, I. J.: Toxins and antitoxins of Bacillus dysenteriae Shiga. *J. exp. Med.*, **31**, 19–33 (1920).
Olitzki, A. L.: Epidemiological and immunological observations in typhoid of man and anthropoids and in experimental salmonellosis of laboratory animals. In: *Infectious Agents and Host Reactions*. Ed. by St. Mudd. Saunders, Philad. pp. 303–323 (1970).
Olitzki, A. L.: Enteric fevers. In: *Causing organisms and host reactions*. S. Karger, Basel. (1972).
Olitzki, A. L., Godinger, D., Geschon, Z.: The uptake of *Salmonella typhi* by phagocytes and its intracellular viability. *Path. Microbiol. (Basel)*, **27**, 175–201 (1964).
Ondráček, J., Simsa, J., Chmelar, V., Morawek, F.: Studium responce shigelového endotoxinu pomoci autoradiografic. *Čas. Lék. čes.*, **105**, 991–993 (1966).
Oppenheimer, J. J., Perry, S.: Effects of endotoxins on cultured leukocytes. *Proc. Soc. exp. Biol.*, **118**, 1014–1019 (1965).
(Orlovsky, A. A.) Орловский, А. А.: Материалы к изучению биологических и патогенных свойств *Bacterii coli communis*. Дисс. Санкт-Петербург, (1897).
Ørskov, F., Ørskov, I., Furowicz, A. J.: Four new *Escherichia coli* O antigens, O148, O151, O152, O153, and one new H antigen, H50, found in strains isolated from enteric diseases in man. *Acta path. microbiol. scand.*, Section B, **80**, 435–440 (1972).
Ørskov, F., Ørskov, I., Jann, B., Jann, K.: Immunoelectrophoretic patterns of extracts from all *Escherichia coli* O and K antigen test strains correlation with pathogenicity. *Acta path. microbiol. scand.*, Section B, **79**, 142–152 (1971).
Ørskov, F., Ørskov, I., Jann, B., Müller-Seitz, E., Westphal, O.: Immunochemistry of *Escherichia coli* O-antigens. *Acta path. microbiol. scand.*, **71**, 339–358 (1967).
Ørskov, F., Sørensen, K. B.: *Escherichia coli* serogroups in breast-fed and bottle-fed infants. *Acta path. microbiol. scand.*, Sect. B., **83**, 25–30 (1975).
Ørskov, I., Ørskov, F., Bettelheim, K. A., Chandler, M. E.: Two new *Escherichia coli* O antigens, O162 and O163, and one new H antigen, H56. Withdrawal of H antigen H50. *Acta path. microbiol. scand.*, Sect. B, **83**, 121–124 (1975*b*).
Ørskov, I., Ørskov, F., Rowe, B.: Three new *Escherichia coli* O antigens, O154, O155 and O156, and one new K antigen, K94. *Acta path. microbiol. scand.*, Sect. B, **81**, 59–64 (1973).
Ørskov, I., Ørskov, F., Rowe, B.: Four new *Escherichia coli* O antigens, O158, O159, O160 and O161, and two new H antigens, H53 and H54. *Acta path. microbiol. scand.*, Sect. B, **83**, 116–120 (1975*a*).
Ørskov, I., Ørskov, F., Smith, H. W., Sojka, W. J.: The establishment of K99, a thermolabile, transmissible *Escherichia coli* K antigen, previously called "Kco", possessed by calf and lamb enteropathogenic strains. *Acta path. microbiol. scand.*, Sect. B, **83**, 31–36 (1975*c*).
Ørskov, J., Jensen, A., Kobayaschi, K.: Studien über Breslauinfektion der Mäuse, mit spezieller Rücksicht auf die Bedeutung des Retikuloendothelialgewebes. *Z. Immun.-Forsch.*, **55**, 34–68 (1928).
Ørskov, J., Kauffmann, K.: Studies on the significance of "Vi-antigen" in the mechanism of typhoid infection in mice. *J. Hyg.*, **36**, 514–524 (1936).
Osada, Y., Nakajo, M., Ogawa, H.: Experimental urinary infection in guinea pigs. *Jap. J. Bact.*, **26**, 553–562 (1971).
Osada, Y., Nakajo, M., Ogawa, H.: Application of experimental keratoconjunctivitis shigellosa in chemotherapeutic evaluation of rifampicin to bacillary disentery. *Jap. J. Microbiol.*, **16**, 329–332 (1972*a*).
Osada, Y., Nakajo, M., Une, T., Ogawa, H., Oshima, Y.: Application of cell culture in studying antibacterial activity of rifampicin to Shigella and enteropathogenic *Escherichia coli*. *Jap. J. Microbiol.*, **16**, 525–533 (1972*b*).
Otto, H. F., Begemann, F.: Vergleichende ultrastructurelle und klinische Studie zum Ablauf des M. Whipple. *Virchows Arch., Abt.* A **350**, 368–388 (1970).
Owen, R. L., Jones, A. L.: Epithelial cell specialization within human Peyer's patches: an ultrastructural study of intestinal lymphoid follicles. *Gastroenterology*, **66**, 189–203 (1974).

Pages, A.: Dynamique da la lyse bactérienne dans la maladie de Whipple. Étude ultrastructurale. *Ann. d'anat. pathol.*, **16**, 511–526 (1971).
Patnaik, B. K., Ghosh, H. K.: Histopathological studies on experimental cholera. *Brit. J. exp. Path.*, **47**, 210–214 (1966).
Patzelt, V.: Der Darm. In: *Handbuch der mikroskopischen Anatomie des Menschen.* Springer, Berlin. Vol **5**, pp. 1–448 (1936).
Peeters, T., Vantrappen, G.: The Paneth cell: a source of intestinal lysozyme. *Gut*, **16**, 553–558 (1975).
Penner, A., Bernheim, A. I.: Studies on the pathogenesis of experimental dysentery intoxication. Production of lesions by introduction of toxin into the cerebral ventricles. *J. exp. Med.*, **76,** 281–282 (1942), **111**, 145–153 (1960).
Penner, A., Klein, S. H.: The pathogenesis of experimental dysentery intoxication. *J. exp. Med.*, **96**, 59–69 (1952).
(Perepelkin, V. S., Zabolotnov, V. I., Vorotyntsev, D. I., Nekrasov, I. L.) Перепелкин, В. С., Заболотнов, В. И., Воротынцев, Д. И., Некрасов, И. Л.: О колиэнтеритах у взрослых и носительстве энтеропатогенных кишечных палочек. *Ж. микробиол. (Москва)*, **8,** 122–124 (1963).
(Perets, L. G.) Перетц, Л. Г.: *Значение нормальной микрофлоры для организма человека.* Медгиз, Москва (1955).
(Perets, L. G.) Перетц Л. Г.: Микрофлора человеческого тела. В кн.: *Многотомное руководство по микробиологии и эпидемиологии инфекционных болезней.* Медгиз, Москва. **1,** 695–761 (1962).
(Petrovskaya, V. G.) Петровская, В. Г.: *Проблема вирулентности бактерий.* Медицина, Москва (1967).
(Petrovskaya, V. G.) Петровская, В. Г.: Проникновение шигелл в эпителиальные клетки как важное условие возможности возникновения инфекционного процесса. *Ж. микробиол. (Москва)*, **9,** 24–27 (1974).
(Petrovskaya, V. G., Blinova, N. I.) Петровская, В. Г., Блинова, Н. И.: Оценка значения типоспецифического антигена для вирулентности шигелл Флекснера 2а при изучении рекомбинантов определенных генетических классов. *Ж. микробиол. (Москва)*, **10,** 69–74 (1971).
(Petrovskaya, V. G., Lycheva, T. A.) Петровская, В. Г., Лычева, Т. А.: Вирулентность гибридов *Sh. flexneri*, полученных при скрещивании с различными штаммами *E. coli* K-12 HFR. *Ж. микробиол. (Москва)*, **5,** 41–47 (1970).
Pfeiffer, A.: Choleraspirillen in der Darmwand. *Dtsch. med. Wschr.*, N **2**, 212–213 (1887).
Pick, L.: Der Paratyphus. In: *Handbuch der speziellen pathologischen Anatomie und Histologie.* Ed. by F. Henke and O. Lubarsch. Berlin. vol. 4/2, pp. 610–767 (1928).
Piéchaud, D., Szturm-Rubinsten, S.: Pouvoir pathogène expérimental des bacilles dysentériques chez le cobaye. *Ann. Inst. Pasteur*, **97**, 511–517 (1959).
Piéchaud, D., Szturm-Rubinsten, S.: Mode de propagation des bacilles dysentériques. II. Étude expérimentale chez le cobaye avec divers sérotypes des sousgroupes A, B et C. *Ann. Inst. Pasteur*, **103**, 306–311 (1962).
Piéchaud, D., Szturm-Rubinsten, S., Charpak, M.: Un nouveau sérotype de "parashigella". *Ann. Inst. Pasteur*, **108**, 125–129 (1965).
Piéchaud, M., Szturm-Rubinsten, S., Piéchaud, D.: Évolution histologique de la kérato-conjonctivité à bacilles dysentériques du cobaye. *Ann. Inst. Pasteur*, **94,** 298–309 (1958).
Pierce, N. F.: Differential inhibitory effects of cholera toxoids and ganglioside on the enterotoxins of *Vibrio cholerae* and *Escherichia coli*. *J. exp. Med.*, **137,** 1009–1023 (1973).
Pierce, N. F., Greenough, W. B., Carpenter, C. C. J.: *Vibrio cholerae* enterotoxin and its mode of action. *Bact. Rev.*, **35**, 1–13 (1971).
Pierce, N. F., Wallace, C. K.: Stimulation of jejunal secretion by a crude *Escherichia coli* enterotoxin. *Gastroenterology*, **63**, 439–448 (1972).
(Pigarevsky, V. E., Ilyin, G. I.) Пигаревский, В. Е., Ильин, Г. И.: Морфологические проявления неспецифической устойчивости организма при действии эзотоксина стафилококка. *Труды Ин-та экспериментальной медицины АМН СССР, Ежегодник, (Ленинград)*, **9** (4), 18–24 (1967).

Pike, R. M., Mackenzie, G. M.:-Virulence of *Salmonella typhimurium*. I. Analysis of experimental infection in mice with strains of high and low virulence. *J. Bact.*, **40,** 171–195 (1940).

Pittman, F. E., El-Hashimi, W. K., Pittman, J.: Studies of human amebiasis. *Gastroenterology*, **65,** 581–603 (1973).

(Planelyes, Kh. Kh.) Планельес, Х. Х.: Первичный очаг при бактериальных инфекциях. *Тр. Акад. мед. наук. СССР*, **5,** 159–171 (1950).

(Planelyes, Kh. Kh., Forshter, Kh. K.) Планельес, Х. Х., Форштер, Х. К.: О внутриклеточном размножении микробов при экспериментальной брюшнотифозной инфекции. *Ж. микробиол. (Москва)*, **4,** 14–25 (1946).

(Planelyes, Kh. Kh., Forshter, Kh. K.) Планельес, Х. Х., Форштер, Х. К.: О патогенезе и иммунологических реакциях при экспериментальной брюшнотифозной инфекции белых мышей. *Ж. микробиол. (Москва)*, **1,** 10–23 (1947).

(Planelyes, Kh. Kh., Forshter, Kh. K.) Планельес, Х. Х., Форштер, Х. К.: Значение регионарных лимфатических желез в развитии экспериментальной инфекции Бреслау. *Труды Акад. мед. наук СССР*, **5,** 181–188 (1950).

(Planelyes, Kh. Kh., Kharitonova, A.) Планельес, Х. Х., Харитонова, А.: *Побочные явления при антибиотикотерапии бактериальных инфекций*. 2-е изд., Медицина, Москва. (1965).

(Planelyes, Kh. Kh., Krasinskaya, S. L.) Планельес, Х. Х., Красинская, С. Л.: К разрешению спора между гематогенной и энтерогенной теориями патогенеза брюшного тифа. *Тр. Акад. мед. наук СССР*. **5,** 172–180 (1950).

Plaut, A. G., Gorbach, S. L., Nahas, L.: Studies of intestinal mucosa. 3. The microbial flora of human small intestinal mucosa and fluids. *Gastroenterology*, **53,** 868–873 (1967).

(Podlevsky, A. F.) Подлевский, А. Ф.: Фагоцитарная активность лейкоцитов у больных брюшным тифом. *Ж. микробиол. (Москва)*, **1,** 57–62 (1962).

Poehlmann, A.: Untersuchungen an Typhus- und Paratyphus Roseolen. *Arch. Derm. Syph.*, **131,** 384–452 (1921).

Poger, M. E., Lamm, M. E.: Localization of free and bound secretory component in human intestinal epithelial cells. *J. exp. Med.*, **139,** 629–642 (1974).

(Pokrovskaya, M. P., Kaganova, L. S.) Покровская, М. П., Каганова, Л. С.: *Цитологический метод изучения механизма иммунитета*. Медгиз, Свердловск. (1947).

(Pokrovskaya, M. P., Kraskina, N. A., Gutorova, N. M., Levenson, V. I., Zhukov, V. G., Allilуev, A. P.) Покровская, М. П., Краскина, Н. А., Гуторова, Н. М., Левенсон, В. И., Жуков, В. Г., Аллилуев, А. П.: Цитологическое изучение процессов выздоровления у животных, иммунизированных Vi-антигеном и зараженных вирулентными брюшнотифозными бактериями. *Ж. микробиол. (Москва)*, **9,** 79–82 (1963).

(Pokrovsky, V. I., Shalygina, N. B.) Покровский, В. И., Шалыгина, Н. Б.: К вопросу об инвазии шигелл в кишечный эпителий и роли этого явления в патогенезе дизентерии. *Ж. микробиол. (Москва)*, **11,** 102–106 (1973).

Policard, A.: *Physiologie et pathologie du système lymphoïde*. Masson, Paris (1963).

(Polonskaya, E. V., Darkshevich, Yu. N.) Полонская, Е. В., Даркшевич, Ю. Н.: К морфологической характеристике колиэнтеритов. В кн.: *Вопросы патологии детского возраста*. Медгиз, Ленинград. 121–128 (1959).

(Polonsky, Yu. V., Voino-Yasenetsky, M. V.) Полонский, Ю. В., Войно-Ясенецкий, М. В.: Осложнения бациллярной дизентерии (по секционным материалам). *Здравоохр. Таджикистана*, **3,** 13–22 (1940).

Polotsky, Yu. E.: Comparative morphological analysis of experimental infectious processes caused with different enteropathogenic and non-enteropathogenic *Escherichia coli*. Thesis. Cand. Med. Sci. Leningrad. (1967).

Polotsky, Yu. E.: Biological importance of *Escherichia coli* organisms under normal conditions and in pathology. *László Kórház Report*, Budapest (1970).

(Polotsky, Yu. E., Arbuzova, V. A.) Полоцкий, Ю. Е., Арбузова, В. А.: Материалы к экспериментальной инфекции, вызываемой энтеропатогенной кишечной палочкой 0124 у лабораторных животных. *Авторефераты Института эпидемиол. микробиол. им. Пастера (Ленинград)*, 8–9 (1965).

(Polotsky, Yu. E., Arbuzova, V. A.) Полоцкий, Ю. Е., Арбузова, В. А.: Особые варианты кишечной палочки в эксперименте на животных. *Труды Ленинградского общества патологоанатомов*, **7,** 145–150 (1966).

(Polotsky, Yu. E., Arbuzova, V. A.) Полоцкий, Ю. Е., Арбузова, В. А.: Экспериментальная инфекция лабораторных животных, вызываемая энтеропатогенной кишечной палочкой O124 : K72(B17). I. Изучение инфекционного процесса у белых мышей при интраназальном заражении. *Ж. микробиол. (Москва)*, **1,** 61–65 (1967).

(Polotsky, Yu. E., Arbuzova, V. A., Ariel, B. M.) Полоцкий, Ю. Е., Арбузова, В. А., Ариэль, Б. М.: Сравнительный анализ биологических кишечных палочек в опытах интраназального заражения белых мышей. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **33,** 17–29 (1968).

(Polotsky, Yu. E., Arbuzova, V. A., Vasser, N. R.) Полоцкий, Ю. Е., Арбузова, В. А., Вассер, Н. Р.: Сравнительный анализ биологических свойств энтеропатогенных кишечных палочек. В кн.: *Микробиология, иммунология, природная очаговость болезней человека, ИЭМ АМН СССР им. Н. Ф. Гамалеи,* Москва. 114–115 (1966).

(Polotsky, Yu. E., Dragunskaya, E. M., Snigirevskaya, E. S., Voino-Yasenetsky, M. V.) Полоцкий, Ю. Е., Драгунская, Е. М., Снигиревская, Е. С., Войно-Ясенецкий, М. В.: Сравнительное изучение патогенных свойств разных возбудителей кишечных инфекций. *Вестник Акад. мед. наук СССР*, **1,** 8–14 (1976).

(Polotsky, Yu. E., Snigirevskaya, E. S., Dragunskaya, E. M.) Полоцкий, Ю. Е., Снигиревская, Е. С., Драгунская, Е. М.: Электронномикроскопическое изучение взаимодействия энтеротоксигенных кишечных палочек с кишечным эпителием. *Бюлл. экспер. биол.*, **6,** 110–113 (1974*a*).

(Polotsky, Yu. E., Snigirevskaya, E. S., Dragunskaya, E. M.) Полоцкий, Ю. Е., Снигиревская, Е. С., Драгунская, Е. М.: Электронномикроскопические данные о способе проникновения шигелл в эпителиальные клетки кишечника. *Бюлл. экспер. биол.*, **2,** 110–114 (1974*b*).

(Polotsky, Yu. E., Vasser, N. R.) Полоцкий, Ю. Е., Вассер, Н. Р.: Экспериментальный кератоконъюнктивит, вызванный энтеропатогенной кишечной палочкой O124 : K72(B17). *Авторефераты Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, 9–13 (1966*a*).

(Polotsky, Yu. E., Vasser, N. R.) Полоцкий, Ю. Е., Вассер, Н. Р.: Результаты энтерального заражения морских свинок энтеропатогенной кишечной палочкой O124 : K72 (B17). *Авторефераты Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, 14–16 (1966*b*).

(Polotsky, Yu. E., Vasser, N. R.) Полоцкий, Ю. Е., Вассер, Н. Р.: Экспериментальная кишечная инфекция, вызванная энтеропатогенной кишечной палочкой 0124 : K72 — возбудителем дизентериеподобных заболеваний взрослых и детей. *Бюлл. экспер. биол.*, **2,** 20–23 (1970*a*).

(Polotsky, Yu. E., Vasser, N. E.) Полоцкий, Ю. Е., Вассер, Н. Р.: Экспериментальный энтероколит морских свинок, вызванный энтеропатогенной кишечной палочкой 0124 : K72(B17). *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **36,** 156–166 (1970*b*).

(Polotsky, Yu. E., Vasser, N. R., Dragunskaya, E. M.) Полоцкий Ю. Е., Вассер Н. Р., Драгунская, Е. М.: Экспериментальный кератоконъюнктивит, вызванный энтеропатогенными кишечными палочками (O124, O143, O28*a*28*c*, O136, O144) — возбудителями дизентериеподобных заболеваний у взрослых и детей. *Ж. микробиол. (Москва)*, **12,** 76–80 (1971).

(Polyansky, N. S.) Полянский, Н. С.: Материалы к уяснению иммунитета при дизентерии Флекснера. *Ж. микробиол. (Москва)*, **3,** 37–40 (1953).

(Popkova, N. F., Rylova, L. I., Beklemisheva, E. D., Shorsher, S. B., Shkrebko, V. A., Pokrovskaya, E. A.) Попкова, Н. Ф., Рылова, Л. И., Беклемишева, Е. Д., Шоршер, С. Б., Шкребко, В. А., Покровская, Е. А.: Характеристика дизентерии, вызванной шигеллами Штуцер—Шмитца. *Ж. микробиол. (Москва)*, **1,** 31–33 (1966).

Porter, P., Kenworthy, R.: Effects of *Escherichia coli* on germ-free and gnotobiotic pigs. II. Serum proteins and antibodies. *J. comp. Path.*, **80,** 233–241 (1970).

Porter, P., Kenworthy, R., Allen, W. D.: Effect of oral immunisation with *E. coli* antigens on post-weaning enteric infection in the young pig. *Vet. Rec.*, **95,** 99–111 (1974).

Porter, P., Noakes, D. E., Allen, W. D.: Secretory IgA and antibodies to *Escherichia coli* in porcine colostrum and milk and their significance in the alimentary tract of the young pig. *Immunology*, **18,** 245–257 (1970).

Prát, V., Losse, H., Koniáková, L., Ritzerfeldt, W.: Experimental ascending *Escherichia coli* pyelonephritis in the rat. *Invest. Urol.*, **8,** 311–318 (1970).

(Pritulin, P. I.) Притулин, П. И.: Деформация сальмонелл на разных стадиях инфекционного процесса. *Ж. микробиол. (Москва)*, **10,** 148 (1963).
Prokopowicz, D., Tomaszko, H.: Experimentelle Salmonellose bei Kaninchen. *Arch. Hyg.*, **152,** 357–359 (1968).
Prost, E., Reimann, H.: Food-borne salmonellosis. *Ann. Rev. Microbiol.*, **21,** 495–528 (1967).
Pryles, C. V., Glagovsky, A.: Serological characterization of *Escherichia coli*. Study in acute and recurrent urinary tract infections in infants and children. *Pediatrics*, **36,** 219–224 (1965).
(Pshenichnov, A. V.) Пшеничнов, А. В.: Роль водного фактора в развитии брюшного тифа и паратифов в уральских условиях. *Ж. микробиол. (Москва)*, **3,** 425–432 (1936).
Punyashthiti, K., Finkelstein, R. A.: Enteropathogenicity of *Escherichia coli*. I. Evaluation of mouse intestinal loops. *Infect. Immunity*, **4,** 473–478 (1971).
Rácz, P.: Morphology and pathogenesis of experimental shigella keratoconjunctivitis. Thesis. Leningrad–Budapest (1963).
Rácz, P., Serény, B.: Early changes in keratoconjunctivitis shigellosa. *Morph.* and *Ig. Orv. Szemle*, **2,** 272–279 (1962).
Rácz, P., Serény, B., Oláh, I.: Kísérleti Shigellosisok pathomorfológiája, különös tekintettel a keratoconjunctivitis shigellosára. (Pathomorphology of experimental shigellosis especially of shigella keratoconjunctivitis). *László Kórház évkönyve*. Budapest (1960).
Rácz, P., Tenner, K., Lennert, K., Serény, B.: Zur Morphologie und Pathogenese der menschlichen Bazillenruhr. *Virchows Arch. path. Anat., Abt. A*, **358,** 309–319 (1973).
Rácz P., Tenner K., Mérő E.: Experimentelle Cystitis listeriosa. *Virchows Arch. path. Anat., Abt. B.*, **8,** 96–105 (1971).
Rácz, P., Tenner, K., Mérő, R.: Experimental listeria enteritis. *Lab. Invest.*, **26,** 694–700 (1972).
Rácz, P., Tenner, K., Szívessy K.: Electron microscopic studies in experimental keratoconjunctivitis listeriosa. I. Penetration of *Listeria monocytogenes* into corneal epithelial cells. *Acta microbiol. Acad. Sci. hung.* **17,** 221–236 (1970).
Raczynsky, J.: Untersuchungen über die Ätiologie der Dysenterie mit Berücksichtigung von zwei Epidemien in Galizien im Jahre 1903. *Wien. klin. Wschr.*, **33,** 897–902 (1904).
Radostits, O. M.: Coliform mastitis in cattle. *Canad. vet. J.*, **2,** 401–405 (1961).
Ralovich, B., Brasch, G., Sélley, E., Málovics, J., Papp, E.: Beitrag zum Pathomechanismus der humanen Salmonellosen. *Z. Immun.-Forsch.*, **136,** 390–403 (1968).
Ralovich, B., Emődy, L.: Observations on primary bacteriaemia. *Acta microbiol. Acad. Sci. hung.*, **16,** 153–158 (1969).
Ralovich, B., Emődy, L., Barna, K., Brasch, Gy., Marton, E., Ternak, G.: Excretion of orally administered intestinal bacteria in humans. *Zbl. Bakt. I. Abt. Orig. A.*, **226,** 82–90 (1974).
Ralovich, B., Rauss, K.: Das Schicksal der Maus-avirulenten Salmonellen bei oraler Verabreichung. *Zbl. Bakt. I. Abt. Orig.*, **211,** 315–334 (1969).
Rantasalo, I., Talanti, S., Hallmann, N.: Über die Pathogenität der *Escherichia coli* O26 : B6. *Ann. Med. exp. Fenn.*, **33,** 378–383 (1955).
Rantz, L. A.: Serological grouping of *Escherichia coli*. Study in urinary tract infection. *Arch. intern. Med.*, **109,** 37–42 (1962).
Rauss, K.: *Dysenteria*. Művelt Nép, Budapest (1955).
Rauss, K.: A dysenteria elleni védőoltás kilátásai (Perspectives of vaccination against dysentery). *Orv. Hetil.*, **104,** 113–117 (1963*a*).
Rauss, K.: A shigellosis bakteriológiájának, pathomechanizmusának és immunológiájának vizsgálata terén elért újabb eredmények (Recent results in the bacteriology, pathomechanism and immunology of shigellosis). *Orv. Hetil.* **104,** 481–487 (1963*b*).
Rauss, K.: Immunitás enterális fertőzésekben. Magyar Mikrobiol. Társ. IV. Kongr. (Immunity in enteral infections. 4th Congress of the Hungarian Microbiological Association). Budapest (1964).
Rauss, K.: Infektionsvorgang und Immunität bei Enterobacteriaceae-Infektionen. In: *Enterobacteriaceae-Infectionen*. Ed. by J. Sedlák und H. Rische. 2. Aufl., Leipzig. pp. 71–96 (1968).

Rauss, K., Kétyi, I.: The rules of homologous and heterologous immunity in the *Sh. flexneri* group. *Acta physiol. Acad. Sci. hung.* **3**, 415 (1952*a*).
Rauss, K., Kétyi, I.: The significance of antibacterial and antiendotoxic effect in dysentery (Flexner) and typhoid immunity. *Acta physiol. Acad. Sci. hung.*, **3**, 619 (1952*b*).
Rauss, K., Kétyi, I.: Immunity in subjects recovered from dysentery. *Acta microbiol. Acad. Sci. hung.*, **2**, 401–407 (1955).
Rauss, K., Kétyi, I.: Beiträge zur antagonistischen Wirkung der Coli-Flora des Darmkanals. *Zbl. Bakt. I. Abt. Orig.*, **177**, 161–175 (1960).
Rauss, K., Kétyi, J., Angyal, T.: Recent results in oral shigella infection of mice. *Proc. 4th. Congr. Hung. Assoc. Microbiol. (Budapest)*, 227–230 (1966*a*).
Rauss, K., Kétyi, I., Angyal, T.: Experimental shigellosis in mice. *Path. Microbiol.*, **29**, 95–110 (1966*b*).
Rauss, K., Kétyi, J., Angyal, T.: Studies on the virulence of *Shigella flexneri*. *Acta microbiol. Acad. Sci. hung.* **14**, 131–142 (1967).
Rauss, K., Verténvi, A.: A new *Escherichia coli* serotype identical in antigenic structure with type 4b of *Shigella flexneri*. *Acta microbiol. Acad. Sci. hung.*, **3**, 307–314 (1956).
Ravin, H. A., Fine, J.: Biological implications of intestinal endotoxins. *Fed. Proc.*, **21**, 65–68 (1962).
Ravin, H. A., Rowley, D., Jenkins, C., Fine, J.: On the absorption of bacterial endotoxin from the gastro-intestinal tract of the normal and shocked animal. *J. exp. Med.*, **112**, 783–792 (1960).
Raynaud, M.: Au sujet de la substance toxique neurotrope du bacille typhique. *Ann. Inst. Pasteur*, **72**, 691–695 (1946); **73**, 191–195 (1947).
Reade, P. C., Bateman, R.: The effect of thorotrast and the resistance of mice to experimental infection with *Escherichia coli* and *Salmonella typhimurium*. *J. Infect. Dis.*, **120**, 471–479 (1969).
Rédey, B.: Magyar Mikrobiol. Társ. nagygyűlése (Conference of the Hungarian Microbiological Association). Budapest (1957).
Rédey, B.: Magyar Mikrobiol. Társ. I. Kongr. (1st Congress of the Hungarian Microbiological Association). Budapest (1960).
Rédey, B.: Magyar Mikrobiol. Társ. IV. Kongr. (4th Congress of the Hungarian Microbiological Association). Budapest (1964*a*).
Rédey, B.: Az enteropathogen *Escherichia coli* baktériumok csoportosítása. Előadás a Magyar Fertőző Szakcsoport ülésén (Classification of enteropathogenic *E. coli* bacteria. Lecture). Budapest (1964*b*).
Rédey, B.: Tengerimalac-keratoconjunctivitist okozó *E. coli* törzsek. Magyar Hygiénikus Társaság Nemzeti Kongresszusa (*E. coli* strains causing keratoconjunctivitis in guinea pigs. National congress of the Hungarian Hygienic Association). Budapest (1970). Cit. by Bakács et al. (1970).
Rédey, B., Csizmazia, F.: The detection of unknown enteric pathogens by conjunctival infection of guinea pigs. *Acta microbiol. Acad. Sci. hung.*, **7**, 11–18 (1960).
Reed, W.: An investigation into the so-called typhoid nodules in the liver in abdominal typhus. *Amer. J. med. Sci.*, **110**, 543–560 (1895).
Rees, T. A.: Studies on *Escherichia coli* of animal origin. I. *Escherichia coli* from natural outbreaks of colibacillosis of calves. *J. comp. Path.*, **68**, 388–398 (1958).
Rees, T. A.: Studies on *Escherichia coli* of animal origin. III. *Escherichia coli* serotypes from pigs. *J. comp. Path.*, **69**, 334–338 (1959).
Reher, H.: Zur Ätiologie des Abdominaltyphus. *Arch. exp. Path. Pharmakol.*, **19**, 420–432 (1885).
Reimann, H. A.: Microbial phagocytosis by enteric epithelial cells. *J. Amer. med. Assoc.*, **192**, 1130–1132 (1965).
Reinert, M., Piroth, M., Höer, P. W., Görsch, H.: Morphologische Veränderungen an den inneren Organen der Ratte im standardisierten reversiblen und irreversiblen Schock. *Virchows Arch. path. Anat.*, **338**, 21–29 (1964).
Rejnek, J., Trávniček, J., Kostka, J., Šterzl, J., Lanc, A.: Study of the effect of antibody in the intestinal tract of germ-free baby pigs. *Folia microbiol. (Praha)*, **13**, 36–42 (1968).
Rejniak, L., Prokopowicz, D., Kurasz, S.: Morphological changes in the course of experimental salmonellosis in rabbits. *Acta morph. Acad. Sci. hung.*, **18**, 155–165 (1970).

Remlinger, P.: Fièvre typhoïde expérimental par contamination alimentaire. *Ann. Inst. Pasteur*, **11**, 829–836 (1897).
Renk, W.: Mastitiden bei Infektionen mit *Escherichia coli*, *Aerobacter aerogenes* und *Klebsiella*. *Zbl. Vet.-Med.*, **2**, 264–281 (1962).
Report of the Enterobacteriaceae Subcommittee of the Nomenclature Committee of the International Association of Microbiological. *Intern. Bull. bact. Nomencl. Taxonomy*, **8**, 25–70 (1958).
Rho, J. M., Josephson, J. E.: Epidemic enteropathogenic *Escherichia coli*, Newfoundland, 1963. Autopsy study of 16 cases. *Canad. med. Ass. J.*, **96**, 392–398 (1967).
Ribbert: Über das Vorkommen von Spaltpilzen in der normalen Darmwand des Kaninchens. *Dtsch. med. Wochenschr.*, **26**, 197–198 (1885).
Rifaat, M. K., Jseri, O. A., Gottlieb, L. S.: An ultrastructural study of the "extraneous coat" of human colonic mucosa. *Gastroenterology*, **48**, 593–601 (1965).
Rigdon, R. H., Leff, W. A.: A gastrointestinal lesion associated with staphylococci infection in man: its production in the rabbit by intravenous infection of staphylococcus toxin. *Arch. Path.*, **21**, 298–307 (1936).
Riley, H. D.: Antibiotic therapy in neonatal enteric disease. *Ann. N. Y. Acad. Sci.*, **176**, 360–370 (1971).
Roantree, R. J.: The relationship of lipopolysaccharide structure to bacterial virulence. In: *Microbial Toxins*. Ed. by S. Kadis, G. Weinbaum, S. Ajl. N. Y. vol. **5**, pp. 1–37 (1971).
Robbins, S. L.: *Pathology*. 3rd ed., Saunders, Philadelphia–London. (1967).
Roberts, D. M., Themann, H., Knust, F. J., Preston, F. E., Donaldson, J. R.: An electron-microscope study of bacteria in two cases of Whipple's disease. *J. Path.*, **100**, 249–255 (1970).
Roelke, K.: *Die Kruse-Sonne (E)-Ruhr*. Wissenschaftl. Verlagsanstalt, Stuttgart (1943).
Rosenthal, L.: Zur Ätiologie der Dysenterie. *Dtsch. med. Wschr.*, **29**, 97–98 (1903).
Ross, C. A.: Cardiovascular responses of unanesthetized rats during traumatic and endotoxin shock. *Proc. Soc. Exp. Biol.*, **96**, 582–587 (1957).
Rössle, R.: Zur Theorie des Typhus abdominalis. *Sitzgsber. dtsch. Akad. Wiss., Math-naturwiss. Kl.*, Nr. I, 3—25 (1948)
Rous, P., Jones, F. S.: The protection of pathogenic microorganisms by living tissue cells. *J. exp. Med.*, **23**, 601–612 (1916).
Rowe, B.: Salmonellosis in simians and other non-human primates. In: *Hazards of Handling Simians*. London. pp. 63—75 (1969).
Rowe, B.: *Escherichia coli* O148 and diarrhoea in adults. *Brit. med. J.*, **3**, 741 (1974).
Rowe, B., Gross, R. J., Allen, H. A.: Enteropathogenic *E. coli* O124 in the United Kingdom. *Lancet*, **1**, 224 (1974).
Rowe, B., Taylor, J., Bettelheim, K. A.: An investigation of travellers' diarrhoea. *Lancet*, **1**, 1–5 (1970).
Rowley, D.: The virulence of strains of *B. coli* for mice. *Brit. J. exp. Path.*, **35**, 528–538 (1954).
Rowley, D.: Stimulation of natural immunity to *E. coli* infections. Observations on mice. *Lancet*, **268**, 232–234 (1955).
Rowley, D.: Endotoxins and bacterial virulence. *J. infect. Dis.*, **183**, 317–327 (1971).
Rowley, D.: Specific immune antibacterial mechanisms in the intestine of mice. *Amer. J. clin. Nutr.*, **27**, 1417–1423 (1974).
(Rubashkina, B. K.) Рубашкина, Б. К.: Клинико-бактериологические наблюдения за сменой типов и видов возбудителя у больных дизентерией, леченных вакциной. *Ж. микробиол. (Москва)*, **3**, 21–24 (1953).
Rubenstein, H. S., Fine, Y., Coons, A. H.: Localization of endotoxin in the walls of the peripheral vascular system during lethal endotexemia. *Proc. Soc. exp. Biol.*, **3**, 458–467 (1962).
Rubin, C. E., Dobbins, W. O.: Peroral biopsy of the small intestine. *Gastroenterology*, **49**, 676–697 (1965).
Rubin, W.: The epithelial "membrane" of the small intestine. *Amer. J. clin. Nutr.*, **24**, 45–64 (1971).
(Rubinshtein, P. L., Skavronskaya, A. G.) Рубинштейн, П. Л., Скавронская, А. Г.: К вопросу о патогенезе острой бактериальной дизентерии. *Ж. микробиол. (Москва)*, **4**, 3–8 (1950).

(Rubtsov, I. V., Belaya, Yu. A., Melnik, E. G., Bershi, E. I.) Рубцов, И. В., Белая, Ю. А., Мельник, Е. Г., Берши, Е. И.: К вопросу о специфической вакцинации против дизентерии живой аттенуированной вакциной. *Ж. микробиол. (Москва)*, **7,** 58–63 (1970).

Rundell, J. O., Lecce, J. G.: Independence of intestinal epithelial cell turnover from cessation of absorption of macromolecules (closure) in the neonatal mouse, rabbit, hamster and guinea pig. *Biol. Neonat. (Basel)*, **20,** 51–57 (1972).

Ruskin, J., McIntosh, J., Remington, J. S.: Studies on the mechanisms of resistance to phylogenetically diverse intracellular organisms. *J. Immunol.* **103,** 252–259 (1969).

Rutter, J. M., Anderson, J. C.: Experimental neonatal diarrhoea caused by an enteropathogenic strain of *Escherichia coli* in piglets: a study of the disease and the effect of vaccinating the dam. *J. med. Microbiol.*, **5,** 197–210 (1972).

Rutter, J. M., Jones, G. W.: Protection against enteric disease caused by *Escherichia coli* — a model for vaccination with a virulence determinant? *Nature (Lond.)*, **242,** 531–532 (1973).

Sack, R. B., Gorbach, S. L., Banwell, J. G., Jacobs, B., Chatterjee, B. D., Mitra, R. C.: Enterotoxigenic *Escherichia coli* isolated from patients with severe cholera-like diseases. *J. infect. Dis.*, **123,** 378–385 (1971).

Sack, R. B., Jacobs, B., Mitra, R.: Antitoxin responses to infections with enterotoxigenic *Escherichia coli*. *J. infect. Dis.*, **129,** 330–335 (1974).

Sacks, A.: Report on investigation into characteristics of new types of non-mannitol-fermenting bacilli isolated from cases of bacillary dysentery in India and Egypt. *J. roy. Army med. Cps.*, **80,** 92–99 (1943).

(Safonova, N. V.) Сафонова, Н. В.: К биологической характеристике энтеропатогенной кишечной палочки серологической группы O1 и обоснованию ее этиологической роли в развитии острых кишечных заболеваний взрослых и детей. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **40,** 109–116 (1973).

(Safronov, A. F., Filippovich, Yu. V.) Сафронов, А. Ф., Филиппович, Ю. В.: К вопросу об использовании культур клеток для выявления патогенности кишечных палочек. *Ж. микробиол. (Москва)*, **1,** 81–82 (1966).

Sainte-Marie, G.: Paraffin embedding technique for studies employing immunofluorescence. *J. Histochem. Cytochem*, **10,** 250–256 (1962).

Saito, K., Akiyama, T., Nakano, M., Ushiba, D.: Interaction between *Salmonella enteritidis* and tissue cultured macrophages derived from immunized animals. *Japan. J. Microbiol.*, **4,** 395–407 (1960).

Saito, K., Ohno, S., Akiyama, T.: The growth of *Salmonella enteritidis* organisms within cultivated mononuclear phagocytes in relation to their virulence. *Jap. J. Bact.*, **12,** 937–947 (1957).

Sakazaki, R., Namioka, S.: Studies on a new *Escherichia coli* type: O136 : K78(B22) : —. *Japan. J. exp. Med.*, **27,** 411–416 (1957).

Sakazaki, R., Tamura, K., Nakamura, A.: Further studies on enteropathogenic *Escherichia coli* associated with diarrhoeal diseases in children and adults. *Japan. J. med. Sci. Biol.*, **27,** 7–18 (1974*a*).

Sakazaki, R., Tamura, K., Nakamura, A., Kurata, T., Gohda, A., Takeuchi, S.: Enteropathogenicity and enterotoxigenicity of human enteropathogenic *Escherichia coli*. *Japan, J. med. Sci. Biol.*, **27,** 19–33 (1974*b*).

Sakazaki, R., Tamura, K., Nakamura, A., Kurata, T.: Enteropathogenic and enterotoxigenic activities on ligated loops in rabbits of *Salmonella* and some other Enterobacteria isolated from human patients with diarrhea. *Japan. J. med. Sci. Biol.*, **27,** 45–48 (1974*c*).

Sakazaki, R., Tamura, K., Prescott, L. M., Bencic, Z., Sanyal, S. C., Sinha, R.: Bacteriological examination of diarrheal stools in Calcutta. *Indian J. med. Res.*, **59,** 1025–1034 (1971).

Sakazaki, R., Tamura, K., Saito, M.: Enteropathogenic *Escherichia coli* associated with diarrhea in children and adults. *Japan. J. med. Sci. Biol.*, **20,** 387–399 (1967).

Sanarelli, G.: *Les entéropathies microbiennes*. Masson, Paris (1926).

Sanarelli, J.: Études sur la fièvre typhoïde expérimentale. *Ann. Inst. Pasteur*, **8,** 193–230 (1894).

Sandritter, W.: Shock-induced lesions of the small intestines in experimental animals. In: *The Small Intestine*. Ed. by A. C. Thockray and F. A. Jones. Oxford. pp. 87–97 (1965).

Sandritter, W., Heymann, G.: Ein Beitrag zur Morphologie der akuten Vergiftung mit bakteriellen Toxinen und Endotoxinen. *Frankfurt. Z. Path.*, **64**, 285–293 (1953).

Sanford, J. P., Noyes, H. E.: Studies on the absorption of *Escherichia coli* endotoxin from the gastrointestinal tract of dogs in the pathogenesis of "irreversible" hemorrhagic shock. *J. clin. Invest.*, **37**, 1425–1435 (1958).

Saphra, J., Winter, J. W.: Clinical manifestations of salmonellosis in man. *New Engl. J. Med.*, **256**, 1128–1134 (1957).

(Sapozhnikova, V. A., Arbuzova, V. A., Safonova, N. V., Krivonosova, K. I., Bartysheva, V. A., Kasatkina, E. N.) Сапожникова, В. А., Арбузова, В. А., Сафонова, Н. В., Кривоносова, К. И., Бартышева, В. А., Касаткина, Э. Н.: К вопросу об этиологии и эпидемиологии так называемой клинической дизентерии. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **36**, 24–31 (1970).

(Sarkisov, D. S., Remezov, P. I.) Саркисов, Д. С., Ремезов, П. И.: *Воспроизведение болезней человека в эксперименте*. Медгиз, Москва (1960).

Sato, J., Tanaka, T., Saito, K., Mitsushashi, S.: Inhibition of *Salmonella enteritidis* ingested in mononuclear phagocytes from liver and subcutaneous tissue of mice immunized with live vaccine. *J. Bact.*, **83**, 1306–1312 (1962).

Savage, D. C.: Localization of certain indigenous microorganisms on the ileal villi of rats. *J. Bact.*, **97**, 1505–1506 (1969).

Savage, D. C.: Associations of indigenous microorganisms with gastrointestinal mucosal epithelia. *Amer. J. clin. Nutr.*, **23**, 1495–1501 (1970).

Savage, D. C.: Survival on mucosal epithelia, epithelial penetration and growth in tissue of pathogenic bacteria. In: *Microbial Pathogenicity in Man and Animals. Symp. Soc. Gen. Microbiol.*, **22**, 25–57 (1972).

Savage, D. C., Blumershine, R. V. H.: Surface-surface associations in microbial communities populating epithelial habitats in the murine gastrointestinal ecosystem: scanning electron microscopy. *Infect. Immunity*, **10**, 240 (1974).

Savage, D. C., Dubos, R., Schaedler, R. W.: The gastrointestinal epithelium and its autochtonous bacterial flora. *J. exp. Med.*, **127**, 67–76 (1968).

Savage, D. C., McAllister, J. S., David, C. P.: Anaerobic bacteria on the mucosal epithelium of the murine large bowel. *Infect. Immun.*, **4**, 492–502 (1971).

Savage, W. G.: Experimental evidence of a heat-resistant gastro-intestinal irritant produced by bacilli of the Salmonella group. *J. Hyg.*, **33**, 233–244 (1933).

Schaedler, R. W., Dubos, R.: Relationship of intestinal flora to resistance. In: *Bacterial Endotoxins*. Ed. by M. Landy, W. Braun. Quinn-Boden, Rayway, New Jersey. pp. 390–396 (1964).

Schmidt, M. B.: Über Typhus abdominalis. *Zbl. allg. Path., path. Anat.*, **18**, 598–602 (1907).

Schmidt, N. J., Lennete, E. H.: Chick embryo technics. In: *Viral and Rickettsial Infections of Man*. Ed. by F. L. Horsfall, J. Tamm. Philad.–Montreal. pp. 1202–1208 (1965).

Schneider, H., Falkow, S.: Characterization of an Hfr strain of *Shigella flexneri*. *J. Bact.*, **88**, 682–689 (1964).

Scholtens, R. T.: Een onbekend type dysenteriebacterie als oorzaak eener epidemie. *Nederl. Tijdschr. Geneesk.*, **84**, 4613–4621 (1940).

Scholtysek, E.: Neue Einblicke in die Parasit-Wirt-Beziehungen mit Hilfe der Elektronenmikroskopie. *Z. Parasitenk.*, **31**, 67–84 (1968).

Schottmüller, H.: Zur Pathogenese des Typhus abdominalis. *Münch. med. Wschr.*, **38**, 1561–1565 (1902).

Schroeder, S. A., Caldwell, J. R., Vernon, T. M., White, P. C., Grauger, S. I., Bennett, J. V.: A waterborne outbreak of gastroenteritis in adults associated with *Escherichia coli*. *Lancet*, **1**, 737–740 (1968).

Sedan, G., Herrman, R.: Note sur l'infection de la cornée par le bacille d'Eberth. *Compt. rend. Soc. biol. et fil.*, **90**, 293–294 (1924).

Sedlák, J., Rische, H.: Escherichia. In: *Enterobacteriaceae-Infectionen*. Ed .by J. Sedlák, H. Rische. G. Thieme, Leipzig. pp. 277–310 (1961).

Seeliger, H.: Ein wenig bekannter Escherichia-Typ als wahrscheinliche Ursache ruhrähnlicher Erkrankungen. *Z. Hyg. Infekt.-Kr.* **135**, 526–535 (1952).
Seeliger, H.: Untersuchungen über die O-Antigene der *Escherichia-coli*-Gruppen O28, O42 und des pathogenen "Katwijk" Typs. *Z. Hyg. Infekt.-Kr.*, **139**, 55–60 (1954).
Seeliger, H.: Antigenverwandtschaft zwischen *Shigella flexneri* Typ 5 und einer neuen *Escherichia coli* — O — Gruppe 129. *Zbl. Bakt. I. Abt. Orig.*, **163**, 7–12 (1955).
Seligmann, E., Saphra, I., Wassermann, M.: Salmonella infection in man — An analysis of 1,000 cases bacteriologically identified by the New York Salmonella Center. *Amer. J. Hyg.*, **38**, 226–249 (1943).
Selter, H.: Über die Dysenteriegifte. *Z. Immun.-Forsch.*, **5**, 458–504 (1910).
Selye, H.: *The story of the adaptation syndrome.* Montreal (1950).
(Seppi, I. V.) Сеппи, И. В.: *Бактериальная дизентерия.* Медгиз, Москва (1963).
Serény, B.: Új állatkísérleti modell a dizentéria problémáinak kutatására. Magyar Mikrobiológiai Társaság ülése (New experimental model for the study of dysentery. Conference of the Hungarian Microbiological Association). Budapest (1954) .
Serény, B.: Experimental keratoconjunctivitis shigellosa. Thesis. Budapest. (1955*a*).
Serény, B.: Experimental Shigella keratoconjunctivitis. *Acta microbiol. Acad. Sci. hung.*, **2**, 293–296 (1955*b*).
Serény, B.: Experimental keratoconjunctivitis shigellosa. *Acta microbiol. Acad. Sci. hung.*, **4**, 367–376 (1957).
Serény, B.: Aetiotropic treatment of experimental shigellosis. *Acta microbiol. Acad. Sci. hung.*, **5**, 179–191 (1958*a*).
Serény, B.: Acquired natural immunity following recovery from keratoconjunctivitis shigellosa. *Acta microbiol. Acad. Sci. hung.*, **5**, 343–358 (1958*b*).
Serény, B.: A new method for the investigation of *Sh. sonnei* cultures. *Acta microbiol. Acad. Sci. hung.*, **6**, 179–189 (1959*a*).
Serény, B.: *Sh. sonnei* cultures fermenting salicin. *Acta microbiol. Acad. Sci. hung.*, **6**, 217–225 (1959*b*).
Serény, B.: Keratokonjunctivitis shigellosa. *Zbl. Bakt. I. Abt. Orig.*, **178**, 325–331 (1960*a*).
Serény, B.: Keratoconjunctivitis shigellosa. In: *Az Országos Közegészségügyi Intézet működése az 1958. évben* (Yearbook of the National Institute for Public Health, 1958). Budapest, 128–137 (1960*b*).
Serény, B.: The dissociation of *Shigella sonnei* on a culture medium containing desoxycholic acid. *Acta microbiol. Acad. Sci. hung.*, **7**, 51–56 (1960*c*).
Serény, B.: Impressions of *Sh. sonnei* colonies on agar culture media. *Acta microbiol. Acad. Sci. hung.*, **7**, 277–284 (1960*d*).
Serény, B.: Shigella törzsek szerotípusának rutinszerű meghatározása (Routine determination of shigella strain serotype). *Egészségtudomány*, **5**, 226–235 (1961*a*).
Serény, B.: Variants of *Sh. sonnei* phase I. *Acta microbiol. Acad. Sci. hung.*, **8**, 159–172 (1961*b*).
Serény, B.: A szerotípus ismeretének jelentősége a dizentéria elleni küzdelemben (Importance of serotype in the treatment of dysentery). *Egészségtudomány*, **6**, 34–42 (1962*a*).
Serény, B.: Gyorsított széklettenyésztési vizsgálat Shigella kimutatására (Rapid faecal culture method for demonstration of shigellae). *Egészségtudomány*, **6**, 274–282 (1962*b*).
Serény, B.: A new method for the measurement of protective potency of dysentery vaccines. *Acta microbiol. Acad. Sci. hung.*, **9**, 55–60 (1962*c*).
Serény, B.: Biochemical reactions and virulence of *Escherichia coli* O124 : K72(B17). *Acta microbiol. Acad. Sci. hung.*, **10**, 11–18 (1963).
Serény, B.: Studies on the pathogenicity of shigellae. *Annal. Immunol. Hung.*, **9**, 261–266 (1966).
(Sergeev, V. V., Mikhailov, I. F., Gleiberman, S. E., Yuditskaya, N. M., Kavtaradze, K. N.) Сергеев, В. В., Михайлов, И. Ф., Глейберман, С. Е., Юдицкая, Н. М., Кавтарадзе, К. Н.: Иммуногенные свойства живой лиофилизированной вакцины в опыте на обезьянах. *Ж. микробиол. (Москва)*, **11**, 26–32 (1968).
(Sergeev, V. V., Taratorina, O. M., Elkina, S. I.) Сергеев, В. В., Тараторина, О. М., Елкина, С. И.: Изучение стрептомицинзависимых мутантов шигелл. *Ж. микробиол. (Москва)*, **9**, 28–32 (1967).

(Sergeevich, E. A.) Сергеевич, Е. А.: О получении экспериментальной модели бактериальной дизентерии на домашних животных. *Ж. микробиол. (Москва)*, **4,** 71–75 (1954).

(Serzhanina, A. N.) Сержанина, А. Н.: Пневмонии. В кн.: Гулькевич Ю. В. — *Перинатальные инфекции*. Беларусь, Минск. 64–82 (1966).

(Shakhnazarova, I. E.) Шахназарова, И. Э.: Морфологическое исследование стенки кишечника мышей, зараженных *Giardia lamblia*. *Мед. паразитол.*, **31,** 694–697 (1962).

(Shantarenko, I. V.) Шантаренко, И. В.: Изучение роли аллергического компонента в патогенезе дизентерии. *Ж. микробиол. (Москва)*, **10,** 141–145 (1959).

(Shantarenko, I. V., Tesla, Z. S.) Шантаренко, И. В., Тесля, З. С.: Некоторые вопросы дизентерии на модели эксперментального дизентерийного кератоконъюнктивита у морских свинок. *Ж. микробиол. (Москва)*, **1,** 71–75 (1960).

(Sharapova, T. A.) Шарапова, Т. А.: Характеристика взаимодействия энтеропатогенных и банальных эшерихий с клеточными культурами почек эмбриона человека. *Ж. микробиол. (Москва)*, **5,** 80–83 (1968).

(Sharapova, T. A., Gavrilyuk, B. K.) Шарапова, Т. А., Гаврилюк, Б. К.: Дифференциация энтеропатогенных и непатогенных кишечных палочек в культурах. *Ж. микробиол. (Москва)*, **8,** 94–96 (1963).

Sharp, G. W. G., Hynie, S.: Stimulation of intestinal adenyl cyclase by cholera toxin. *Nature, (London)*, **229,** 266–269 (1971).

(Shastina, G. V.) Шастина, Г. В.: Пневмония, вызванная кишечными палочками. Автореферат дисс. канд. Ленинград. (1966).

(Shastina, G. V., Leontyev, V. Ya.) Шастина, Г. В., Леонтьев, В. Я.: Сочетанные поражения кишечника при дизентерии у детей. *Арх. пат. (Москва)*, **6,** 39–43 (1970).

(Shastina, G. V., Mikhaylova, E. N.) Шастина, Г. В., Михайлова, Е. Н.: Изменения легких у белых мышей при заражении их кишечными палочками. *Бюлл. экспер. биол.*, **6,** 111–118 (1967).

Shaughnessy, H. J., Milzer, A., Neal, J., Levinson, S. O.: Production of potent inactivated vaccines with ultraviolet irradiation. IV. Vaccination against bacillary dysentery. *J. infect. Dis.*, **78,** 69–78 (1946*a*).

Shaughnessy, H. J., Olsson, R. C., Rass, K., Friewer, F., Levinson, S. O.: Experimental human bacillary dysentery. Polyvalent dysentery vaccine in its prevention. *J. Amer. med. Assoc.*, **132,** 362–368 (1946*b*).

Shepard, C. C.: Nonacid-fast bacteria and HeLa cells: their uptake and subsequent intracellular growth. *J. Bact.*, **77,** 701–714 (1959).

Sherr, H. P., Banwell, J. G., Rothfeld, A., Hendrix, T. R.: Pathophysiological response of rabbit jejunum to *Escherichia coli* enterotoxin. *Gastroenterology*, **65,** 895–902 (1973).

(Shestopalova, N. M., Avakyan, A. A., Reingold, V. N., Tkal, V. V.) Шестопалова, Н. М., Авакян, А. А., Рейнгольд, В. Н., Ткаль, В. В.: Электронномикроскопическое изучение пластических процессов кишечного эпителия некоторых млекопитающих. *Арх. анат. (Ленинград)*, **3,** 34–44 (1960).

(Shifrin, A. I., Khavkin, T. N.) Шифрин, А. И., Хавкин, Т. Н.: К вопросу о реактогенности Ку-вакцины. В кн.: *Вопросы инфекционной патологии. ИЭМ АМН СССР*, Ленинград. 92–96 (1962).

Shiga, K.: Über den Disenteriebacillus (Bacillus dysenteriae). *Zbl. Bakt., I. Abt.*, **24,** 817–828, 870–874, 913–918 (1898).

Shmilovitz, M.: Anaerogenic serotype 147 as an aetiologic agent of dysentery. *Ann. Inst. Pasteur*, **119,** 446–455 (1970).

(Shneerson, F. S., Ivanova, A. A., Livshits, E. B.) Шнеерсон, Ф. С., Иванова, А. А., Лившиц, Е. Б.: О роли дизентерийных возбудителей в этиологии детских поносов. *Ж. микробиол. (Москва)*, **5–6,** 181–183 (1941).

Shnitka, T. K.: Current concepts on the pathogenesis and pathology of inflammatory lesions in the intestine. *Canad. med. Ass. J.*, **91,** 7–22 (1964).

Shore, E. G., Dean, A. G., Holik, K. L., Davis, B. R.: Enterotoxin-producing *Escherichia coli* and diarrheal disease in adult travelers: a prospective study. *J. infect., Dis.*, **129,** 577–582 (1974).

(Shtriter, V. A., Barats, M. M.) Штритер, В. А., Барац, М. М.: Сравнительное изучение патогенных и непатогенных кишечных палочек при экспериментальном заражении белых мышей. *Тр. Ин-та вакцин и сывороток, Уфа*, **7,** 339–350 (1962*a*).

(Shtriter, V. A., Barats, M. M.) Штритер, В. А., Барац, М. М.: Опыт использования куриных эмбрионов для сравнительного изучения кишечных палочек различных серотипов. *Тр. Ин-та вакцин и сывороток, Уфа,* **7,** 351–360 (1962*b*).

(Shur, I. V.) Шур, И. В.: *Заболевания сальмонеллезной этиологии.* 2-е изд. Медицина, Москва. (1970).

(Sidorov, M. A., Polyakova, O. A.) Сидоров, М. А., Полякова, О. А.: О профилактике колибактериоза новорожденных телят. *Ветеринария,* **3,** 53–55 (1974).

Siegmund, H.: Einfache Entzündungen des Darmrohres. In: *Handbuch der spez. path. Anatomie und Histologie.* Ed. by F. Henke und O. Lubarsch. Berlin. vol. **3,** pp. 261–370 (1929).

Šikl, H.: Bakteriologische Untersuchungen am pathologisch-anatomischen Material von Bacillenruhr. *Z. Hyg.*, **90,** 337–386 (1920).

(Sinay, T.) Синай, Т.: *Вопросы профилактики и лечения дизентерии.* Медгиз, Москва. (1950).

(Sinay, T. Ya., Korshakova, A. C., Dobronitskaya, S. V.) Синай, Т. Я.: Коршакова, А. С., Доброницкая, С. В.: Проницаемость слизистой оболочки кишечника для бактерий группы Флекснера в экспериментальных условиях. *Ж. микробиол. (Москва),* **4,** 58–61 (1951).

Sinha, R., Deb, B. C., Ghosh, A. K., Shrivastava, D. L.: Virulence of *Vibrio cholerae* strains, isolated from carriers, in the rabbit ileal loop. *Indian. J. med. Res.*, **57,** 1636–1640 (1969).

(Siroko, I. A.) Сироко, И. А.: Биопроба на морской свинке при идентификации дизентерийных бактерий. *Военно-мед. ж.,* **9,** 54–56 (1957).

(Siroko, I. A.) Сироко, И. А.: Кератоконъюнктивит у морской свинки как биопроба при идентификации дизентерийных бактерий. *Ж. микробиол. (Москва),* **4,** 71–77 (1958).

(Siroko, I. A., Verkholomov, E. E.) Сироко, И. А., Верхоломов, Е. Е.: Вирулентность шигелл при длительном переживании в воде. II. *Ж. микробиол. (Москва),* **3,** 138–139 (1965).

(Sirotinin, N. N., Brodskaya, E. A.) Сиротинин, Н. Н., Бродская, Е. А.: Некоторые вопросы патогенеза дизентерии. В кн.: *Дизентерия.* Киев 57–61 (1959).

(Sirotinin, N. N., Gromashevskaya, L. L., Ovsievskaya, N. V., Botsman, N. E.) Сиротинин, Н. Н., Громашевская, Л. Л., Овсиевская, Н. В., Боцман, Н. Е.: Об экспериментальной модели дизентерийного процесса. В кн.: *Дизентерия.* Киев 62–71 (1959).

(Sirotinin N. N., Ovsievskaya, N. V., Brodskaya, E. A., Gromashevskaya, L. L.) Сиротинин, Н. Н., Овсиевская, Н. В., Бродская, Е. А., Громашевская, Л. Л.: Об экспериментальной модели дизентерийного процесса. *Ж. микробиол. (Москва),* **3,** 14–18 (1958).

Sirotinin, W.: Die Übertragung von Typhusbacillen auf Versuchstiere. *Z. Hyg.*, **1,** 465–488 (1886).

(Skvortsov, M. A.) Скворцов, М. А.: *Патологическая анатомия важнейших заболеваний детского возраста.* 4-е изд. Медгиз, Москва. (1946).

Smadel, J. E.: Intracellular infections. *Bull. N. Y. Acad. Sci.*, **39,** 158–172 (1963).

(Smirnov, B. N.) Смирнов, Б. Н.: *Антибиотикотерапия и иммунитет при паратифозной инфекции.* Медицина, Ленинград. (1966).

(Smirnova, A. M.) Смирнова, А. М.: Экспериментальные исследования паратифозной инфекции, вызванной палочками Гертнера. Автореф. дисс. канд. мед. наук Ленинград, (1950).

(Smirnova, A. M.) Смирнова, А. М.: Экспериментальные исследования паратифозной инфекции, вызванной палочкой Гертнера. *Ж. микробиол. (Москва),* **2,** 76–80 (1955).

(Smirnova, L. A.) Смирнова, Л. А.: К биологической характеристике энтеропатогенной кишечной палочки серотипа 301 серологической группы O25. I. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград),* **36,** 203–210 (1970).

(Smirnova, L. A.) Смирнова, Л. А.: К биологической характеристике эталонных культур кишечных палочек по данным легочной модели. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград),* **40,** 121–126 (1973).

(Smirnova, L. A.) Смирнова Л. А.: Материалы к биологической характеристике *Escherichia coli,* обладающих энтеротоксигенными свойствами. Дисс. канд., Ленинград, (1974).

(Smirnova, L. A., Dragunskaya, E. M.) Смирнова, Л. А., Драгунская, Е. М.: Изучение специфичности инфекционного процесса, развивающегося в легких белых мы-

шей, интраназально зараженных пассированными культурами энтеропатогенной кишечной палочки серологической группы O25 (серотип 301). *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **40,** 151–154 (1973*a*).

(Smirnova, L. A., Dragunskaya, E. M.) Смирнова, Л. А., Драгунская, Е. М.: К стабильности способности пассированных культур энтеропатогенной кишечной палочки серологической группы O25 (серотип 301) размножаться в легких интраназально зараженных мышей. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **40,** 155–158 (1973*b*).

(Smirnova, L. A., Mamushkin, V. I., Pidyak, E. T.) Смирнова, Л. А., Мамушкин, В.И., Пидяк, Е. Т.: Результаты идентификации и изучения биологических свойств штамма энтеропатогенной кишечной палочки серологической группы O32 (условное обозначение «серотип A-12»), выделенного в Алма-Ате в 1973 году. В кн.: *Эпидемиол., диагностика и профилактика кишечных, респираторных и природно-очаговых инфекций.* Ин-т эпидемиол. микробиол. им. Пастера, Ленинград. 69–71 (1975).

Smith, A. L.: *Microbiology and Pathology.* 8th ed., Mosby, St. Louis (1964).

Smith, H.: The use of bacteria grown *in vivo* for studies on the basis of their pathogenicity. *Ann. Rev. Microbiol.*, **12,** 77–102 (1958).

Smith, H.: Biochemical challenge of microbial pathogenicity. *Bact. Rev.*, **32,** 164–184 (1968).

Smith, H., Taylor, J. (ed.): *Microbiol Behaviour "in vitro" and "in vivo".* 14th Symposium of the Soc. Gen. Microbiol., Cambridge. (1964).

Smith, H. W.: Observations on the aetiology of neonatal diarrhoea (scours) in calves. *J. Path. Bact.*, **84,** 147–168 (1963).

Smith, H. W., Gyles, C. L.: The relationship between two apparently different enterotoxins produced by enteropathogenic strains of *Escherichia coli* of porcine origin. *J. Med. Microbiol.*, **3,** 387–401 (1970*a*).

Smith, H. W., Gyles, C. L.: The effect of cell-free fluids prepared from cultures of human and animal enteropathogenic strains of *Escherichia coli* on ligated intestinal segments of rabbits and pigs. *J. Med. Microbiol.*, **3,** 403–409 (1970*b*).

Smith, H. W., Halls, S.: Observations by the ligated intestinal segment and oral inoculation methods on *Escherichia coli* infections in pigs, calves, lambs and rabbits. *J. Path. Bact.*, **93,** 499–529 (1967*a*).

Smith, H. W., Halls, S.: Studies on *Escherichia coli* enterotoxin. *J. Path. Bact.*, **93,** 531–543 (1967*b*).

Smith, H. W., Jones, J. E. T.: Observations on the alimentary tract and its bacterial flora in healthy and diseased pigs. *J. Path. Bact.*, **86,** 387–412 (1963).

Smith, H. W., Linggood, M. A.: Observations on the pathogenic properties of the K88, Hly- and Ent-plasmids of *Escherichia coli* with particular reference to porcine diarrhoea. *J. Med. Microbiol.*, **4,** 467–485 (1971).

Smith, H. W., Linggood, M. A.: Further observations on *Escherichia coli* enterotoxins with particular regard to those produced by atypical piglet strains and by calf and lamb strains: the transmissible nature of these enterotoxins and of a K antigen possessed by calf and lamb strains. *J. Med. Microbiol.*, **5,** 243–250 (1972).

Smith, N. W., Sack, R. B.: Immunologic cross-reactions of enterotoxins from *Escherichia coli* and *Vibrio cholerae*. *J. infect. Dis.*, **127,** 164–170 (1973).

Smith, T.: Hydropic stages in the intestinal epithelium of new-born calves. *J. exp. Med.*, **41,** 81–88 (1925).

Smith, T., Little, R. B.: The significance of colostrum to the new-born calf. *J. exp. Med.*, **36,** 181–198 (1922).

Smith, T., Little, R. B.: Studies on pathogenic *B. coli* from bovine sources. I. The pathogenic action of culture filtrates. *J. exp. Med.*, **46,** 123–131 (1927).

Smith, T., Orcutt, M. L.: The bacteriology of the intestinal tract of young calves with special reference to early diarrhea ("scours"). *J. exp. Med.*, **41,** 89–106 (1925).

Söderlind, O.: Studies on *Escherichia coli* in pigs. II. Serological investigations. *Zbl. Vet.-Med., B,* **18,** 569–590 (1971).

Sojka, W. J.: *Escherichia coli in Domestic Animals and Poultry.* Commonwealth Agricultural Bureau, Farnham Royal, England. (1965).

(Sokolov, M. I.) Соколов, М. И.: Культивирование вирусов в куриных эмбрионах. В кн.: *Руководство по лабораторной диагностике вирусных и риккетсиозных болезней.* Ред. П. Ф. Здродовский и М. И. Соколов. Медицина, Москва. 89–96 (1965).

Sollazzo, G.: Untersuchungen an per os mit Paratyphus-bacillen infizierten Mäusen. *Z. Hyg.*, **110**, 306–323 (1929).

(Solodova, T. L.) Солодова, Т. Л.: Микрофлора при острых пневмониях. *Труды Саратов. мед. ин-та*, **65,** 88–93 (1970).

(Solovyev, M. M.) Соловьев, М. М.: Морфологические и биологические особенности лямблий в связи с их обитанием на щеточной каемке кишечного эпителия. *Acta protozool. (Warszawa)*, **6**, 365–376 (1968).

(Solovyev, V. N.) Соловьев, В. Н.: *Действие антибиотиков в тканях организма.* Медицина, Москва. (1968).

(Solovyeva, O. I., Moryasch, Ts. E., Novgorodskaya, E. M., Itsekson, B. L.) Соловьева, О. И., Морьяш, Ц. Е., Новгородская, Э. М., Ицексон, Б. Л.: К вопросу о судьбе дизентерийной палочки в инфицированном организме. *Тр. Ин-та эпидемиол. микробиол. им Пастера (Ленинград)*, **6,** (1938).

South, M. A.: IgA in neonatal immunity. *Ann. N. Y. Acad. Sci.*, **176**, 40–48 (1971).

Sprinz, H.: Morphologic response of the intestinal mucosa to enteric bacteria and its implication for sprue and asiatic cholera. *Fed. Proc.*, **21**, 57–64 (1962).

Sprinz, H.: Pathogenesis of intestinal infections. *Arch. Path.*, **87**, 556–562 (1969).

Sprinz, H., Gangarosa, E. J., Williams, M.: Histopathology of the upper small intestines in typhoid fever. Biopsy study of experimental disease in man. *Amer. J. dig. Dis.* **11**, 615–624 (1966).

Sprunt, D. H., Martin, D. S., Williams, J. F.: Intestinal pneumonia. II. *J. exp. Med.*, **62**, 449–456 (1935).

Staley, T. E., Corley, L. D., Bush, L. J.: Colibacillosis in ungulants: uptake of *Escherichia coli* and proteins by calf intestinal epithelium. In: *19th World Vet. Congress.* vol. **1**, pp. 308–311 (1970*a*).

Staley, T. E., Corley, L. D., Bush, L. J., Jones, E. W.: The ultrastructure of neonatal calf intestine and absorption of heterologous proteins. *Anat. Rec.*, **172**, 559–579 (1972*a*).

Staley, T. E., Corley, L. D., Jones, E. W.: Permeability of the intestinal epithelium of neonatal puppies to *Escherichia coli*. In: *19th Gaines Veterinary Symposium: The newer knowledge about dogs.* Gaines Dog Research Center. New York, 3–8 (1969*a*).

Staley, T. E., Corley, L. D., Jones, E. W.: Early pathogenesis of colitis in neonatal pigs monocontaminated with *Escherichia coli*. I–II. *Amer. J. dig. Dis.*, **15**, 923–952 (1970*b*).

Staley, T. E., Corley, L. D., Jones, E. W.: Malabsorption in neonatal pigs monocontaminated with *Escherichia coli* (O55B5). *Amer J. dig. Dis.*, **17**, 239–247 (1972*b*).

Staley, T. E., Jones, E. W., Corley, L. D.: Attachment and penetration of *Escherichia coli* into intestinal epithelium of the ileum in newborn pigs. *Amer. J. Path.*, **56,** 371–392 (1969*b*).

Staley, T. E., Jones, E. W., Corley, L. D.: The fine structure of the duodenal absorptive cell in the newborn pig before and after feeding of colostrum. *Amer. J. vet. Res.*, **30**, 567–581 (1969*c*).

Staley, T. E., Jones, E. W., Corley, L. D.: Intestinal monocontamination in the neonatal pig: microbial and microscopic studies. In: *Germ-Free Biology: experimental and clinical aspects.* Ed. by E. A. Mirand and N. Back. New York, pp. 65–73 (1969*d*).

Staley, T. E., Jones, E. W., Corley, L. D., Anderson, I. L.: Intestinal permeability to *Escherichia coli* in the foal. *Amer J. vet. Res.*, **31**, 1481–1483 (1970*c*).

Staley, T. E., Jones, E. W., Marschall, A. E.: The jejunal absorptive cell of the newborn pig: an electron microscopic study. *Anat. Rec.*, **161**, 497–515 (1968).

(Stasilevich, Z. K.) Стасилевич, З. К.: Экспериментальное изучение инфекции, вызванной патогенными типами кишечной палочки. В кн.: *Вопросы инфекционной патологии в эксперименте на обезьянах.* Под ред. Б. А. Лапина. Институт экспериментальной патологии и терапии АМН СССР, Сухуми. 145–158 (1958*a*).

(Stasilevich, Z. K.) Стасилевич, З. К.: Экспериментальное воспроизведение сальмонеллезной инфекции Гейдельберг. В кн.: *Вопросы инфекционной патологии в эксперименте на обезьянах.* Под ред. Б. А. Лапина. Институт экспериментальной патологии и терапии АМН СССР, Сухуми. 173–178 (1958*b*).

(Stasilevich, Z. K.) Стасилевич, З. К.: Экспериментальный сальмонеллез у обезьян. *Ж. микробиол. (Москва)*, **2,** 70–74 (1961).

Šťastná, L.: *Escherichia coli* as the aetiological agent of human diseases. In: *Proc. 5th Sci. Meet. Fac. Hyg.* 1969, Charles Univ., Prague. pp. 181–186 (1971).
Steinhaus, E. A.: *Insect Microbiology.* Comstock, New York (1947).
Steinhaus, E. A.: *Principles of Insect Pathology.* McGraw-Hill, New York, Toronto, London (1949).
Stenzel, W.: Untersuchungen über die Harnblasenpathogenität einiger Enterobacteriaceen-Gruppen im Bingelschen Versuch. *Z. Hyg. Infekt.-Kr.*, **147**, 123–132 (1960).
Stenzel, W.: Über die pathogenetische Bedeutung des thermolabilen Eiwesstoxins der *Sh. dysenteriae* 1. *Z. Hyg. Infekt.-Kr.*, **147**, 240–245 (1961*a*).
Stenzel, W.: Serologische und toxikologische Untersuchungen der Lipopolysaccharide schleimhautpathogener und apathogener Shigella-Stammpaare. *Z. Hyg. Infekt-Kr.*, **147**, 391–398 (1961*b*)
Stenzel, W.: *Esch. coli*-Shigella-Hybriden und ihre Schleimhautpathogenität. *Z. Hyg. Infekt.-Kr.*, **147**, 521–528 (1961*c*).
Stenzel, W.: Über den Einfluss der Streptomycinresistenz auf die Virulenz von Shigellen und Coli-Shigella-Hybriden. *Z. Hyg. Infekt.-Kr.*, **148**, 152–156 (1961*d*).
Stenzel W.: *Shigella scholtensii* und *Shigella manolovii* — zwei zur Untergruppe D zu stellende provisorische Shigella-Typen. *Z. Hyg. Infekt.-Kr.*, **148**, 433–444 (1962*a*).
Stenzel, W.: Untersuchungen über die Spezifizität des gewebsimmunogenen Faktors schleimhautpathogener Enterobacteriaceen. *Z. Hyg. Infekt.-Kr.*, **148**, 512–516 (1962*b*).
Stenzel, W.: Die Schleimhautpathogenität der bakteriellen Ruhrerreger im Tierversuch. *Zbl. Bakt. I. Abt. Orig.*, **187**, 195–207 (1962*c*).
Stenzel, W.: Katalase-Aktivität und Virulenz bei Shigellen. *Arch. Hyg. (Berlin)*, **147**, 172–176 (1963*a*).
Stenzel, W.: Untersuchungen über die Hybridisierung zwischen *E. coli* und *Shigella in vivo. Arch. Hyg. (Berlin)*, **147**, 444–451 (1963*b*).
Stenzel, W.: Classification of the "coliform" serotypes 792 and 147. *Intern. Bull. Bacteriol. Nomencl. Taxon.*, **14**, 15–16 (1964).
Stenzel, W.: Zur Kenntnis der Ruhrerregender *Escherichia coli* Serotypen. *Zbl. Bakter. I. Abt. Orig.*, **195**, 482–488 (1965).
Stenzel, W.: Über Beziehungen zwischen Allgemein- und Schleimhautpathogenität der Schigellen. *Path. Microbiol. (Basel)*, **29**, 236–242 (1966).
Stenzel, W.: Der Schleimhautpathogenitätsverlust der Ruhrerreger in vitro. *Zbl. Bakt. I. Abt. Orig.* **216**, 54–66 (1971*a*).
Stenzel, W.: Zur Genetik der Schleimhautpathogenität bei Ruhrerregern. *Zbl. Bakt. I. Abt. Orig.*, **216**, 67–68 (1971*b*).
Šterzl, J., Kostka, J., Mandel, L., Riha, I., Holub, M.: Development of the formation of gammaglobulin and of normal and immune antibodies in piglets reared without colostrum. In: *Mechanisms of Antibody Formation.* Prague. pp. 130–145 (1960).
Stevenson, J.: *B. coli* D 433 in cases diarrhoea in adults. *Brit. med. J.*, **2**, 195–196 (1950).
Stoerk, O.: Über Cholera. *Beitr. path. Anat.*, **62**, 121–174 (1916).
Story, P., Hansbury, W. J.: Morphological changes in *Salmonella typhimurium* infections. *J. Path.*, **73**, 433–450 (1957).
(Strongovskaya, N. V.) Стронговская, Н. В.: Экспериментальное изучение реакции нарастания фага. *Ж. микробиол. (Москва)*, **2**, 25–28 (1963).
(Strukov, A. I.) Струков, А. И.: *Патологическая анатомия.* 2-е изд. Медицина, Москва, (1971).
(Strukov, A. I., Maksimovits, N. A., Tishchenko, M. A., Tretyakova, B. L.) Струков, А. И., Максимович, Н. А., Тищенко, М. А., Третьякова, Б. Л.: Патоморфология местных и общих изменений при дизентерии у маленьких детей. *Арх. пат. анат. и пат. физиол.*, **1—2**, 99–104 (1940).
Stuart, A. E.: *The Reticuloendothelial System.* Edinburgh–London. (1970).
Stuart, A. E., Clark, J., Boulton, J., Colloe, J. G.: Auto-phagocytosis in mouse typhoid. *J. Path.*, **97**, 93–98 (1969).
Stypulkowska, H.: Zastosowanie keratoconjunctivitis shigellosa u morskich swined. Do badan odpornosci poszczepiennej. *Med. Doswiad. Mikrobiol.* **14**, 113–121 (1962).
Sunshine, P., Herbst, J. J., Koldovsky, O., Kretchmer, N.: Adaptation of the gastrointestinal tract to extrauterine life. *Ann. N. Y. Acad. Sci.*, **176**, 16–29 (1971).

Suter, E.: Some aspects of intracellular parasitism of pathogenic microorganisms. A review. *Internat. J. Leprosy*, **22**, 1–11 (1954).
Suter, E., Ramseier H.: Cellular reactions in infection. *Adv. Immunol.*, **4**, 117–173 (1964).
Suzuki, Y.: Clinical studies on the diarrheal disease caused by pathogenic *Escherichia coli*. I. Diarrheal disease due to *Escherichia coli* O124. *J. Japan. Ass. Infect. Dis.*, **32**, 859–863 (1959).
(Sviridov, L. P.) Свиридов, Л. П.: Влияние сверхчастотных излучений различной интенсивности на течение сальмонеллезной инфекции у белых мышей. *Ж. микробиол. (Москва)*, **3**, 10–14 (1970).
(Sviridova, M. P.) Свиридова, М. П.: Токсические субстанции кишечной палочки патогенного серотипа O111 : B4. Автореф. Дисс. канд. мед. наук, Харьков, (1965).
Szturm-Rubinsten, Sz., Piéchaud, D.: Facteur kératoconjonctival des Shigella. *Ann. Inst. Pasteur.*, **95**, 763–767 (1958).
Szturm-Rubinsten, S., Piéchaud, D.: Observations sur la localisation de *Sh. flexneri* 3 dans les cellules épithéliales de la vessie du cobaye lors d'une cystite expérimentale. *Ann. Inst. Pasteur*, **105**, 785–792 (1963).
Szturm-Rubinsten, S., Piéchaud, D.: Pouvoir pathogène et ubiquité des souches "intermédiaires" entre Shigella et Alkalescens-Dispar. Importance taxonomique. *Ann. Inst. Pasteur*, **121**, 763–767 (1971).
Szturm-Rubinsten, S., Piéchaud, D., Charpak, M.: Shigella, Alkalescens-Dispar: souches intermédiaires. *Ann. Inst. Pasteur*, **106**, 122–126 (1964).
Szturm-Rubinsten, S., Piéchaud, D., Floch, T.: Quelques observations sur les entérobactéries proches des Shigella, souches dites intermédiaires. *Ann. Inst. Pasteur*, **113**, 656–659 (1967).
Szturm-Rubinsten, S., Piéchaud, D., Thibault, P.: Inoculation conjonctivale au cobaye de bacilles dysentériques. *Ann. Inst. Pasteur*, **93**, 463–467 (1957).
Takaki, J.: Experimentelles zur Typhusbazillenträgerfrage. *Zbl. Bakt., I. Abt. Ref.*, **85**, 94–95 (1927).
Takano, J., Yardley, J. H.: Jejunal lesions in patients with giardiasis and malabsorption. *Bull. Johns Hopk. Hosp.*, **116**, 413–429 (1965).
Takasaka, A., Honjo, Sh., Imaizumi, K., Ogawa, H., Nakaya, R., Nakamura, A., Mise, K.: Experimental infection with *Shigella sonnei* in cynomolgus monkeys (Macaca iris). *Japan. J. med. Sci. Biol.*, **22**, 389–393 (1969).
Takeuchi, A.: Electron-microscopic studies of experimental Salmonella infection. I. *Amer. J. Path.*, **50**, 109–136 (1967).
Takeuchi, A.: Penetration of the intestinal epithelium by various microorganisms. *Curr. Top. Path.*, **54**, 1–27 (1971).
Takeuchi, A., Formal, S. B., Sprinz, H.: Experimental acute colitis in the rhesus monkey following peroral infection with *Shigella flexneri*. *Amer. J. Path.*, **52**, 503–529 (1968).
Takeuchi, A., Jervis, H. R., Nakazawa, H., Robinson, D. M.: Spiral-shaped organisms on the surface colonic epithelium of the monkey and man. *Amer. J. clin. Nutr.*, **27**, 1287–1296 (1974).
Takeuchi, A., Sprinz, H.: Electron-microscopic studies of experimental Salmonella infection in the preconditioned guinea pig. II. *Amer. J. Path.*, **51**, 137–161 (1967).
Takeuchi, A., Sprinz, H.: Spirochetes in the colon of the Rhesus monkey. *Fed. Proc.*, **28**, 513 (1969).
Takeuchi, A., Sprinz, H.: Intestinal spirochetosis. *Amer. J. Path.*, **59**, 46a (1970).
Takeuchi, A., Sprinz, H., LaBrec, E., Formal, S. B.: Experimental bacillary dysentery. An electron-microscopic study of the response of the intestinal mucosae to bacterial invasion. *Amer. J. Path.*, **47**, 1011–1044 (1965).
Takeuchi, A., Sprinz, H., Sohn, A.: Intestinal spirochetosis in the monkey and man. *Lab. Invest.*, **24**, 450 (1971).
Takeuchi, A., Zeller, J. A.: Ultrastructural identification of spirochetes and flagellated microbes at the large intestinal epithelium of the rhesus monkey. *Infect. Immunity*, **6**, 1008–1018 (1972).
(Tallmeyster, E. T., Raudsik, T. A.) Таллмейстер, Э. Т., Раудсик, Т. А.: К изучению патогенных свойств некоторых свежевыделенных ЭПКП различных серологических типов. *Ж. микробиол. (Москва)*, **7**, 21–24 (1965).

Tanaka, N., Nishimura, T., Yamaguchi, H., Yoshiyuki, T.: Histochemical studies on experimental typhoid by means of fluorescein labeled antibody. II. *Japan. J. Microbiol.*, **4**, 35–51 (1960*a*).

Tanaka, N., Yamaguchi, H., Nishimura, T., Yoshiyuki, T.: Histochemical studies on experimental typhoid by means of fluorescein-labeled antibody. III. *Japan. J. Microbiol.*, **4**, 433–449 (1960*b*).

Tannock, G. W., Blumershine, R. V. H., Savage, D. C.: Association of *Salmonella typhimurium* with, and its invasion of, the ileal mucosa in mice. *Infect. Immunity*, **11**, 365–370 (1975).

Tannock, G. W., Smith, J. M. B.: A Salmonella carrier state involving the upper respiratory tract of mice. *J. infect. Dis.*, **123**, 502–506 (1971).

(Tarasov, V. I.) Тарасов, В. И.: К вопросу о бактериологической диагностике гастроинтестинальных форм сальмонеллеза. *Ж. микробиол. (Москва)*, **12**, 28–33 (1971).

(Tarasova, A. P.) Тарасова, А. П.: Морфологические изменения в легких при коклюше. *Труды Ленинград. сан.-гиг. мед. ин-та*, **41**, 165–177 (1958).

(Tarasova, A. P.) Тарасова, А. П.: Морфологические изменения во внутренних органах у детей при колибациллярной инфекции. *Труды Педиатрического мед. ин-та. (Ленинград)*, **29**, 45–54 (1962).

(Tarasova, A. P.) Тарасова, А. П.: К вопросу о поражениях кишечника у детей, вызванных патогенными штаммами кишечной палочки. *Труды Ленинград. сан.-гиг. мед. ин-та*, **72**, 66–73 (1963).

Taylor, J.: Discussion on infantile gastroenteritis. *Proc. roy. Soc. Med.*, **44**, 516–519 (1951).

Taylor, J.: *Escherichia coli* as an enteropathogen. *Zbl. Bakt., I. Abt. Orig.*, **174**, 357–363 (1959).

Taylor, J.: Host specificity and enteropathogenicity of *Escherichia coli*. *J. appl. Bact.*, **24**, 316–325 (1961).

Taylor, J.: Host-parasite relations of *Escherichia coli* in man. *J. appl. Bact.*, **29**, 1–12 (1966).

Taylor, J.: Discussion. *Ann. N. Y. Acad. Sci.*, **176**, 136–137 (1971).

Taylor, J., Bettelheim, K. A.: The action of chloroform-killed suspensions of enteropathogenic *Escherichia coli* on ligated rabbit-gut segments. *J. gen. Microbiol.*, **42**, 309–313 (1966).

Taylor, J., Charter, R.: Discussion on the Meeting of the Society for General Microbiology. *J. gen. Microbiol.*, **12**, 372–374 (1955).

Taylor, J., Maltby, M. P., Payne, J. M.: Factors influencing the response of ligated rabbit-gut segments to injected *Escherichia coli*. *J. Path. Bact.*, **76**, 491–499 (1958).

Taylor, J., Powel, R. W., Wright, J.: Infantile diarrhoea and vomiting. *Brit. med. J.*, **2**, 117–125 (1949).

Taylor, J., Wilkins, M. P.: The effect of Salmonella and Shigella on ligated loops of rabbit gut. *Indian J. med. Res.*, **49**, 544–549 (1961).

Taylor, J., Wilkins, M. P., Payne, J. M.: Relation of rabbit gut reaction to enteropathogenic *Escherichia coli*. *Brit. J. exp. Path.*, **42**, 43–52 (1961).

Temme, N., Giffels, V., Heyer, R.: Auffälliger Fundort (Scheide) von *Shigella flexneri*. *Med. Mschr.*, **23**, 321–322 (1969).

Tenner, K., Rácz, P., Mérő, E.: Significance of "intracellular parasitism" in experimental salmonella cystitis. *Acta microbiol. Acad. Sci. hung.*, **18**, 219–229 (1971*c*).

Tenner, K., Rácz, P., Rédey, B.: Significance of the "epithelial phase" in experimental salmonella conjunctivitis. *Acta microbiol. Acad. Sci., hung.*, **18**, 167–177 (1971*a*).

(Tenner, K., Rácz, P., Serény, B.) Теннер, К., Рац, П., Шерень, Б.: Сравнительное электронномикроскопическое изучение дизентерийного и листериозного конъюнктивита *Арх. пат. (Москва)*, **11**, 12–16 (1970).

Tenner, C., Rácz, P., Serény B.: Significance of "intracellular parasitism" in experimental Shigella cystitis. *Acta microbiol. Acad. Sci. hung.*, **18**, 179–189 (1971*b*).

Tenner, K., Serény, B., Rácz, P., Orbán, I.: Mitigált virulenciájú Shigella törzs által tengerimalac szemén okozott elváltozások. Magyar Mikrobiol. Társaság ülése (Lesions of guinea pig eye caused by a shigella stain of mitigated virulence. Conference of the Hungarian Microbiological Association). Budapest (1969).

(Ter-Grigorova, E. N.) Тер-Григорова, Е. Н.: Патологоанатомические данные о колитах в детском возрасте в Азербайджане. *Педиатрия* **3**, 20–23 (1938).

Teveli, Z.: Vulvovaginitis dysenterica. *Arch. Kinderheilk.*, **104,** 69–71 (1934).
Thal, E., Dinter, Z.: Zur Pathogenität der Stammgruppe "455" (Enterobacteriaceae) für die Maus. *Nord. Veterinärmed.*, **5,** 855–858 (1953).
Thom, R., Schlicht, J.: Die Ultrastructur der Rattenleber bei Tetrachlorkohlenstoffvergiftung und protrahierter Hypothermie. *Virchows Arch. Abt. A.*, **349,** 115–123 (1970).
Thomson, S.: The role of certain varieties of Bacterium coli in gastro-enteritis of babies. *J. Hyg. (Cambridge)*, **53,** 357–367 (1955).
Thorpe, B. D., Marcus, S.: Phagocytosis and intracellular fate of Pasteurella tularensis. *J. Reticuloendoth. Soc.*, **4,** 10–23 (1967).
(Tikhonova, N. M., Nedozorova, V. K.) Тихонова, Н. М., Недозорова, В. К.: Патологоанатомическое исследование при колибациллезах у детей. *Труды Томск. Ин-та вакцин и сывороток и Мед. ин-та,* **16,** 110–114 (1965).
Tillotson, J. R., Lerner, A. M.: Pneumonias caused by Gram-negative bacilli. *Medicine (Baltimore)*, **45,** 65–76 (1966).
Tillotson, J. R., Lerner, A. M.: Characteristics of pneumonias, caused by Escherichia coli. *New Engl. J. Med.*, **277,** 115–122 (1967*a*).
Tillotson, J. R., Lerner, A. M.: Pneumonia caused by Escherichia coli and Pseudomonas. *Antimicrobial Agents and Chemotherapy-1966*, 198–201 (1967*b*).
(Timakov, V. D., Petrovskaya, V. G.) Тимаков, В. Д., Петровская, В. Г.: *Биология и генетика шигелл Флекснера.* Медицина, Москва, (1972).
(Timashkevich, T. B.) Тимашкевич, Т. В.: Кишечник. В кн.: *Клеточное обновление.* Под ред. Л. Д. Лиознера. Медицина, Ленинград. 105–118 (1966).
Tizzoni, G., Cattani, G.: Recherches sur le choléra asiatique. *Beitr. path. Anat.*, **3,** 191–237 (1888)
Tlaskalová, H., Kamarýtová, V., Mandel, L., Prokešová, L., Kruml, J., Lanc, A., Miller, I.: The immune response of germ-free piglets after peroral monocontamination with living *Escherichia coli* strain O86. *Folia biol. (Praha)*, **16,** 177–187 (1970).
Tlaskalová, H., Medlin, J., Šterzl, J.: Ontogenetic development of antibody formation in response to different doses of Gram-negative microorganisms in young rabbits. *Folia microbiol. (Praha)*, **14,** 578–587 (1969).
(Tolybekov, A. S., Krasnik, F. I.) Толыбеков, А. С., Красник, Ф. И.: Морфология экспериментальной орнитозной инфекции. *Воп. вирусол.*, **1,** 84–90 (1966).
Tomasi, T. B., Jr.: Structure and function of mucosal antibodies. *Ann. Rev. Med.*, **21,** 281–298 (1970).
Tomasi, T. B., Grey, H. M.: Structure and function of immunoglobulin A. *Progr. Allergy*, **16,** 81–213 (1972).
Toner, P. G.: Cytology of intestinal epithelial cells. *Int. Rev. Cytol.*, **24,** 233–343 (1968).
Trabulsi, L. R.: Revelação de colibacilos associados à diarréias infantis pelo método da alça ligada do intestino do coelho. *Rev. Inst. Med. trop. S. Paulo*, **6,** 197–203 (1964).
Trabulsi, L. R.: Experimental kerato-conjunctivitis of the guinea-pig by Enterobacteria. *Rev. Inst. Med. trop. S. Paulo*, **7,** 16–23 (1965).
Trabulsi, L. R., Fernandes, M. R. F.: *Escherichia coli* serogroup O115 isolated from patients with enteritis. Biochemical characteristics and experimental pathogenicity. *Rev. Inst. Med. trop. S. Paulo*, **11,** 358–362 (1969).
Trabulsi, L. R., Fernandes, M. R. F., Zuliani, M. E.: Novas bacterias patogênicas para o intestino do homen. *Rev. Inst. Med. trop. S. Paulo*, **9,** 31–39 (1967).
Trabulsi, L. R., Zuliani, M. E., Serrano, J. A.: On two new enterobacteria pathogenic to the guinea-pig eye (cultures 185T-64 and 193T-64). *Rev. Inst. Med. trop. S. Paulo*, **7,** 241–246 (1965).
Trávníček, J., Kostka, J., Lanc, A., Rejnek, J., Šterzl, J.: The effect of specific antibodies in the intestine of germfree piglets monoinoculated with the pathogenic strain of *Escherichia coli* O55. In: *Advances in Germfree Research and Gnotobiology.* London-Cleveland. pp. 247–252 (1968).
Trier, J. S.: Structure of the mucosa of the small intestine as it relates to intestinal function. *Fed. Proc.*, **26,** 1391–1404 (1967).
Trier, J. S., Phelps, P. S., Edelman, S., Rubin, C. E.: Whipple's disease. *Gastroenterology*, **48,** 687–707 (1965).

Trier, J. S., Rubin, C. E.: Electron microscopy of the small intestine: a review. *Gastroenterology*, **49**, 574–603 (1965).
(Trifonova, A. G.) Трифонова, А. Г.: Изучение так называемых «временных» дизентерийных и «анаэрогенных» серотипов группы кишечных бактерий. *Труды Ин-та эпидемиологии и микробиологии.* София, **7**, 55–63 (1960).
Trifonova, A.: Über die Taxonomie zweier Vertreter der Familie Enterobacteriaceae. *Zbl. Bakt. I. Abt. Orig.*, **193**, 386–393 (1963).
Trifonova, A.: Über die taxonomische Stellung einiger Vertreter der Familie Enterobacteriaceae. *Zbl. Bakt. I. Abt. Orig.*, **195**, 470–482 (1965).
Trillat, A., Kaneko, R.: Activité de l'infection par voie aérienne. *C. R. Acad. Sci. (Paris)*, **173**, 109–113 (1921).
(Troitsky, V. L.) Троицкий, В. Л.: Об основных задачах научно-исследовательской работы в области дизентерии. *Ж. микробиол. (Москва)*, **5**, 41–47 (1952).
Troitsky, V. L.: Experimental research on immunity to dysentery. *J. Hyg. Epidem. (Praha)* **2**, 257 (1958*a*).
(Troitsky, V. L.) Троицкий, В. Л.: Итоги и перспективы изучения дизентерии в опытах на обезьянах. *Вестн. Акад. мед. наук СССР*, **3**, 39–41 (1958*b*).
(Troitsky, V. L., Tumanyan, M. A.) Троицкий, В. Л., Туманян, М. А.: О всасывании антигена дизентерийных бактерий. В кн.: *Труды по применению радиоактивных изотопов в медицине.* Изд. 2-е, Медгиз, Москва. 139–147 (1955).
(Troitsky V. L., Tumanyan, M. A., Fridenstein, A. Ya.) Троицкий, В. Л., Туманян, М. А., Фриденштейн, А. Я.: Исследования о влиянии ионизирующих излучений на естественный иммунитет. *Ж. микробиол. (Москва)*, **6**, 3–9 (1958).
(Tsimbler, I. V.) Цимблер, И. В.: Актуальные вопросы клиники и лечения колиэнтеритов. В кн.: *Колиэнтериты.* Под ред. О. В. Барояна. Медгиз, Москва. 167–176 (1962).
(Tsinzerling, A. V.) Цинзерлинг, А. В.: О морфологии экспериментального кандидоза легких. Ежегодник (*Труды Ин-та экспер. мед. АМН СССР*) за 1957 г., 359–362 Ленинград, (1958).
(Tsinzerling, A. V.) Цинзерлинг, А. В.: *Острые респираторные инфекции.* Медицина, Ленинград, (1970).
(Tsinzerling, A. V.) Цинзерлинг А. В.: Внутриэпителиальное размножение энтеробактерий и его роль в патогенезе дизентерии и кишечной колиинфекции. *Ж. микробиол. (Москва)*, **3**, 11–15 (1973*a*).
(Tsinzerling, A. V.) Zinserling, A.: Dysentery and colienterocolitis. Some aspects of pathogenesis and pathological anatomy. *Virchows Arch., Abt. A.*, **361**, 19–30 (1973*b*).
(Tsinzerling, A. V., Tarasova, A. P., Pribluda, B. A.) Цинзерлинг, А. В., Тарасова, А. П., Приблуда, Б. А.: О патологической анатомии колиэнтерита 0111. *Арх. пат. (Москва)*, **1**, 46–51 (1968).
(Tsinzerling, V. D., Tsinzerling, A. V.) Цинзерлинг, В. Д., Цинзерлинг, А. В.: *Патологическая анатомия острых пневмоний разной этиологии.* Медгиз, Ленинград, (1963).
(Tur, A. F.) Тур, А. Ф.: Особенности клиники пневмоний у новорожденных и недоношенных детей. *Вопросы педиатрии*, **4**, 3–13 (1947).
Turck, M., Fournier, M. R., Petersdorf, R. G.: Laboratory and clinical studies of *Escherichia coli* infection. In: *Antimicrobial Agents and Chemotherapy-1961*, 113–120 (1962).
Turck, M., Petersdorf, R. G.: The epidemiology of nonenteric *Escherichia coli* infections: prevalence of serological groups. *J. clin. Invest.*, **41**, 1760–1765 (1962).
Turgeon, F., Borduas, A. G., Frappier, A.: Pathogénéité, pour l'embryon du poulet et de la souris, de quelques souches d'*Esch. coli* entéropathogènes. *Rev. Canad. Biol.*, **19**, 417–424 (1960).
(Ugleva, A. I.) Углева, А. И.: Экспериментальная паратифозная инфекция, обусловленная сальмонеллами группы «С». Автореф. дисс. канд. мед. наук, Ленинград, (1953).
Ugolev, A. M.: Membrane (contact) digestion. *Physiol. Rev.*, **45**, 555–595 (1965).
(Ugolev, A. M.) Уголев, А. М.: *Физиология и патология пристеночного (контактного) пищеварения.* Наука, Ленинград, (1967).
Újváry, G.: Über die ätiologische Rolle der *Escherichia-coli*-Gruppe bei extraenteral lokalisierten Infektionen. *Zbl. Bakt. I. Abt. Orig.*, **170**, 395–405 (1958).

Undritsov, M. I.) Ундрицов, М. И.: Некоторые вопросы патогенеза бактериальной дизентерии. *Материалы обл. научн. конферен. врачей Куйбышева*, 107–109 (1955).
Ushiba, D.: Phagocytosis of bacteria and their intracellular multiplication in experimental typhoid. *Tohoku J. exp. Med.*, **76**, 133–143 (1962).
Ushiba, D.: Two types of immunity in experimental typhoid; "cellular immunity" and "humoral immunity". *Keio J. Med.*, **14**, 45–56 (1965).
Vaillard, L., Dopter, Ch.: De dysentérie épidémique. *Ann. Inst. Pasteur*, **17**, 486–491 (1903).
(Valdman, A. A.) Вальдман, А. А.: Экспериментальные исследования о паратифозных заболеваниях. *Арх. биол. наук (Ленинград)*, **29**, 561–572 (1929*a*).
(Valdman, A. A.) Waldman, A. A.: Pathologisch-histologische Untersuchungen über die experimentelle Paratyphusinfection der weissen Maus. *Krankheitsforsch.*, **7**, 307–320 (1929*b*).
(Valdman, A. A.) Waldman, A. A.: Experimentelle Untersuchungen über die Breslau-Infection bei Kaninchen. *Beitr. path. Anat.*, **85**, 591–610 (1930).
(Valdman, A. A.) Вальдман, А. А.: Экспериментальное исследование тифозных и паратифозных заболеваний у кроликов. *Арх. биол. наук.* **31**, 1, 17–24 (1931*a*).
(Valdman, A. A.) Waldman, A. A.: Experimentelle Untersuchungen über die Typhus- und Paratyphusinfektion bei Kaninchen. *Zbl. Bakt. I. Abt. Orig.*, **120**, 52–58 (1931*b*).
(Valdman, A. A.) Вальдман, А. А.: Морфологические изменения в кишечнике мыши при разных способах заражения паратифом. *Бюлл. Всесоюз. ин-та экспер. мед.*, **5**, 59–64 (1935).
(Valdman, A. A.) Вальдман, А. А.: *Паратифозная инфекция*. Медгиз, Ленинград, (1955).
(Valdman, A. A.) Вальдман, А. А.: *Опыт экспериментального анализа инфекционного процесса*. Медицина, Ленинград, (1964).
(Valdman, A. A., Mendelson, A. I.) Вальдман, А. А., Мендельсон, А. И.: Экспериментальные исследования паратифозной инфекции, вызванной сальмонеллой Гейдельберг. *Ж. микробиол. (Москва)*, **11**, 52–55 (1953).
(Valdman, A. A., Rostova E. N.) Waldman, A. A., Rostowa, E. N.: Über Darmveränderungen bei Mäusen nach verschiedenen Infizierungsarten mit Bac. enteritidis Breslau. *Zbl. Bakt. I. Abt. Orig.*, **136**, 49–54 (1936).
(Vasilyeva, N. G., Polyak, N. S.) Васильева, Н. Г., Поляк, М. С.: Микрофлора мокроты при пневмониях и ее чувствительность к антибиотикам. *Антибиотики (Москва)*, **6**, 527–530 (1967).
(Vasser, N. R.) Вассер, Н. Р.: Материалы к изучению биологической характеристики энтеропатогенной кишечной палочки 0124 : К72(В17). *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **33**, 49–68 (1968).
(Vasser, N. R., Polotsky, Yu. E.) Вассер, Н. Р., Полоцкий, Ю. Е.: Испытание биологических свойств возбудителей острых кишечных инфекций на модели изолированной петли кишечника кролика. *Авторефераты Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, 34–36 (1970*a*).
(Vasser, N. R., Polotsky, Yu. E.) Вассер, Н. Р., Полоцкий, Ю. Е.: Экспериментальный кератоконъюнктивит, вызванный энтеропатогенной кишечной палочкой 0124 : К72 (В17). *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **36**, 149–155 (1970*b*).
(Vasser, N. R., Polotsky, Yu. E.) Вассер, Н. Р., Полоцкий, Ю. Е.: Опыт сравнительного изучения биологических свойств энтеробактерий на модели перевязанной петли кишечника кролика. I. Анализ результатов микробиологического исследования. *Ж. микробиол. (Москва)*, **7**, 66–70 (1972).
(Vasser, N. R., Semenova, O. A.) Вассер, Н. Р., Семенова, О. А.: К биологии и циркуляции энтеропатогенной кишечной палочки серологической группы 01. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **40**, 117–120 (1973).
(Vedibeda, D. K., Sakun, T. L., Zadnepryany, A. A.) Ведибеда, Д. К., Сакун, Т. Л., Заднепряный, А. А.: Роль костного мозга в формировании носительства тифозных бактерий в эксперименте. *Ж. микробиол. (Москва)*, **4**, 71–77 (1972).
(Vedmina, E. A., Kaverina, N. V., Navashin, S. M.) Ведьмина, Е. А., Каверина, Н. В., Навашин, С. М.: Биомицин и биомицин в сочетании с экмолином при экспериментальной инфекции. В кн.: *Биомицин*, сб. **2**, под ред. З. В. Ермольевой и А. Ф. Билибина. Медгиз, Москва. 46–48 (1956).

Veress, B., Baintner, K., Jr.: Morphological and histochemical features of protein absorption in newborn animals. *Acta morph. Acad. Sci. hung.*, **18**, 237–248 (1970).
(Veselkin, P. N.) Веселкин, П. Н.: *Лихорадка.* Медгиз, Москва, (1963).
Vigari, G., Olitzki, A. L., Olitzki, Z.: The action of the thermolabile toxin of *Shigella dysenteriae* on cells cultivated *in vitro*. *Brit. J. exp. Med.*, **41**, 179–189 (1960).
(Vilner, L. M.) Вильнер, Л. М.: О применении куриных эмбрионов в качестве экспериментальной модели для изучения вирулентности и иммуногенности штаммов *S. typhi*. *Ж. микробиол. (Москва)*, **10**, 133–137 (1958).
(Vilner, L. M.) Вильнер Л. М.: Культивирование тифозных микробов в развивающихся куриных эмбрионах. *Ж. микробиол. (Москва)*, **6**, 17–19 (1960).
Vincent, H.: Sur la sérothérapie anticolibacillaire. *Compt. Rend. Acad. Sci. (Paris)*, **187**, 407–410 (1928).
Violle, H., Crendiropoulo, A.: Note sur le choléra expérimental. *C. R. Soc. Biol. (Paris)*, **78**, 331–332 (1915).
(Vishnevetskaya, L. O.) Вишневецкая, Л. О.: К вопросу о катаральных формах дизентерийного колита. *Педиатрия*, **5**, 58–62 (1941).
(Vishnevetskaya, L. O., Voit, E. B., Kotysheva, A. V., Rabinovich, D. Ya., Fridman, E. E., Shalevich, M. F.) Вишневецкая, Л. О., Войт, Е. Б., Котышева, А. В., Рабинович, Д. Я. Фридман, Э. Е., Шалевич, М. Ф.: К морфологии кишечных заболеваний у детей первых месяцев жизни, вызванных патогенными штаммами кишечной палочки. *Педиатрия*, **4**, 27–31 (1960).
(Voino-Yasenetskaya, M. K.) Войно-Ясенецкая, М. К.: О роли бактерий дизентерийной группы в этиологии кишечных заболеваний. Дисс. канд. мед. наук, Сталинабад, (1944).
(Voino-Yasenetskaya, M. K.) Войно-Ясенецкая, М. К.: Опыт изучения дизентерийной инфекции у лабораторных животных. *Ж. микробиол. (Москва)*, **4**, 65–69 (1957).
(Voino-Yasenetskaya, M. K.) Войно-Ясенецкая, М. К.: Интраназальное заражение мышей дизентерийными бактериями как метод изучения действия антибиотиков. *Тр. Ин-та эпидемиол. микробиол. им. Пастера (Ленинград)*, **18**, 282–289 (1958).
(Voino-Yasenetskaya, M. K., Voino-Yasenetsky, M. V.) Войно-Ясенецкая, М. К., Войно-Ясенецкий, М. В.: Об этиологии колитов. *Врач. дело (Киев)*, **3**, 249–254 (1948).
(Voino-Yasenetskaya, M. K., Voino-Yasenetsky, M. V., Polonsky, Yu. V.) Войно-Ясенецкая, М. К., Войно-Ясенецкий, М. В., Полонский, Ю. В.: Изменения кишечника при бациллярной дизентерии. *Сов. медицина*, **7**, 22–25 (1942).
(Voino-Yasenetsky, M. V.) Войно-Ясенецкий, М. В.: Длительное хранение материала для гистологических исследований. *Лабор. практика*, **12**, 1–2 (1940).
(Voino-Yasenetsky, M. V.) Войно-Ясенецкий, М. В.: Колиты и малярия. *Клин. мед. (Москва)*, **7—8**, 61–66 (1943).
(Voino-Yasenetsky, M. V.) Войно-Ясенецкий, М. В.: *Патологическая анатомия и некоторые вопросы патогенеза малярии.* Москва, (1950).
(Voino-Yasenetsky, M. V.) Войно-Ясенецкий, М. В.: Локализация возбудителей дизентерии в кишечнике. *Ж. микробиол. (Москва)*, **11**, 56–60 (1963).
(Voino-Yasenetsky, M. V.) Войно-Ясенецкий, М. В.: Амебиаз. Балантидиаз. В кн.: *Многотомное руководство по патологической анатомии*, **9**, 674–680, 722–725. Медицина, Москва, (1964*a*).
(Voino-Yasenetsky, M. V.) Войно-Ясенецкий, М. В.: Развитие и исход сальмонеллезной инфекции при интраназальном заражении белых мышей. II. Морфологические исследования. *Тр. Ин-та эпидемиол. и микробиол. им. Пастера (Ленинград)*, **27**, 106–124 (1964*b*).
Voino-Yasenetsky, M. V.: Biological basis of specificity in pathogenesis of infectious processes. In: *9th Internat. Congress Microbiol. Symposia (Engl.)*. Moscow pp. 227–236 (1966*a*).
(Voino-Yasenetsky, M. V.) Войно-Ясенецкий, М. В.: Сущность поражений кишечника при дизентерии. *Арх. пат. (Москва)*, **10**, 3–15 (1966*b*).
(Voino-Yasenetsky, M. V.) Войно-Ясенецкий, М. В.: Оценка эксперимента в инфекционной патологии. *Вестн. АМН СССР*, **11**, 40–48 (1966*c*).
(Voino-Yasenetsky, M. V.) Войно-Ясенецкий, М. В.: Моделирование инфекционных процессов. В. кн.: *Моделирование в биологии и медицине.* Медицина, Ленинград. 130–139 (1969).

(Voino-Yasenetsky, M. V.) Войно-Ясенецкий, М. В.: Кишечные инфекции и проблема внутриклеточного паразитирования микробов. *Арх. пат. (Москва)*, **11,** 3–11 (1970).
(Voino-Yasenetsky, M. V.) Войно-Ясенецкий, М. В.: О роли внутриклеточного паразитирования шигелл в патогенезе дизентерии. *Ж. микробиол. (Москва)*, **7,** 43–46 (1974).
(Voino-Yasenetsky, M. V., Karpova, E. M.) Войно-Ясенецкий, М. В., Карпова, Е. М.: Сущность очаговых изменений селезенки при возвратном тифе. *Арх. пат. (Москва)*, **11,** 30–38 (1959).
(Voino-Yasenetsky, M. V., Khay, L. M.) Войно-Ясенецкий, М. В., Хай, Л. М.: Патологическая анатомия экспериментальной коклюшной инфекции. В кн.: *Вопросы патогенеза и патологической анатомии инфекционных болезней*. Ленинград. 203–212 (1957).
(Voino-Yasenetsky, M. V., Khay, L. M.) Войно-Ясенецкий М. В., Хай Л. М.: Развитие инфекционного процесса в зависимости от условий заражения. *Ж. гиг. эпид., микробиол., иммунол. (Прага)*, **4,** 412–420 (1960).
(Voino-Yasenetsky, M. V., Khavkin, T. N.) Войно-Ясенецкий, М. В., Хавкин, Т. Н.: Изучение внутриэпителиальной локализации возбудителей дизентерии при помощи флуоресцирующих антигел. *Ж. микробиол. (Москва)*, **4,** 98–100 (1964).
(Voino-Yasenetsky, M. V., Snigirevskaya, E. S., Belyanin, V. L.) Войно-Ясенецкий, М. В., Снигиревская, Е. С., Белянин, В. Л.: Спирохеты на слизистой оболочке кишечника. *Арх. пат. (Москва)*, **5,** 34–40 (1975).
(Voino-Yasenetsky, M. V., Voino-Yasenetskaya, M. K.) Войно-Ясенецкий, М. В., Войно-Ясенецкая, М. К.: Экспериментальная пневмония, вызываемая бактериями дизентерийной группы. В кн.: *Вопросы инфекционной патологии*. Изд. ИЭМ АМН СССР, Ленинград. 49–66 (1962*a*).
Voino-Yasenetsky, M. V., Voino-Yasenetskaya, M. K.: Experimental pneumonia caused by bacteria of the Shigella group. *Acta morph. Acad. Sci. hung.*, **11,** 439–453 (1962*b*).
(Voino-Yasenetsky, M. V., Yampolskaya, S. A.) Войно-Ясенецкий, М. В., Ямпольская, С. А.: Гистопатология склеромы. *Арх. пат. (Москва)*, **4,** 34–42 (1956).
(Voino-Yasenetsky, M. V., Zhabotinsky, Yu. M.) Войно-Ясенецкий, М. В., Жаботинский, Ю. М.: *Источники ошибок при морфологических исследованиях*. Медицина, Ленинград, (1970).
Vörös, S., Rédey, B., Czizmazia, F.: Antigen structure of a new enteropathogenic *Escherichia coli* strain. *Acta microbiol. Acad. Sci. hung.*, **11,** 125–129 (1964).
Vosti, K. L., Goldberg, L. M., Monto, A. S., Rantz, L. A.: Host-parasite interaction in patients with infections due to *Escherichia coli*. I. The serogrouping of *E. coli* from intestinal and extraintestinal sources. *J. clin. Invest.*, **43,** 2377–2385 (1964).
(Vygodchikov, G. V.) Выгодчиков, Г. В.: Микробиология пневмоний. *Вестн. Акад. мед. наук СССР*, **9,** 3–10 (1960).
(Vysokovich, V. K.) Высокович, В. К.: О прохождении бактерий через легкие. *Еженед. клинич. газ.*, **9,** 595, 621, 643 (1889).
Walker, W. A., Isselbacher, K. J.: Uptake and transport of macromolecules by the intestine. Possible role in clinical disorders. *Gastroenterology*, **67,** 531–550 (1974).
Walters, E. W., Cooper, M. L., Keller, H. M.: The microscopical detection of colonies of Shigella in stool cultures. *J. Bact.* **67,** 247–251 (1954).
Ward, M. W., Watt, P. J.: Adherence of *Neisseria gonorrhoeae* to uretral mucosal cells. *J. infect. Dis.*, **126,** 601–605 (1972).
Watkins, H. M. S.: Some attributes of virulence in Shigella. *Ann. N. Y. Acad. Sci.*, **88,** 1167–1186 (1960).
Watson, J. H. L., Haubrich, W. S.: Bacilli bodies in the lumen and epithelium of the jejunum in Whipple's disease. *Lab. Invest.*, **21,** 347–357 (1969).
Waxler, G. L., Christie, B. R., Drees, D. T.: Use of the gnotobiotic pig in the study of *Escherichia coli* infection. *Ann. N. Y. Acad. Sci.*, **176,** 141–149 (1971).
Weil, A. J., Volentine, J. A.: Infection of the developing chick embryo with dysentery bacilli. *Proc. Soc. exp. Biol., Med.*, **44,** 160–161 (1940).
Weil, M. H., Spink, W. W.: A comparison of shock due to endotoxin with anaphylactic shock. *J. Labor. Clin. Med.*, **50,** 501–515 (1957).
Wentworth, F. H., Brock, D. W., Stulberg, C. S., Page, R. M.: Clinical, bacteriological and serological observations of two human volunteers following ingestion of *Escherichia coli* O127 : B8. *Proc. Soc. exp. Biol. Med.*, **91,** 586–589 (1956).

Wernet, P., Breu, H., Knop, J., Rowley, D.: Antibacterial action of specific IgA and transport of IgM, IgA and IgG from serum into the small intestine. *J. infect. Dis.*, **124**, 223–226 (1971).

Wessel, W., Rácz, P.: Elektronenmikroskopische Untersuchungen der Cornea bei experimenteller Shigellen-Keratitis. *Virchows Arch. path. Anat.*, **342**, 109–128 (1967).

Westenhöfer, M.: Über primäre noduläre Ruhr. *Berlin. klin. Wschr.*, **47**, 1119–1122 (1918).

Westphal, O., Kauffmann, F., Lüderitz, O., Stierlin, H.: Zur Immunochemie der O-Antigene von Enterobacteriaceae. III. Analyse der Zuckerbausteine Kreuzreagierender Salmonella-Arizona- und Escherichia-O-antigene. *Zbl. Bakter. I. Abt. Orig.*, **179**, 336–342 (1960).

Wheeler, K. M., Stuart, C. A.: The mannitol-negative Shigella group. *J. Bact.*, **51**, 317–325 (1946).

Whipp, S. C., Moon, H. W.: Modification of enterosorption induced by a heat labile *Escherichia coli* enterotoxin in rabbit intestinal loops. *Proc. Soc. exp. Biol., Med.*, **140**, 395–398 (1972).

Whipple, G. H.: A hitherto undescribed disease characterized anatomically by deposits of fat and fatty acids in the intestinal and mesenteric lymphatic tissues (Intestinal lypodystrophy). *Bull. Johns Hopk. Hosp.*, **18**, 382–391 (1907).

Whitby, J. R., Rowley, D.: The role of macrophages in the elimination of bacteria from the mouse peritoneum. *Brit. J. exp. path.* **40**, 358–370 (1959).

Wichert, P., von: Erfahrungen in der bakteriologischen Diagnostik der Pneumonie. *Z. inn. Med.*, **14**, 930–933 (1959).

Wiebecke, B., Heybowitz, R., Lohrs, U., Eder, M.: Der Einfluss des Hungers auf die Proliferationskinetik der Dünn- und Dickdarmschleimhaut der Maus. *Arch. Zell. Path.*, **4**, 164–175 (1969).

Wiener, E., Levanon, D.: The *in vitro* interaction between bacterial lipopolysaccharide and differentiating monocytes. *Lab. Invest.*, **19**, 584–590 (1968).

Wilder, M. S., Elberg, J.: Interaction of virulent and avirulent *Listeria monocytogenes* with cultures of mouse peritoneal macrophages. *Infect. Immunity*, **8**, 409–415 (1973).

Willmer, E. N.: Tissues in culture and in the body. In: *Models and Analogues in Biology, Symposia of the Soc. Exp. Biol.*, vol. 14, pp. 28–40 (1960).

Wilson, G. S., Miles, A. A.: The reaction that follow the intraperitoneal injection of bacteria or of suspensions of inert particles into normal animals. In: *Topley and Wilson's Principles of Bacteriology and Immunity*. 5th ed., London. vol. **2**, pp. 1273–1274 (1964).

Winter, S. T.: The age factor in acute diarrhea during childhood. *Clin. Pediatrics*, **13**, 17–19 (1974).

Woods, D. D., Foster, M. A.: Metabolic considerations relating to the life of bacteria *in vivo*. In: *Microbial Behaviour "in vivo" and "in vitro"*. Ed. by H. Smith and J. Taylor. Cambridge. pp. 30–43 (1964).

Wostmann, B. S.: Defence mechanisms in germfree animals. Part I. Humoral defence mechanisms. In: *The Germ-Free Animal in Research*. Ed. by M. E. Coates, H. A. Gordon, B. S. Wostmann. London–New York. pp. 197–209 (1968).

Wostmann, B. S., Reddy, B. S., Bruckner-Kardoss, E., Gordon, H. A., Singh, B.: Causes and possible consequences of cecal enlargement in germfree rats. In: *Germfree Res. Biol. Effect Gnotobiol. Environ.*, New York–London. pp. 261–270 (1973).

Wray, C., Thomlinson, J. R.: Anaphylaxis in calves and the development of gastrointestinal lesions. *J. Path.*, **98**, 61–73 (1969).

Wundt, W.: Experimentelle Erzeugung von Krankheiten durch Salmonellen und Shigellen. In: *Handbuch der experimentellen Pharmakologie*. Springer, Berlin–New York. vol. **16**, pp. 144–243 (1966).

(Yabrov, A. A., Anatoly, S. A., Savitskaya, O. V., Bodazhkova, O. M., Kruglikhina, E. M., Dudkina, M. I.) Ябров, А. А., Анатолий, С. А., Савицкая, О. В., Бодажкова, О. М., Круглихина, Э. М., Дудкина, М. И.: Выявления цитопатического действия некоторых микробных токсинов на культуру клеток. *Ж. микробиол. (Москва)*, **8**, 81–84 (1967).

(Yagud, S. L., Barshtein, Yu. A.) Ягуд, С. Л., Барштейн, Ю. А.: Вивчения локализации черевнотифозного антигена при экспериментальной инфекции методом флуоресциючих антител. *Мікробіологічный ж. (Київ)*, **33**, 641–646 (1971).

Yahagi, H.: Early features of infection in ligated loops of the rabbit small intestine inoculated with *Shigella flexneri* 3a, enteropathogenic *Escherichia coli* and *Vibrio parahaemolyticus*. III. Study of bacterial invasiveness with the fluorescent antibody technique. *Keio J. Med.*, **16**, 133–146 (1967).

Yahagi, H., Ghoda, A., Sasaki, S.: Early features of infection in ligated loops of the rabbit small intestine inoculated with *Shigella flexneri* 3a, enteropathogenic *Escherichia coli* and *Vibrio parahaemolyticus*. II. Gross appearance and histological findings of ligated loops after inoculation. *Keio J. Med.*, **16**, 119–132 (1967).

(Yakhnina, N. A., Shatrov, I. I., Mordvinova, N. B., Kuznetsova, N. S., Shaposhnikova, R. P., Shulman, E. A., Kazachina, K. N., Perova, L. B., Salamandra, E. G., Sinay, A. Ya., Sherishevskaya, E. F., Shabad, A. T., Golubeva, T. V.) Яхнина, Н. А., Шатров, И. И., Мордвинова, Н. Б., Кузнецова, Н. С., Шапошникова, Р. П., Шульман, Э. А., Казачина, К. Н., Перова, Л. Б., Саламандра, Э. Г., Синай, А. Я., Шеришевская, Е. Ф., Шабад, А. Т., Голубева, Т. В.: Биологические свойства возбудителей, выделенных при различных клинических формах дизентерии. *Ж. микробиол. (Москва)*, **3**, 128 (1960*a*).

(Yakhnina, N. A., Khomitskaya, T. A., Shatrov, I. I.) Яхнина, Н. А., Хомицкая, Т. А., Шатров, И. И.: Экспериментальное изучение колиэнтеритов. *Ж. микробиол. (Москва)* **6**, 77–81 (1960*b*).

(Yakhnina, N. A., Kuznetsova, N. S.) Яхнина, Н. А., Кузнецова, Н. С.: Значение экспериментального кератоконъюнктивита как модели для изучения иммунитета при дизентерии. *Ж. микробиол. (Москва)*, **2**, 98–102 (1959).

(Yakhnina, N. A., Shatrov, I. I., Mordvinova, N. B.) Яхнина, Н. А., Шатров, И. И., Мордвинова, Н. Б.: Эпидемиология колиэнтеритов. В кн.: *Колиэнтериты*. Под ред. О. В. Барояна. Медгиз, Москва. 63–79 (1962).

(Yakobson, D. A.) Якобсон, Д. А.: К оценке методов изучения вирулентности энтеропатогенных кишечных палочек. *Ж. микробиол. (Москва)*, **3**, 137 (1965).

(Yakovleva, L. A.) Яковлева, Л. А.: К вопросу о патолого-анатомических проявлениях дизентерии у обезьян. В кн.: *Вопросы инфекционной патологии в эксперименте на обезьянах*. Под ред. Б. А. Лапина. Институт экспериментальной патологии и терапии АМН СССР, Сухуми. 39–53, (1958*a*).

(Yakovleva, L. A.) Яковлева, Л. А.: К вопросу о патолого-анатомических изменениях при экспериментальной дизентерии Флекснера. В кн.: *Вопросы инфекционной патологии в эксперименте на обезьянах*. Под ред. Б. А. Лапина. Институт экспериментальной патологии и терапии АМН СССР, Сухуми. 65–72(1958*b*).

(Yakovleva, L. A.) Яковлева, Л. А.: Патологоанатомическая характеристика спонтанной дизентерии у обезьян. *Арх. пат. (Москва)*, **7**, 61–69 (1958*c*).

(Yakovleva, L. A.) Яковлева, Л. А.: Морфологическая характеристика экспериментальных сальмонеллезов у обезьян. *Арх. патол. (Москва)*, **2**, 78–81 (1966).

Yamada, M., Ogawa, H.: A microcinematographic observation on the infection of dysentery bacilli HeLa cells. *J. Japan. Soc. Cancer Therapy, Sept. Suppl.*, 94–95 (1967).

Yamagata, S., Tanifuji, K., Fukumi, H., Nakaya, R.: An outbreak of diarrhea due to enteropathogenic Escherichia. The 11th Meeting of Japanese Society of Public Health, Nagoya (1956). — cited by Sakazaki et al. (1967).

Yamamoto, I., Hampton, J. C.: Phagocytosis of *Salmonellae enteritidis:* dense material surrounding the microorganisms in the phagosomes of host cells. *3th intern. congr. electr. micr. Kyoto*, 735–736 (1966).

Yardley, I. H.: Giardiasis. *Gastroenterology*, **52**, 301–304 (1967).

Yee, R. B., Buffenmyer, C. L.: Infection of cultured mouse macrophages with *Shigella flexneri*. *Infect. Immunity* **1**, 459–463 (1970).

(Zadvornyak, P. V., Dzhumanbaeva, A. A., Udalova, L. P.) Задворняк, П. В., Джуманбаева, А. А., Удалова, Л. П.: Этиология пневмоний у новорожденных и детей грудного возраста. *Ж. микробиол. (Москва)*, **8**, 23–28 (1968).

Zaleski, S., Ceprynska-Ciekawa, M.: Paleszki okreźnicy typow serologicznych O111:B4, O55 : B5 i O26 : B6 u malych zwierzat dómowych. Chorobotwórczość i wystepowanie. *Roczn. Panstw. Zakl. Hig.*, **7**, 163–176 (1956).

Zamsheck, N. *et al.*: Histology and ultrastructure of the parasite and intestinal mucosa in human giardiasis. *Gastroenterology*, **44**, 860 (1963).

(Zarudin, V. V.) Зарудин, В. В.: О морфологических изменениях при острых ко-

либациллярных желудочно-кишечных заболеваниях у детей раннего возраста. *Вопр. охр. мат. дет.*, **3**, 27–35 (1961).

(Zemskov, V. M.) Земсков, В. М.: Адъювантное действие нуклеиновых кислот и бактериальных эндотоксинов. *Ж. микробиол. (Москва)*, **3**, 16–23 (1972).

(Zhdanov, V. M.) Жданов, В. М.: *Эволюция заразных болезней человека*. Медицина, Москва, (1964).

Zoeller, C., Bastouil: Kératite Éberthienne et immunité. Kératite à bacilles paratyphiques A et B. *C. R. Soc. Biol. (Paris)*, **90**, 1154–1156 (1924).

Zoeller, C., Manoussakis: De la kérato-conjonctivité expérimentale dysentérique. *C. R. Soc. Biol. (Paris)*, **91**, 257–258 (1924*a*).

Zoeller, Ch., Manoussakis: De la conjonctivo-kératite diphtérique expérimentale. *C. R. Soc. Biol. (Paris)* **90**, 1399–1400 (1924*b*).

APPENDIX

Since the completion and submitting of the manuscript some new data, mostly concerning different enteropathogenic *Escherichia coli* (EEC), in particular enterotoxigenic *E. coli* (ETEC) and their enterotoxins, as well as other enterotoxigenic organisms and their enterotoxins, have been published. Besides, some works of the contributors have also been completed. In the light of recent observations the significance of data reported by workers who have or have not been mentioned in the above Chapters has changed. Thus additional consideration of some points seems to be necessary.

Recent observations have shown that human diarrhoea may be induced not only by ETEC producing both heat-stable (ST) and heat-labile (LT) enterotoxins but also by those producing either only ST (D. Sack et al., 1975; Morris et al., 1976) or only LT (Morris et al., 1976). This has been revealed by means of intragastric inoculation of mice, a method permitting the detection of low-molecular-weight (1,000–10,000 daltons) ST enterotoxin only (Jacks and Wu, 1974; Giannella, 1976) as well as by recently introduced tests which only reveal LT enterotoxin. Great progress has been achieved in the selective detection of LT enterotoxin, further in its comparison with choleragen using tissue culture assays (mentioned in Chapter 20). The most adequate tests make use of Y1 mouse adrenal tumour cells (Donta, 1974*a*, *b*, 1976; Donta et al., 1974; Donta and Viner, 1975) and Chinese hamster ovary (CHO) cells (Guerrant et al., 1974; Finkelstein et al., 1976*a*). The determination of adenylate cyclase activity in human embryonic intestinal and other cells is more laborious, but provides a good tool for studying the molecular mechanism of action of LT enterotoxins (Kantor, 1975). D. Sack and R. Sack (1975) have developed a miniculture variant of the Y1 adrenal cell test. Keusch and Donta (1975) used Y1 adrenal cells for detecting cytotonic effect (in terms of increased steroidogenesis due to adenyl cyclase activation) and HeLa cells for detecting cytotoxic effect (in terms of detachment of cells from glass surface due to cell death). They classified enterotoxins of *Vibrio cholerae* and *E. coli* as cytotonic, while those of *Sh. dysenteriae* 1 and *Cl. perfringens* as cytotoxic. Cytotoxicity of *Cl. perfringens* enterotoxin has been confirmed *in vivo* on strength of the shedding of the epithelium of the villi in exposed rabbit ileal loops (McDonel and Duncan, 1975).

Cell culture tests based on the above cytotonic effect, as well as the intradermal "vascular permeability factor" (PF) assay according to Evans et al.

(1973) proved to be suitable for screening LT-producing ETEC. When purified, this heat-labile *E. coli* enterotoxin was found to have a high molecular weight, between 35,000 and 100,000 daltons (Dorner et al., 1976; Finkelstein et al., 1976*a*). Finkelstein et al. (1976*a*) have shown that the LT enterotoxin (represented by single polypeptide chains) is almost 10^6 times less active than purified choleragen in causing morphologic alterations of CHO cells, approximately 1,000 times less active in skin PF test, and at least 100 times less active in rabbit ileal loops. These authors assumed that accessory or host-derived factors are required for the LT effect. Indeed, Rappaport et al. (1976) have reported on activation of ETEC LT enterotoxin by trypsin. Further studies of LT enterotoxin may be facilitated by enhanced release of the toxin in response to Mitomycin C which leaves it unchanged (Isaacson and Moon, 1975).

Recent studies of travellers' diarrhoea have resulted in the isolation of new ETEC strains (Gorbach et al., 1975; D. Sack et al., 1975; R. Sack, 1975; Morris et al., 1976; Finkelstein et al., 1976*b*); other new ETEC have been isolated from children with diarrhoea (R. Sack et al., 1975*a*). These new ETEC have been serotyped as O5 : H27; O6 : H-; O8 : H9; O8 : H?; O15 : H8; O15 : H-; O20 : H-; O21 : H21; O25 : H-; O42 : H37; O44 : H28; O48 : H26; O63 : H-; O64 : H-; O78 : H-; O109 : H21; O109 : H48; O112a 112b : H21; O114 : H-; O115 : H40; O115 : H21; O115 : H-; O127 : H-; O128 : H7; O128 : H12; O142 : H7; O163 : H19; some others are still untyped (F. Ørskov et al., 1976). Some ETEC producing only ST (from serogroups O8, O50, O128 and untypable) or only LT (from serogroups O5, O8, O25, O40, O75) should be added to this list (Morris et al., 1976).

As regards shigella-like EEC-II, Stenzel (1975) has found that serotype 185 described by Trabulsi as EEC O115 (see Chapter 19) should be considered as belonging to *E. coli* O152. To reveal such EEC, often called "invasive" *E. coli* after DuPont et al. (1971), a number of workers tried to test isolates of different origin. Thus, Park et al. (1974), like Marier et al. in 1973, examined strains from cheese. Park and co-workers disclosed rabbit-gut-loop-positive and partly even keratoconjuctivitis-positive strains; in contrast to the strains of O124 found by Marier and co-workers, these were not shigella-like EEC-II, but EEC-I (O18 : B20, O128 : B12 and O125 : B15) though Park et al. regarded them as "invasive". These results contradict all available information on EEC-I pathogenic properties [see Chapters 19 and 20, as well as a recent study by Goldschmidt and DuPont (1976)] and should be confirmed by morphological examinations (investigating the capacity of the strains to multiply in the epithelial cells). Wachsmuth et al. (1975) looked for "invasive" and enterotoxin-producing strains among the isolates from non-enteric *E. coli* infections (NEEC strains — see Chapter 19) and failed to detect any "invasiveness" or enterotoxigenicity in strains from urine and blood. With similar purposes Rudoy and Nelson (1975) screened *E. coli* isolates from diarrhoeal and healthy babies, using HEp-2 cells, Serény's test and infant mice. They referred to an abstract of Ainbender et al. (1973) who observed HeLa "invasion" by *E. coli* strains from healthy babies (in half of the babies aged from 3 months to 6 years). Rudoy and Nelson reported that 30 per cent of their untypable strains from diarrhoeal and 12 per cent from

healthy babies invaded HEp-2 cells; the workers regarded the strains as "invasive", though Serény's test yielded negative results. These authors also described that the majority of strains from sick (86 per cent) and many from healthy (41 per cent) babies were enterotoxigenic, however, as compared with other investigators' results (Dean et al., 1972; D. Sack et al., 1975; Giannella, 1976; Goldschmidt and DuPont, 1976; Morris et al., 1976), the values of the suckling-mouse assay considered positive were too low. In addition, it is no use applying the term "invasiveness" to *E. coli* strains based only on the results of cell culture tests, because both EEC-I and shigella-like EEC-II multiply therein (see Chapter 20). Moreover, it is often rather difficult to distinguish extracellular bacteria (which multiply abundantly in the culture medium, particularly when the latter is not changed every 2–3 hours) from those adhering to the cells of a monolayer. Without several positive and negative controls this may even be impossible.

The question whether the so-called "classic" enteropathogenic *E. coli* serotypes, i.e. EEC-I, are capable of producing enterotoxins, was raised by many authors. Zinkernagel and Colombini (1975) observed accumulation of moderate or insignificant amounts of fluid in rabbit gut loops challenged with EEC-I (inhibited by immune bovine lactoserum), but EEC-I supernatant or whole cell lysate preparations failed to give distended loops. Revealing a number of ETEC strains in Apache children R. Sack et al. (1975*a*) stressed that none of the 64 strains of EEC-I from 43 sick children gave positive ileal-loop reaction. Results of examinations for antibodies to LT enterotoxin suggested rather frequent occurrence of ETEC in Apache children in that locality (R. Sack et al., 1975*b*). Gross et al. (1976) used Y1 adrenal and CHO cell tests for detecting LT, and the infant-mouse test for detecting ST enterotoxin in 6 strains of EEC-I that had caused infantile enteritis epidemics in Great Britain. Their attempts failed, and the authors concluded that either EEC-I produce enterotoxin undetectable by the available techniques or the pathogenesis of infantile enteritis differs from that of travellers' diarrhoea. Goldschmidt and DuPont (1976) studied 48 EEC-I strains to examine their pathogenic properties using Serény's test, rabbit gut loops, suckling mice and Y1 adrenal cells. The supernatants of only three strains (two of eight O26 : B6 and one of four O111 : B4) out of 48 EEC-I strains did induce pronounced fluid accumulation and dilatation of exposed rabbit gut loops. All other tests gave negative results. The workers suggested the lack of correlation between *E. coli* serotypes and presently known virulence properties in test systems; they also expressed doubts about the value of serotyping *E. coli* isolates from sporadic cases of diarrhoea. Nevertheless, rejecting such proposals, Rowe et al. (1976) recommended to continue serotyping *E. coli* and the study of their pathogenic properties. Reviewing the problem of enteropathogenicity of different *E. coli*, Neter (1975) also pointed out the necessity of further studies. Ørskov et al. (1976) noted that in the Copenhagen WHO *E. coli* Centre collection there are only a few enterotoxigenic strains belonging to EEC-I serogroups. Complete serotyping with H-antigen detection is necessary for any comparison of EEC-I and ETEC. ETEC strains mostly belong to certain special serotypes. The

authors emphasized that both full serotyping and enterotoxin indication would be necessary for future diagnostics.

The morphologic studies described in Chapter 22 have been continued and extended. It has recently been shown that *V. cholerae* El Tor and ETEC attach to the enterocyte surface and multiply there in a similar manner (Polotsky et al., 1977*a*, *c*). Thereafter the living organisms as well as their supernatants or whole cell lysates induce pronounced hypersecretion of the intestinal epithelium indicated by nonspecific stimulation of the cells, enhanced merocrine and apocrine secretion of absorptive, low-differentiated midvillial and undifferentiated crypt cells, as shown by electron microscopy. Such drastic changes of enterocytes exposed to ETEC or *V. cholerae* El Tor are apparently induced by absorption of their enterotoxins. Grady et al. (1975) and Strombeck and Harrold (1975) have shown that cholera enterotoxin is absorbed by enterocytes and is degraded by acid proteases in the cells.

The importance of the attachment of *V. cholerae* and human and porcine ETEC has also been emphasized by other workers (Evans et al., 1975; Hohmann and Wilson, 1975; Sellwood et al., 1975; Freter and Jones, 1976; Knop and Bellamy, 1976; Nagy et al., 1976; Rutter et al., 1976; Schrank and Verwey, 1976). Evans et al. (1975) have found a plasmid-controlled surface-associated colonization factor in human ETEC. Spontaneous loss of this plasmid (60×10^6 daltons in molecular weight) or neutralization of the factor by hyperimmune antiserum resulted in loss of pathogenicity of the organisms, though they continued to produce potent equivalent enterotoxin as detected by responses in infant or adult rabbits. The significance of plasmid-controlled K88 antigen for the attachment of porcine ETEC to the gut epithelium is well known (see Chapters 19 and 22, as well as a recent review of Moon, 1974). Besides, peculiar morphologic structures have been revealed on the surface of porcine ETEC strains lacking K88 antigen (Hohmann and Wilson, 1975; Nagy et al., 1976). By means of these structures bacterial attachment to the surface of enterocyte microvilli was also accomplished. In addition, porcine ETEC attachment to the gut epithelium is influenced by host-derived factors; there exist two different pig phenotypes (Sellwood et al., 1975).

The studies described in Chapter 22 have been complemented with a search for enterotoxin producing EEC-I strains in infantile diarrhoea cases. Two strains of O26 : K60 : H11 serotype out of 23 EEC-I strains tested were found to be enterotoxin producing (Polotsky et al., 1977*b*). Their living cultures, supernatants and whole cell lysates evoked pronounced fluid accumulation and dilatation of exposed rabbit gut loops. The enterotoxins of these two strains also gave marked positive reaction in intradermal rabbit PF assay, similarly to standard ETEC enterotoxins and purified choleragen. Like other loop-negative EEC-I strains (which behaved as described in Chapter 22)—or even more extensively than these—the organisms of enterotoxigenic EEC-I strains adhered to the brush border, intruded between the microvilli and multiplied on the outer epithelial cell membrane. They made close contact with the latter and caused shedding of the microvilli, penetrated the enterocytes becoming enclosed in membrane-bound phagosome-like vacuoles, appeared in the lamina propria and elicited mild

focal polymorphonuclear infiltration. All this differed markedly from ETEC behaviour. In addition, however, enterotoxin producing EEC-I organisms as well as their supernatants and whole cell lysates induced intense epithelial hypersecretion like ETEC. The conclusion was drawn that even very rare EEC-I organisms possessing the capacity to produce enterotoxin(s) differ from ETEC unable to penetrate the epithelium.

The attention payed to enterotoxins and improvement of the methods invoved have recently resulted in the discovery of new enterotoxigenic organisms causing tropical sprue. Thus, Klipstein et al. (1973) isolated *Klebsiella pneumoniae*, *Enterobacter cloacae* and *E. coli* O1 : H31 from jejunal aspirates of sprue patients and observed abundant isotonic fluid accumulation in ligated rabbit gut loops exposed to their culture filtrates. The effect of the bacteria and their enterotoxins on intestinal structure (Klipstein and Schenk, 1975) and transport (Klipstein et al., 1975) was studied. Enterotoxins of *Kl. pneumoniae* (Klipstein and Engert, 1975, 1976*a*) and *Ent. cloacae* (Klipstein and Engert, 1976*b*) were purified. They proved to be heat-stable low-molecular-weight (1,000—10,000 daltons) enterotoxins similar to ST enterotoxin of ETEC. Klipstein et al. (1976) have described isolates of *Kl. pneumoniae*, *Kl. ozaenae* and *E. coli* (O4 : H32 and untypable) in jejunal aspirates of patients with tropical sprue. Cell-free preparations of randomly selected strains of each of the coliform bacteria contained either ST or LT, or both forms of enterotoxin. It should be mentioned that the same biotype of *E. coli* (O4 : H32) produced LT toxin only in one instance and both forms of toxin in the other. Wadström et al. (1976) have recently reported that 37 per cent of infants and children admitted to a paediatric clinic in Addis-Abeba harboured enterotoxigenic bacteria as shown by the rabbit ileal loop, rabbit skin (PF) and adrenal cell tests. Thirty-eight per cent of the isolated strains were *E. coli* (see Ørskov et al., 1976), the others belonged to *Klebsiella* (15 per cent), *Enterobacter cloacae* (12 per cent), *Citrobacter* (11 per cent), *Aeromonas* (11 per cent), *Proteus* (7 per cent), *Serratia* (2 per cent) and *Pseudonomas* (1 per cent). Among the enterotoxigenic *E. coli* only one strain was of known EEC-I serotype.

Takeuchi (1975) has recently reviewed the problem of penetration of the gut epithelium by *Salmonella typhimurium* and compared them with other enteropathogens, including protozoal and viral ones, which were studied in different models (Takeuchi and Philipps, 1975, 1976; Takeuchi and Hashimoto, 1976; Takeuchi et al., 1976).

Giannella et al. (1975, 1976) extending the studies of *S. typhimurium* have further shown that invasion of the instestinal mucosa by salmonellae and the resulting inflammation do not substantially alter the permeability characteristics of the mucosa; fluid accumulation is induced by active secretory process, the activation of adenylate cyclase. However, the intestinal fluid secretion is blocked by treatment with indomethacin, which is a rapid, intense inhibitor of prostaglandin synthesis, though neither the invasiveness of salmonellae nor their peripheral dissemination are affected. This means that hypersecretion is apparently caused by endogenic prostaglandin rather than by an enterotoxin-like substance. Similar, but less significant inhibition of intestinal fluid secretion, also without affecting the

invasiveness and inflammation, was found in experiments with *Shigella flexneri* 2a (Gots et al., 1974). In monkeys challenged with *Shigella flexneri* 2a Rout et al. (1975) observed mucosal invasion by shigellae in the colon (as described in Chapter 11) and net jejunal secretion though neither bacterial invasion, nor any significant small bowel alteration were in evidence. Despite the difference between monkey salmonellosis (see Chapter 17) and shigellosis (shigella inability to invade the small intestine and damage ileal transport) net jejunal secretion occurred similarly in both of these invasive diarrhoeas. The possible importance of endogenic factors like prostaglandins in the gut exposed to shigellae was suggested. Continuing these studies, nevertheless, Kinsey et al. (1976) have failed to demonstrate watery diarrhoea (with fluid jejunal secretion) in monkeys challenged intracaecally with *Sh. flexneri* 2a. This means that some undefined interaction between the monkey's jejunal mucosa and shigellae, passing through the small intestine, does occur.

The situation is quite different with *Sh. dysenteriae* 1 producing enterotoxin of a special type. According to Keusch and Jacewicz (1975) Shiga enterotoxin and neurotoxin are closely related or even identical proteins, while cytotoxin is another protein, though it may be a subunit of the larger toxin that is capable of acting directly on the HeLa cells. As regards the mechanism of action of shigella enterotoxin in the rabbit ileum, Flores et al. (1974) and Donowitz et al. (1975) noted cytotoxic effect and no marked increase in the activity of the adenylate cyclase system. However, Charney et al. (1976) observed activation of intestinal mucosal adenylate cyclase by *Sh. dysenteriae* 1 enterotoxin employing concentrations of ATP greater than those required to demonstrate adenyl cyclase activation by choleragen. Therefore the authors suggested that alterations in fluid and electrolyte transport induced by *Sh. dysenteriae* 1 enterotoxin may, in part, be mediated by the adenylate cyclase system.

REFERENCES TO THE APPENDIX*

Aibender, E., Zepp, H. D., Hevizy, M., Hodes, H. L.: *E. coli* typing by HeLa cell invasion. *Pediatr. Res.*, **7,** 370 (1973).

Charney, A. N., Gots, R. E., Formal, S. B., Giannella, R. A.: *Shigella dysenteriae* 1 enterotoxin. *Gastroenterology,* **70,** 1085–1090 (1976).

Donowitz, M., Keusch, G. T., Binder, H. J.: Effect of shigella enterotoxin on electrolyte transport in rabbit ileum. *Gastroenterology,* **69,** 1230–1237 (1975).

Donta, S. T.: Neutralization of cholera enterotoxin-induced steroidogenesis by specific antibody. *J. infect. Dis.*, **129,** 284–288 (1974*a*).

Donta, S. T.: Differentiation between the steroidogenic effects of cholera enterotoxin and adrenocorticotropin through use of a mutant adrenal cell line. *J. infect. Dis.*, **129,** 728–731 (1974*b*).

Donta, S. T.: Interactions of choleragenoid and G_{M1} ganglioside with enterotoxins of *Vibrio cholerae* and *Escherichia coli* in cultured adrenal cells. *J. Infect. Dis.*, **133,** *suppl.*, S115–S119 (1976).

Donta, S. T., Moon, H. W., Whipp, S. C.: Detection of heat-labile *Escherichia coli* enterotoxin with the use of adrenal cells in tissue culture. *Science,* **183,** 334–336 (1974).

Donta, S. T., Viner, J. P.: Inhibition of the steroidogenic effects of cholera and heat-labile *Escherichia coli* enterotoxins by G_{M1} ganglioside; evidence for a similar receptor site for the two toxins. *Infect. Immun.* **11,** 982–985 (1975).

Dorner, F., Jaksche, H., Stöckl, W.: *Escherichia coli* enterotoxin: purification, partial characterization, and immunological observations. *J. infect. Dis.*, **133,** *suppl.*, S142–S156 (1976).

Evans, D. G., Evans, D. J. Jr., Gorbach, S. L.: Identification of enterotoxigenic *Escherichia coli* and serum antitoxin activity by the vascular permeability factor assay. *Infect Immun.* **8,** 731–735 (1973).

Evans, D. G., Silver, R. P., Evans, D. J. Jr., Chase, D. G., Gorbach, S. L.: Plasmid-controlled colonization factor associated with virulence in *Escherichia coli* enterotoxigenic for humans. *Infect. Immun.*, **12,** 656–667 (1975).

Finkelstein, R. A., LaRue, M. K., Johnston, D. W., Vasil, M. L., Cho, G. J., Jones, J. R.: Isolation and properties of heat-labile enterotoxin(s) from enteropathogenic *Escherichia coli. J. infect. Dis.*, **133,** *suppl.*, S120–S137 (1976*a*).

Finkelstein, R. A., Vasil, M. L., Jones, J. R., Anderson, R. A., Barnard, T.: Clinical cholera caused by enterotoxigenic *Escherichia coli. J. clin. Microbiol.*, **3,** 382–384 (1976*b*).

Flores, J., Grady, G. F., McIver, J., Witkum, P., Beckman, B., Sharp, G. W. G.: Comparison of the effects of enterotoxins of *Shigella dysenteriae* and *Vibrio cholerae* on the adenylate cyclase system of the rabbit intestine. *J. infect. Dis.*, **130,** 374–379 (1974).

Freter, R., Jones, G. W.: Adhesive properties of *Vibrio cholerae:* nature of the interaction with intact mucosal surfaces. *Infect. Immun.*, **14,** 246–256 (1976).

Giannella, R. A.: Suckling mouse model for detection of heat-stable *Escherichia coli* enterotoxin: characteristics of the model. *Infect. Immun.*, **14,** 95–99 (1976).

* Items also cited in the main bibliography (pp. 331–397) have not been repeated here.

Giannella, R. A., Gots, R. E., Charney, A. N., Greenough, W. B., Formal, S. B.: Pathogenesis of salmonella-mediated intestinal fluid secretion. Activation of adenylate cyclase and inhibition by indomethacin. *Gastroenterology*, **69**, 1238–1245 (1975).

Giannella, R. A., Rout, W. R., Formal, S. B., Collins, H.: Role of plasma filtration in the intestinal fluid secretion mediated by infection with *Salmonella typhimurium*. *Infect. Immun.*, **13**, 470–474 (1976).

Goldschmidt, M. C., DuPont, H. L.: Enteropathogenic *Escherichia coli:* lack of correlation of serotype with pathogenicity. *J. infect. Dis.*, **133**, 153–156 (1976).

Gorbach, S. L., Kean B. H., Evans, D. G., Evans, D. J., jr., Bessudo, D.: Travellers' diarrhea and toxigenic *Escherichia coli*. *N. Engl. J. Med.*, **292**, 933–936 (1975).

Gots, R. E., Formal, S. B., Giannella, R. A.: Indomethacin inhibition of *Salmonella typhimurium*, *Shigella flexneri*, and cholera-mediated rabbit ileal secretion. *J. infect. Dis.*, **130**, 280–284 (1974).

Grady, G. F., Keusch, G. T., Deschner, E. E.: Kinetics of absorption of toxin of *Vibrio cholerae*. *J. infect. Dis.*, **131**, 210–216 (1975).

Gross, R. J., Scotland, S. M., Rowe, B.: Enterotoxin testing of *Escherichia coli* causing epidemic infantile enteritis in the U. K. *Lancet*, **1**, 629–631 (1976).

Guerrant, R. L., Brunton, L. L., Schnaitman, T. C., Rebhun, L. I., Gilman, A. G.: Cyclic adenosine monophosphate and alteration of Chinese hamster ovary cell morphology: a rapid, sensitive *in vitro* assay for the enterotoxins of *Vibrio cholerae* and *Escherichia coli*. *Infect. Immun.*, **10**, 320–327 (1974).

Hohmann, A., Wilson, M. R.: Adherence of enteropathogenic *Escherichia coli* to intestinal epithelium *in vivo*. *Infect. Immun.*, **12**, 866–880 (1975).

Isaacson, R. E., Moon, H. W.: Induction of heat labile enterotoxin synthesis in enterotoxigenic *Escherichia coli* by Mitomycin C. *Infect. Immun.* **12**, 1271–1275 (1975).

Jacks, T. M., Wu, B. J.: Biochemical properties of *Escherichia coli* low-molecular-weight, heat-stable enterotoxin. *Infect. Immun.*, **9**, 342–347 (1974).

Kantor, H. S.: Enterotoxins of *Escherichia coli* and *Vibrio cholerae:* tools for the molecular biologists. *J. infect. Dis.*, **131**, *suppl.*, S22–S32 (1975).

Keusch, G. T., Donta, S. T.: Classification of enterotoxins on the basis of activity in cell culture. *J. infect. Dis.*, **131**, 58–63 (1975).

Keusch, G. T., Jacewicz, M.: The pathogenesis of shigella diarrhea. V. Relationship of Shiga enterotoxin, neurotoxin, and cytotoxin. *J. infect. Dis.*, **131**, *suppl.*, S33–S39 (1975).

Kinsey, M. D., Formal, S. B., Dammin, G. J., Giannella, R. A.: Fluid and electrolyte transport in rhesus monkeys challenged intracecally with *Shigella flexneri* 2 a. *Infect. Immun.*, **14**, 368–371 (1976).

Klipstein, F. A., Engert, R. F.: Enterotoxigenic intestinal bacteria in tropical sprue. III. Preliminary characterization of *Klebsiella pneumoniae* enterotoxin. *J. infect. Dis.*, **132**, 200–203 (1975).

Klipstein, F. A., Engert, R. F.: Purification and properties of *Klebsiella pneumoniae* heat-stable enterotoxin. *Infect. Immun.*, **13**, 373–381 (1976*a*).

Klipstein, F. A., Engert, R. F.: Partial purification and properties of *Enterobacter cloacae* heat-stable enterotoxin. *Infect. Immun.*, **13**, 1307–1314 (1976*b*).

Klipstein, F. A., Holdeman, L. V., Corcino, J. J., Moore, W. E. C.: Enterotoxigenic intestinal bacteria in tropical sprue. *Ann. intern. Med.*, **79**, 632–641 (1973).

Klipstein, F. A., Horowitz, I. R., Engert, R. F., Schenk, E. A.: Effect of *Klebsiella pneumoniae* enterotoxin on intestinal transport in the rat. *J. clin. Invest.*, **56**, 799–807 (1975).

Klipstein, F. A., Schenk, E. A.: Enterotoxigenic intestinal bacteria in tropical sprue. II. Effect of the bacteria and their enterotoxins on intestinal structure. *Gastroenterology*, **67**, 642–655 (1975).

Klipstein, F. A., Short, H. B., Engert, R. F., Jean, L., Weaver, G. A.: Contamination of the small intestine by enterotoxigenic coliform bacteria among the rural population of Haiti. *Gastroenterology*, **70**, 1035–1041 (1976).

Knop, J., Bellamy, J. E. C.: Supression of growth of *Vibrio cholerae* in the intestine of immunized mice. *Med. Microbiol. Immunol.*, **162**, 153–158 (1976).

McDonel, J. L., Duncan, C. L.: Histopathological effect of *Clostridium perfringens* enterotoxin in the rabbit ileum. *Infect. Immun.*, **12**, 1214–1218 (1975).

Moon, H. W.: Pathogenesis of enteric diseases caused by *Escherichia coli*. *Adv. Vet. Sci. Comp. Med.*, **18**, 179–212 (1974).

Morris, G. K., Merson, M. H., Sack, D. A., Wells, J. G., Martin, W. T., Dewitt, W. E., Feeley, J. C., Sack, R. B., Bessudo, D. M.: Laboratory investigation of diarrhea in travellers to Mexico: evaluation of methods for detecting enterotoxigenic *Escherichia coli*. *J. clin. Microbiol.*, **3**, 486–495 (1976).

Nagy, B., Moon, H. W., Isaacson, R. E.: Colonization of porcine small intestine by *Escherichia coli:* ileal colonization and adhesion by pig enteropathogens that lack K 88 antigen and by some acapsular mutants. *Infect. Immun.*, **13**, 1214–1220 (1976).

Neter, E.: Enteropathogenicity of *Escherichia coli*. *Am. J. Dis. Child.* **129**, 666–667 (1975).

Ørskov, F., Ørskov, I., Evans, D. J. Jr., Sack, R. B., Sack, D. A., Wadström, T.: Special *Escherichia coli* serotypes among enterotoxigenic strains from diarrhoea in adults and children. *Med. Microbiol. Immunol.*, **162**, 73–80 (1976).

Park, C. E., Todd, E. C. D., Purvis, U., Laidley, R.: Disease traced to cheese. *Lancet*, **1**, 172–173 (1974).

Polotsky, Yu. E., Dragunskaya, E. M., Samostrelsky, A. Yu., Vasser, N. R., Efremov, V. E., Snigirevskaya, E. S., Seliverstova, V. G.: Interaction of *Vibrio cholerae* El Tor and gut mucosa in ligated rabbit ileal loop experiment. *Med. Biol. (Helsinki)*, *55*, (1977*a*) — to be published.

Polotsky, Yu. E., Dragunskaya, E. M., Seliverstova, V. G., Avdeeva, T. A., Chakhutinskaya, M. G., Kétyi, I., Verténvi, Á., Ralovich, B., Emődy, L., Málovics, I., Safonova, N. V., Snigirevskaya, E. S., Karyagina, E. I.: Pathogenic effect of enteropathogenic *Escherichia coli* and *Escherichia coli* causing infantile diarrhoea. *Acta microbiol. Acad. Sci. hung.*, **24**, (1977*b*) — to be published.

Polotsky, Yu. E., Dragunskaya, E M., Snigirevskaya, E. S., Seliverstova, V. G.: Interaction of the intestinal epithelium with cholera vibrios and *Escherichia coli* causing cholera-like diseases. *Arkh. Patol. (Moscow)*, **2**, 10–17 (1977*c*).

Rappaport, R. S., Sagin, J. F., Pierzchala, W. A., Bonde, G., Rubin, B. A., Tint, H.: Activation of heat-labile *Escherichia coli* enterotoxin by trypsin. *J. infect. Dis.*, **133**, *suppl.*, S41–S54 (1976).

Rout, W. R., Formal S. B., Giannella, R. A., Dammin, G. J.: Pathophysiology of shigella diarrhea in the rhesus monkey: intestinal transport, morphological and bacteriological studies. *Gastroenterology*, **68**, 270–278 (1975).

Rowe, B., Gross, R. J., Scotland, S. M.: Serotyping of *Escherichia coli*. *Lancet*, **ii**, 37–38 (1976).

Rudoy, R. C., Nelson, J. D.: Enteroinvasive and enterotoxigenic *Escherichia coli*. Occurrence in acute diarrhea of infants and children. *Am. J. Dis. Child.*, **129**, 668–672 (1975).

Rutter, J. M., Jones, G. W., Brown, G. T. H., Burrows, M. R., Luther, P. D.: Antibacterial activity in colostrum and milk associated with protection of piglets against enteric disease caused by K88-positive *Escherichia coli*. *Infect. Immun.*, **13**, 667–676 (1976).

Sack, D. A., Merson, M. H., Wells, J. G., Sack, R. B., Morris, G. K.: Diarrhoea associated with heat-stable enterotoxin-producing strains of *Escherichia coli*. *Lancet*, **ii**, 239–241 (1975).

Sack, D. A., Sack, R. B.: Test for enterotoxigenic *Escherichia coli* using Y1 adrena cells in miniculture. *Infect. Immun.*, **11**, 334–336 (1975).

Sack, R. B.: Human diarrheal disease caused by enterotoxigenic *Escherichia coli*. *Ann. Rev. Microbiol.*, **29**, 333–353 (1975).

Sack, R. B., Hirschhorn, N., Brownlee, I., Cash, R. A., Woodward, W. E., Sack, D. A.: Enterotoxigenic *Escherichia coli*-associated diarrheal disease in Apache children. *N. Engl. J. Med.*, **292**, 1041–1045 (1975*a*).

Sack, R. B., Hirschhorn, N., Woodward, W. E., Sack, D. A., Cash, R. A.: Antibodies to heat-labile *Escherichia coli* enterotoxin in Apaches in Whiteriver, Arizona. *Infect. Immun.*, **12**, 1475–1477 (1975*b*).

Schrank, G. D., Verwey, W. F.: Distribution of cholera organisms in experimental *Vibrio cholerae* infections: proposed mechanisms of pathogenesis and antibacterial immunity. *Infect. Immun.*, **13**, 195–203 (1976).

Sellwood, R., Gibbons, R. A., Jones, G. W., Rutter, J. M.: Adhesion of enteropatho-

genic *Escherichia coli* to pig intestinal brush borders: the existence of two pig phenotypes. *J. Med. Microbiol.*, **8**, 405–411 (1975).
Stenzel, W.: Identität des Serotyps 185 Trabulsi mit *Escherichia coli* O152. *Zbl. Bakt. Hyg., I Abt. Orig. A*, **232**, 473–476 (1975).
Strombeck, D. R., Harrold, D.: Comparison of the rate of absorption and proteolysis of ^{14}C-choleragen and ^{14}C-bovine serum albumin in the rat jejunum. *Infect. Immun.*, **12**, 1450–1456 (1975).
Takeuchi, A.: Electron microscope observations on penetration of the gut epithelial barrier by *Salmonella typhimurium*. In:*Mircobiology* 1975. Ed. by D. Schlessinger. Am. Soc. Microbiol., Washington, D. C. pp. 174–181 (1975).
Takeuchi, A., Binn, L. N., Jervis, H. R., Keenan, K. P., Hildebrandt, P. K., Valas, R. B., Bland, F. F.: Electron microscope study of experimental enteric infection in neonatal dogs with a canine coronavirus. *Lab. Invest.*, **34**, 539–549 (1976).
Takeuchi, A., Hashimoto, K.: Electron microscope study of experimental enteric adenovirus infection in mice. *Infect. Immun.*, **13**, 569—580 (1976).
Takeuchi, A., Phillips, B. P.: Electron microscope studies of experimental *Entamoeba histolytica* infection in the guinea pig. I. Penetration of the intestinal epithelium by trophozoites. *Am. J. trop. Med. Hyg.*, **24**, 34–48 (1975).
Takeuchi, A., Phillips, B. P.: Electron microscope studies of experimental *Entamoeba histolytica* infection in the guinea pig. II. Early cellular and vascular changes accompanying invasion of the lamina propria. *Virchows Arch. B Cell Path.*, **20**, 1–13 (1976).
Wachsmuth, I. K., Stamm, W. E., McGowan, J. E. Jr.: Prevalence of toxigenic and invasive strains of *Escherichia coli* in a hospital population. *J. infect. Dis.*, **132** 601–603 (1975).
Wadström, T., Aust Kettis, A., Habte, D., Holmgren, J., Meeuwisse, G., Möllby, R., Söderlind, O.: New enterotoxigenic bacteria isolated. *Brit. Med. J.*, **1**, 1401 (1976).
Zinkernagel, R. M., Colombini, A.: Passive oral immunization with bovine immunoglobulins: enteropathogenic *Escherichia coli* from infants and bovine anti-*E. coli* lactoserum assayed in the rabbit ileal loop model. *Med. Microbiol. Immunol.*, **162**, 1–7 (1975).

*

Finkelstein, R. A.: The cholera enterotoxin — Robert Koch revisited. *Zbl. Bakt. Hyg. I. Abt. Orig. A* **235**, 13–19 (1976).
Finkelstein, R. A.: Progress in the study of cholera and related enterotoxins. In: *Selected Topics in Bacterial Toxicology*. Ed. by A. Bernheimer. John Wiley, New York (1976).
Formal, S. B., Gemski, P.: Studies on the pathogenesis of intestinal disease caused by invasive bacteria. *Zbl. Bakt. Hyg. I. Abt. Orig. A* **235**, 9–12 (1976).
Myers, L. L., Guinée, P. A. M.: Occurrence and characteristics of enterotoxigenic *Escherichia coli* isolated from calves with diarrhea. *Infect. Immun.*, **13**, 1117–1119 (1976).
Neter, E.: Factors of pathogenicity: Gram-negative bacteria. *Zbl. Bakt. Hyg. I. Abt. Orig. A* **235**, 3–8 (1976).
Schenkein, I., Green, R. F., Santos, D. S., Maas, W. K.: Partial purification and characterization of a heat-labile enterotoxin of *Escherichia coli*. *Infect. Immun.*, **13**, 1710–1720 (1976).
Sinelnikova, M. P., Logacheva, L. S., Ovchinnikova, E. A., Seppi, I. V.: Intraepitelial shigella invasion in the intestinal mucosa of patients with acute *Sh. sonnei* dysentery. *Zh. Mikrobiol. (Moscow)*, **10**, 20–24 (1976).
Stanislavsky, E. S., Volynsky, M. Ya.: Enterotoxins of enterobacteria. *Zh. Mikrobiol. (Moscow)*, **10**, 3–9 (1976).
Steinrück, H.: Die Enterotoxine von *Escherichia coli*. Vorkommen, Nachweis, Eigenschaften und Bedeutung. *DDR-Med.-Rep.*, **5**, 195–245 (1975).
Tabachnik, A. L., Girshovich, E. S., Temper, P. M.: Enterotoxigenic *Escherichia coli*. *Zh. Mikrobiol. (Moscow)*, **3**, 31–38 (1977).
Voino-Yasenetsky, M. V.: Methodological approaches to study of infectious processes' morphology. *Arkh. Pat. (Moscow)*, **2**, 3–10 (1977).
Voino-Yasenetsky, M. V., Ismailov, F. N., Dragunskaya, E. M., Ravdonikas, L. E., Grabovskaya, K. B.: Experimental salmonella conjunctivitis and keratitis. *Zh. Mikrobiol. (Moscow)*, **7**, (1977) — to be published.

SUBJECT INDEX*

* Items listed in the Contents are not repeated in the Index.