Symposia Biologica Hungarica 5

MODERN TRENDS IN NEUROMORPHOLOGY

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Akadémiai Kiadó, Budapest

MODERN TRENDS IN NEUROMORPHOLOGY

International Conference
on Neuromorphology
held in Commemoration
of the Centenary of the Birth
of Mihály Lenhossék

Edited by
J. SZENTÁGOTHAI

(Symposia Biologica Hungarica 5)

At the centenary of the birth of Mihály Lenhossék, outstanding Hungarian anatomist, in July 1964, an international conference was held in Budapest, the complete material of which is included in the present volume. The papers written by internationally well-known Hungarian and foreign scientists deal with the following subjects: texture of receptors, structure of the vegetative nervous system, motorial end organs, central nerve courses and synapses, as well as neurosecretion.

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AKADÉMIAI KIADÓ, BUDAPEST 1965

International Conference on
Neuromorphology
held on 5 and 6 July 1963 in Budapest, in Commemoration
of the Centenary of the Birth of

MIHÁLY LENHOSSÉK

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LIST OF PARTICIPANTS

Australia
ECCLES, J. C., Canberra

Austria
LASSMANN, G., Wien

Bulgaria
KADANOFF, D., Sofia

France
PICARD, D., Marseille

German Democratic Republic
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MÜLLER, W., Köln—Lindenthal

Hungary
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BENKE, B., Budapest
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KRIEG, W. J. S., Chicago, Illinois

USSR
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GONCHARENKO, L. YE., Kiev

The following authors' papers were presented in their absence
BRODAL, A., Oslo, Norway
MILOCHIN, A. A., Leningrad, USSR
SÁVAY, GY., Szeged, Hungary

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Symp. Biol. Hung., **5**, 9—13 (1965)

TO THE MEMORY OF MIHÁLY LENHOSSÉK

Opening Address

by

I. TÖRŐ

DEPARTMENT OF HISTOLOGY, UNIVERSITY MEDICAL SCHOOL BUDAPEST, HUNGARY

To remember the great personalities of the past is an obligation of posterity. But commemoration is not only a duty but also a source of power. The life of every individual is interesting and may reflect a series of deeds worthy of imitation, but that of the great ones is exemplary. The history of Hungarian medical science is rich in great names, but even among these that of Mihály Lenhossék, once Professor of Anatomy at the University of Budapest, who made Hungarian morphology famous the world over, occupies a place of distinction.

I have had only limited personal experiences regarding Mihály Lenhossék's personality and character. My image of him has been gathered from writings and hearsay. My personal contact with him had mostly been confined to discussions following the lectures which I, then a young assistant from the country, held here in Budapest. I remember details of a rather long discussion that followed one of my lectures dealing with the regeneration of the lens, a topic of particular interest to him as one deeply involved in the investigation of the histological structure of the eye.

A keen judgement and exactness in observation were characteristic to Lenhossék. The intimate friendship he maintained with Cajal could be attributed to his keen sense of criticism. At first he was rather sceptical in his statements on Cajal's work, but later revised his view, which was duly acknowledged by Cajal in his papers.

His universal culture and wide knowledge were not only the source but also the basis of this professional work.

He was brought up in a family with high traditional ambitions, thence imbibed an ardent love for humanity, enthusiasm for science and culture. His father's devotion to science and his mother's love of music raised a tender and intimate atmosphere around him, in which he developed into the highly cultured and refined personality known from his writings and habits.

His family originates from Pozsony. He was born on the 28th of August, 1863 in Budapest. His father, József, was Professor of Anatomy first at Kolozsvár, later in Budapest. His grandfather was also a university man, Professor of Anatomy and Physiology, which subjects were joined to one and the same chair at that time.

Originally he wanted to follow studies in the history of literature and philosophy. However, after his matriculation in 1881, he gave way to his father's persuasion and changed for medicine. As disclosed in his memoirs, he himself could give no other reason for this decision but that he

was spurred by an ambition to catch up with his father and grandfather. Even as a child he was rather happy with a book, later as a student he enjoyed the microscope and therefore his figure was not of the athletic type. A high forehead, lively but sometimes somewhat dreamy glance gave his face a fascinating expression. An imperial adorned his lower lip, as seen on his portrait. His fetching personality won him many friends and disarmed his enemies. As a medical student he was more engaged in the theoretical pursuits of sciences than their practical result. He was conscious of his contemplative nature, somewhat lacking in the practical way of thinking. His main interest centered around anatomy, pathological anatomy, microscopy and problems of embryology. In histology he was a self-taught man. When a junior, he investigated the histology of the intervertebral ganglia in the frog and without showing his work to anyone sent it in for publication to the *Archiv für Mikroskopische Anatomie*, where it was accepted. This work was elaborated at the Physiological Department, since at the Department of Anatomy they had no immersion objective. As a senior he worked in the Pathological Department under Professor Scheuthauer, who greatly impressed him by his proficiency in cultural history. Most probably this initiated an intimate contact between master and disciple, both of them being attracted by exhanges of ideas beyond mere professional discussions. In the fifth year of his medical curriculum he was admitted to his father's department as demonstrator of anatomy. He qualified in medicine in 1886 and became Second Assistant (Prosector). In 1888 he won Venia Legendi in Anatomy.

According to his memoirs, he would have succeeded his father, had the latter lived a few more years. However, at the death of József Lenhossék, on December 2, 1888 he was too young, only 25. He was entrusted with the direction of the Department, and it seemed possible that the situation might prevail for some years. Later, however, the opinion still was held that even in some years he would be too young for the Chair of Anatomy and, therefore, Lajos Thanhoffer from the Physiological Department of the Veterinary Highschool was appointed as professor. Although at home in histology, Thanhoffer was no anatomist, but at the moment there was no one more suitable at hand. In the distance of many years the real causes which precluded the realization of the first and sound project are unknown. We regret to read that Mihály Lenhossék was advised by some persons to quit anatomy and attempts were made to persuade him to become a surgeon. But feeling no inclination for medical practice, he loyally adhered to his profession, even at the expense of leaving the country. At the invitation of Professor Kollmann he accepted the post of Prosector at the Department of Anatomy in Basle in 1889. He spent three years and a half there in satisfaction. On November 21, 1891, his achievements on the anatomy of the spinal chord (Über die Fortschritte der Rückenmarksanatomie) brought him the title of Honorary Lecturer. He could work in peace in Basle. From here he was invited by Professor W. His to Leipzig, where he would have proceeded gladly. However, Professor His was unable to refuse the request of Professor Kölliker in the interest of a Prosector at Würzburg and so advised Lenhossék to accept the vacant post at Würzburg. As Lenhossék's final and steady aim was to return to his native country, he thought that work at a German department would be an

advancement to that end, therefore he accepted the offer. Later he was disappointed, as he had been invited to Leipzig as an Associate Professor, while in Würzburg he was employed only as Honorary Lecturer. However, he resigned himself to his fate. He never imagined that as compared to the active co-operation in faculty life in Switzerland, things would be so different in Würzburg. He became painfully conscious of a rigid subordination prevailing there and realized an unbridgeable abyss existing between the Regius Professor and the Honorary Lecturer. Lack of intimacy with his Chief, and with university life in general, explained his enthusiasm to accept the post of Prosector at the Department of Anatomy in Tübingen under the leadership of Professor Froriep. He spent four years at Tübingen and had many pleasant memories from that period. He participated in concerts as soloist and became an honoured and well-known member of that community.

However, homesickness was ever present in him. With Ernő Jendrássik and Otto Petrik he kept close contact. Owing to these connections, in 1897 he was elected Corresponding Member of the Hungarian Academy of Sciences. In 1899 Géza Mihálkovics, Director of the Department I of Anatomy in Budapest, unexpectedly died at the age of 55, and Mihály Lenhossék was appointed as his successor. He assumed leadership of the Department on January 1, 1900.

The major part of his activities fell to this period. He wrote several textbooks, published many scientific papers, but took his share also in the propagation of morphological, anthropological and biological research. Twice he was elected Dean of the Faculty and once Rector Magnificus of the University. As he himself put in his memoirs, the leading motive of his activity was to give the best morphological basis of the medical science for his students. In his opinion a bare enumeration of the details of anatomical structures of the organs without addition of further comments could not result in an appropriate scientific standard and could not even meet the needs of the students either. He likewise regarded it unsatisfactory that his lectures on anatomy would serve merely the requirements of surgery, as the students' activities would rather be exercised in the field of medicine and not in surgery. His view-point of medical science was that the demonstration of anatomy as a biological science was of primary importance to the students.

The biological view should — in his opinion — be predominant in morphology, where the description of structures should always refer to a connection with function. This, of course, was valid also in the reverse, as proved by the University at that time when the joint instruction of morphology and physiology served for the fundament of medical curriculum. This concept was always more in force in histology than in macroscopic anatomy. It was Lenhossék who transferred the concept to the latter field, and for this very fact his sound reasonings and treatment of the subject are of special value. In this respect the following statement is found in his memoirs: "Es gibt eine Menge physiologischer Dinge, die überhaupt nur von Anatomen, im unmittelbaren Anschluß an die Vorweisung der betreffenden anatomischen Einzelheiten vollkommen klar gemacht werden können. Ich habe meine Aufgabe im Unterricht stets in diesem Sinne aufgefaßt und dieser meiner Auffassung gemäß auch mein 1922 erschienenes ungarisches Lehrbuch der Anatomie

reichlich mit physiologischen Hinweisen durchflochten." Apart from his research on the nervous system — which will in the following be discussed by Professor Környei — he achieved lasting results in numerous other fields. For instance: the behaviour of centrioles during spermiogenesis, where he proposed that the names Spermatogenese, Spermatogonie, Spermatocyte, Spermatide so far used be substituted by the following nomenclature: Spermiogenese, Spermiogonie, Spermiocyte, Spermide.

He was first to describe the importance of the basal bodies of the ciliated cells, which later was confirmed by his disciple György Rényi. Much of his interest was directed also to the interstitial cells of the testicle and the microcentres of smooth muscle.

He sought the factors determinmg sex in the ovum, as far as the ovary produces male and female ova in a definite ratio. He treated the subject in *Der Überschuß an Knabengeburten und seine biologische Bedeutung*. He regarded sex chromosomes as the sign of sex but not as its factor.

The striking work of his *Histogenese des Glaskörpers* as he himself said, was his 'scientific problemchild' (wissenschaftliches Schmerzenskind). He considered the vitrous body as the product of the lense. In his work *Über die Entwicklung der Zonulafasern* he deals with developmental problems of the eyes.

Some of his anthropological works appeared between 1915 and 1920. After the death of Professor Török he occupied the chair of anthropology. His publications *Aufgaben der physischen Antropologie*, *Nahtverknöcherungen im Kindesalter*, *The internal surface (relief) of the ramus mandibulae*, *The anatomy of the teeth*, *On the optic nerve of snakes*, *Geschlechtsverhältnis der menschlichen Früchte vom 4—10 Schwangerschaftsmonat* witness his versatility.

Mihály Lenhossék lived and lectured for 10 years in Germany. German science and German scientists were his ideals, but in his heart he remained a true Hungarian. The constituents of his greatness were: brains, heart and character. These are reflected also in his scientific world. His parents brought him up as a Catholic, but he first turned to be a materialist, later an agnostic, though never arrived at the recognition of the materialistic foundations of life. He not only condemned war, but it shocked him to his core.

He was an outstanding international scientist who as a researcher, lecturer, writer and personality made an indelible imprint on the history of Hungarian science.

Huzella said with reference to Lenhossék: "As a representative of national scientific culture, a scientist finds the culminating intellectual expression of his nationality and the deepest patriotism in the internationalism of science."

However eminent and independent the culture of a small state may be, it is unable to construct out of its own power a complete whole of science without shortcomings. Such states are compelled to seek connections with the research and educative institutes of more developed states, to import knowledge what enables them to fill gaps and keep in step with the progress of international science. Owing to historical circumstances, Hungarian science acquired these intellectual reinforcements for a long time mainly from German sources, and Lenhossék was one of those gifted

Hungarians who endeavoured to satisfy his aspirations at the stimulus of that school. The period spent in Germany made him able to master the language and appropriate habits of life surrounding him there to such a degree that German anatomists regarded him as one of themselves. However, he did never forget his nationality, and never for one minute did he keep those around him in the belief that he had disowned his country. His patriotism was increasingly confirmed by the recognition of his vocation, realizing his duty to raise Hungarian anatomy to the international level, to which he devoted his full strength from the moment a post became vacant for him in one of the departments of anatomy in Hungary.

Now the appreciation of science is high and dynamically rising in Hungary. From year to year the Government takes effective measures for the promotion of scientific research work. Owing to this, Hungarian morphology keeps up with the international standard and proceeds in the field which had been made accessible by Mihály Lenhossék for his disciples, who by this symposium strive to return to the international science the interest of the fund Lenhossék had gathered in intellectual wealth beyond the borders of his country.

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MIHÁLY LENHOSSÉK AND THE DEVELOPMENT OF THE NEURONE THEORY

by

St. Környey

DEPARTMENT OF NEUROLOGY AND PSYCHIATRY, UNIVERSITY MEDICAL SCHOOL PÉCS, HUNGARY

The conception of the nerve cell and its processes as a cellular unit was first proposed by embryologists and neuropathologists. The idea appeared as early as 1857 in a paper of Kupffer's, but it was only in the eigthies that His, in a systematic work on the histogenesis of the nervous system, demonstrated the initial development of the axon from the neuroblast. In neuropathology Forel referred to the facts revealing that characteristic of the neurone which was later termed trophic unity. Thus the two principal theses — the building up of a leading system by a subsequent articulation of cellular units, and the dependence of the nerve fibre on the cell body — later contracted in one formula by Ramón y Cajal as the fourfold unity of the neurone—had already been known when Lenhossék joined the new research trend.

This pioneer work was carried out with the histological methods of the time. However, the neurone theory could not be provided with a solid morphological basis before the Golgi impregnation was applied to the systematic investigation of nervous structures by Santiago Ramón y Cajal. It was not until 1889 that these results were first recognized by the scientific world.

In Lenhossék's youth the atmosphere at the University of Budapest was undoubtedly conducive to interest in neuroanatomical research. His father, József Lenhossék, was the professor of anatomy and one of the first authors to describe the microscopic anatomy of the human bulb. The Neurological Institute of the University of Vienna still has a box containing his preparations cut with razor and stained only by chrome, yet, even in their present condition, visualizing the main structures. The chair of topographical anatomy and embryology was held be Victor Mihálkovics whose monograph on brain development is still remembered, almost 90 years after its publication. It was probably due to their influence that three of their disciples from the same generation devoted themselves to research in neurohistology. They were Mihály Lenhossék (Michael von Lenhossék in the German lit.), Stephen Apáthy, later a most passionate opponent to the doctrine of neurone, and Charles Schaffer, a principal representative of the doctrine of neurone in neuropathology for decades.

Lenhossék's first contribution to neurohistology was disclosed when the Golgi method had not yet been available for the neuronists. In his first paper, in 1886, he supported the pseudo-unipolar character of the spinal ganglion cells and furnished the decisive proof for the assumption of Ranvier that the process of the neurone of the spinal ganglion divides

into two rami, taking one of them for the central and the other the peripheral direction. Today this characteristic of the spinal ganglion cells is accepted without reserve, but a perusal of the controversies of the early eighties, including Retzius's opinion that Ranvier's statement seemed to be very probable though unproved, will suffice to show the difficulties it encountered.

In this opening period Lenhossék was the first suggesting a division of the cellular complex of the tuber cinereum into individual nuclei. He described the exact extension of the formation which, as a result of his paper, has become known as supraoptic nucleus. In comparative myelogenetic studies of the spinal cord he partly extended and also limited the validity of Stieda's discovery, according to which the pyramidal tract in the cord of certain rodents was situated not in the lateral but in the posterior column, and gave a detailed description of the posterior column.

But it was the Golgi method which found one of its principal exponents in Lenhossék. For the rapid propagation and recognition of Cajal's work we are highly indebted to Kölliker, one of the leading German histologists, who was among the first to apply the Golgi method and evaluate its results in a systematic description of the nervous structures in his treatise on histology. Besides him and another distinguished morphologist, G. Retzius, two young scientists, Lenhossék and van Gehuchten, made their names associated with the development of the new doctrine both by the amount and importance of their contributions.

Lenhossék's work with the Golgi method was restricted to the years from 1889 to 1896. In 1890 he proved the existence of centrifugal fibers in the posterior roots of the hen, a discovery made about the same time also by Cajal. Lenhossék, always concerned with functional understanding, indicated the relationship of this observation to the regulatory influence of the posterior roots on vasodilatation, as demonstrated experimentally by Stricker and others. A further discovery in which Lenhossék shared priority with another leading scientist, this time with Retzius, was the description and interpretation of the sensory cells of the lumbricus. His attention was given to sensory cells and sensory endings in studies of the sense buds of the mucosa of the mouth, of Jacobson's organ, and the maculae and cristae acusticae. Again at the same time as Retzius, he showed that the sensory cells in the taste buds did not possess an axon and that in the vertebrates only the olfactory sensory cells preserved the invertebrate character of a sense cell.

Lenhossék published his results concerning some sensory and vegetative ganglia, and some nerve endings in a volume entitled *Beiträge zur Histologie des Nervensystems und der Sinnesorgane*. He studied the development of the cells of the spinal ganglia and demonstrated that unipolarization of the originally bipolar neurones takes place in consequence of withdrawal of the cell body from the processes, while formerly a fusion of the proximal parts of the processes was believed to occur. He supported the view of Retzius that the geniculate ganglion was equivalent to the sensory ganglia, and showed that the processes of its unipolar cells become the fibers of the nervus intermedius; he also analysed other connections of this ganglion. This work was reassumed and furthered by Szentágothai almost four decades later. In another paper in the same volume Lenhossék proved

the vegetative character of the sphenopalatine ganglion and described its connections with the trigeminal nerve.

Lenhossék was the first to study the development of the neuroglia in the human embryo with the Golgi method. The terms astrocyte and astroblast were proposed by him. It may be mentioned that while calling attention to Golgi's statement that some gliomas were made up of astrocytes, he suggested the name astroma, a forerunner of modern terminology, as recognized by Bailey and Cushing.

In 1892 Lenhossék gave a comprehensive description of the results of his work on the spinal cord, which he completed with a review on the general results of the new trends in neurohistology. Waldeyer's report published one year earlier became renowned for introducing the term neurone. The only world language in which Lenhossék published was German, but his small book *Der feinere Bau des Nervensystems im Lichte neuester Forschungen* was translated into French. A second German edition entirely recast and enlarged followed three years later.

One of the most interesting works of Lenhossék concerns the retina and the optic lobe of the Cephalopoda, a study which was later supported and complemented by Cajal.

In 1891 Lenhossék gave a detailed description of the early development of the spinal ganglia in the human embryo.

Several papers of Lenhossék contribute to the structure of the nerve cell, especially to that of the spinal ganglion cells in which he discovered the centrosome. In these papers he proposed some technical improvements. Thus, after being reintroduced by Lenhossék, the use of toluidin blue first proposed by Mann, replaced the original Nissl staining with saponised methylene blue. In these papers Lenhossék also proposed two terms which became generally known, although less preferred, namely tigroid for Nissl substance and amphicyte for the capsular cells of the spinal ganglia.

In later years Lenhossék returned to problems of the histogenesis and histology of the nervous tissue. To prove the centrogenic growth of the nerve fibres and the ectodermal origin of Schwann's cells on the basis of the current histological stains and silver preparations, in 1906 he studied the histogenesis of the peripheral nerve fibres. However, a purely morphological work like his was at that time already overshadowed by the experimental studies of Harrison. Nevertheless, the terms lemmocyte and lemmoblast proposed by Lenhossék have been generally adopted. He interpreted the early development of neurofibrils in the neuroblast in the sense that they serve as a mechanical support of the outgrowing axon.

Later his work was dedicated to cytological structure, in particular to the synapses, of the autonomic cranial ganglia. He recognized the differences distinguishing the nerve cells of these ganglia from those of the sympathetic system, which was an important contribution to the morphological distinction between the parasympathetic and the sympathetic systems. In recent years the interesting synaptic formations in the ciliary ganglion have become the starting points of investigations with methods of experimental physiology and histochemistry.

In his last neurohistological paper, Lenhossék described the structural

peculiarity of the optic nerve of the serpents, given in the fact that the myelinated fibres are embedded in a neuroglial syncytium.

Lenhossék's scientific work was characterized by a rigorous criticism of himself as well as others. When writing his autobiography, his modesty induced him to restrict his own merits to having helped the neurone theory to recognition. It is interesting to follow the progress of the neurone theory to recognition. The neurone theory has never been seriously contested in French or Anglo-Saxon science. Owing to Sherrington's work and his syntheses, it has become the basis of modern neurophysiological investigation, in which every principal fact proves it to be the only anatomical conception compatible with physiological observations. Yet the neurone theory was passionately contradicted by most German representatives of the various fields of the neurological research and by Lenhossék's fellow-countryman Apáthy. The opposition lasted nearly to our day, while recently even exponents of the generation directly influenced by the most enthusiastic antineuronists Nissl, Held and Bethe seem to accept the neurone theory without reservation.

In periods of scientific development when two divergent theories are fighting one another, literature is usually abundant in polemic papers, often based, instead of the description of new findings, on pure speculation which hardly seems justified to later generations, since either the reasoning of one party, or the whole question, has lost importance in the meantime. The combat between neuronists and antineuronists was not exempt from this weakness of scientific attitude. Lenhossék himself was rather reserved in taking part in the heated controversies in the literature. In his works he generally described the facts established by him, and in their light he examined conflicting opinions.

SECTION ONE

RECEPTORS

Symp. Biol. Hung., **5**, 21—41 (1965)

THE VESTIBULAR SENSORY CELLS AND THEIR INNERVATION*

by

H. ENGSTRÖM

UNIVERSITY OF GÖTEBORG, SWEDEN

H. W. ADES

U.S. NAVAL SCHOOL OF AVIATION MEDICINE
PENSACOLA, FLORIDA,

J. E. HAWKINS, JR.**

UNIVERSITY OF GÖTEBORG, SWEDEN

The basic study of the ampullar cristae of the guinea pig by Wersäll (1956) greatly advanced our knowledge of the vestibular epithelia. Further studies by Smith (1956), Engström and Wersäll (1958), Bairati (1961), Engström (1958, 1961), Wersäll (1961) and others confirmed a principle that could be recognized in all vestibular sensory epithelia of higher mammals. It is now generally accepted that there are two distinct types of hair cells in the vestibular sensory epithelia. During the last few years, however, it has become evident that the description of the vestibular sensory cells has been oversimplified and that important features of their structure have been overlooked. Especially is this true of the innervation of the hair cells; therefore we considered it of interest to describe these sensory cells and their nerve endings once again in the light of recent observations.

MATERIAL AND TECHNIQUE

We have had at our disposal a large number of inner ears from both guinea pigs and squirrel-monkeys, fixed in 1.5% veronal-buffered osmic acid and embedded in acrylate or epoxy resins. Unstained sections were examined by phase contrast microscopy, and sections stained with uranyl acetate or lead hydroxide were studied with the RCA EMU 3c electron microscope. Particular attention was devoted to the macular epithelia, but further observations have shown that the findings are valid for the cristae as well.

OBSERVATIONS

There are two distinct kinds of sensory cells in all the vestibular sensory epithelia we have studied, and their characteristic features can be recognized in Figs 1, 2 and 4. Besides these there are several cells of intermediate type, as well as groups of cells which are enclosed in a single nerve calyx. Compared with our schematic drawings in earlier publications, Figs 1, 2 and 4 show considerable differences, especially in the arrangement of the sensory hairs and in the innervation of the hair cells.

* This study has been supported by the Office of Naval Research (Contract No. 62558—3647) and by the Swedish Medical Research Council.

** Special fellow of the USA Public Health Service. Present address: Kresge Hearing Research Institute, University of Michigan, Ann Arbor.

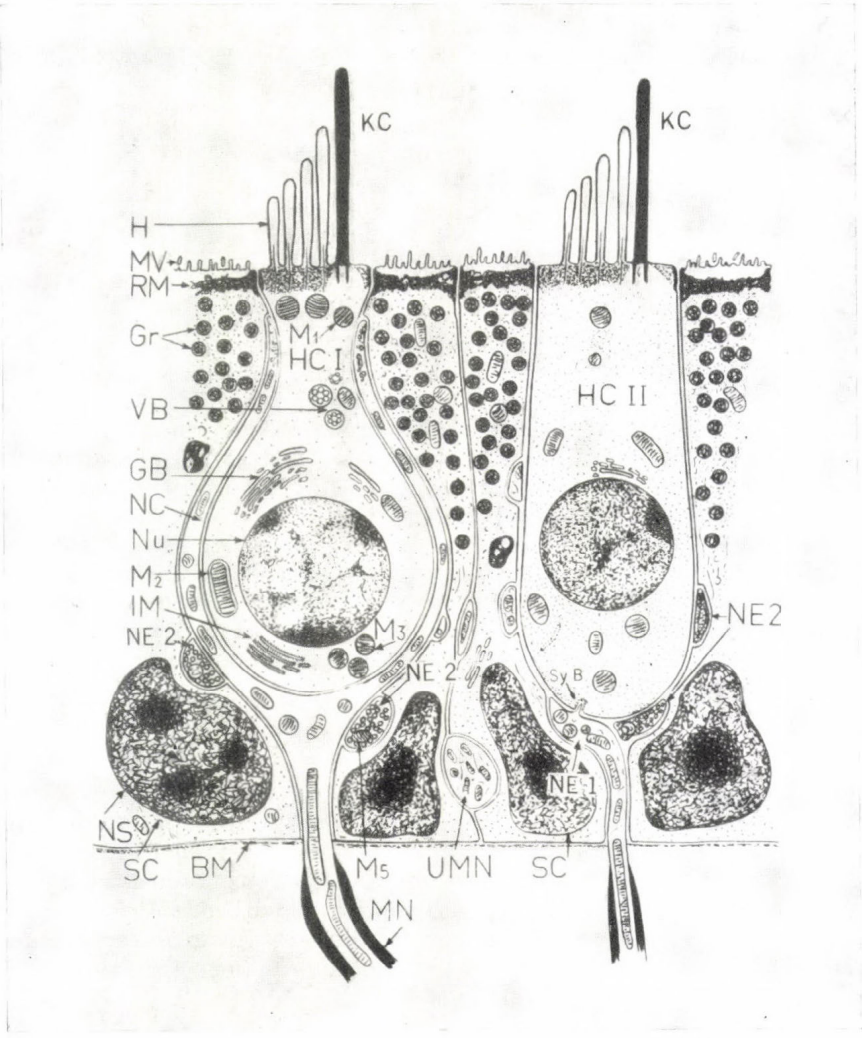

Fig. 1.—Schematic drawing of two vestibular sensory cells with surrounding supporting cells. HC I = haircell Type I, HC II = haircell Type II, H = stereocilia, KC = kinocilia, MV = microvilli, RM = reticular membrane, Gr = granules in supporting cells, VB = vesiculated bodies, GB = Golgi complex, NC = nerve calyx, Nu = nucleus, IM = intracellular membranes with rhibosomes, NE 1 = sparsely granulated nerve endings, NE 2 = richly granulated nerve endings presumably with efferent function, Sy B = synaptic bar, NS = nucleus of a supporting cell, BM = basement membrane, M_1—M_5 = mitochondria, SC = supporting cell, UMN = unmyelinated nerve, MN = nerve with myelin sheath

In a recent publication on the structure and functions of the inner ear sensory hairs, Engström, Ades and Hawkins (1962) described the two distinct kinds of sensory hairs on vestibular sensory cells, each of which is equipped with about 70 stereocilia and a single kinocilium. These were

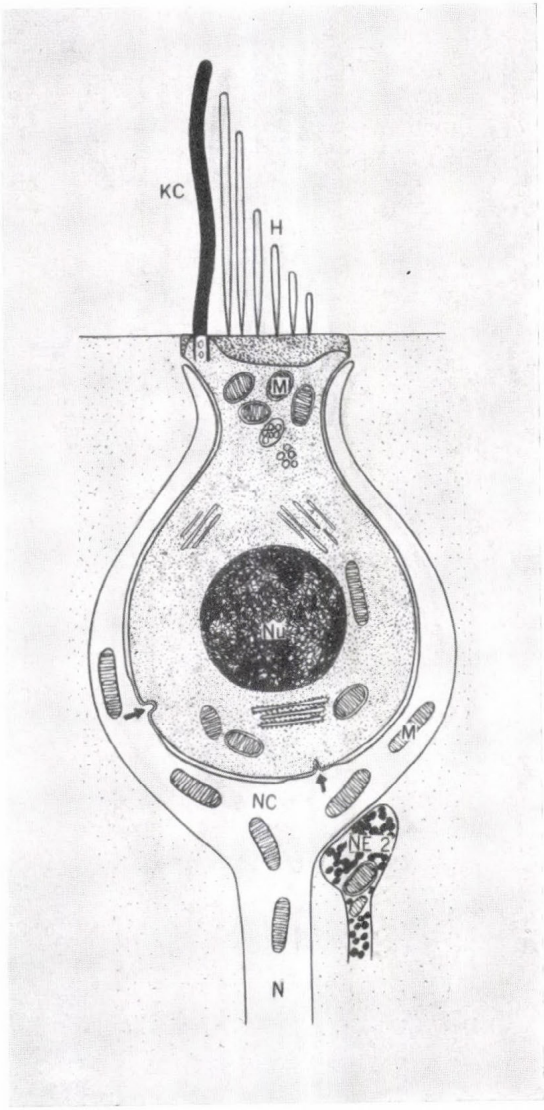

Fig. 2.—Schematic drawing of a vestibular hair cell of Type I. Observe the oblique arrangement of the hairs (H), increasing in length towards the kinocilium (KC). The invaginations (arrows) from the nerve calyx (NC) indicate regions with thinner synaptic areas. NE 2 is a richly granulated nerve ending along the nerve calyx

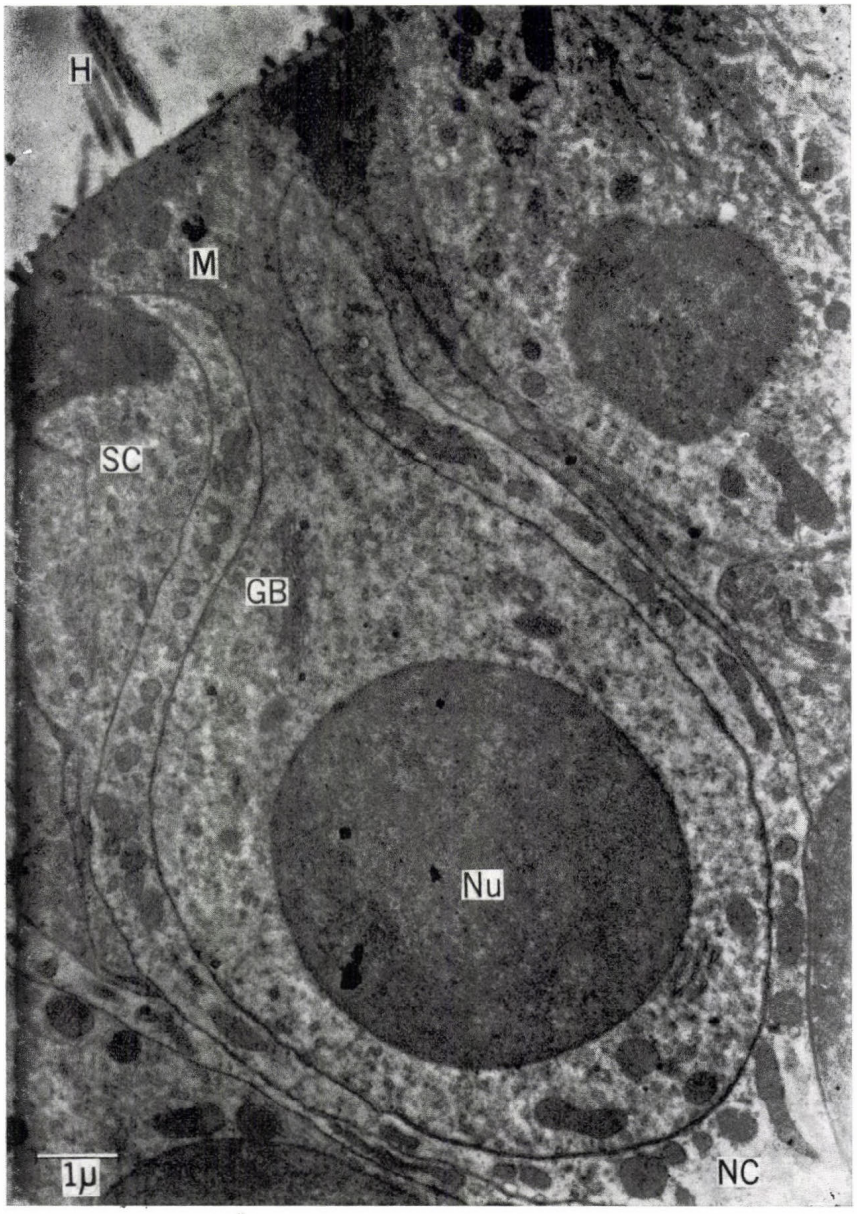

Fig. 3.—Electron micrograph of a hair cell of Type I. The large nucleus (Nu) is located in the basal portion of the cell, the Golgi body (GB) is typically located above the nucleus and groups of mitochondria (M) under the cuticle with the hairs (H). SC is a supporting cell

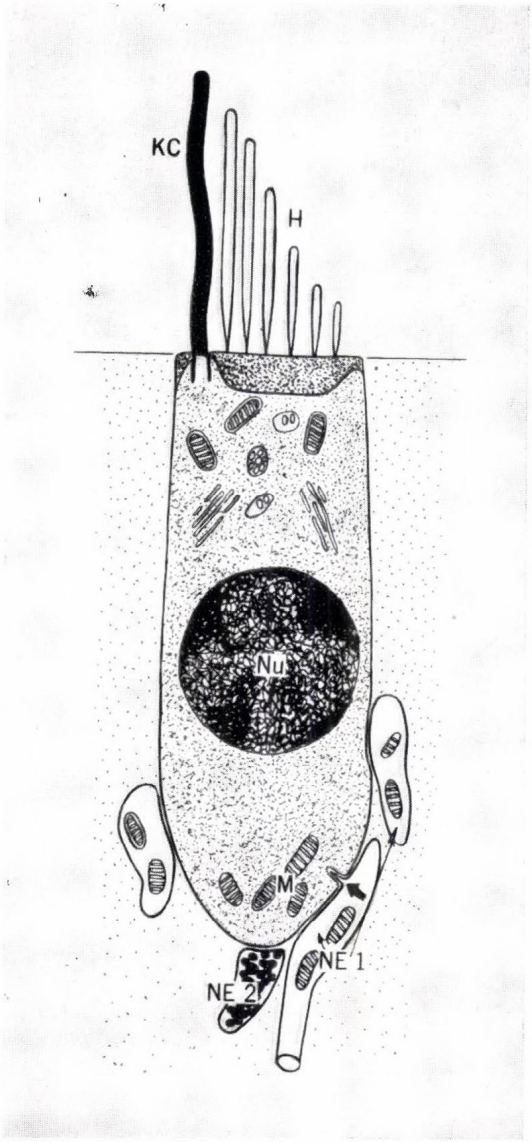

Fig. 4.—Schematic drawing of a hair cell of Type II. Also here the hairs have an increasing length toward the kinocilium (KC). There are two kinds of nerve endings (NE 1 and NE 2). The endings of Type 1 show distinct invaginations in the cell forming 'synaptic bars'

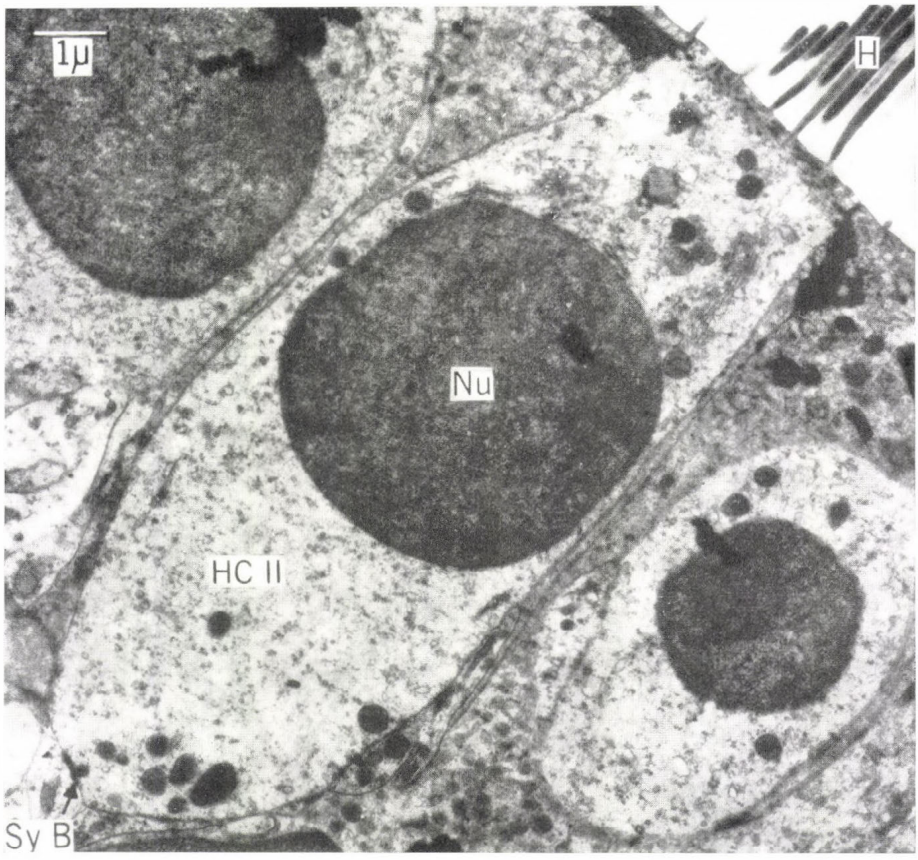

Fig. 5.—Electron micrograph of a Type II cell (cf. Fig. 4)

known before, but the fact had been overlooked that the sensory hairs of the stereocilium type have different lengths, and that they gradually increase in length toward the kinocilium. This gradation in lengths is very pronounced, and it can readily be seen by both phase and electron microscopy (Figs 2, 4 and 7). Actually it was hinted at by Retzius, but it has been overlooked in the discussion of the physiology of the vestibular sensory cells, although the pattern is quite characteristic, and the cells are distinctly polarized over large areas. The polarization of the kinocilium in one direction has been noted also in the neuromasts of fish (Trujuillo-Cenóz 1961), in the lateral line of Xenopus (Kalmijn, Dijkgraaf 1963), and in the lateral line of fish (Flock et al 1962), but it is not stated whether the stereocilia have different lengths. It has also been clearly established that the polarization of the cells has a clearly defined physiological importance, and that bending of the hairs toward the kinocilium corresponds to a rise in activity. Dijkgraaf (1963) noted the importance of this polarization. Interest in the kinocilium has been further increased since it has

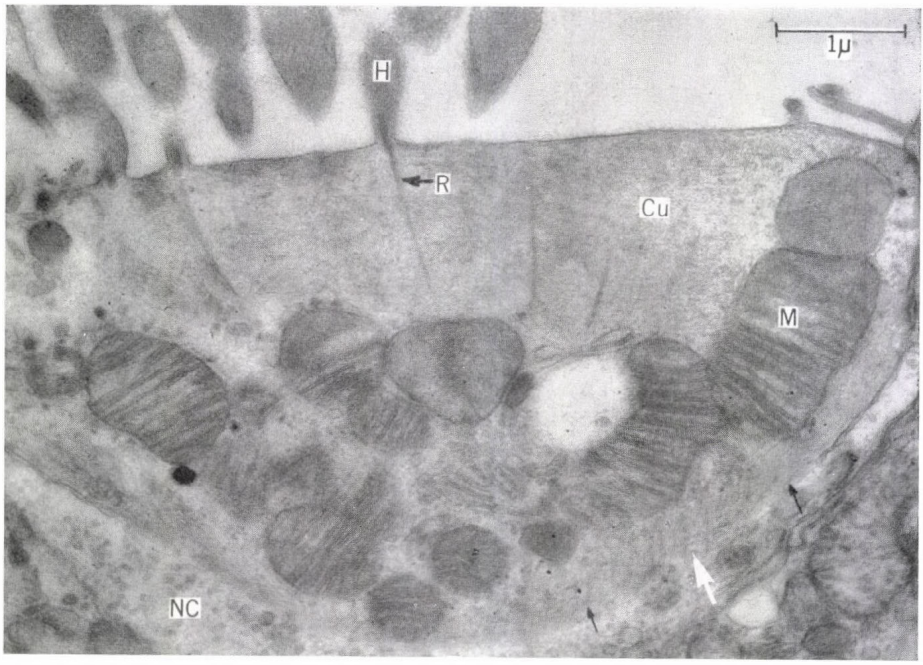

Fig. 6.—Cuticular plate (Cu) of a Type I hair cell from the Macula utriculi of a guinea-pig. The hairs (H) have very long roots (R), which penetrate the cuticle, and possibly reach to the mitochondrial region (M). NC is the nerve calyx. The black and white arrows to the right indicate regions with periodical arrangement of structures first observed by Friedmann

been shown that the primitive otolith of the ascidian tadpole is enclosed in what seems to be a modified kinocilium, and that no stereocilia are present on the cell (Dilly 1962). In this connection it is of great interest that a modified kinocilium is also present on every cochlear hair cell, as shown by Flock et al (1962) and by us (1962). These observations and several others have prompted us to consider the kinocilium as a most important sensory hair. We no longer regard the bending of the stereocilia as directly responsible for excitation of the hair cell, but only indirectly because of a mechanical effect on the kinocilium and the basal body. It is extremely interesting to note that modified kinocilia are now being found on most sensory cells and even inside the central nervous system. In the vestibular sensory epithelia, with the rather stiff stereocilia increasing in length toward the very long kinocilium, it must be assumed that either a sidewise movement of the statoconia or an increased pressure upon the hair-bearing surface must be met by a gradual increase in resistance, which could result in a graded form of response or could be an important safeguard to prevent damage to the sensitive organelles inside the cell. It will be of great interest to see if the staircase pattern in the arrangement of stereocilia is present also in the side-line organ of, for instance, Xenopus, where adjacent rows of sensory cells are oppositely polarized.

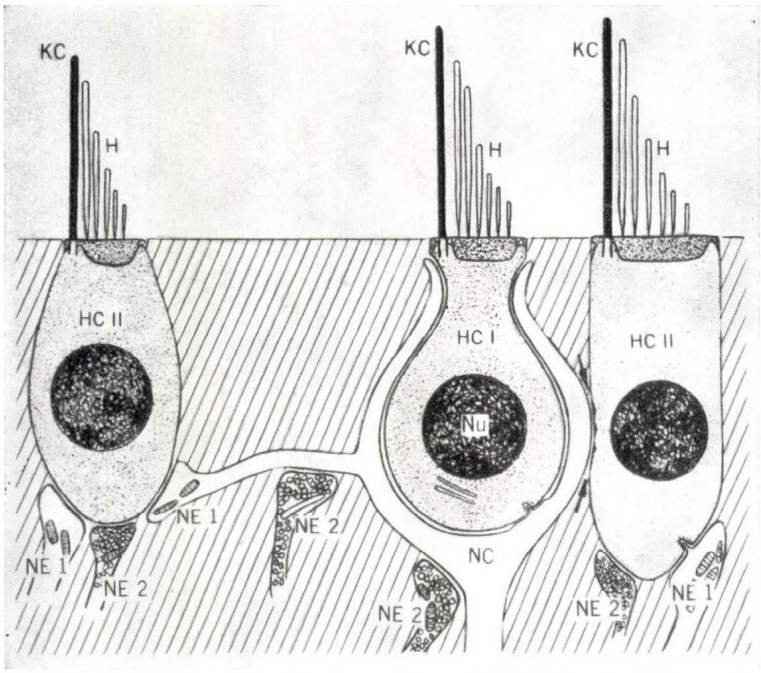

Fig. 7.—Schematic drawing showing the interrelation of some vestibular sensory cells to each other. It is quite clear that a nerve calyx can innervate a Type I cell as well as a Type II cell. A nerve calyx may also send an extra branch to a Type II cell. The figure also shows the location of the richly granulated endings (NE 2) relative to the sparsely granulated ones (NE1)

In hair cells of Type I (Figs 1, 2 and 3) the infracuticular region is richly provided with mitochondria (Fig. 6) indicating a high enzymatic and metabolic activity. It is important to establish the exact function of these mitochondria. In the outer hair cells of the guinea pig and squirrel monkey cochlea there is a marked accumulation of mitochondria in the region under the basal body, the structure corresponding to the kinocilium of the vestibular cells. There it seems probable that the modified kinocilium has a close functional relationship to the nearby mitochondria, as these often are grouped around the basal body. It is also essential to determine whether the rootlets of the vestibular stereocilia penetrate the cuticle and make contact with the large mitochondria. It is quite evident that the rootlets very nearly if not entirely penetrate the cuticle. (Fig. 6).

The hair cells of Type I usually have a characteristic arrangement of the cytoplasmic organelles, as can be seen from Figs 1 and 2, but as very little of importance can be added at present, we refer the reader to earlier publications. The hair cells of Type II show considerable variation in form. In the utricular and saccular maculae some of the cells are short and bulky, but the majority have the form shown in Figs 4 and 5. The cells of the cristae are often taller and thinner. The endoplasmatic reticulum and the

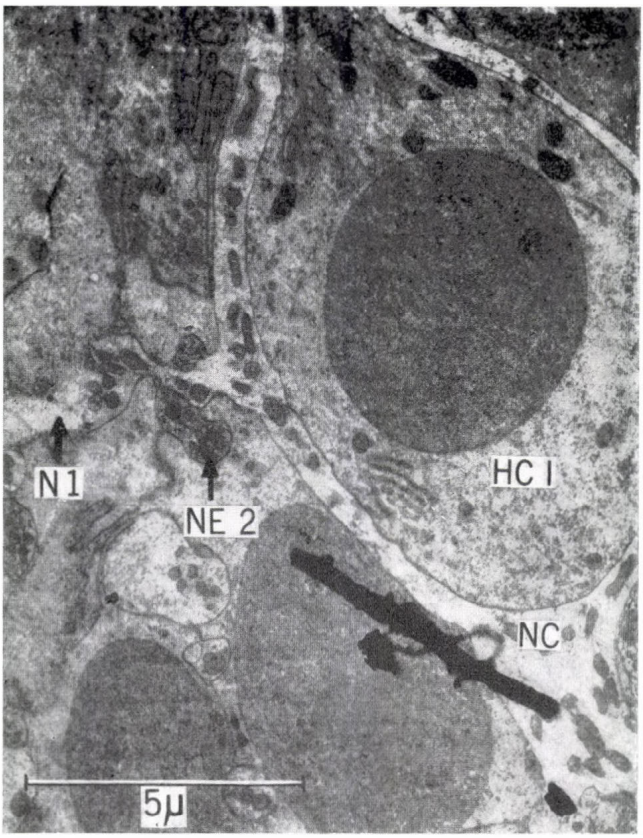

Fig. 8.—Type I hair cell (HC I) where the nerve calyx (NC) sends a nerve branch (NE 1) to another cell. This branch is also provided with a granulated nerve ending (NE 2)

location of the cytoplasmic organelles differ considerably from the pattern seen in the cells of Type I (cf. Fig. 3 with Fig. 5).

The most striking and significant differences among vestibular cells lie in the mode of innervation. It was originally supposed by Wersäll (1956) and by Engström and Wersäll (1958) that the two types of sensory cells were innervated by nerve fibers of different calibre, and this is perhaps still true to a certain extent; however, the innervation of the sensory cells is more complicated than was at first believed. A clear overlapping of the innervation takes place, so that Type I and Type II cells may be innervated by the same nerve fiber. Specifically a Type II cell can apparently be innervated by collateral twigs from a nerve calyx that surrounds a Type I cell (cf. Figs 7, 8). Our evidence shows that the nerve calyx on its inner side may innervate a Type I cell and on its outer side can make close contact with a Type II cell. In this contact area structures resembling synapses can be observed (Figs 9, 10, 11). There is now reason to believe that the Type II cell is phylogenetically the primary cell. Since this cell also has

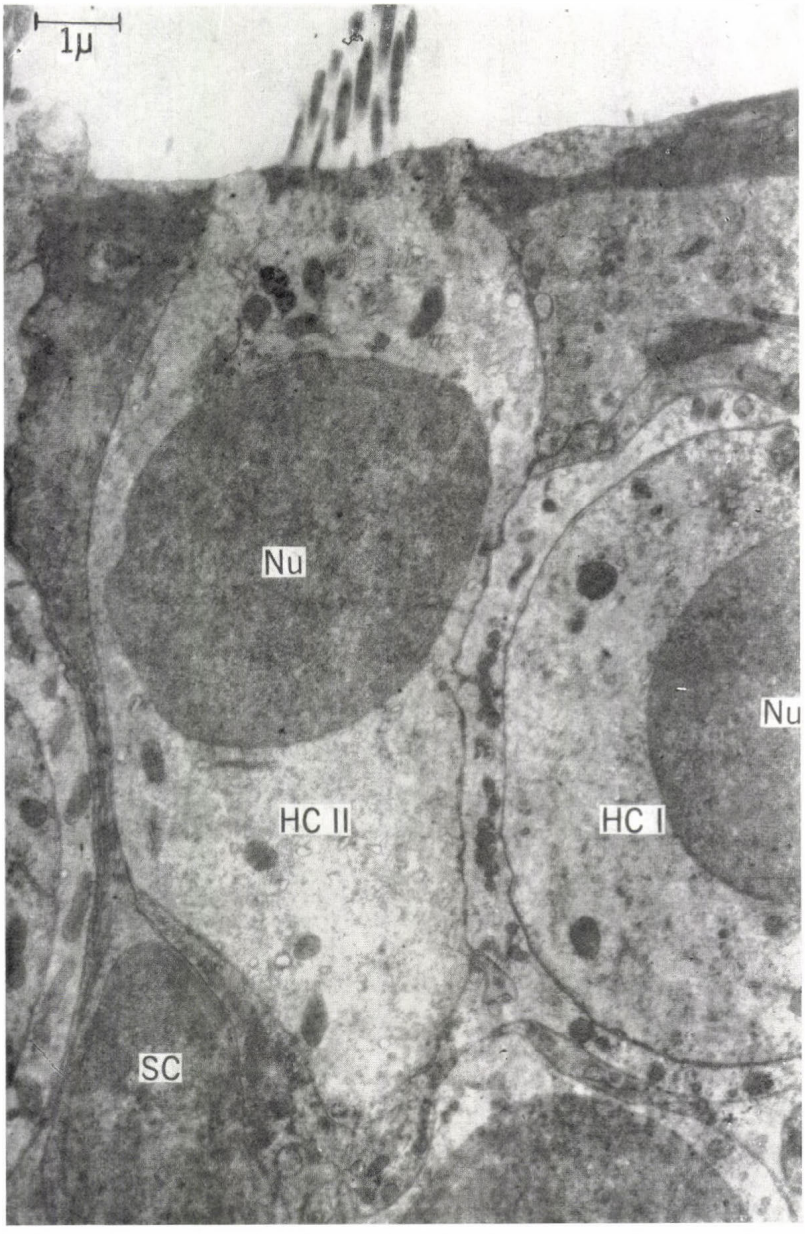

Fig. 9.—A hair cell of Type I (HC I) and one of Type II (HC II) divided only by a nerve calyx. As can be seen from the following Fig. 10, both types of cells form synaptic regions with the nerve calyx

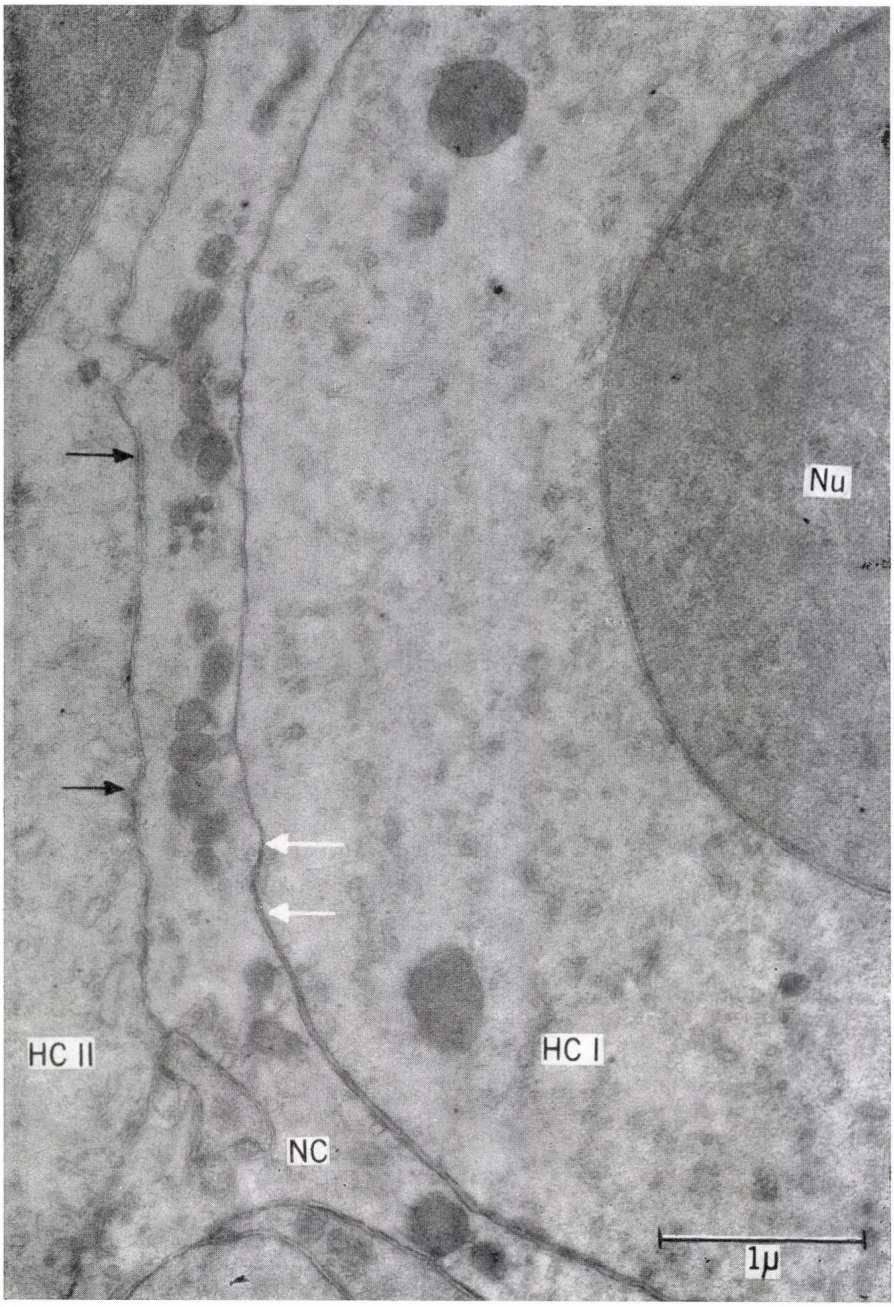

Fig. 10.—Detail from Fig. 9. HC I is a Type I cell where the contact with the nerve calyx (NC) has a different thickness (white arrows). HC II is a Type II cell where black arrows indicate regions of supposed synaptic contact. Observe also the nuclear membranes and the nuclear pores

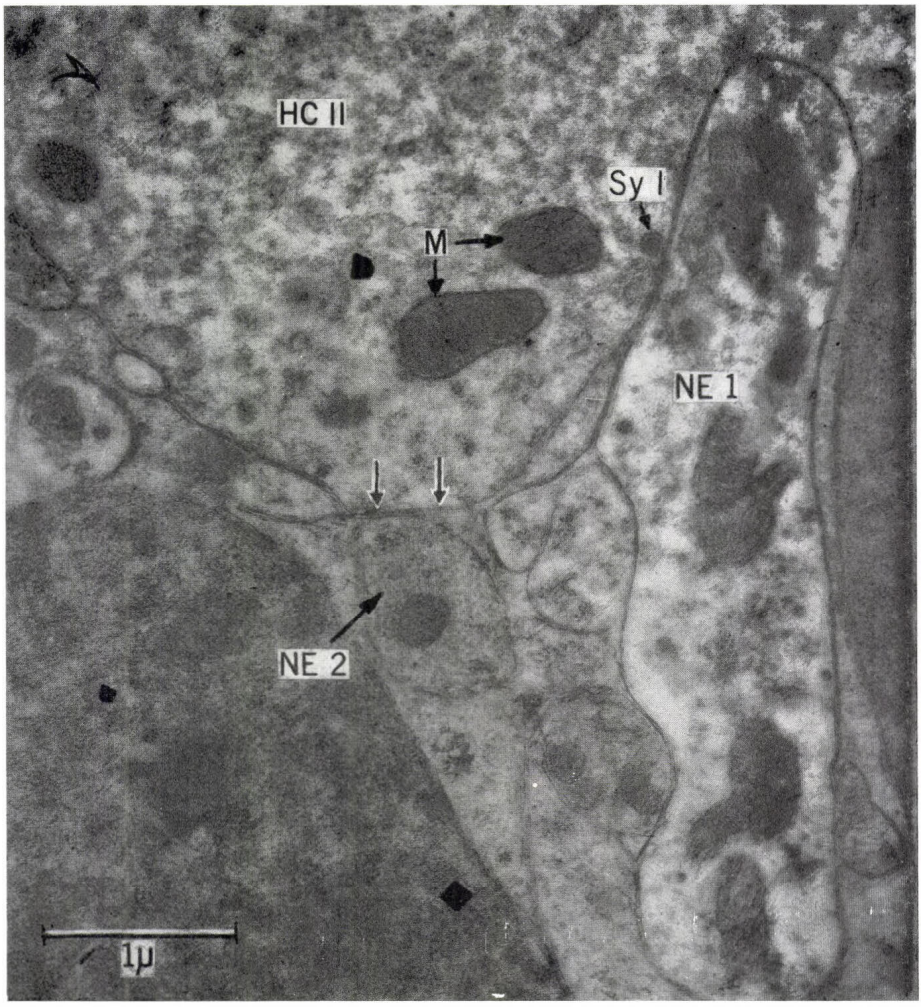

Fig. 11. — Hair cell of Type II (HC II) with a large nerve ending of the little granulated type (NE 1) with a distinct synaptic invagination (Sy I). The richly granulated nerve ending (NE 2) has also a distinct synaptic contact (black arrows)

a simpler form of innervation, it will be best to begin by considering this first.

The hair cell of Type II is innervated by at least two different kinds of nerve endings. One of these, called nerve ending Type 1, is sparsely granulated and forms a synaptic contact with the hair cell plasma membrane. The synaptic region often shows a small indentation into the vestibular cell, and in some instances the nerve ending may be partially engulfed by the sensory cell. The synaptic indentation varies in form and size. In some cases it is smooth and rounded as in Fig. 11 while in others it is oblique showing a few vesicles inside the sensory cell. In still other cases it is very

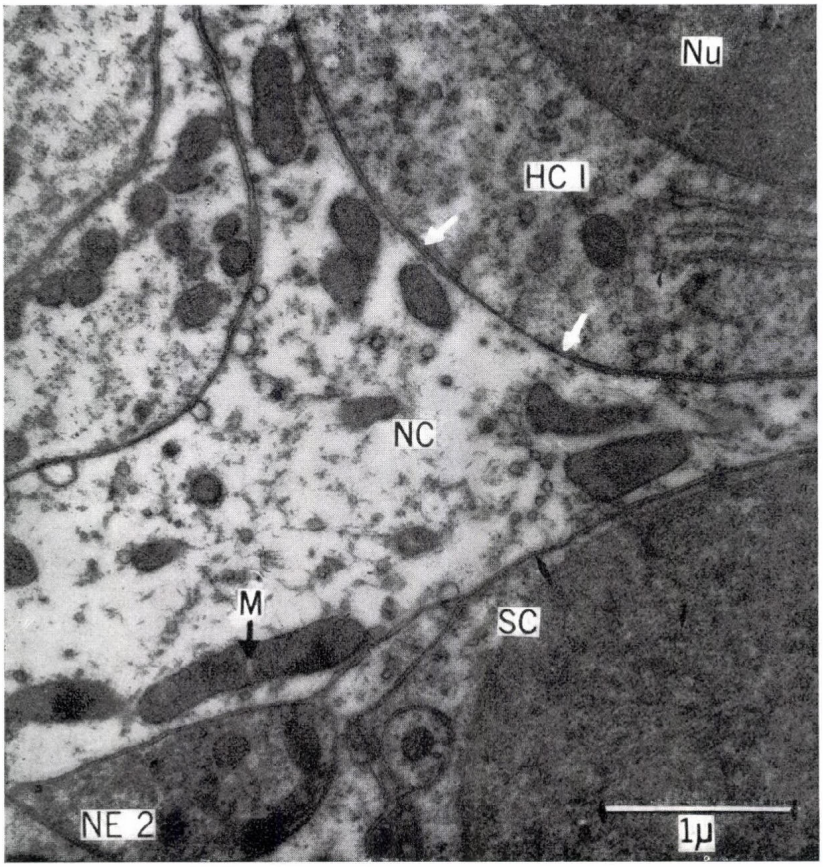

Fig. 12.—Hair cell Type I (HCI) with a thin synaptic region (white arrows) to the nerve calyx (NC). The granulated NE 2 is very distinct. SC is the supporting cell

similar to what Smith and Sjöstrand have called a 'synaptic bar', but as far as we have found, there is always an invagination or indentation of the sensory cell's plasma membrane with sometimes a denser region on both sides of the invagination. As mentioned above, vesicles are sometimes seen on the same side as the sensory cell, but in other cases the appearance is more that of short spines or radiating denser structures of about 200 Å in length. (cf. Robertson's studies on the structure of synapses). Further studies are necessary to clarify these structural complexities. The general character of these endings indicates that they are postsynaptic, which further implies centripetal conduction. Along one cell surface there are found several endings of this type varying both in size and form. Some are distinctly bouton-shaped, while others are long and slender and make extended contact with the sensory cells. Some of these endings are so large as to resemble a half of a nerve calyx such as will be described for Type I cells.

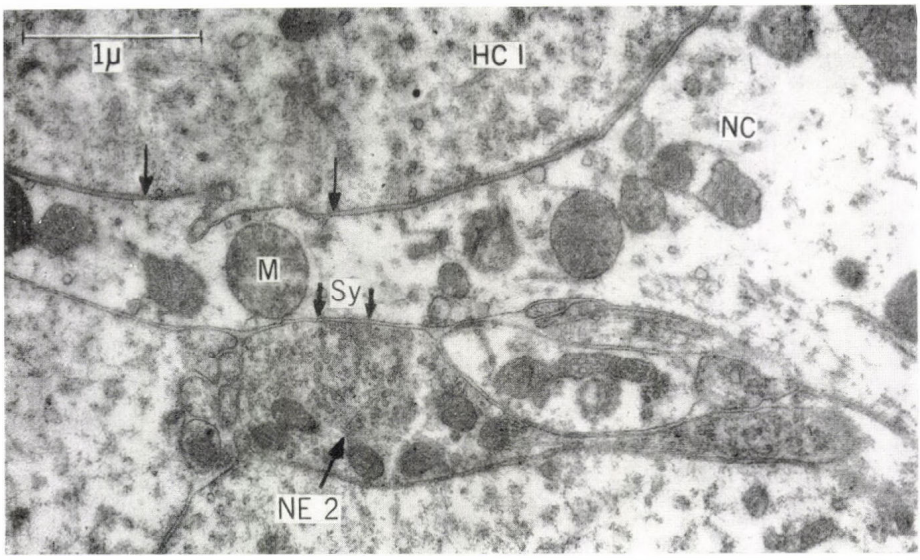

Fig. 13.—Hair cell Type I (HC I) with a thin synaptic region (black arrows) and surrounding nerve calyx (NC). The nerve ending of Type 2 (NE 2) is richly granulated and forms a distinct synaptic region (Sy)

The other kind of nerve ending is less numerous. They are often bouton-shaped and very densely granulated (Fig. 11), differing considerably from the sparsely granulated form. They are referred to as Type 2. These granulated endings are in direct contact with the sensory cell surface and there is often a distinct thickening of the plasma membrane of both sensory cell and nerve ending in the area of contact. There is evidence that granulated nerve endings of this type may also establish synaptic contacts with nerve fibers belonging to Type 1, a similar kind of granulated ending being found in contact with the nerve calyces surrounding vestibular sensory cells of Type I.

The vestibular sensory cell of Type I is surrounded almost totally by a sparsely granulated nerve calyx (Figs 1 and 2). Calyx and cell are separated by a distance of 250—300 Å. It was earlier supposed that this distance was uniform all around the cell, except for the neck region creating the impression that only the neck region should be regarded as the synaptic area. This impression was strengthened by the observation that the nerve calyx in the neck region contained vesicles similar to the synaptic vesicles. (C. Smith 1956).

Our studies have clearly shown, however, that the distance between the sensory cell and the nerve calyx is not uniform all the way around the cell. Instead there are well-defined areas of narrowing of the space between calyx and cell (Fig. 12). In the center of these areas an invagination can often be seen and sometimes this is a narrow invagination similar to C. Smith's synaptic bar (Fig. 13). Small vesicles with a diameter of 200—400 Å are often found inside the thin regions, especially in contact with the invaginations. These invaginations can sometimes be rather large (Fig. 14). Often

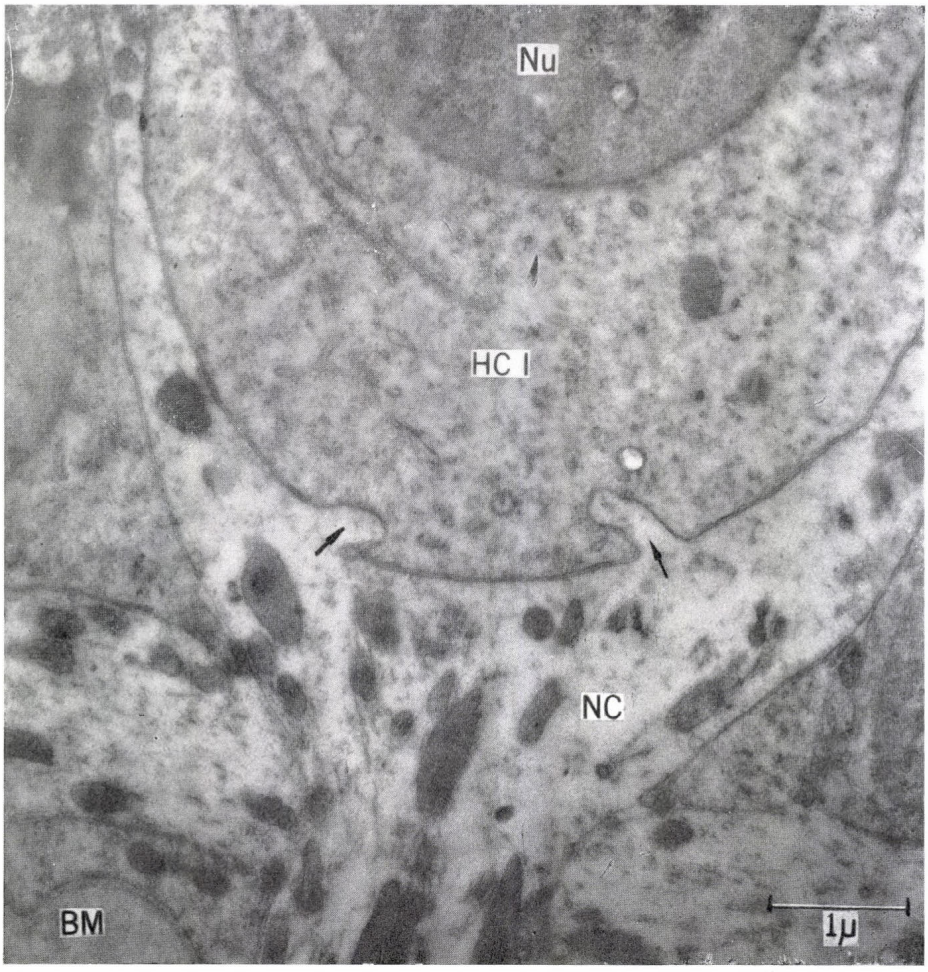

Fig. 14.—Hair cell of Type I with very large invaginations. Cf. studies by Wersäll et al

a densely granulated nerve ending of Type 2 can be found outside the nerve calyx in the same region. One or more of such densely granulated endings are almost always found outside the nerve calyx, as previously described by Engström (1958, 1961).

In our earlier publications we assumed that sensory cells of Types I and II were innervated by different types of nerve fibers. The present study has clearly shown that the outer surface of the nerve calyx is often in contact with a sensory cell of Type II, and that osmiophilic 'synaptic regions' can clearly be seen along the contact surface, furthermore, a nerve calyx has often been seen to give off a branch to a nearby Type II cell (Fig. 8). Along such a side branch, which, in all cases we have seen, terminates with a sparsely granulated ending, i. e. an ending of Type 1, there is usually

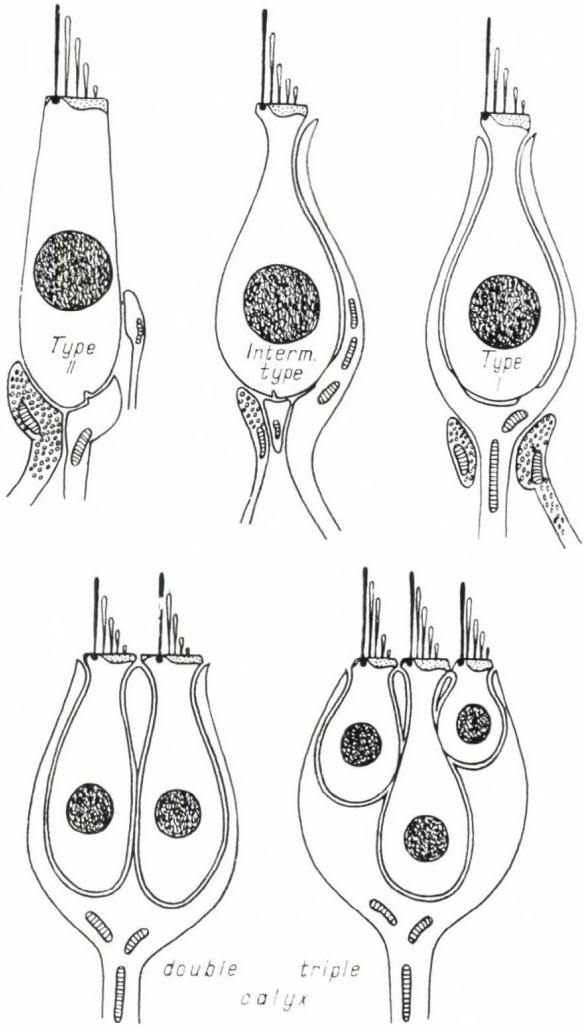

Fig. 15.—Different types of innervation of vestibular sensory cells. There are many different varieties from the Type II over an intermediate form to Type I and to groups of sensory cells in one calyx

seen a densely granulated ending of Type 2. Our studies have also shown that in both maculae and cristae nerve calyces may be found which contain two, three, or sometimes even four sensory cells (Fig. 15).

The present study has clearly shown that the contact between a vestibular sensory cell and its centripetal nerve fiber follows a regular pattern with characteristic regions containing invaginations, in which granular or vesicular structures are very sparse or absent. Other kinds of endings show

Fig. 16.—Section of eponembedded crista amp. where it is possible to get a rather good impression of the structure of the epithelium already at low magnification

a clearly different form and are filled with synaptic vesicles. There is every reason to suppose that the two morphological varieties of synapses are correspondingly different with respect to function.

It is possible that the synaptic contact between sensory cell and centripetal nerve ending signifies an electrical form of transmission, while the granulated endings act with a chemical transmittor. Such an idea has also been expressed by Eccles in a discussion of one of our papers.

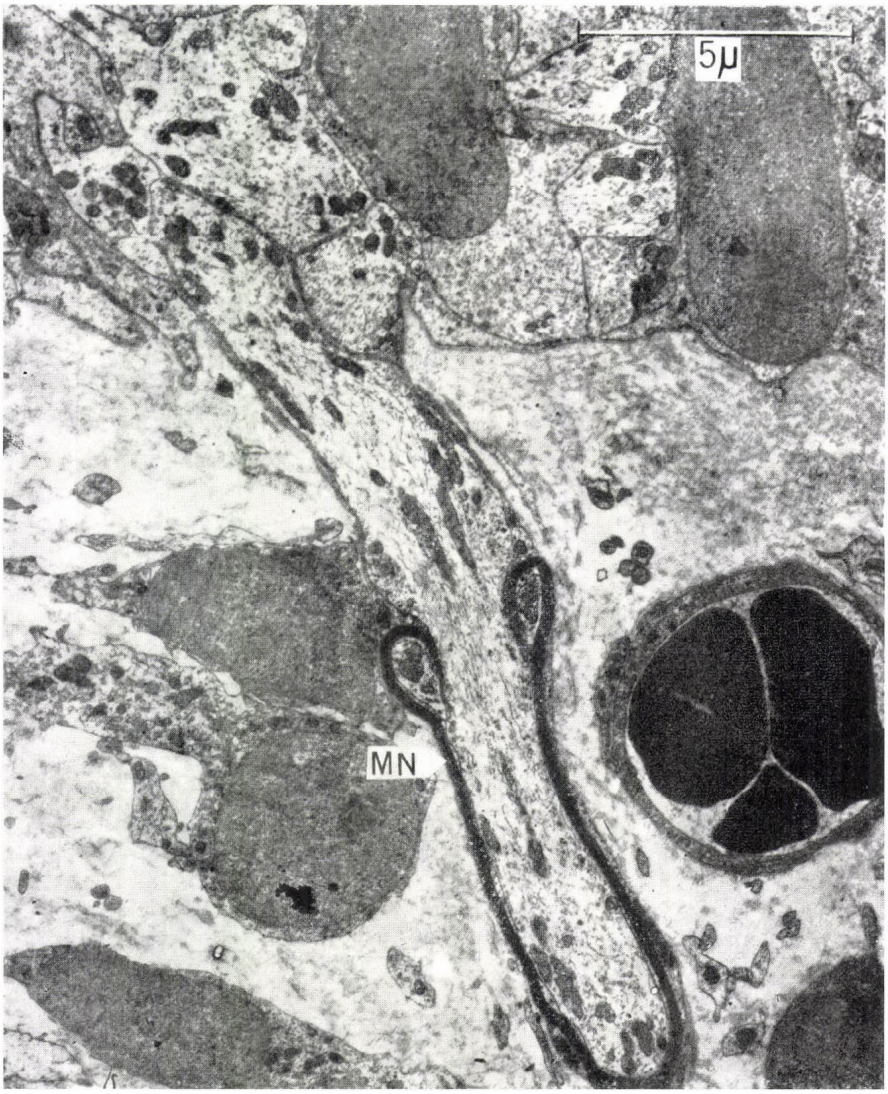

Fig. 17.—The epithelial and subepithelial region of a macula utriculi showing the infolding of the myelin (MN) and a rich accumulation of mitochondria where the myelin ends

Fig. 18.—Complicated infoldings of the myelin in regions of demyelinization under the macular epithelium. Some unmyelinated fibers can also be seen

SUMMARY

The present study has shown that the vestibular sensory cells are of two main types, but that cells may be found which form an intermediate variety, presumably a partial differentiation from the phylogenetically older Type II cell into a cell of Type I.

This study has also clearly established that sensory cells of both types may be innervated by one and the same nerve fiber, and that the innervation pattern of the vestibular sensory cells is far more complicated than earlier believed.

Our study has further shown that well-defined synaptic regions are found between the nerve calyx and base of the sensory cell of Type I. These bear a clear resemblance to the supposed synaptic regions between nerve endings of the sparsely granulated type and the sensory cells of Type II.

DISCUSSION

Eccles: Dr Engström's beautiful pictures have very clearly indicated to me that the transmission from receptor cell to afferent terminal is electrical. There is no sign of structural features characteristic of chemically transmitting synapses, such as synaptic vesicles and the membrane thickenings. On the other hand, he showed the invaginations of membrane and the very close contact and even fusion of membranes that resemble the presumed electrical transmitting regions that Robertson has demonstrated in the electrical transmitting synapses between crustacean giant fibres and motor fibres. There can, I think, be no doubt that the heavily vesiculated terminals of the efferent olivo-cochlear bundle are chemically transmitting. However, I did not see many of the 'active zones' that according to Couteaux, Gray, Palay and others are the specialized areas of chemical transmission. These active zones are characterized by membrane thickenings and accumulation of vesicles. The close apposition of a vesiculated structure is not a sufficient guarantee of functional synapse. I would like to ask Dr Engström whether he finds such active zones with contacts of vesiculated terminals both onto the receptor cells and the afferent nerve terminals. Desmedt's experiment certainly indicates that the olivocochlear bundle inhibits both the afferent nerve terminals, which would be postsynaptic inhibition, and also the receptor cell, which would be presynaptic inhibition. I think that Dr Engström has demonstrated both types of synapses.

Walberg: Referring to what Professor Eccles has just said, I think that one of the few ways to get a disappearance of synaptic vesicles in boutons is to cut their fibres. If you do so, the vesicles disappear, but this process may take several days. Thus, in the adult cat vesicles are present in degenerating boutons even on the 7th day after operation. Obviously, therefore, the synaptic vesicles are structures of great importance for the normal function of boutons. Probably they are not easily removed, even though a continuous stimulation of the fibers is made in physiological experiments.

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DIE NERVENENDIGUNGEN DER GESCHMACKSKNOSPEN

von

D. Kadanoff

ANATOMISCHES INSTITUT DER UNIVERSITÄT
SOFIA, BULGARIEN

Die ersten Untersuchungen über Form und Lage der Nervenelemente der Geschmacksknospen und des sie umgebenden Epithels fallen auf die letzten Jahre des vorigen Jahrhunderts. G. Retzius (1892) und M. v. Lenhossék (1892 und 1893 a), die die Golgi-Methode benutzten, haben die Nervenendigungen der Knospen bei einigen Arten der Fische, Amphibien und Säugetiere beschrieben. Diesen Studien folgten weitere Arbeiten, die an verschiedenen Tieren ausgeführt wurden (Arnstein (1893), Jacques (1893), Ploschko (1897), Dogiel (1897), Botezat (1910), Cajal (1904), Kolmer (1910), Boeke (1921). Vor meinen Untersuchungen (1953) wurden spezielle Untersuchungen über die Nervenendigungen der Geschmacksknospen beim Menschen unter Anwendung von neueren Silberimprägnationsmethoden nicht durchgeführt. Nur im Kapitel über das Geschmacksorgan im Handbuch der mikroskopischen Anatomie (herausgegeben von W. v. Möllendorff 1927) hat Kolmer (1910) diese Endigungen beim Menschen in zwei Abbildungen gezeigt. Doch sind diese im Vergleich zu den bereits bekannten Endigungen bei anderen Säugern spärlich und liegen nur im basalen Bereich der Knospen.

Die Knospen der Fische ähneln in ihrer Struktur sehr stark denen der Säugetiere. Nach v. Lenhossék (1893 a, b) bildet eine der Nervenfasern, die zu jeder Knospe hinziehen, unter der Basis derselben durch Verzweigung und Verbindung ihrer Ästchen eine dichte Verflechtung, die vom Autor als Cupula bezeichnet wurde (Abb. 1). Von der der Knospe zugekehrten Seite der Cupula treten kurze Ästchen aus, die zum Teil im Basalabschnitt der Knospe selbst in kleinen Knöpfchen enden. Die anderen Nervenelemente der Knospe, die wahrscheinlich durch die Teilung einer oder zweier dicken, vom subepithelialen Nervengeflecht ausgehenden markhaltigen Nervenfasern entstanden sind, treten in die Knospe ein und verzweigen sich dort. Sie wurden von Lenhossék (1892 und 1893 a, b) als intragemmale Fasenr bezeichnet. Die intragemmalen Fasern weisen stellenweise kleine knötchenartige Verdickungen (Varikositäten) auf. Die Nervenfasern, die unter dem Epithel der Zunge liegen, geben Äste ab, die in das Epithel zwischen den Knospen eindringen, sich als perigemmale oder intergemmale Fasern weiterverzweigen und zwischen den Epithelzellen enden. Beim Kaninchen hat v. Lenhossék (1893 a, b) keine Cupula an der Basis der Geschmacksknospen der Papillae foliatae gefunden. Die intragemmalen Fasern (Ästchen) legen sich den Zellen der Geschmacksknospen an und enden nach Lenhossék (1893 a, b) frei, d. h. sie sind nicht als ihre Fortsätze zu betrachten. Er unterstützt die Auffassung, daß man, wenn ein perigem-

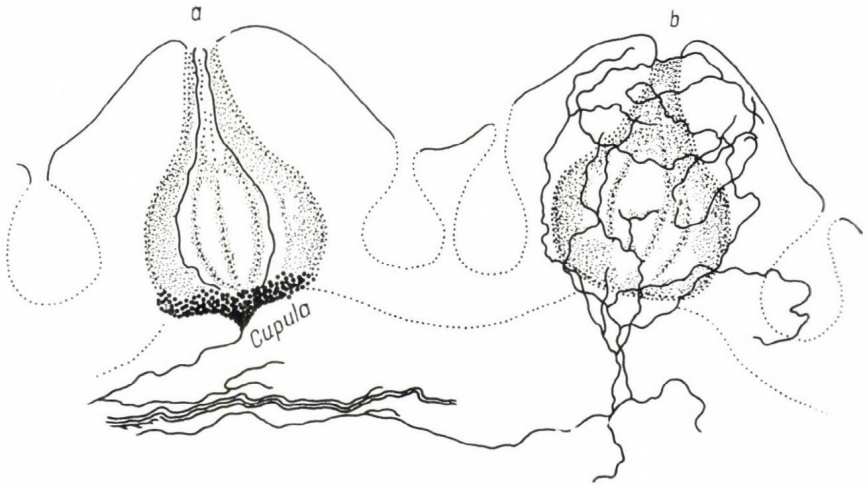

Abb. 1.—Zwei Endknospen aus der Mundschleimhaut eines Meeraales. Golgi-Methode. Originalzeichnung von M. v. Lenhossék (aus *Anat. Anz. 8*, 1893). *a* = Zu der Endknospe tritt eine Nervenfaser, die die Cupula (subgemmales Büschel) an der Basis der Knospe bildet. Von der Cupula gehen terminale Ästchen ab, die in der Knospe als intragemmale Nervenfasern verlaufen. *b* = Perigemmale (intergemmale) Nervenfasern um eine Endknospe

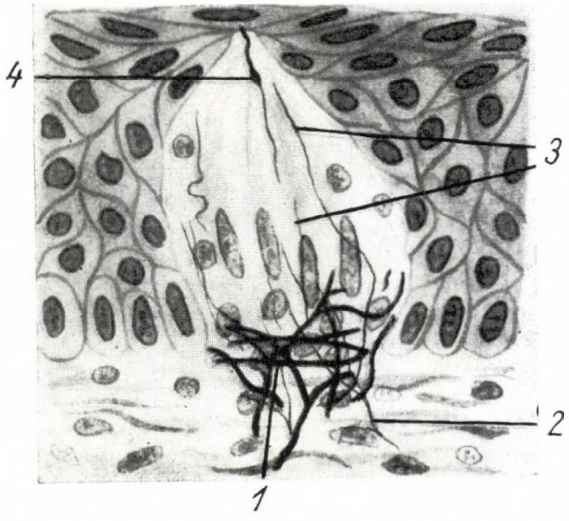

Abb. 2.—Geschmacksknospe in einer Papilla fungiformis des Menschen. Dichtes subgemmales Knäuel. Pyridinmodifikation der Silberimprägnationsmethode von Bielschowsky. Zeichnung, Obj. Ölimmersion 1/12, Ok. 1. *1* = Subgemmales Nervenknäuel, *2* = selbständige dünne Nervenfaser, die intragemmale Nervenästchen abgibt, *3* = intragemmale Nervenfasern, *4* = Varikosität einer intragemmalen Nervenfaser

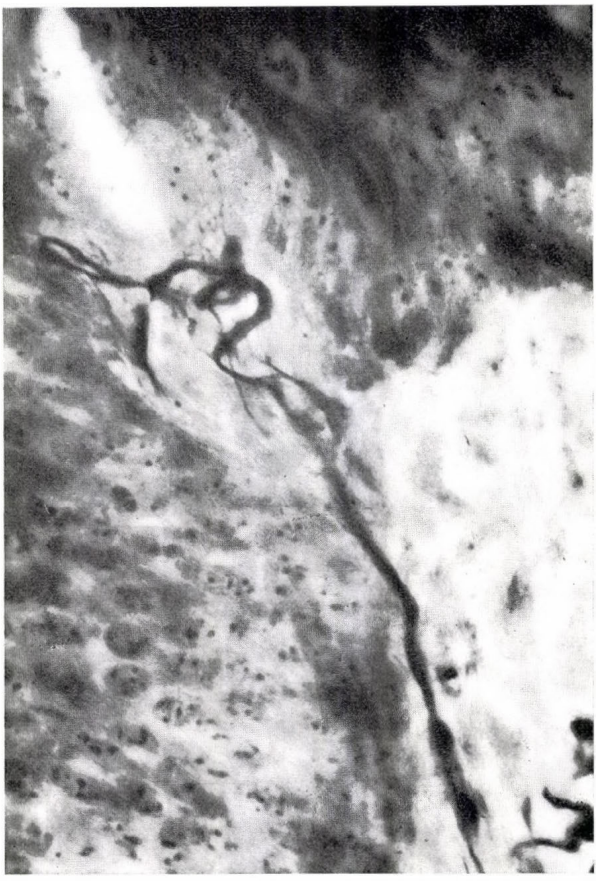

Abb. 3. — Geschmacksknospe in einer Papilla fungiformis des Menschen. Lockeres subgemmales Knäuel mit intragemmalen Nervenfasern. Pyridinmodifikation der Silberimprägnationsmethode von Bielschowsky. Mikrophotographie, Reichert, Obj. 40, Ok. 8

males Mäntelchen um die Knospen tatsächlich vorhanden ist, dessen Fasern zu den intergemmalen rechnen muß.

Die von Lenhossék vor 70 Jahren erzielten vorzüglichen Resultate beweisen, daß man mit einer nicht immer eindeutige Ergebnisse sichernden Methode, wie die Golgi-Methode, die feine Struktur der rezeptorischen Vorrichtungen des peripherischen Nervensystems aufdecken kann, was oft auch mit modernen histochemischen Methoden nicht gelingt.

Nach meinen Untersuchungen (1953) liegen die Nervenelemente der Geschmacksrezeptoren und ihre Endigungen beim Menschen subgemmal und intragemmal. Bei einem Teil der Knospen durchflechten und verbinden sich die Verzweigungen der Hauptnervenfaser (oder Fasern) unter der Basis der betreffenden Knospe, wodurch ein ungleichmäßig dichtes subgem-

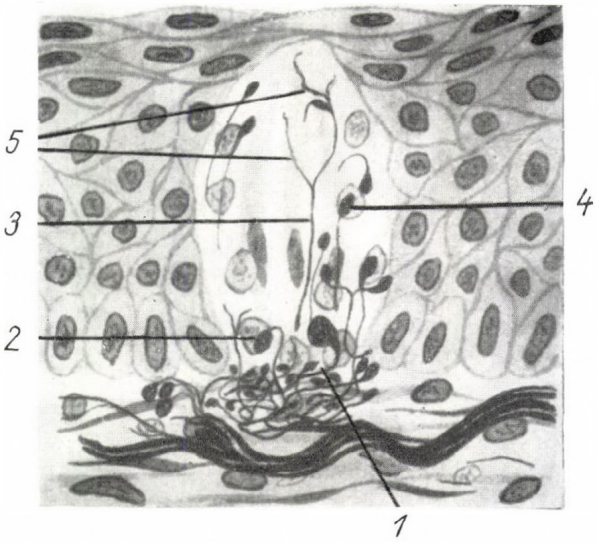

Abb. 4. — Geschmacksknospe an der dorsalen Seite des Kehldeckels des Menschen. Subgemmales Nervenbüschel. Pyridinmodifikation der Silberimprägnationsmethode von Bielschowsky. Zeichnung, Obj. Ölimmersion 1/12, Ok. 1. *1* = Subgemmales Nervenbüschel, *2* = neurofibrilläres Endästchen im basalen Bezirk der Geschmacksknospe, *3* = intragemmale Nervenfaser, *4* = kugelförmige Nervenendigungen einer intragemmalen Nervenfaser, *5* = durchschnittenes intragemmales Nervenfaserästchen

males Endgebilde entsteht (Abb. 2—4). In andere Knospen treten die Nervenfaserästchen nach 1—2 Windungen ein, ohne subgemmale Endgebilde zustande zu bringen. Es sind zwei Formen des subgemmalen Gebildes zu unterscheiden: Knäuel und Büschel. Die Knäuel teilen sich in dichte und lockere. Die dichten Knäuel entstehen durch enge Verflechtungen der Nervenfaserästchen (Abb. 2). Die lockeren Knäluel bestehen aus einigen sich kreuzenden Schlingen, die durch Windungen der Äste ihrer Hauptnervenfasern zustande kommen (Abb. 3). Die Bestandselemente eines Teiles der Knäuel geben einzelne Verzweigungen ab, dringen in die Knospen ein und endigen dort in kleinen Knöpfchen und Kügelchen.

Die Büschel entstehen meist durch die mehrfache Teilung einer dicken markhaltigen Nervenfaser, deren terminale Äste kurz sind und in Endverdickungen verschiedener Form—kugelförmig, oval oder schaufelartig—auslaufen (Abb. 4). Manche dieser Äste dringen in den basalen Teil der Knospe ein, wo sie zwischen deren Zellen in Knöpfchen endigen.

Im Kehldeckel liegen die Nervenelemente der subgemmalen Knäuel und besonders der subgemmalen Büschel sehr oft in Gruppen von dicht angehäuften Zellen (Spezialzellen), die sich dunkel färben und meistens quer übereinander gelagert sind (Abb. 5). Bei den Knospen des Kehlkopfes trifft man nicht selten auch vielfach verflochtene subgemmale Knäuel

Tabelle 1

Fälle von Nekrospermie im Krankenmaterial der Urologischen Klinik

	Karth. Nº	Alter	ml	pH	Spermien-zahl	Motile Spermien	Hoden	Prostata	Diagnose	Fruktose mg %	Pathol. Formen	Bemerk.
1	1324	33	0,5 0,5		15 Mill/ejac	ϕ	fest	normal, drüsig	Nekrosp. total + Hypospermie	ungenüg.	allgem.	—
2	1614	25	2,7 4,0	7,0 8,0	86 Mill./ejac	einmal: 1-1	fest	normal, drüsig	Nekrosp. maxim. + Oligospermie	392	50 %	—
3	1555	26	3,1		1-2/Blfd.	ϕ	schlaff			abwesend	allgem.	—
4	1729	28	1,2	6,8	8-10/Blfd.	ϕ	schlaff			128	allgem.	—
5	1788	45	2,4	7,5	vereinz. 1-1	ϕ	atroph.		Total Nekrosp. +	187	allgem.	—
6	1103	25	3,4		3-6/Blfd.	ϕ.	fest	normal, drüsig	Hyposp. maxim.	abwesend	teratoide	niedrigere 17-Ketosteroid-Werte
7	1194	26	3,2 1,8		8-10/Blfd.	ϕ	fest	normal, flach		abwesend	allgem.	
8	1746	27	1,8 2,2	6,8 6,8	2-4/Blfd. 4-5/Blfd.	ϕ	fest	normal, drüsig		36	allgem.	—
9	1329	24	1,4 2,0	7,5	4-5/Blfd.	einmal: 1	fest	normal, flach		530	teratoide	Biopsie
10	1587	34	3,1 3,5		6-8/Blfd. 12-15/Blfd.	sehr vereinz. 1-1	fest	normal, drüsig		480	80 %	—
11	1787	32	1,3 1,8	7,4 7,7	vereinz. 1-1	sehr vereinz. 1-1	fest	normal, drüsig	Maxim. Nekrosp. +	350	allgem.	—
12	1768	38	2,2 2,7	7,4 7,4	vereinz. 1-1	sehr vereinz. 1-1	fest		Hyposp. maxim.	148	allgem.	—
13	1758	32	4,2 5,0		2-3/Blfd.	sehr vereinz. 1-1	fest	normal, drüsig		33	allgem.	nach 250 mg Testosteron Fructosew: 265
14	1350	31	1,2		1-1/Blfd.	einmal: sehr vereinz. 1-1	fest	normal, drüsig		abwesend	allgem.	Biopsie

Bei dem einzigen Kranken mit relativ hoher Spermienzahl und totaler Nekrospermie (Fall Nr. 1) vermochten wir trotz wiederholter Untersuchung die Fruktose nicht zu bestimmen, weil die Spermamenge zweimal nicht mehr als 0,5 ml ausmachte und wegen ihres mukösen Zustandes kaum Plasma ergab.

In einem anderen oligospermischen Ejakulat (Fall Nr. 2) sahen wir bei einer Untersuchung nur eine einzige (sich träge bewegende) Spermie. Der Fruktosewert war 392 mg%.

In den Fällen Nr. 3, 4 und 5 gesellte sich zur totalen Nekrospermie maximale Hypospermie. Die Spermien sämtlicher drei Kranken verfügten im allgemeinen über pathologische Struktur, doch trat die testikuläre Herkunft des Leidens auch bei der physischen Untersuchung zutage: *die Hoden dieser Kranken waren stark atrophisch.* Ihre Fruktosewerte waren zwar niedriger, aber höher als die untere Grenze des Normalwertes. In diesen Fällen war es denkbar, daß die Akinese tatsächlich dem extremen Grad der Hypokinospermie entsprach, d. h. der Fehler ausschließlich bei den Spermien lag.

Bei den Patienten Nr. 6 und 7 bestand totale Nekrospermie und maximale Hypospermie, während ihre Hoden dem Normalzustand entsprachen. Es waren frühere Kranke, die wir zur Nachuntersuchung bestellten, zu der sie leider nicht erschienen, weil sie sich angeblich unbekannten Orts aufhielten.

In den Fällen Nr. 9, 10, 11, 12, 13 und 14 tauchte bei maximaler Hypospermie und Akinese hier und da eine sich — wenn auch schlecht — bewegende Spermie auf. In sämtlichen Fällen dominierten die pathologischen Formen. Die Hoden zeigten einen anscheinend einwandfreien Status. Bei 3 Kranken war der Fruktosegehalt im Spermaplasma hoch, während dieser Wert im Fall Nr. 12 148 mg% betrug und im Fall Nr. 13 auffallend niedrig war, nämlich 33 mg%. Allerdings stieg dieser Wert nach Verabreichung von 250 mg Testosteron auf 265 mg%, doch trat in den Bewegungsverhältnissen keine Veränderung ein.

Auf Grund der schweren Hypospermie muß trotz der scheinbaren Intaktheit der Hoden auch in obigen Fällen auf die Läsion des germinativen Gewebes als Ursache geschlossen werden. Der Mangel an Bewegung beruhte nicht auf dem Spermaplasma, sondern auf der strukturellen Läsion der Spermien. Wir erachten es als einen Fehler, daß wir bei diesen Kranken — mit Ausnahme von zweien — nicht auf der Durchführung der Hodenbiopsie bestanden haben, deren Ergebnis unsere Behauptung besser dokumentiert hätte.

Während in den bisher beschriebenen Fällen die Aufrechterhaltung des hypo- oder akinetischen Zustands auf die Spermien zurückgeführt werden konnte, muß der Fall des Kranken Nr. 8 als kombiniertes Bild bezeichnet werden. Auch hier lag maximale Hypospermie bei normalem Genitalzustand vor. Der Fruktosespiegel machte indessen nur 36 mg% aus, ein Wert, der bedeutend unter dem normalen liegt. Zugleich war die Verschiebung der Reaktion in Säurerichtung auffallend: pH = 6,8. Durch diesen niedrigen Wert wird die Bewegung bereits an und für sich stark beeinträchtigt. Sicherlich ergab sich die Säurekomponente nicht aus den milchsäureartigen Derivaten des abgebauten Kohlenhydrats, da ja kaum Spermien anwesend waren, welche die Fruktolyse ermöglicht hätten. Möglicherweise beruhte diese Verschiebung auf den schwach sauren Produkten der Prostata. In diesem Fall dürfte also die Akinese mehrere Gründe gehabt haben: 1. die pathologische Spermatogenese, 2. den niedrigen Fruktosespiegel, 3. das saure Medium. (Sekret der Prostata: Aplasie der Vesicula semin. oder Obliteration ihrer Ausführungs-

gänge?) Dies wäre somit der einzige Fall, wo auch die Zusammensetzung des Spermaplasmas für den Ausfall der Bewegung verantwortlich zu machen war.

Unsere Zusammenstellung ist angesichts des seltenen Vorkommens der Nekrospermie bescheiden. Wenn überhaupt von einer Schlußfolgerung gesprochen werden darf, so läßt sich sagen, *daß die Ursache des Ausfalls bei Nekrospermie vor allem in den Spermien gesucht werden muß. Der defizitäre Zustand des Spermaplasmas als eine die Unbeweglichkeit aufrechterhaltende Ursache kommt viel seltener vor.* Dessenungeachtet müssen die Fruktoseverhältnisse in jedem Fall geklärt werden. Wenn die Untersuchung den Mangel an diesem Kohlenhydrat bestätigt, so befinden wir uns in bezug auf die Korrektion in einer günstigeren Lage. Mit Androgen läßt sich dieser Wert gegebenenfalls (wie in unserem Fall Nr. 11) erhöhen, und im Falle einer entsprechenden Spermienzahl und Zellstruktur erscheinen gewisse Hoffnungen in bezug auf die Fertilität gerechtfertigt. Leider ist dies der seltenere Fall. Die Normalisierung der geschädigten Spermien, d. h. die Korrektion der germinativen Läsion, ist eine viel schwerere Aufgabe und mit Hilfe der üblichen Therapie (Gonadotropine, Rebound- Verfahren usw.) im allgemeinen kaum zu lösen.

Wir glauben, *die Nekrospermie in der überwiegenden Mehrzahl der Fälle berechtigterweise auf eine Schädigung der Spermien zurückführen zu müssen.*

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DIE KLINISCHE BEDEUTUNG DER AMINOSÄUREN IM MENSCHLICHEN EJAKULAT

R. DOEPFMER

UNIVERSITÄTS-HAUTKLINIK, BONN

Zusammenfassung

Mit der von KRAMPITZ ausgearbeiteten säulenchromatographischen Methode wurden im menschlichen Ejakulat qualitativ und quantitativ basische, saure und amphotere Aminosäuren bestimmt. Die eigenen Ergebnisse wichen qualitativ und quantitativ wesentlich von den Resultaten mit der papierchromatographischen Methode ab. Beim gleichen Individuum war der Gehalt der Aminosäuren weitgehend konstant, sofern der Spermaliquor zu bestimmten gleichen Zeiten nach der Ejakulation untersucht wurde. Bei Oligo-Astheno-Teratospermien und bei Azoospermien mit Leydigzellinsuffizienz sowie einer Aspermie infolge Mißbildung der akzessorischen Geschlechtsdrüsen wiesen die verschiedenartigen Aminosäuren einen deutlichen Abfall gegenüber Normospermien auf. Der Gehalt der Aminosäuren dürfte ebenso wie der Fruktosegehalt weitgehend von einer normalen Funktion der Leydigzellen abhängen. Primäre Hodenschäden mit Oligo-Astheno-Teratospermien oder Azoospermien ohne Leydigzellinsuffizienz ließen keine wesentlichen Abweichungen von Normospermien erkennen. Die Anfertigung eines Aminosäurendiagramms erfordert etwa 2 Tage Dauer. Die Bestimmung der Aminosäuren ist daher für Routineuntersuchungen wie die Fruktosebestimmung zunächst nicht geeignet.

Über die Rolle der Motilität und insbesondere der Qualität der Motilität, d. h. der Intensität der Vorwärtsbewegung beim Zustandekommen einer Befruchtung besteht ebenso wie vor 4 Jahrzehnten bei der Geburt der Spermatologie keine Einigkeit. Einige Autoren [4, 16 usw.] nehmen an, daß auch unbewegliche Spermien infolge von Muskelkontraktionen bis in die Tube vordringen können. Die Ergebnisse an einem sehr großen Krankengut von MACLEOD und GOLD [9] sprechen jedoch dafür, daß die Qualität der Motilität doch ein wesentliches, wenn nicht das wesentlichste Charakteristikum für die Bedeutung der Zeugungsfähigkeit darstellt.

Wenig erforscht ist bis heute, ob Spermien aus dem Spermaliquor, aus den Zervixsekreten oder aus den Sekreten des Uterus oder der Tube Substanzen als Energiequelle aufnehmen können. Bei einem normalen Geschlechtsverkehr wandern bei normaler Gonadentätigkeit des Mannes und der Frau die Spermien in wenigen Sekunden oder Minuten in das Zervixsekret. Da mit Sicherheit der Spermaliquor nicht durch das Zervixsekret zu dringen vermag, ist in der Regel der Spermaliquor nicht als Energiequelle für die Motilität der Spermien zu betrachten.

Zahlreiche biochemische Untersuchungen des Ejakulats [10, 14, 17] brachten keine sicheren darstellerischen Hinweise über die Befruchtungsfähigkeit der Spermien. Lediglich der Fruktose- und Inositgehalt des Spermaliquors gab Aufschlüsse über die Funktion der Leydigzellen, und zeigte eine mögliche Ursache der Infertilität auf [14]. Im Gegensatz zu den Ergebnissen von

Tierversuchen [10] fanden SCHIRREN [14] und NIERMANN [12] beim Menschen keine sicheren Beziehungen zwischen dem Zitronensäuregehalt des Spermaliquors und der Leydigzellenfunktion. Die Bestimmung der Fruktolyse, d. h. des Fruktoseverbrauchs und somit des Abbaues der Fruktose in einem gewissen Zeitabschnitt nach der Ejakulation gab gewisse Aufschlüsse über das Stoffwechselgeschehen der Spermien, zeigte jedoch ebensowenig wie die Bestimmung der Hyaluronidase oder anderer Fermentkomplexe sichere Anhaltspunkte für die Konzeptionsfähigkeit der Spermien.

Über die Bedeutung der Aminosäuren im Spermaliquor liegen beim Menschen nur wenige Untersuchungen vor.

ADAM und KORTING [1], JACOBSSEN [3] und LUNDQUIST [8] bestimmten die Aminosäuren im menschlichen Sperma qualitativ und KEUTEL und GABSCH [5] quantitativ mit der papierchromatographischen Methode. SAKAR, LUECKE und DUNCAN [13] trennten die Aminosäuren mikrobiologisch. Nach GASSNER nahmen die freien Aminosäuren im Spermaliquor ebenso wie die Fruktose und die Aminosäuren nach der Kastration ab; durch Substitution von Testosteron konnte jedoch der Aminosäuregehalt nicht wieder normalisiert werden.

Nach TYLOR und LORD ROTHSCHILD bewirkte der Zusatz von Aminosäuren zur Suspension in Seewasser eine starke Verlängerung der Dauer der Beweglichkeit und Befruchtungsfähigkeit von Seeigeln. Die Aminosäuren wurden bei diesem Geschehen durch Stoffwechselvorgänge nicht verbraucht. Es trat jedoch ein langsamer Abbau ein, so daß möglicherweise das eigentlich wirksame Agens das hierbei ständig gelieferte Ammoniak war.

In diesem Zusammenhang sind auch die Ergebnisse von DOEPFMER und FREIHOFF zu erwähnen, die durch Zusatz von Aminosäurechlorid die Motilität und die Motilitätsdauer von Hodenspermien von Meerschweinchen auf 56 Stunden und von Menschen auf 30 Stunden verlängern konnten.

Unsere Untersuchungen galten den Fragen, ob der Gehalt der verschiedenartigen Aminosäuren ähnlich wie der Gehalt der Fruktose oder des Inosit beim Menschen von einer normalen Funktion der Leydigzellen abhängig ist. Weiterhin prüften wir den Aminosäuregehalt bei Patienten mit Normospermien, Oligo-Astheno-Teratospermien und Azoospermien und insbesondere die quantitativen Mengen der verschiedenen Aminosäuren in kurzen Zeitabständen nach der Ejakulation.

Die quantitativen Bestimmungen der Aminosäuren im menschlichen Ejakulat sind kompliziert und außerordentlich kostspielig. Bisher wurden für die Erfassung der Aminosäuren durch Trennung der Eiweißbausteine folgende Methoden angewandt:

1. Das mikrobiologische Verfahren, bei dem das Wachstum bestimmter Testbakterien geprüft wird, die zu ihrer Existenz gewisse, von Art zu Art verschiedene Aminosäuren benötigen. Dieses Verfahren ist sehr empfindlich und spezifisch, läßt sich jedoch nur zur qualitativen Auswertung heranziehen.

2. Das papierchromatographische Verfahren eignet sich vorwiegend nur für qualitative Untersuchungen.

Nach KOFRANYI [6] soll die Fehlerbreite bei einer quantitativen papierchromatographischen Bestimmung bei etwa $\pm 30\%$ liegen. Die papierchromatographische Methode ist einfach und schnell durchführbar, jedoch für quantitative Auswertungen in der Regel nicht geeignet.

3. Das säulenchromatographische Verfahren dürfte nach den heutigen Kenntnissen für die quantitative Bestimmung der Aminosäuren die Methode der Wahl sein.

Die Technik dieses Verfahrens wurde eingehend von KRAMPITZ [7] dargestellt. Die Genauigkeit dieser Methode liegt nach unseren Erfahrungen bei $\pm 1\%$. Die Nachteile dieses Verfahrens sind durch den hohen Kostenpunkt und den riesigen Zeitaufwand gegeben. Zur Durchführung einer Vollanalyse von komplizierten Aminosäurengemischen wie sie im Sperma vorhanden sind, benötigt man 2 Tage.

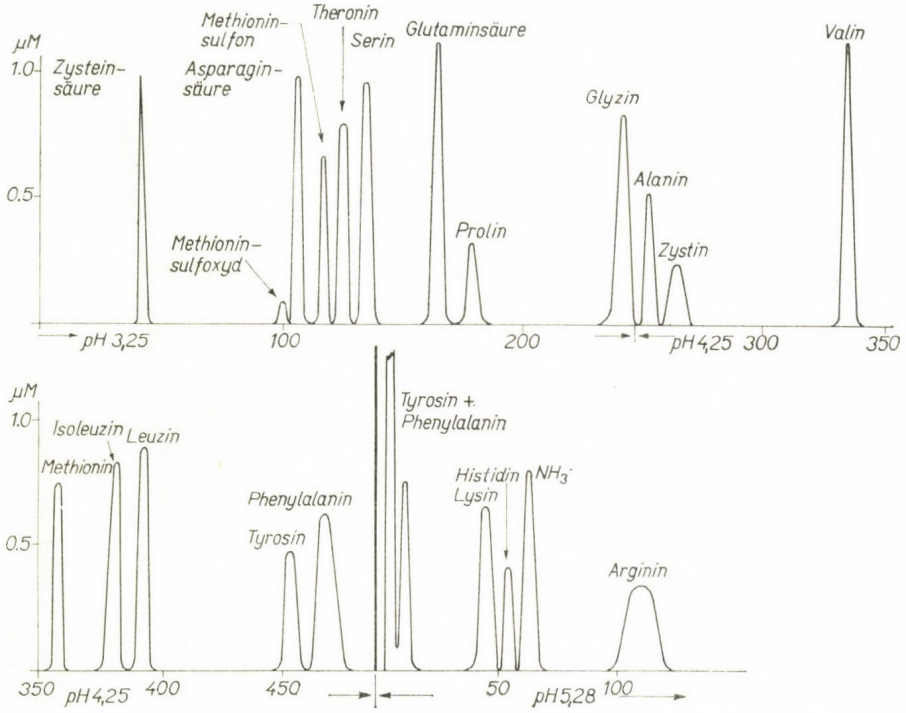

Abb. 1. Normales Diagramm der verschiedenen Aminosäuren im menschlichen Ejakulat

Für unsere eigenen, gemeinsam mit KRAMPITZ durchgeführten Untersuchungen versetzten wir etwa 4 bis 6 Stunden nach der Ejakulation den Spermaliquor mit einem Überschuß an 1%iger Pikrinsäure, um die vorhandenen Proteine zu entfernen. Wir untersuchten 10 Patienten mit Normospermien, 5 mit Oligo-Astheno-Teratospermien, 5 mit Azoospermien und 1 Patienten mit Aspermie, die durch eine Mißbildung der samenabführenden Wege und Bläschendrüsen bedingt war.

Die Abbildung 1 zeigt ein Diagramm, auf dem die verschiedenen Aminosäuren aus einem Spermaliquor eines Patienten mit einer Normospermie dargestellt sind.

In der Tabelle 1 haben wir unsere Ergebnisse den papierchromatographisch ermittelten Befunden von KEUTEL und GABSCH [5] sowie den säulen-

Tabelle 1

Aminosäuren im menschlichen Ejakulat und Blutplasma

Amphotere Aminosäuren mg/ccm	KEUTEL und GABSCH [5]	Eigene Untersuchungen				
	Normospermie	Normospermie	Oligospermie	Azoospermie	Aspermie bei Mißbildung der Bläschendrüsen + Prostata	Blutplasma
Glutathion	0,17	—*	—	—	—	—
Methionin	0,047	0,038	0,021	0,007	0,011	0,0008
Zystin	11,7	0,034	0,002	0,006	0,015	0,010
Zystein	9,0	—**	—	—	—	—
Taurin	19,8	—***	—	—	—	0,004
Glyzin	6,4	0,589	0,251	0,230	0,071	0,018
Oxyprolin	11,6	—	—	—	—	—
Alanin	0,52	0,291	0,141	0,096	0,043	0,036
Prolin	2,2	0,269	0,100	0,139	—	0,016
Tyrosin	0,8	0,514	0,207	0,232	0,101	0,009
Valin	0,8	0,498	0,240	0,134	0,100	0,037
Phenylalanin	0,15	0,282	0,146	0,164	0,112	0,009
—Diaminobuttersäure	0,55	—	—	—	—	—
Threonin	4,15	0,477	0,282	0,190	0,115	0,012
Tryptophan	0,076	—	—	—	—	—
Serin	0,75	1,116	0,560	0,429	0,104	0,010
β-Alanin	—	0,360	0,109	0,158	0,152	—
β-Aminoisobuttersäure	—	0,317	0,171	0,053	—	—
Isoleuzin }		0,624	0,259	0,191	0,087	0,023
Leuzin }	0,3	0,967	0,506	0,332	0,228	0,013
	69,013	6,426	2,995	2,361	1,139	0,1978
Säure, Aminosäuren, mg/ccm						
Asparaginsäure	5,6	0,998	0,458	0,336	0,187	0,007
Glutaminsäure	0,95	1,797	1,193	1,552	0,397	0,094
	6,55	2,795	1,651	1,988	0,584	0,101
Basische Aminosäuren mg/ccm						
Arginin	1,88	0,790	0,310	0,295	0,094	0,014
Lysin	0,8	1,521	0,587	0,580	0,183	0,027
Ornithin	0,47	—[1]	—	—	—	0,007
Histidin	1,97	1,091	0,443	0,398	0,079	0,014
	5,12	3,402	1,340	1,773	0,356	0,062

* hydrolisiert
** wird als Zystin bestimmt
*** Bestimmung ungenau
[1] nicht gefunden

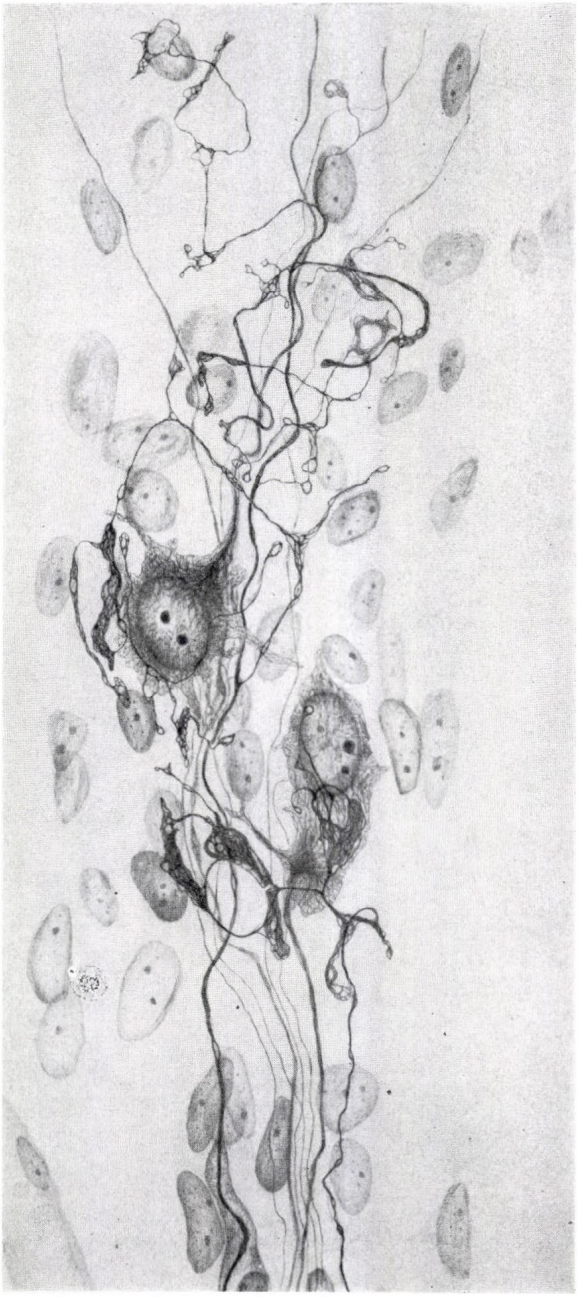

Fig. 1*a*—Sensory apparatus terminal twigs interrelations with ganglion cells. A detail of Fig. 1

Fig. 2.—Typical sensory apparatus in the proventriculus muscular coat of a duck (*Anas platyrhynchos*). Bielschowsky—Gros method, Zeiss, obj. HJ 90/I. 30, oc. K10

All the observations submitted here for consideration have been carried out by the Bielschowsky-Gros method on the intrinsic myenteric plexus ganglia of some wild ducks' *(Anas platyrhynchos, Netta rufina)* stomach (proventriculus).

The object was taken for investigation, because in the myenteric plexus of the oesophagus and the stomach of the ducks chosen, in addition to large ganglia we may find also small ones with loosely scattered nerve cells in them and even separate single neurons, which make it possible to follow the interrelation of afferent nerve fibers with nerve cells with great efficiency.

In Fig. 1 a preparation of the proventriculus muscular coat of a wild duck *(Anas platyrhynchos)* is given.

In the preparation one can clearly see a myelinated fiber passing in slender nerve bundle of the myenteric plexus. On loosing its myelin sheath, it divides into two chief branches. The right-hand branch of the myelinated fiber, on curving in semi-circle, comes out of the nerve bundle and enters the intermuscular loose connective tissue, where it divides into a number of still smaller branches. Going on dividing, they terminate in fine tender terminal branches having peculiar flat plates (lamella) and ringlets of different size. All the end ramifications are encircled by a big number of large transparent nuclei of the so-called auxiliary (or special) cells, which are very characteristic for certain kinds of sensory nerve endings.

This complex nerve formation made by the right-hand branch of the myelinated fiber has a structure fully characteristic for the sensory apparatuses that are met in large quantities in the loose connective tissue of the proventriculus muscular coat of the birds investigated. For comparison, Fig. 2 shows a standard sensory apparatus of the same proventriculus muscular coat of the wild duck. As can be seen in Figs 1 and 2, both formations are built identically. And it is the fact, one of the branches of myelinated nerve fiber described (Fig. 1) forms a typical sensory apparatus that makes the afferent (sensory) character of the fiber absolutely certain.

But what is most important is that the other large branch of the same myelinated afferent fiber (the left-hand one in Fig. 1) proceeds to a small myenteric plexus ganglion consisting only of two neurons, and here it breaks up into its terminal twigs. These are shaped exactly as those at the bottom sensory apparatus, formed by the right-hand branch of the afferent fiber, and those of the sensory apparatus given in Fig. 2, which allows to distinguish them easily enough from the preganglionic fiberend ramifications.

As seen in Figs 1 and 1*a*, the sensory terminal twigs are distributed in the nerve bundle, where a ganglion is located, and also in the surrounding loose connective tissue. One of these terminal twigs goes to the ganglion cell. Having surrounded it, it penetrates, through layers of satellite-cells, till the very nerve cell and comes into touch with its body and its dendrites by some of its lamellae and ringlets.

Two other identical twigs (Fig. 1*a*) spread along the axon of the same nerve cell and end in ringlets on its axon hillock.

To the second ganglion neuron there proceeds a separate thin nerve fiber which starts from the branches of the bottom sensory apparatus (Figs 1 and 1*a*). Not far from the nerve cell it breaks up into several terminal

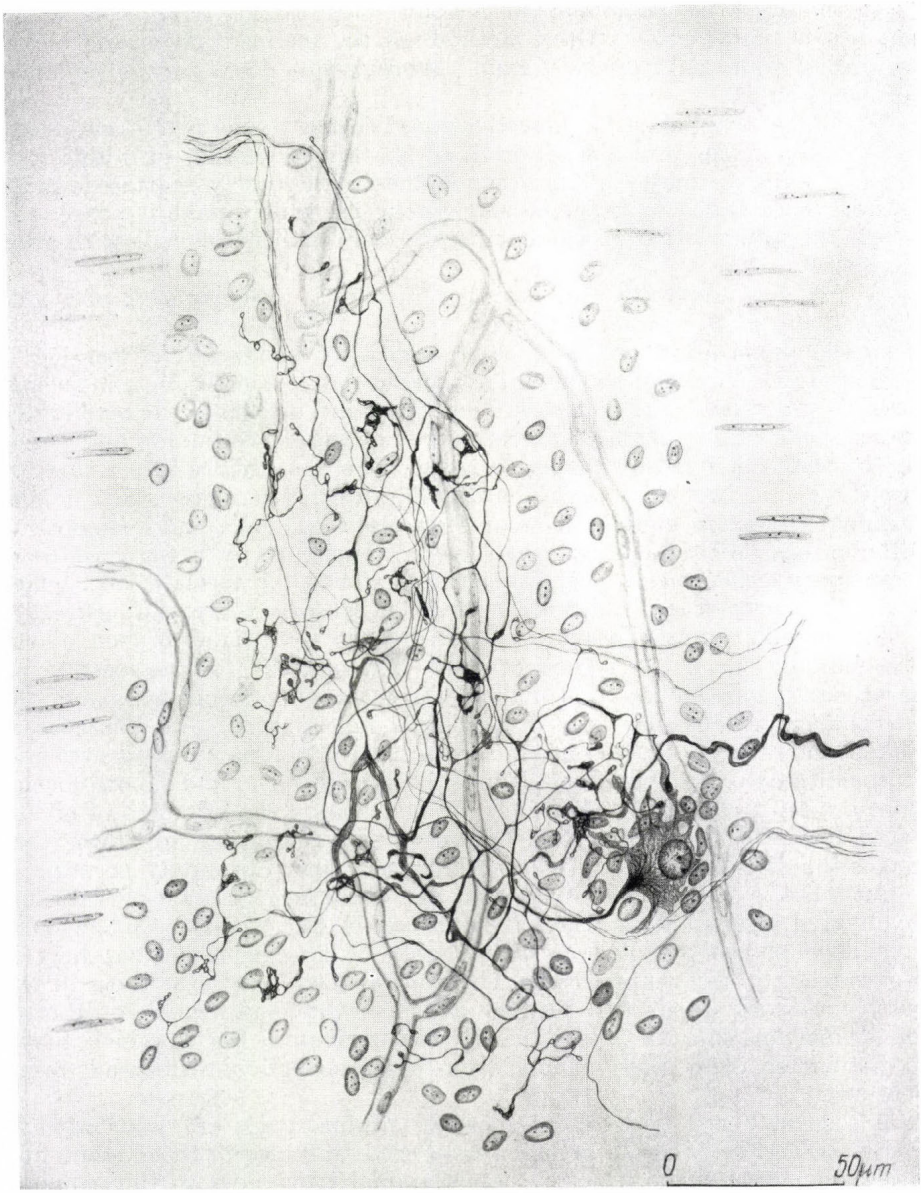

Fig. 3.—Nerve cell in a sensory apparatus field. Proventriculus muscular coat of a duck *(Anas platyrhynchos)*. Bielschowsky—Gros method, Zeiss, obj. HJ 90/I. 30 oc. K10

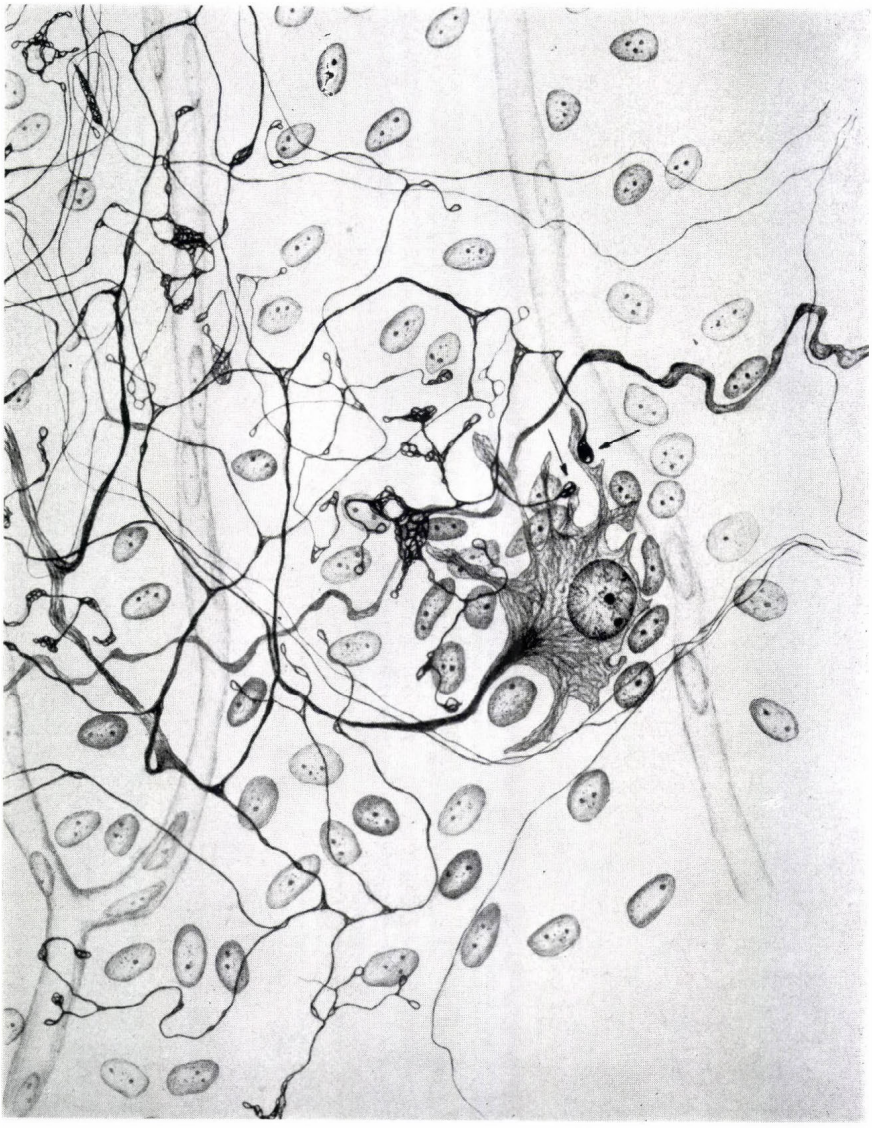

Fig. 3*a*.—Sensory terminal twigs' relation to a nerve cell. Their direct coming into touch with a nerve cell's dendrites is well seen (arrows). A detail of Fig. 3

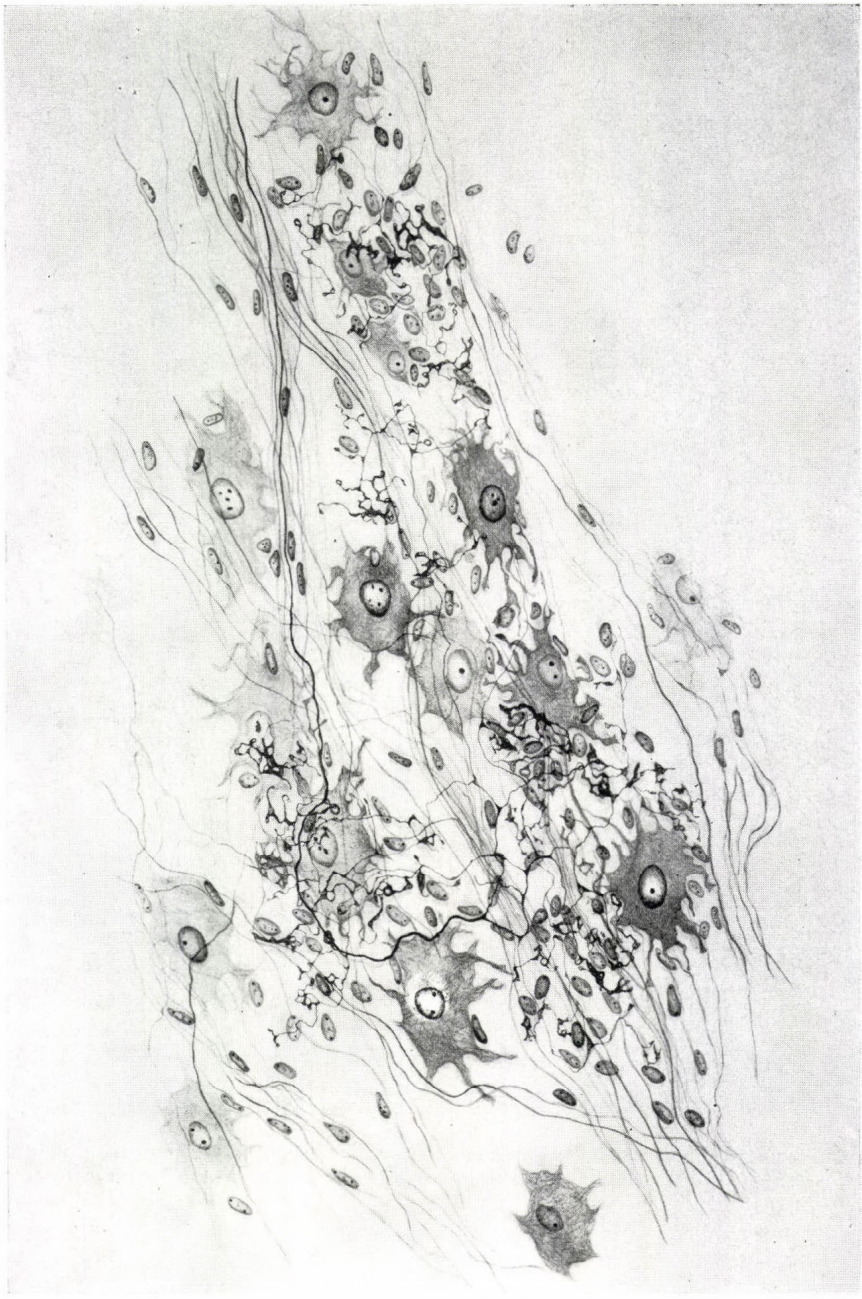

Fig. 4.—Sensory apparatus in myenteric plexus ganglion. Proventriculus of a duck (*Anas platyrhynchos*). Bielschowsky—Gros method, Zeiss, obj. HJ 90/I. 30, oc. K10

twigs having wide end lamellae so typical of certain sensory apparatuses. The lamella of one of these terminal twigs is closely adjacent to the nerve-cell body, while going off the lamella of the thinnest end-nerve twigs spread all over the nerve-cell body surface.

So, in the preparation one can see the nerve fiber, whose afferent character is beyond doubt, enter into a direct contact with the myenteric plexus nerve-cell bodies and their processes (dendrites and axons). I suppose this observation to be the most convincing proof of the afferent (sensory) innervation of the autonomic ganglion cells.

And this is not an exceptional phenomenon. I have at my disposal some scores of preparations where one can see similar relation of the afferent nerve fibers to the autonomic ganglion cells.

Fig. 3 shows a typical sensory apparatus of the proventriculus muscular coat of a duck *(Anas platyrhynchos)*. There is a single nerve cell in the field of this sensory apparatus. Some terminal twigs of the sensory apparatus near it. A part of them terminate in ringlets or lamellae on the neuroglial cells around the neuron, while two terminal twigs enter into a direct contact with its dendrites, (see also the arrows in Fig. 3*a*).

The axon of the neuron passes through the whole of the sensory apparatus field, coming more than once into contact with its terminals.

I have observed similar interaction between the afferent nerve fibers and the nerve cells in the large ganglia of the myenteric plexus as well.

Fig. 4 shows a section of a ganglion of the myenteric plexus proventriculus of a wild duck *(Anas platyrhynchos)*.

Within the ganglion the afferent nerve fiber ramifies intensively, making a true sensory apparatus. Its numerous terminal twigs carry wide lamellae and end ringlets very typical for a sensory apparatus. Several of these terminal twigs lie in the ganglion connective tissue stroma and among the neuroglial cells, but some of them come close up to the nerve cells and terminate by their lamellae or ringlets in their bodies and dendrites. This is very well seen in the preparation. But, to be still more certain of the direct contact of the afferent nerve fiber terminal twigs with the ganglion neurons, 9 have studied their interrelations also on thin sections through a ganglion (10—15 μ). In such sections the afferent nerve fiber's end ramifications are badly damaged by the knife, and naturally, fragments of sensory apparatus only remain, but then the close tie of their terminal twigs with the ganglion cells are sharply outlined. (Figs 5 and 5*a*).

So the morphological observations mentioned here have shown that around the autonomic ganglion cells there are sensory appratuses, the terminal twigs of which come into the closest relation with the nerve cells. This suggests that these sensory apparatuses are functionally tied up with the autonomic ganglion cells' specific activity, and hence these nerve cells must possess receptive fields of their own.

The notions I have about the autonomic ganglion neurons' afferent innervation by the central nervous system are schemetically shown in Fig. 6.

An autonomic ganglion neuron is represented here, the axon (postganglionic fiber) of which innervates the inner organs' effectors (smooth muscles, glands and the like).

If formely such neurons were considered to have connection with the central nervous system only by efferent nerve tracks (through preganglio-

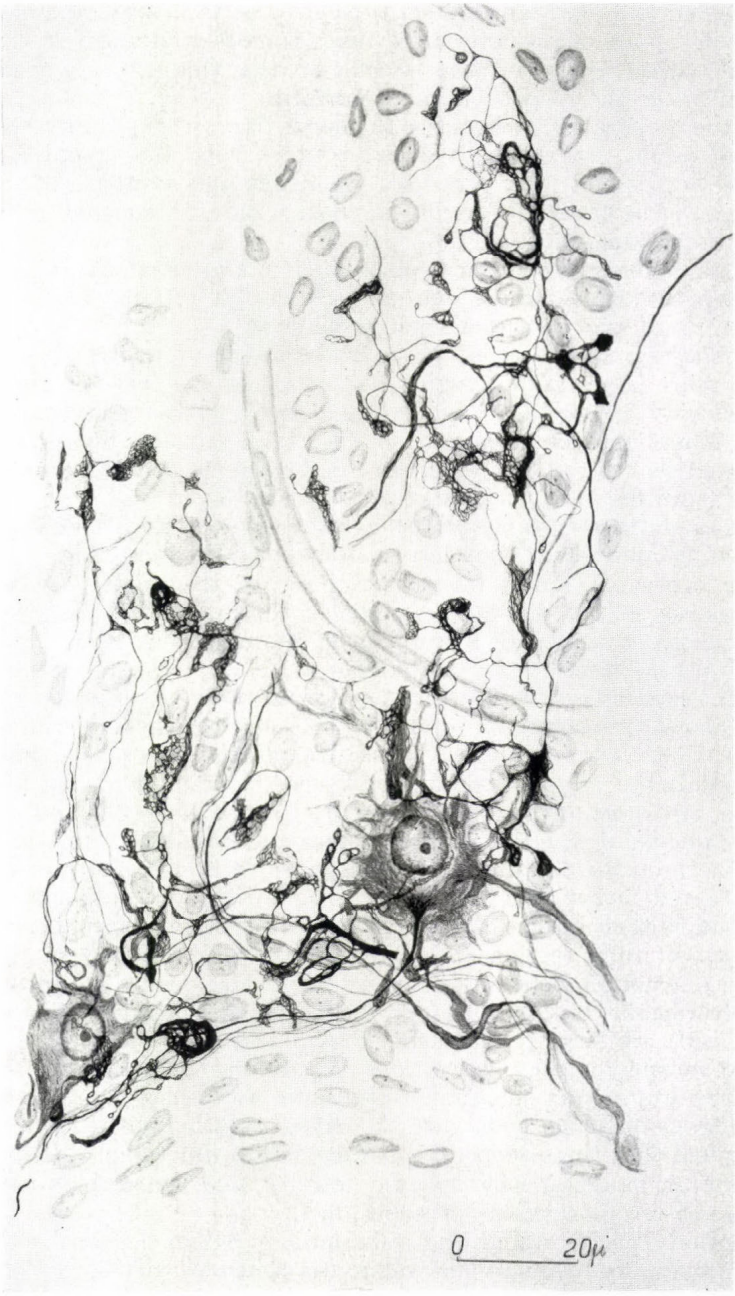

Fig. 5.—Sensory apparatus fragments as seen in a thin section (15μ) across the myenteric plexus ganglion. Proventriculus of a duck (*Anas platyrhynchos*). Bielschowsky—Gros method, Zeiss, obj. HJ 90/I.30, oc. K10

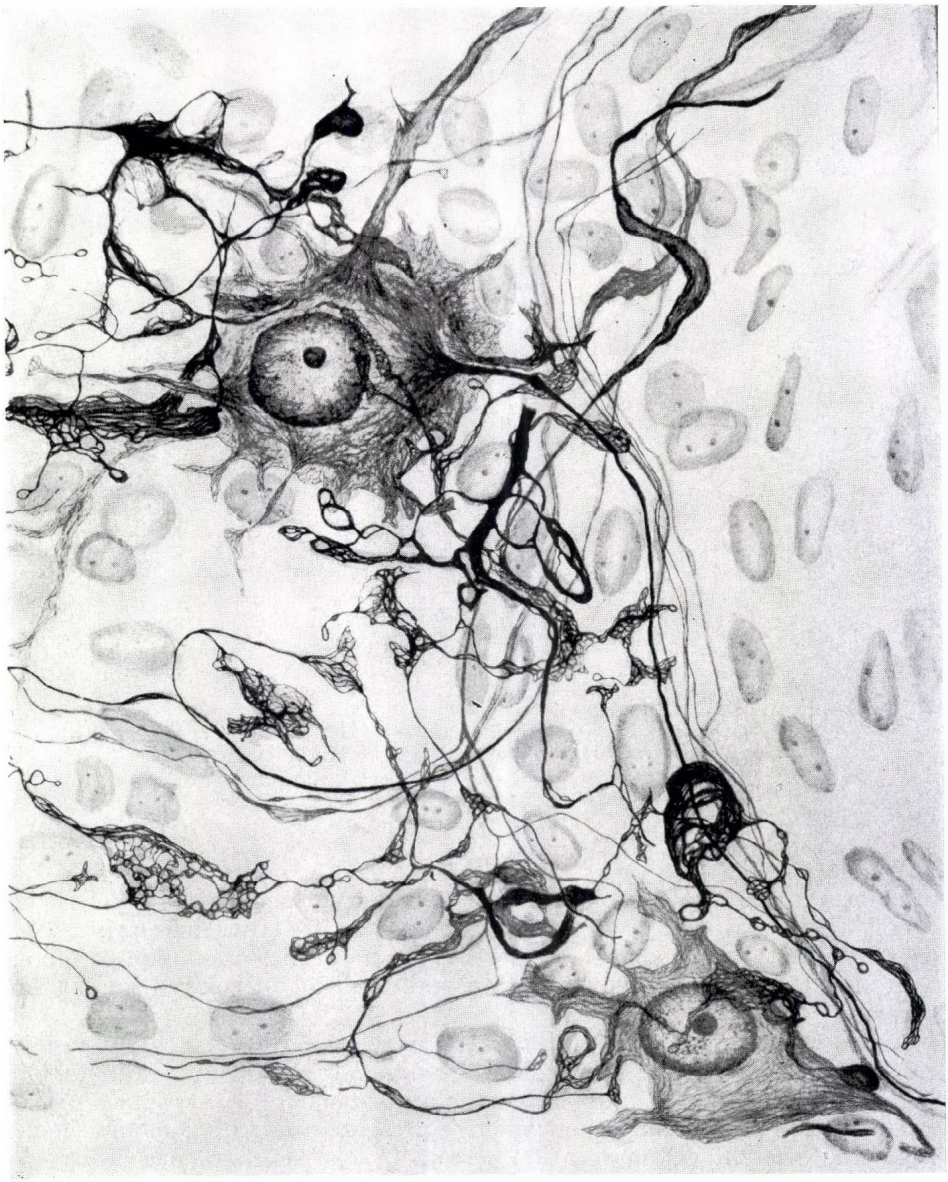

Fig. 5*a*.—Sensory terminal twigs coming into touch with the nerve cell's body and its processes (dendrites and axon) is well seen. A detail of Fig. 5

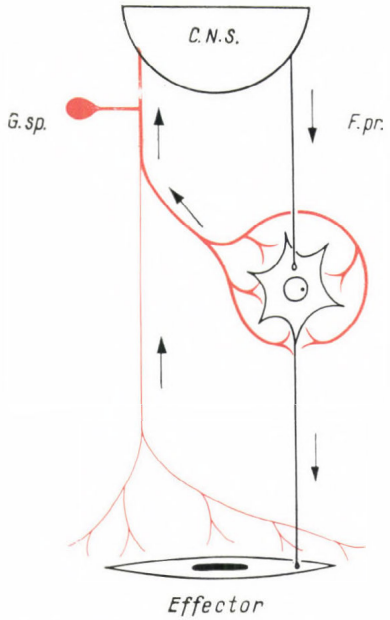

Fig. 6.—Schematic rendering of an autonomic ganglion cell's afferent (sensory) innervation by the central nervous system. C. N. S. = central nervous system, G. sp. = spinal ganglion (or analogous to it, N. vagus sensory ganglions), F. pr. = preganglionic fiber

nic fibers), now the morphological findings mentioned above are direct proofs of the existence of an afferent innervation of these. This newly established afferent nerve pathway proceeding from the autonomic ganglion neuron to the central nervous system is indicated by a thick red line in the schema.

And now the connection of the central nervous system to the autonomic ganglion neurons should be considered as effected by two communication system, i.e. the efferent system and the afferent one.

The efferent communication system is presented by preganglionic fibers, while the afferent communication system by the sensory nerve fibers of cerebrospinal or vagal origin which form sensory apparatuses around and on the autonomic ganglion cells. It is only if these two communication systems are present that it is posible to understand the central regulation of the autonomic ganglion neurons and their synaptic junctions' functional state; such a regulation would be impossible without a steady stream of sensory information about their functional state into the central nervous system.

Of the kinds of stimulants most adequate for the nerve cell's sensory apparatuses we have no positive knowledge as yet. According to morpho-

logical observations, showing the closest relations of the sensory apparatus's terminal twigs with the neurons, only the processes accompanying the neurons' specific activity can be considered as adequate stimulants for them. This may be either chemical mediator substance liberation or electrostatic field vibrations around a neuron.

Here opens a wide scope for an entirely new physiological study of the autonomic ganglion neurons as possessing their own receptive fields. Inasmuch as these neurons possess their own receptive field (which is definitely shown by the morphological data), any change in the neurons' functional state may cause some reflex influences within the nervous system, as well as in the whole organism.

Evidently, a large number of thorough and intricate physiological experiments are necessary to make the functional purpose of the sensory apparatuses around neurons fully known. But at present, there is one thing certain: the morphological observations have definitely proved the afferent (sensory) innervation of the nerve cells.

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SECTION TWO

VEGETATIVE NERVOUS SYSTEM

Symp. Biol. Hung., **5**, 69—83 (1965)

THE STRUCTURE OF THE SYNAPSES IN THE HEART OF THE EUROPEAN POND TURTLE (EMYS ORBICULARIS)

by

A. Ábrahám

DEPARTMENT OF GENERAL ZOOLOGY AND BIOLOGY, JÓZSEF ATTILA UNIVERSITY
SZEGED, HUNGARY

The structure of the intracardiac ganglion synapses can by no means be considered as adequately cleared. There is no agreement on structure, origin and function of the nerve-fiber plexus formations surrounding the ganglion cells. We have similarly controversial and uncertain information on the finer structural details as regards the intimate connexion between the cardiac muscle cells and the nerve fibers. Since decades we have been engaged in the examination of both problems. Results on all classes of vertebrates have been treated in detail in *Die Innervation des Herzens und der Blutgefäße von Vertebraten*, a monograph edited by the Publishing House of the Hungarian Academy of Sciences in 1964.

The results summarized in this paper on the synapses of the intracardiac ganglia, or intracardiac nerve cells on the one hand, and on myoneural junctions of the cardiac muscle fibers, on the other, have been obtained through investigations of the heart of *Emys orbicularis* by means of modifications of the Bielschowsky method. In the course of our investigations vagotomy has been performed in many cases and the vagal branches running to the heart were cut through either on the right, or on the left, or on both sides. The operation was generally well tolerated by the animals. Transection of one vagus did not produce any symptoms. The animals ate and moved in the habitual way, and no special reaction in their organs of sense could be observed. In the course of dissection we found that after transection of one vagus the gullet showed a stomach-like dilatation at some places, and the blood supply strongly decreased throughout the whole region of the intestinal channel. After cutting through both vagus nerves, the animals rejected food completely. In spite of this, they survived about 32 days in all three experiments of ours. Most assuredly, the operation produced morphological changes discernible in different organs, but this time we intend to deal only with the symptoms, resp. changes appearing in the nervous system of the cardiac wall, in which connection it has to be mentioned first that, in compliance with previous observations in other areas, the operation induced an extremely strong increase in the argyrophilia of the nerve fibers. The other phenomenon to be emphasized is that the synapses appeared in a special abundance and with an extraordinary clearness on both the isolated nerve cells and the cardiac muscle cells. Our observations can briefly be summarized as follows.

In the region of the cardiac wall of the turtle, the nerve cells are, as a rule, divided into two groups. One consists of the nerve cells localized along the large nerve stems running in the right atrial epicardium that surrounds

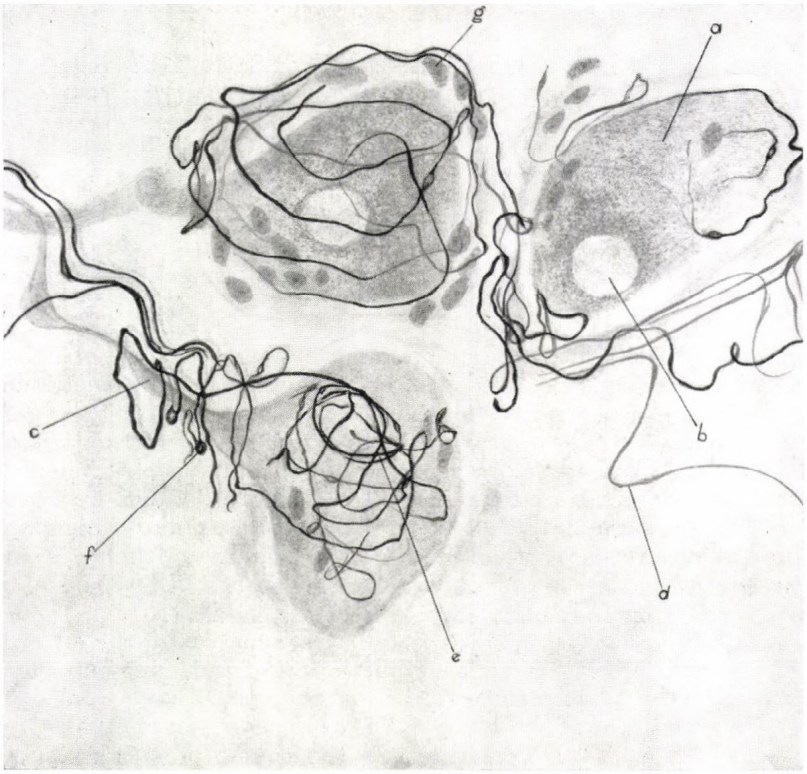

Fig. 1.—*Emys orbicularis*, heart. Nerve cells from the right atrial epicardium. *a* = nerve cell, *b* = nucleus of the nerve cell, *c* = cellular process, *d* = preganglionlic fiber, *e* = pericellular plexus, *f* = nerve ending, *g* = nucleus of the satellite cell. Bielschowsky–Ábrahám stain, × 1600

the entrance of the large veins and in the atrial septum. These cells form an almost continuous row of ganglion cells along both sides of the nerve stems. They are on the whole of spherical or slightly elliptical shape. The relatively large cells have round nuclei, their centrally localized nucleolus is dense and strongly refractive. There is a single, sometimes slightly granulated process—well discernible all the same—emerging from the cytoplasma. The processes of the cells enter the nerve bundles where they appear to proceed without division. The cells are definitely unipolar and are in most cases stained faintly (Fig.1). Judged by their position and structure, they may be parasympathetic cells. They resemble somewhat the unipolar cells of the cerebrospinal ganglia, differing, however, markedly by always being surrounded by a conspicuous and rich basket of pericellular fibers of denser or looser structure. The fibers of the baskets have often spheroid varices and terminate in well-discernible dense end knobs, or more seldom in terminal rings (Figs 2 and 3). At sites where one, two or even three fine pericellular fibers terminate, a smaller depression of the cell surface occurs, which is easy to recognize by its clear-cut outlines.

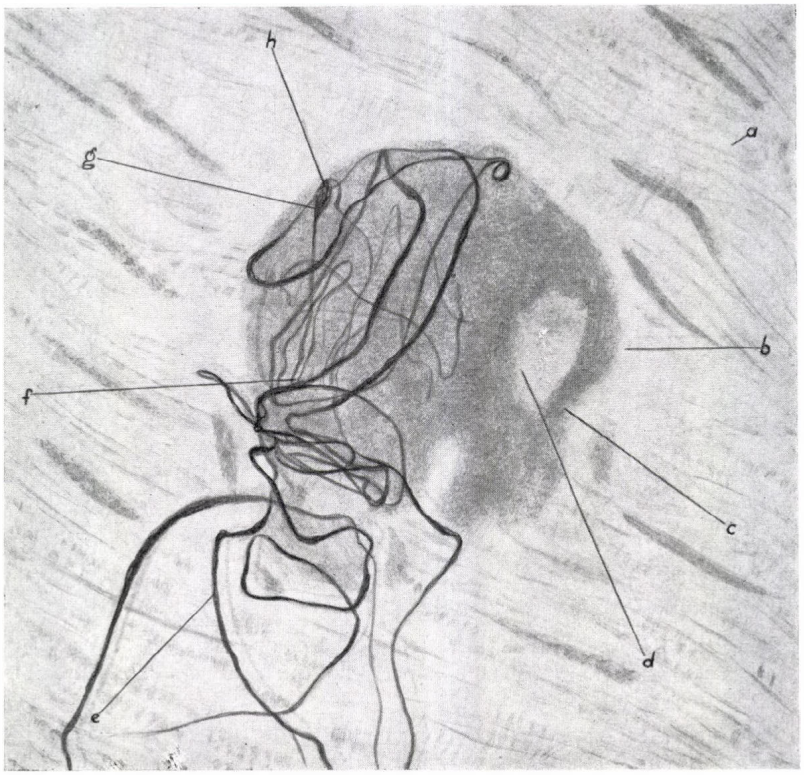

Fig. 2. — *Emys orbicularis*, heart. Nerve cell from the right atrial epicardium. *a* = myocardium, *b* = epicardium, *c* = nerve cell, *d* = nucleus of the nerve cell, *e* = nerve fiber, *f* = preganglionic fiber, *g* = pericellular plexus, *h* = nerve ending. Bielschowsky–Ábrahám stain, × 1600

When examining the pericellular plexus in thin sections, of course, we have no possibility to trace the origin of the fibers and their connexions with nerve trunks or fiber bundles. In order to establish the connexions, we had to choose relatively thick sections, in which the origin and connexion of the nerve fibers entwining the cells could be well revealed. Apart from smaller variations occurring in almost every cell, the connexions may be characterized as follows. A homogeneous smooth-bordered nerve fiber branches off from a rich nerve bundle toward the margin of the cell. Before reaching the cell, this nerve fiber forms numerous looser or denser spirals. At some places the breadth of these spirals is uniform, at others it may ramify or proceed undivided to its endings in the above-mentioned terminal circles or terminal knobs (Figs 4, 5 and 6). There occur, of course, more complicated and extreme cases as well, e.g. when the fiber emerging from the nerve stem bifurcates so that one of the branches entwines one, and the other an other nerve cell. In other cases the nerve fiber bifurcates on the body of the nerve cell, and one of the branches entwines that cell on

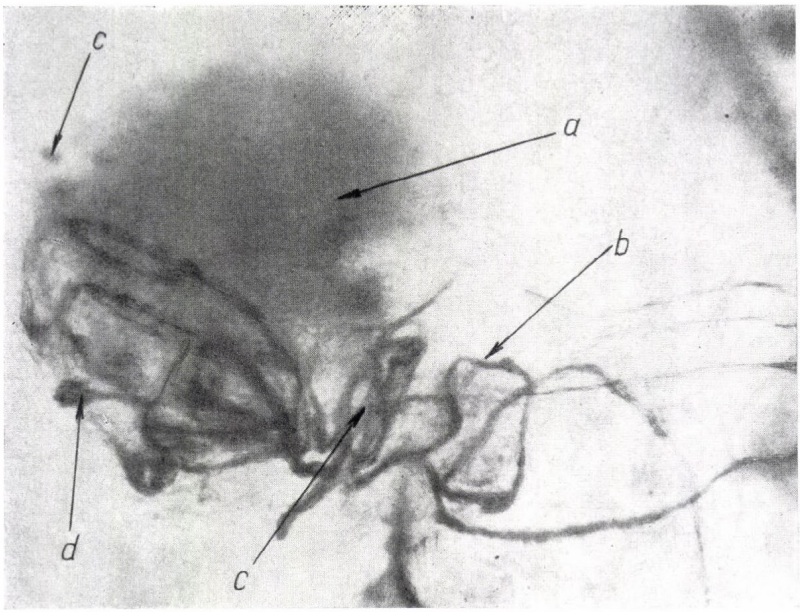

Fig. 3.—*Emys orbicularis*, nerve cell in the right atrial epicardium. *a* = nerve cell, *b*= preganglionic fiber, *c* = pericellular plexus, *d* = nerve ending

which it ramified, whereas the other proceeds and forms a terminal arborization around the plasma of another nerve cell.

Seeing these lustrous terminal formations of the nerve fibers around the nerve cells, one cannot help speculating as to the function of these arborizations and where the fibers forming them might originate from? In the case of the cardiac ganglia we presume that the pericellular synapses are either the terminal apparatuses of the vagus fibers of central origin, or of the processes of unipolar cells situated somewhere in the preceding portion of the nerve stems. The first assumption, although most logical and in accordance with our knowledge of the cardiac innervation, is inconsistent with our observation that the pericellular plexuses remain intact even for 28 to 32 days after the transection of the vagi. Thus the possibility remains that the pericellular baskets connect different nerve cells of the nerve trunks. This might account for the fact that degeneration fails to come about after vagotomy.

Chabarowa, relying on experiments performed on cats, believes that the intracardiac pericellular baskets are afferent synapses belonging to inform higher centers of the central nervous system about the processes occurring in the cardiac wall. Chabarowa removed the first thoracic spinal ganglion of cats and found that the pericellular plexuses around the nerve cells of the cardiac ganglia underwent degeneration. Since we have performed no exstirpation of spinal ganglia hitherto, we cannot commit ourselves on such an interpretation of the question.

The cells belonging to the other group of nerve cells are of the sympathetic type. They are mostly multipolar, and in a smaller part unipolar,

Fig. 4. — *Emys orbicularis*, heart. Nerve cell from the right atrial epicardium. *a* = myocardium, *b* = epicardium, *c* = nerve cell, *d* = the nucleus of the nerve cell, *e* = nerve stem, *f* = preganglionic fiber, *g* = pericellular plexus, *h* = nerve ending. Bielschowsky-Ábrahám stain, ×1600

but bipolar forms are not unusual either. Pericellular baskets are never seen around these cells. Their synapses localized on the cell body, however few in number, are essentially minute rings. That the end rings, whose connexion with the nerve fibers could be observed but rarely, are synapses indeed, has been verified most convincingly by a few propitious findings. In some cases it is distinctly visible that the nerve fiber, passing along the surface of the cell, gives off a side branch in about the central region of the cell body. This side branch finally terminates on the plasma of the cell (Fig. 7) as a slightly elongated end disc. This is, of course, not the only form of termination. There are also cases in which semivesicular formations of different size may be seen on the cellular surface. A wall of well-defined protoplasm separates these formations from the plasma of the cell. In these semispherical depressions occasionally nerve-fiber terminations are to be found in the form of minute, dense globules, whose connexions with the preganglionic fiber may be established as well.

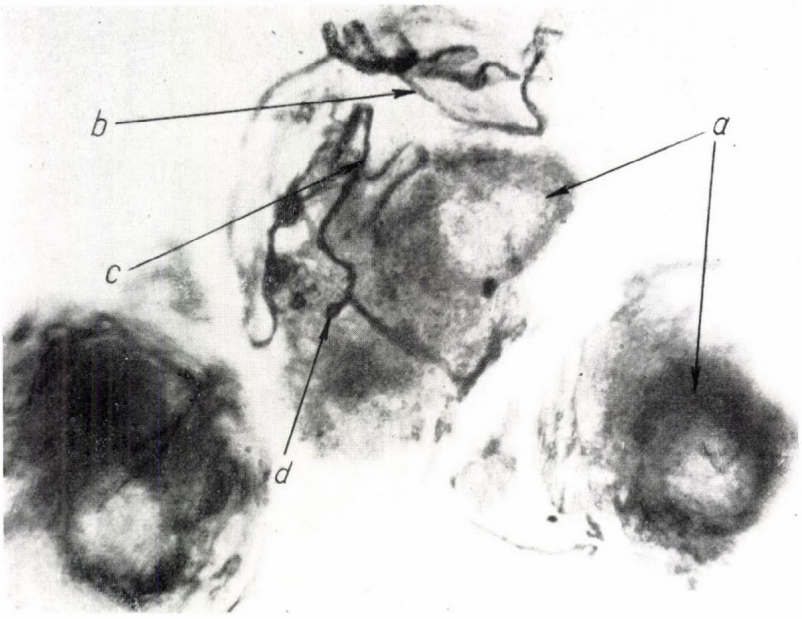

Fig. 5.—*Emys orbicularis*, nerve cells in the right atrial epicardium. *a* = nerve cell, *b* = preganglionic fiber, *c* = pericellular plexus, *d* = nerve ending

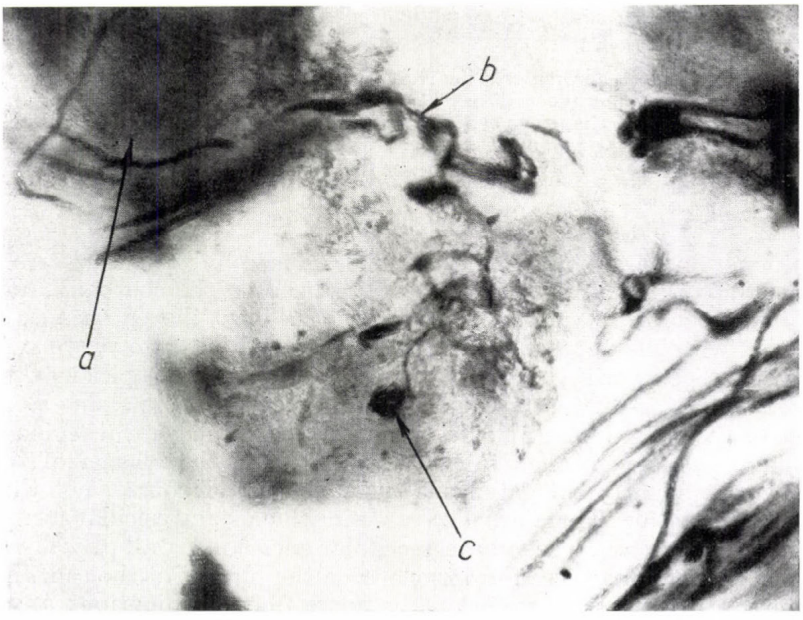

Fig. 6.—*Emys orbicularis*, nerve cell in the right atrial epicardium. *a* = nerve cell, *b* = preganglionic fiber, *c* = nerve ending

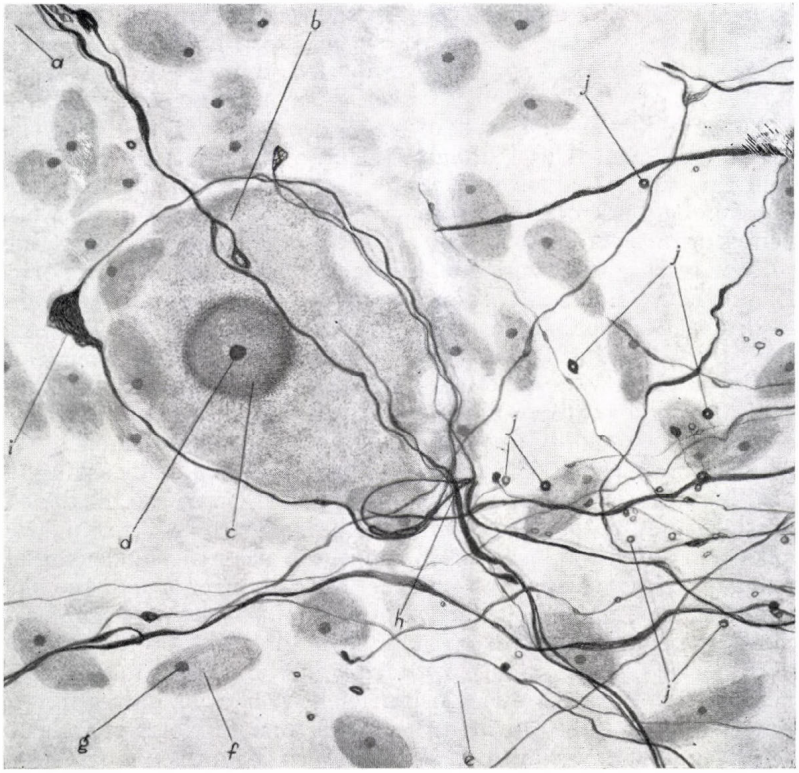

Fig. 7.—*Emys oribcularis*, the ventricular wall of the heart. Nerve cell in the epicardium, end rings of the nerves in the myocardium. a = epicardium, b = nerve cell, c = the nucleus of the nerve cell, d = the nucleolus of the nerve cell, h = nerve plexus, i = neurofibril, j = terminal ring. Bielschowsky–Ábrahám stain, × 1600

In spite of the numerous investigations, the structure of the nerve endings occurring in the region of the cardiac muscle fibers is still far from being known. This appears also from the fact that opinions have never so far agreed concerning the mode of connexion between nerve elements and muscle cells. Some authors supported the assumption of definite terminations of the nerve fibers,while others—denying any form of individual terminations—surmised the contact between nerve elements and cardiac muscle cells to be effected by some terminal neurofibrillar network, or terminal reticulum. If we want to get a better understanding of the state of the problem, we also have to review the earlier literature.

In the course of this examinations with methylene-blue, Smirnow (1900) found that single fibers originating from bulky nerve plexuses turn into fine fibrils that ramify and terminate on the surface of the muscle fiber, without entering into the protoplasm itself. Later several authors extended their examinations on all classes of vertebrates. These investigations revealed a dense nerve plexus in the myocardium (Gerlach 1876), without showing

any trace of free nerve endings. Most of the researchers were of the opinion that the apparently spherical nerve endings seen at times in the preparations were only artefacts, or due to imperfect impregnation. Dogiel (1907) found a plexus of rather fine fibers around the cardiac muscle fibers of the turtle, he had, however, but one preparation, in which fine neural end knobs could be seen. Also Hofmann (1902) and Fukutake (1925) speak of a simple fine nerve plexus and pretend that the formations deemed by some authors to be 'end knobs of nerves' are not really anything else than varicosities or fragments of cut nerve fibers. Against these authors, however, many other researchers supported Smirnow's statement and reported that the efferent nerve fibers supplying the myocardium have free terminations in the form of terminal knobs. Free myocardiac nerve endings have been described by Michailow (1908), and the presence of such formations was verified by Ramon y Cajal (1904). Boeke (1927, 1932) found free nerve endings in the myocardium of the turtle, of some mammals, and especially of the sheep. The examinations of Boeke have been confirmed by Wollard (1926), Tudor Jones (1927) and Lawrentjew (1929). According to this latter author, the fine fibrils issuing—in the cat—from the plexus of nerve fibers penetrate into the cardiac muscle fibers, where they terminate in the protoplasm in small loops situated sometimes near the nucleus. Still later Boeke examined the cardiac musculature of the hedgehog and the blackbird and found that some side branches of the nerve plexus terminate intraplasmatically near the nucleus of the muscle fiber. Beside the free terminal knob-like nerve endings, rarely appearing in convincing form, the section always showed "ein zartes, aus feinsten, leicht varikösen, immer miteinander anastomosierenden Neurofibrillenzügen aufgebautes Netzwerk, das sowohl mit den Blutgefäßen, wie mit den Muskelfasern zusammenhängt." A similar fine, anastomosing plexus of neurofibrils has been described by Fukutake (1925) in the endocardium of dogs, further in the heart of birds and here especially in the vascular wall. Boeke fully supports the statements of Fukutake and emphasizes that a 'ground plexus' may be observed in the myocardium, consisting of fine anastomosing neurofibrils and that this ground-plexus is in continuity with the neurofibrillar plexus situated in the adventitia of the vessels. In his opinion a plasmodium containing nuclei and loose bundles of anastomosing neurofibrils has to be considered as the histological substratum of impulse transmission in the myocardium.

According to Fattorusso (1943), some of the nerve fibers form a plexus in the myocardium, while others, to which the thicker fibers belong, have free endings. Tcheng (1950) confirms the assumption of free endings.

Thus the myoneural junction in the myocardium is a problem which has not been elucidated hitherto in spite of all the relevant valuable publications. Meyling (1948, 1953), Akkeringa (1949), Jabonero (1952, 1954), Field (1951), Mitchel (1953) are generally of the opinion that the fine neurofibril-like nerve fibers resulting from the terminal ramifications of the efferent fibers are anastomosing with the processes of the so-called interstitial cells, and that the interstitial cells form the transmitting links between the nervous impulses of central origin and the cardiac muscle. This viewpoint has been emphatically maintained by Meyling (1953), who, as can be read in Mitchel's book "asserts that the so-called free, loop and bulb endings are artefacts produced by incomplete staining; and he believes the networks

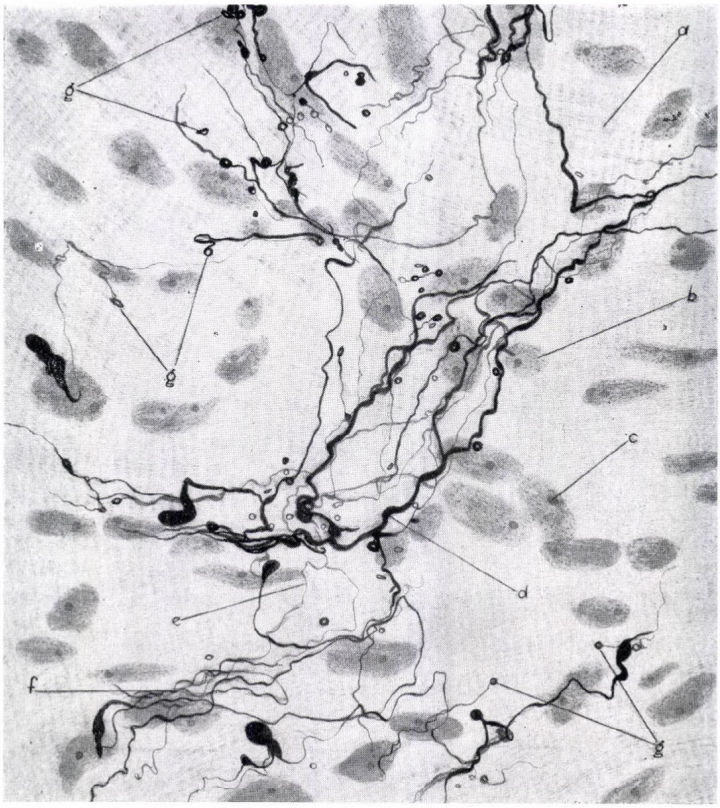

Fig. 8.—*Emys orbicularis*, heart. Nerve-fiber plexus and nerve endings in the ventricular myocardium. *a* = striated muscle cell, *b* = nucleus of muscle cell, *c* = nucleolus, d = thick nerve fiber, *e* = thin nerve, fiber, *f* = nervefiber plexus, *g* = terminal ring of the nerve. Bielschowsky–Ábrahám stain, ×1200

formed by the interstitial cells intervene between the efferent fibers and the effector structures."

According to Stöhr (1957), the myoneural junction in the cardiac muscle is effected by the so-called 'terminalreticulum': ". . . das sich mit Muskelfasern und Blutkapillaren in gleicher Weise plasmatisch verbindet. Elemente aus Vagus und Sympathicus müssen in diesem Netz enthalten sein und die Tätigkeit der Herzmuskulatur in harmonischer Zusammenarbeit regeln."

Although having not succeeded in convincingly staining the nerve endings, Ábrahám (1937, 1938, 1939) from the very beginning held the view—based on analogy—that the nerve fibers supplying the myocardium might form delicate preterminal plexuses, from which single fibers are branching off and terminate with free endings on the muscle fibers, or within them. In the course of further examinations (1959, 1961, 1962) of the heart of submammal vertebrates, he succeeded in getting numerous preparations proving his views in the myocardium of fishes, amphibians, reptiles and birds.

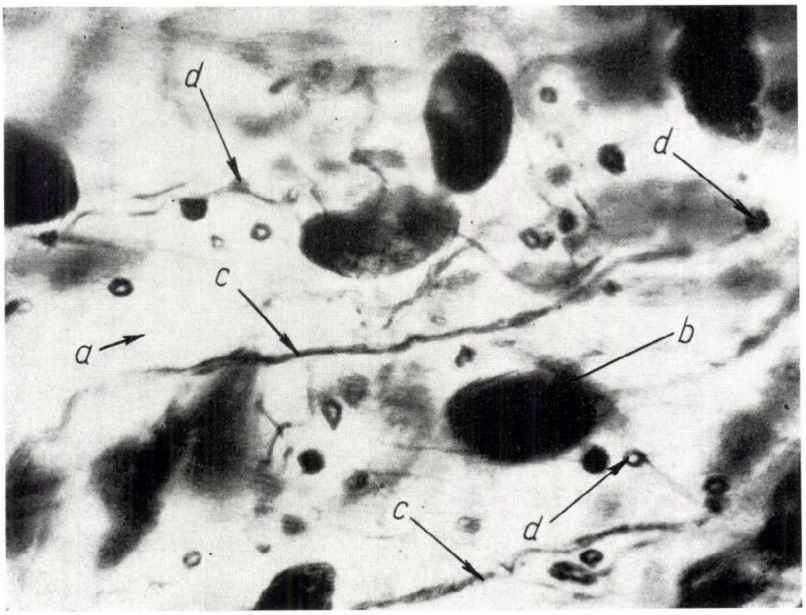

Fig. 9.—*Emys orbicularis*, nerve endings in the ventricular myocardium. *a* = striated muscular tissue, *b* = nucleus of the striated muscle, *c* = nerve fiber, *d* = nerve ending

Concerning the efferent innervation of the myocardium, four different interpretations prevail in the relevant literature. According to that represented by Smirnow, Nonidez, Lawrentjew and Ábrahám, the efferent fibers terminate freely on the muscle fibers often in sublemmal end knobs, occasionally quite close to the nucleus.

According to Boeke, as the most important representative of the second view, a lesser number of the efferent fibers terminate in end knobs in the protoplasm of the muscle fibers in a way that the protoplasm of the muscle fiber forms a distinct net work around the end bulb. The fibrous elements of this plexus are said to be in uninterrupted connexion with the axonal neurofibrils. According to this assumption, the neurofibrils, the constituents of the nerve fibers and the axoplasm are in continuity with the protoplasm of the muscle fiber. This means that the elements of the nerve fiber are continuous with the sarcoplasmatical thread-like formations surrounding the end knobs. According to Boeke such forms of endings may be found relatively rarely only in particularly well-impregnated preparations. The large majority of the nerve fibers become thinner by tapering and branching into fine fibrils and are embedded into a plasmodium of Schwann cells. These nerve fibers anastomose at spots and form a reticulum with minute meshes, covering the muscle fibers and the capillaries between them like a veil. Boeke believes the free nerve terminations are vagal endings, and the reticulum is the end system of the sympathetic nerve fibers.

The third view-point is linked up with the names of Stöhr (1951) and Seto (1936) who deny any kind of free nerve ending and consider the terminal

reticulum of nerve fibers as the only and exclusive means by which the neuromuscular junction is effected. Fibers of vagal and sympathetic origin are thought to participate jointly in the reticulum.

The fourth view-point is that of Meyling, who insists upon a connexion system existing between the executive organs and the nervous system in any area of the vegetative nervous system. In his opinion this system consists of multipolar large cells—the interstitial cells mentioned by Gerlach and Cajal—anastomosing among each other. According to Meyling, in the myocardium there are no free nerve endings at all.

It is beyond doubt that the four different views are due to some extent to different technics, but they perhaps even more represent predilections of their proponents, and it would not be an easy task to select with certainty the correct explanation. Everybody who ever tries to work with these delicate terminal structures of the nervous system has to be aware of hazards of impregnation. Examining a number of good preparations from all classes of vertebrates, one is likely to obtain random results as may induce one to support, seemingly with good reason, one or the other opinion. It may even occur that a researcher in possession of an abundance of preparations deems all the four aspects correct and acceptable in part or as a whole, respectively. Although not without any reservation, I myself have always been of the opinion that one need not search for some particular form of connexion either in the smooth musculature or in the myocardium, because the nerve fibers sure arrive here from somewhere, and if they come separately and keep their independence at their end, they must also have terminations. I succeeded in proving this first in teleostei (1959), then in amphibians (1961), in reptiles (1961) and in birds (1962).

Most recently I was fortunate enough to encounter in the myocard of *Emys orbicularis* an excellent material in which the termination of all nerve fibers, by means of free terminal rings both within the walls of the atria and of the ventricles, could be demonstrated with excellent clarity. In the following I give a brief report on these findings.

The myocardium of the pond turtle shows a rather considerable abundance in nerve fibers. A similarly rich system of nerve fibers could only be found in the atrial wall of the heart of carps and in the muscular valves of birds. Thicker sections of the cardiac wall help to reveal the myocardiac plexus system with an almost confusing complexity. These plexuses, though dense and rich everywhere, are especially abundant in the vetricular rings of the atrium, in the transverse section of which the unmyelinated nerve fibers of different thickness appear in dense strands. In the plexuses, no matter how thick or thin the sections may be, the different terminal circles are seen in enormous masses and with unusual clearness. The connexions of these terminal circles with the nerve fibers may be verified convincingly in a considerable percentage of the rings (Figs 8 and 9). There are bigger, smaller, and quite little ones among the circles, substantiating that they belong to fibers of different thickness, and also evidencing that all efferent fibers of this area of the myocardium end in terminal circles. To obtain appropriate information about the place of the plasma of the muscle fibers, the most successful ones of our impregnated frozen sections were embedded and cut into section series of 5 to 10 microns. In such preparations the terminal rings and their connexions with the terminal fibers are to be seen

very clearly. It may readily be observed, too, that there are bigger and quite small ones among the circles. It could be established further that some of the rings are situated near to the surface of the cell, whereas others are often found near the nucleus. Pictures have fairly often been observed in which the muscle fibers appear to bear several terminal rings near the nucleus. No structure whatever was found around the circles to indicate that the adjacent protoplasm was of a radial arrangement or the rays were in continuity with the circumference of the rings, as described at that time by Boeke. According to our observations, the terminal rings are clearly separated from the protoplasm. From light microscopical pictures we concluded that the situation here might be similar to that revealed recently by electron microscopical examinations on the connexion between the vegetative fibers and the smooth muscle cells. (Fig. 9.)

Knowing the parts of the myocardium of the turtle that make material best suited for our purpose, it will not be too difficult to clarify by electron microscope investigation the actual relation between the ring-like nerve terminations and the structural details (mitochondria, vesicles, etc.) on both sides of the junction. Investigations along these lines are now in progress, and I hope to be soon able to report on the results. By the analysis of the light microscopical pictures of the highest magnification, we surmise that the terminal circles and the sarcoplasm are clearly separated from each other.

In the parietal system of the heart no other nerve terminations or forms of connexions were found. The preparations have been carefully studied, and in the course of our examinations special attention has been given to the reticular arborizations mentioned in the literature, as well as to the interstitial cells considered by some authors as connecting links between nerve fibers and muscle. We have, however, to stress most emphatically that no nervous formations whatever of these kinds could be observed. The preterminal fibers arborizing and finally terminating in rings keep their independence to their very endings, and show no signs of anastomoses. The cells described in the literature as interstitial cells (Gerlach, Cajal, Meyling) are, in our opinion, connective tissue cells and are by no means involved in the transmission of impulses. The free nerve terminations, in the present case the rings, are the only elements that have some role in the transmission of impulses. The bigger ones are, in our opinion, terminations of the fibers of vagal and the smaller ones of sympathetic origin. This inference had to be drawn from the fact that while finding no other impulse-transmitting apparatus in the cardiac wall of the turtle, the rings, especially in some areas, appear in almost unimaginable numbers. By this evidence one may surmise several nerve fibers terminating on one muscle fiber, and one might even venture to say that two nerve fibers of different origin terminate on the same nerve fiber, one belonging to the vagus, while the other to the sympathetic system. If this holds true, we have sufficient morphological explanation of the excitatory and inhibitory influences exercised directly on the heart muscle.

SUMMARY

(1) By different modifications of the Bielschowsky procedure, the synapses were examined in the heart of the pond turtle *(Emys orbicularis)*. As a result of these investigations we could establish that in the cardiac wall the nerve cells belong to two big groups. The cells in one group situated along the nerve stems running in the sinus venosus, in the right atrium and in the atrial septum are spheroid, large unipolar cells of the parasympathetic type, the processes of which enter the nerve stem in which they proceed further.

(2) The other group consists of multipolar, larger cells, which are to be found in the atrial epicardium, in the atrial and ventricular myocardium, constituting at some places ganglia of dense structure, while elsewhere they are situated isolatedly in loose plexuses of nerve fibers.

(3) The parasympathetic cells are surrounded by looser or denser, pericellular baskets, the free ends of which are terminating on the cellular surface.

(4) The terminations are circular or elongated shovel-shaped formations, which usually are halfway embedded in a well-defined vesicular depression of the cell surface.

(5) The fibers that participate in the pericellular baskets emerge from thick nerve stems and occasionally form spirals around the origin of the ganglion cell process.

(6) In our opinion the pericellular baskets or plexuses are interneuronal synapses.

(7) There are no pericellular baskets around the nerve cells of the sympathetic type. Their synapses consist of terminal bulbs of various size, some of which are embedded into a semi-vesicular depression of the cell plasma.

(8) The synapses of the muscle fibers are terminal rings seen everywhere in the cardiac wall and are to be found in enormous numbers on the atrioventricular boundary.

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FINE STRUCTURE OF SYNAPSES AND THEIR DISTRIBUTION ON THE VEGETATIVE NEURON

by

V. P. Babmindra

LABORATORY OF MORPHOLOGY, PAVLOV INSTITUTE OF PHYSIOLOGY OF THE SOVIET ACADEMY OF SCIENCES
LENINGRAD, USSR

Knowledge of fine structure and distribution of synapses on the neuron is highly important for the elucidation of their functions.

In the present paper the structure of the pericellular apparatus in the sympathetic trunk of man, cats and rabbits is described. The material was impregnated according to Bielschowsky—Gros, or with Deineka's modification of the Golgi method, or stained with methylene blue.

Extensive information has been obtained concerning the presence of neuroplasm ('Kontaktplasma' or perifibrillar substance) in the synaptic end structures. This fact was first established by means of impregnation techniques (Dogiel 1908; Kolossow 1954; Babmindra 1957; Kirsche 1958) and later confirmed by histochemical and electron microscope studies (Szentágothai 1957; Couteaux 1958; Boycott, Gray and Guillery 1961). Yet papers still appear which fully neglect these findings.

In the present work two components can be clearly distinguished in the synaptic endings: (1) neurofibrillar framework and (2) neuroplasm of the ending. The neurofibrillar framework consists of a dense mass of neurofibrils which are in continuity with the fibrils of the presynaptic fibre. The neurofibrillar component has ring, bulb, or irregular shape with a complex network of fibrils. The fibrils are finer and thinner in this region, as compared to the presynaptic fibre.

The neuroplasm of the ending appears as a clear area around the neurofibrillar framework. Its amount in the synaptic ending varies from an almost imperceptible zone to a mass 2 or 3 times larger than the neurofibrillar component of the pericellular apparatus.

Fig. 1 shows a synaptic ending on a neuron from the upper cervical ganglion of man. The neuroplasm of the ending is clearly seen around the neurofibrillar framework which is homogenously stained and appears as a black mass. The neuroplasm usually follows the contours of the fibrillar component, but in certain cases it may be larger on one side.

The perifibrillar substance is denser than the body of the neuron. This is revealed in cases when deep impressions are seen on the cell body without any distortion of the perifibrillar substance. Fig. 2 shows a similar situation. The end bulb with a clear zone of neuroplasm penetrates deeply into the cell body. We succeeded in observing a very thin membrane, most probably the continuation of the neurilemma, which separates the neuroplasm of the synaptic ending from adjacent parts. The neuroplasm of the ending contains scarce fine radial fibrils running from the network to the membrane (Fig. 3). The neurofibrillar framework of the synaptic ending may

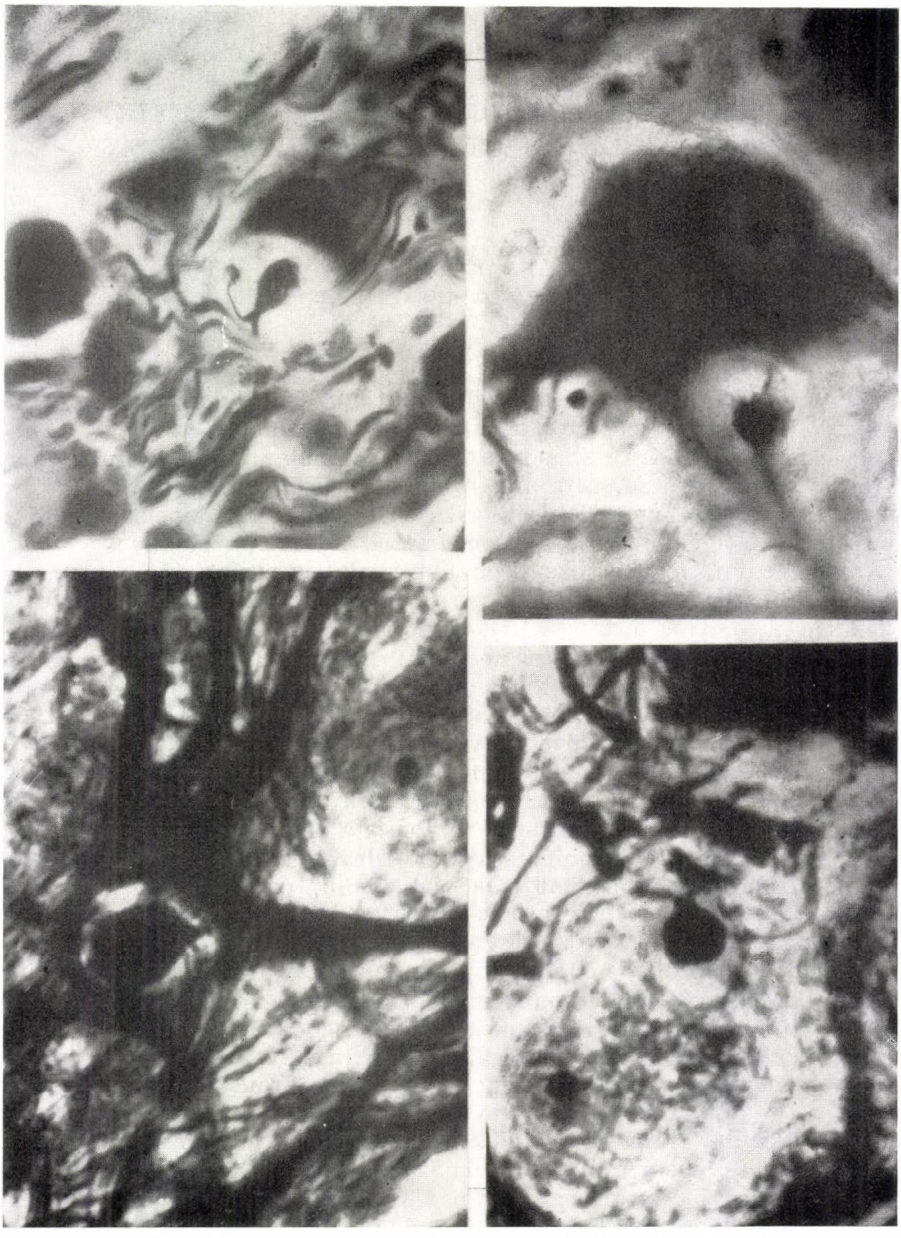

Fig. 1.—Axosomatic synaptic ending with clear zone of 'Kontaktplasma'. Gangl. cervic. super. of man, age 47. Bielschowsky—Gros method, imm. obj. 60, oc. 7

Fig. 2.—Axosomatic synaptic end bulb with a clear zone of neuroplasm is deeply impressed into cell body. Gangl. cervic. super. of man, age 42. Bielschowsky—Gros method. imm. obj. 60, oc. 10

Fig. 3.—Synaptic ending with thin membrane which separates the neuroplasm of the end bulb from the adjacent parts. Gangl. stellatum of woman, age 34. Bielschowsky–Gros method, imm. obj., 100, oc. 5

Fig. 4.—Complex structure of the synaptic ending. Gangl. stell. of cat. Golgi–Deineka method, imm. obj. 60, oc. 12.5

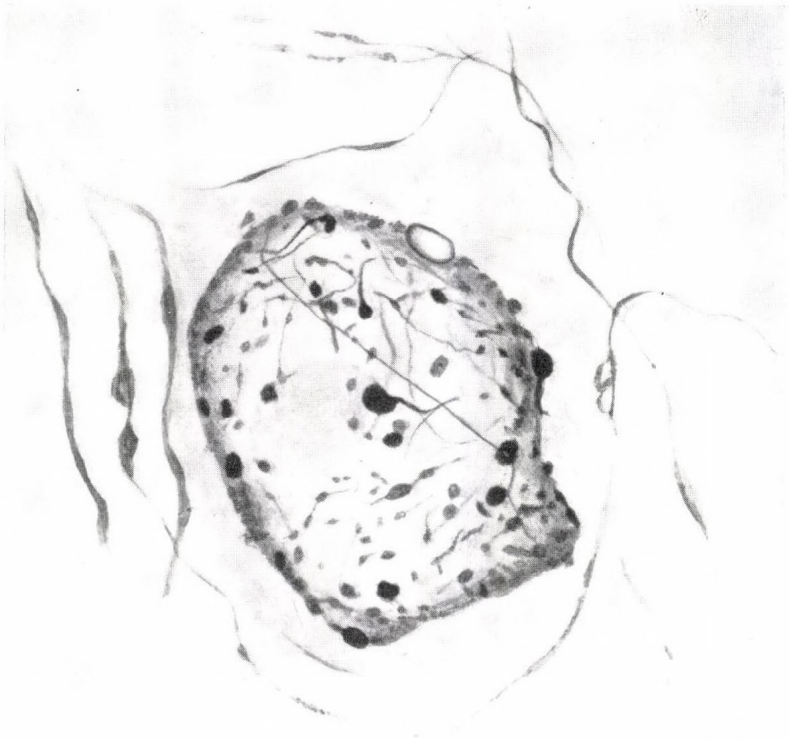

Fig. 5.—Pericellular apparatus on the cell body of the neuron. Gangl. stell. of dog. Methylen-blue method, imm. obj. 90, oc. 15

reveal a complex structure. In Fig. 4 we can see a very fine fibril which splits off from the main mass of neurofibrillar framework and ends at a distance of 4 microns from it. Both are surrounded by a large mass of perifibrillar substance.

The second contact may occur at a still greater distance than the first. In the contact region the perifibrillar substance is clearly seen. Differences are further observed in the degree of contact with the cell body. In certain cases the endings may establish only a slight contact with it, but sometimes they markedly penetrate the cell body.

Methylene-blue reveals the pericellular apparatus in a more complete form, which, however, highly differs from the pictures observed in impregnated preparations. Fig. 5 shows a large amount of vesicles and buds on the surface of a lightly stained neuron. Many buds are connected by very fine fibrils. When discussing the role of synapses in the transmission of nervous impulses we cannot dispense with the knowledge of their distribution over the body and dendrites of the neuron. Synapses are observed both on the perikaria (axosomatic synapses) and on the dendrites (axodendrite synapses).

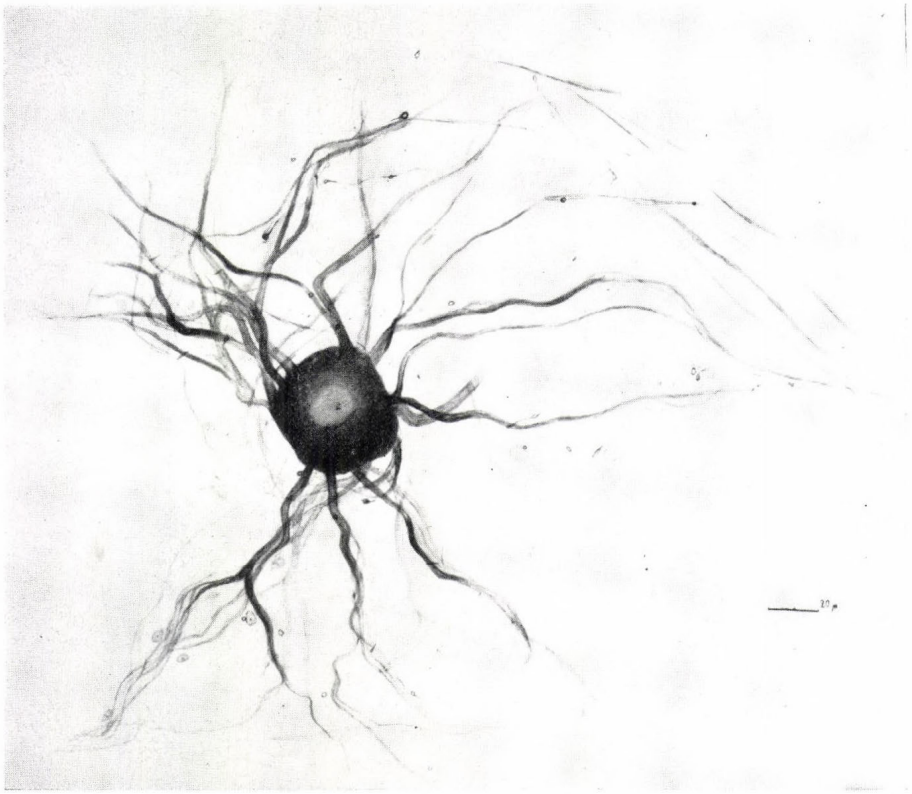

Fig. 6.—Axodendritic synapses. Gangl. stell. of cat. Golgi—Deineka method, imm. obj. 60, oc. 12.5

We have been particularly interested in the synapses to be found on the dendrites. But no exact measurements are available in the literature as to the distance of the dendritic synapses from the cell body. The authors who so far published drawings and micrographs suggest a maximal distance of 30 to 70 microns. We, however, have come across synapses on the dendrites at a distance of 225 microns from the cell body. These synapses showed no difference in size or form from the axosomatic synapses (Fig. 6). They sometimes have twice as large diameter as that of the dendrite. The vast majority of the synapses are located on the dendrites of sympathetic neuron (not on the cell body).

In accordance with other authors we also experienced synaptic endings in the dendritic glomeruli (Fig. 7). The role of the dendritic glomeruli has not yet been clarified, so the distribution of synapses is highly interesting. The contact between dendrites is especially close in these structures. These close contacts are perhaps responsible for the transmission from one dendrite to another.

This assumption has been supported by recent publications of Whittaker and Gray (1962) and Walberg (1963) reporting on dendrodendritic contacts studied with the electron microscope.

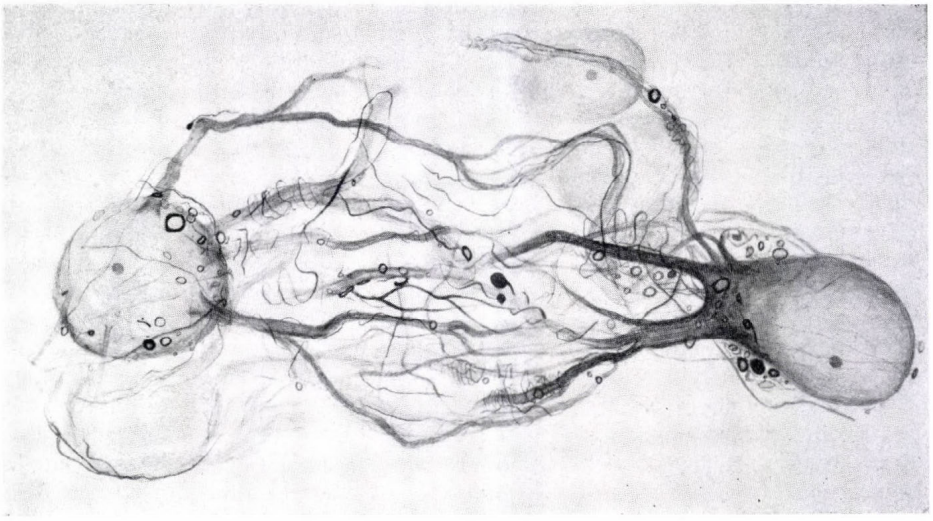

Fig. 7.—Axosomatic and axodendritic synapses. Dendritic glomerule in gangl. cerv. super. of man, age 44. Bielschowsky—Gros method, imm. obj. 60, oc. 7

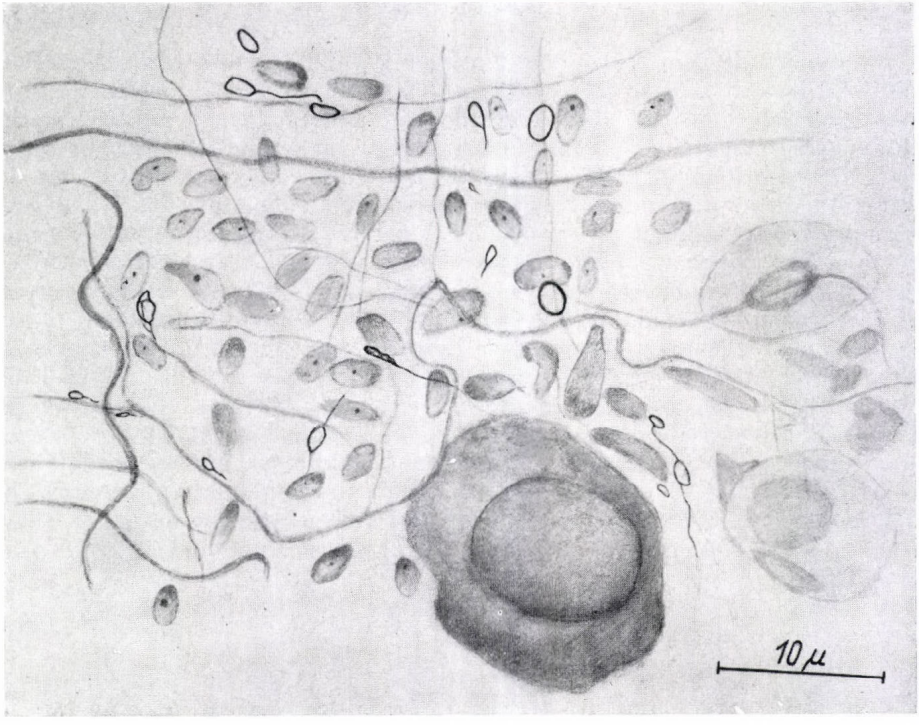

Fig. 8.—Synaptic endings between glial cells. Gangl. stell. of cat. Bielschowsky—Gros method, imm. obj. 100, oc. 7

'Free' synapses were constantly found at great distances from the cell body. These synapses do not establish any contact with the neurons, but often adhere to glial elements (Fig. 8). The adherence may be so marked as to cause deformation to the glial nuclei. The following circumstance observed in our laboratory should, however, be born in mind (Kolossow 1954; Kolossow and Milochin 1962). Sensory endings may be present in the immediate neighbourhood of the perikaria and the processes of neurons. These endings are similar to the endings of the preganglionic fibres and might be misidentified as synaptic structures when demonstrated as isolated structures without their myelinated fibres. No reliable criteria are available for their differentiation. On the basis of our observations we might state that synaptic endings have thinner preterminal fibres and denser endings with distinct and smooth margins. Finally, we wish to stress the importance of a detailed study of the distribution of synapses over the perikaria and dendrites, and the necessity of comparing these results with similar studies on the intercalary neurons of the sensory system (e.g. the nuclei of Goll, Burdach, Clark), as well as on the neurons of the motor system (motoneurons of the anterior horn) and the neurons of the reticular formation. This approach may contribute to the elucidation of the role of the dendrites and their synapses, and the work of the whole neuron.

DISCUSSION

Kadanoff: Zu dem Vortrag des Kollegen Babmindra möchte ich bemerken, daß die in den Imprägnationspräparaten gezeigte hell gefärbte Substanz (Axoplasma?) um die aus Neurofibrillen bestehenden synaptischen Endigungen mir zu groß erscheint. Es ist möglich, daß dies einer Schrumpfung der Neurofibrillennetze der Endigungen zuzuschreiben ist. Auch um die Endigungen der afferenten Neurone in verschiedenen Geweben — Epithel, Bindegewebe, Muskulatur — habe ich hell gefärbte Höfe (1928—30) beobachtet und beschrieben. Sie sind aber viel schmaler und bestehen höchstwahrscheinlich aus einer enzymatischen Substanz (1962—63 nachgewiesen).

Szentágothai : I could completely agree with your interpretation of the clear zone often seen around the impregnated (neurofibrillar) part of the ending. This is probably the region where the synaptic vesicles are concentrated. According to electron microscope pictures, the amount of filamentous material and of synaptic vesicles is subject to extreme variations, some endings being very rich in filaments and poor in vesicles and the reverse. The non-silver-staining part of one ending might, therefore, be a large part or conversely an insignificant fraction of the ending.

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HISTOLOGICAL AND HISTOCHEMICAL EXAMINATIONS ON THE CILIARY GANGLION OF MAMMALS

by

A. Stammer

DEPARTMENT OF GENERAL ZOOLOGY AND BIOLOGY, JÓZSEF ATTILA UNIVERSITY
SZEGED, HUNGARY

Among the vegetative ganglia the ciliary ganglion shows the most structural varieties. Lenhossék (1910, 1912) was the first to call attention to the specifities of the ciliary ganglion of birds and reptiles. Szakáll (1902) reported on the anatomical differences of the ciliary ganglia in mammals. The first data on their microscopical structure were found in the works of Pines (1928). Ernyei (1934), Rossi (1936) Seto (1931) and Warwick (1954) contributed a great deal to the knowledge of the ganglionic structure. In spite of all these examinations and results we still cannot say that the structure, central and peripheral connexions of the ciliary ganglion are well known. The cognition is rendered more difficult by the fact that the ganglia show rather essential differences not only according to the classes, but also within the same group of vertebrates. That is what we had to establish on the basis of comparative anatomical and histological examinations carried out in different species of birds (1954). In the course of the histological and cholinesterase-activity examinations in mammals, we recently succeeded in demonstrating the above said even more definitely.*

MATERIAL AND METHODS

In selecting our material we endeavoured to represent every order of mammals by one species. The ciliary ganglia of the following mammals were examined: urchin *(Erinaceus europeus)*, common flittermouse *(Myotis myotis)*, cat *(Felis domestica)*, dog *(Canis familiaris)*, fox *(Vulpes vulpes)*, brown hare *(Lepus europeus)*, guinea pig *(Cavia cobaya)*, pig *(Sus scrofa domestica)*, roe *(Capreolus capreolus)***, deer *(Cervus cervus)***, cattle *(Bos taurus)* and of the horse *(Equus caballus)*.

After fixation in 10 % formalin, freezing microtome sections were prepared. The material was impregnated according to Bielschowsky's method modified by Schultze—Gros, Ábrahám and Jabonero. The thickness of the section varied from 10 to 20 microns.

Using Gerebtzoff's modification of the Koelle—Friedenwald method, the specifical cholinesterase localization was examined histochemically in the ciliary ganglia of the dog and the cat. Incubation time varied from 2 to 5 hours, thickness of the frozen sections was 5 to 15 microns.

* Comparative examination of the ciliary ganglion is a common work with Professor A. Ábrahám

** I was helped by the Management of the Zoological Garden in getting these two species otherwise difficult to obtain.

DIFFERENCES IN THE MACROSCOPICAL STRUCTURE

The most essential macromorphological differences in the ciliary ganglia of mammals are due to the nerve branches entering into, i. e. emerging from the ganglion. The form of the ganglia and their internal structure depend on these nerves. In the course of examinations we could establish that the form and quantity of the few entering and rather numerous emerging nerves varied even within one and the same species of animals. On the comparative basis, however, only two essential anatomical differences were found. The ciliary ganglion of some of the mentioned species (of *Erinaceus, Myotis, Canis, Vulpes, Cavis, Lepus)* is—like that of reptiles and birds—closely connected to the root of the oculomotorius. These ganglia have an elongated form and are much broader at the entrance of the myelin-sheathed nerve branch (radix brevis seu motoria), emerging from the oculomotor nerve and conveying preganglionic fibers, than at the emergence of the postganglionary fibers (Fig. 1a). In the above species the postganglionic nerve stem emerges as a unified stem from the ganglion, then ramifies into numerous ciliary branches (nervi ciliares breves), which join the sensory fiber branches (nervi ciliares longi) of trigeminal origin before their entrance into the bulb. Even with the best binocular microscope it is rather difficult to trace the connexions of the two kinds of nerves.

It should be emphasized, however, that not a single thick fiber can be seen in the postganglionic stem emerging from the ganglia of these species, whereas in the nerve stems entering into the eyeball, resp. in those running in the sclera, thick fibers will always appear. No sympathetic roots can be spoken of in any of the above species, though the entrance of vegetative fibers into the ganglia by the vascular way is by no means precluded.

In the rest of the species *(Felis, Sus, Capreolus, Cervus, Ovis, Bos, Equus)* the ganglia are multiangular, star-shaped (Fig. 1b), owing to the considerable number of the entering and emerging nerve stems. The quality of the nerve fibers only may reveal, on the microscopical picture, whether the discernible stems are entering or emerging ones. In the ganglia of this multiangular type the three roots may be more easily surmised. Their differentiation, however, meets many difficulties even by microscopical examinations, namely the well-preparable entering branches (oculomotor and trigeminus) are equally built up of thin and thick nerve fibers, and there is no morphological proof for the differentiation of the numerous postganglionic stems from the surmised sympathetic root. On the other hand, it is only in man that the authors acknowledge the connexion between the sympathetic root and the ganglion is definitely proved.

The dimension of the ciliary ganglia depends on the size of the body and the development of the vegetative nervous system. In animals the size of the ciliary ganglion is related in general to the size of the body. As a striking feature it has to be mentioned, however, that in the cat, whose vegetative nervous system is known to be well developed, the ciliary ganglion is of unusually large size, so there is hardly any difference as compared to that in *Bos* or in *Equus*.

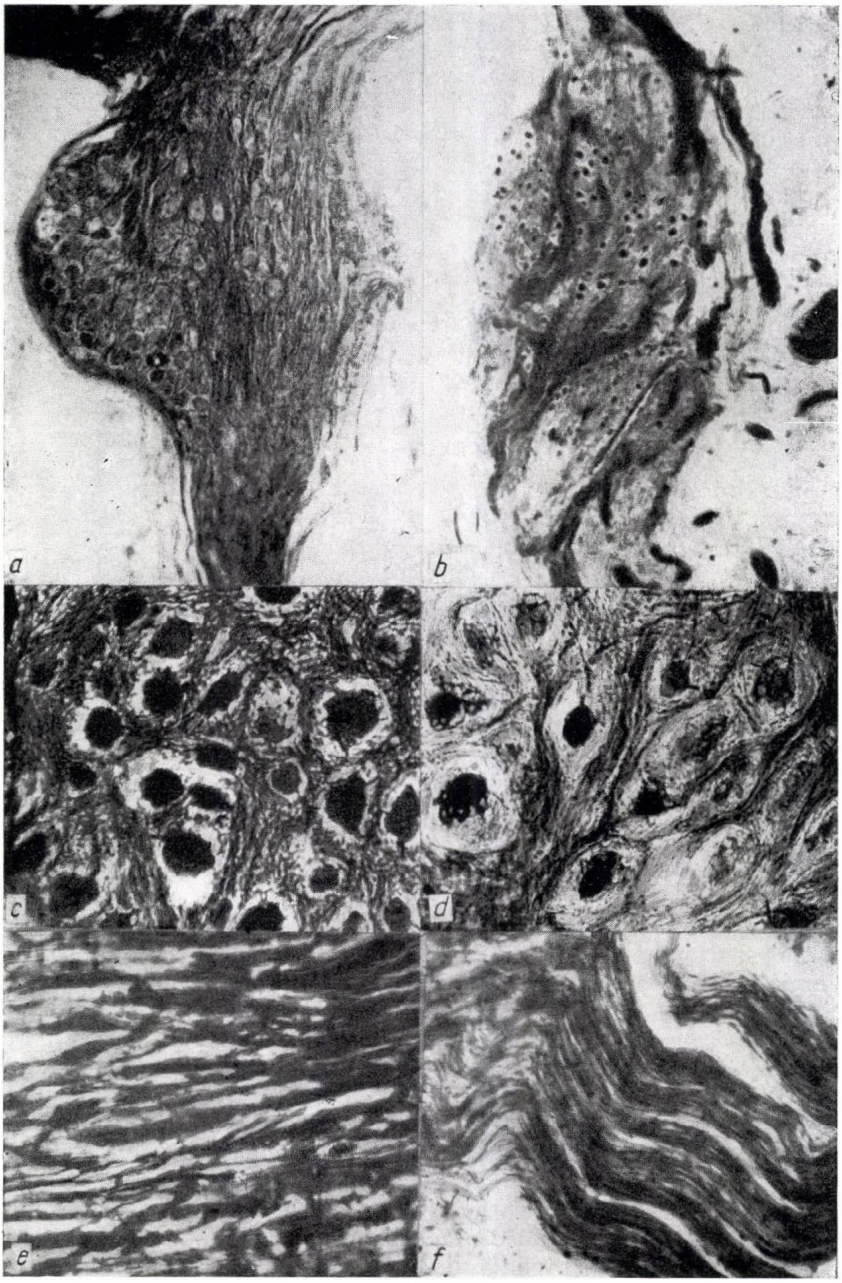

Fig. 1.—*a* = *Canis familiaris*, ciliary ganglion, *b* = *Ovis aries*, ciliary ganglion, *c* = *Vulpes vulpes*, ciliary ganglion, *d* = *Bos taurus*, ciliary ganglion, *e* = *Canis familiaris*, ciliary ganglion. Preganglionic fibers of the oculomotorius, *f* = *Ovis aries*, ciliary ganglion, postganglionic fibers

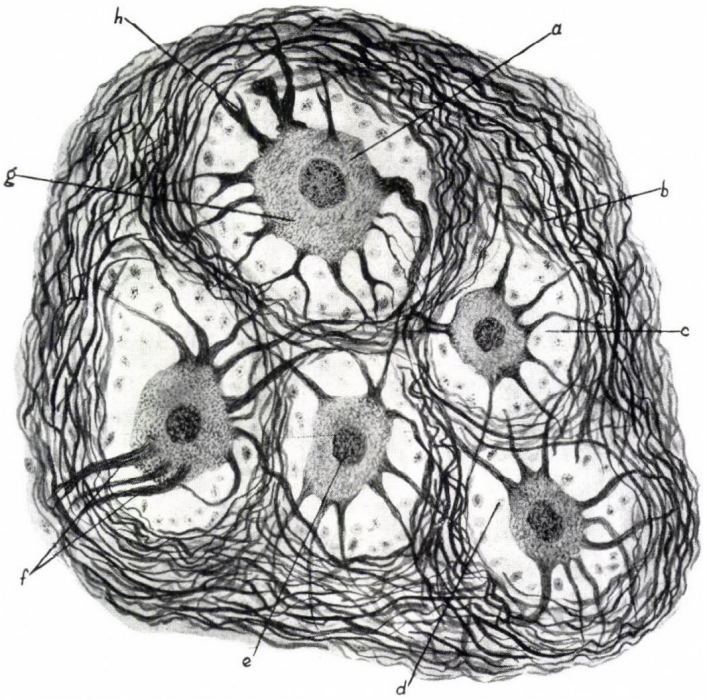

Fig. 2. — *Felis domestica*, ciliary ganglion. Multipolar cells with long processes. *a* = nerve cell, *b* = fiber system, *c* = intercellular space, *d* = pericyte (glia) nucleus, *e* = nucleus, *f* = processes, *g* = cell body, *h* = fork-like ramification. Bielschowsky–Ábrahám method, ×400

THE DIFFERENCES IN THE MICROSCOPICAL STRUCTURE

The microscopical structure of the ciliary ganglia in mammals show interesting features as to the size and type of cells, and the quality of the ganglionic nerve-fiber plexus. The comparative investigation revealed that in the examined species of *Insectivora*, *Chiroptera*, *Carnivora* the size of the ganglionic cells shows an almost complete conformity with the ganglionic dimensions of the *Ungulata* species, which have much bigger ciliary ganglia. Owing to the almost identical size of the ganglion cells, also their number is almost the same. Where the ganglia are of a greater size, larger intercellular spaces may be observed. This can be well demonstrated by comparing the ciliary ganglia of the fox and the cattle (Fig. 1c,d) on microscopical pictures of identical magnification. It is, however, the age that makes the most striking dimensional differences among ganglionic cells, i. e. the cells of the embryonic ganglia are much smaller and less differentiated than they are in developed age. The dense structure of the embryonic ganglia, with the mass of cell bodies practically communicating with each other, substantiates that the number of the ciliary ganglion cells in animals does not change in the course of life. The *cell types* of the ciliary ganglia are multipolar, their shapes, however, are diverse. Among the shapes,

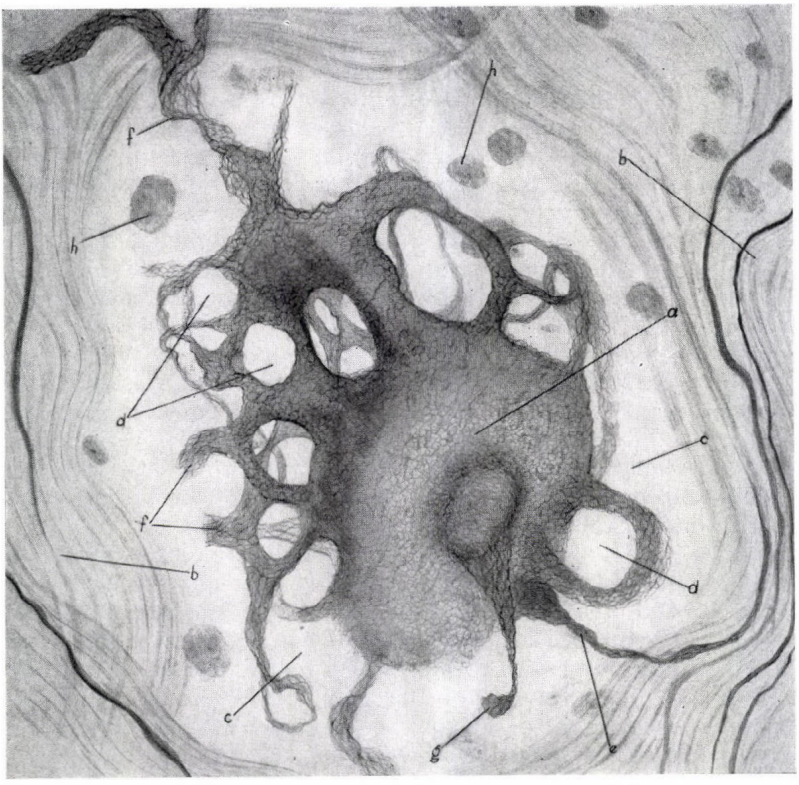

Fig. 3.—*Bos taurus*, ciliary ganglion. Fenestrated multipolar cell. *a* = nerve cell, *b* = fiber system, *c* = intercellular space, *d* = fenestrae, *e* = neurite, *f* = dendrite, *g* = denritic plate, *h* = pericyte (glia) nucleus. Bielschowsky–Ábrahám method, × 1,800

two main types may be distinguished. One is a stellate multipolar cell, provided with long processes, the other is a fenestrated multipolar cell.

The first cell type has many—about 10 to 24—processes, most of which enter into the neighbouring intracellular plexus, some show fork-like ramifications (Fig. 2). In this cell type differentiation between the long processes, especially of dendrites and neurites, like in most other vegetative ganglia, is almost impossible.

Pines (1928) was first to describe the other cell type with the fenestrated shape. We found various shapes of this type in the different species. The fenestrated processes arising from the bending back of the dendrites into the plasma may surround the whole surface of the cellular body (totally fenestrated), but most often appear only at one end of the cell. In this cell type—to be called semifenestrated—the neurite, as a process staining darker, emerges from the pole opposite to the fenestrated part. In most of the species these windows appear only as quite simple auricles, but in some species, e. g. in the cattle, fenestration is so considerable that the whole cellular body assumes a lace-like structure (Fig. 3). There are

many transition forms between these two main types. There are cells on which, apart from 10 to 16 long processes, 2 or 3 small fenestrae appear *(Felis)*, in other instances there are more fenestrae and, except for the neurites, only very few long dendritic branches to be observed *(Canis)*.

As to the occurence of the cell types, it can be most definitely established that either cells provided with long processes or the fenestrated ones prevail in the ganglia of the different species. The multipolar cells with long dendrites formed the ciliary ganglia in *Canis*, *Bos*, *Ovis*, *Capreolus* and *Cervus*. Most of the transition types may be found in the ganglia of *Vulpes* and *Sus*. According to the average appearance of the processes, the ganglionic cells of the pig belong to the main type with long processes, while those of the fox to the main fenestrated type.

The cells provided with dentritic slabs found in the ciliary ganglion of old dogs (Seto 1937) could be demonstrated in the ganglion of aged cattle as well. Such cells are only frequent in aged animals, and can never be found in the ganglia of young or developed animals, that is why they may be considered as pathological cell form. In our opinion the fusing of the dendritic endings into slabs seems to affect the cell function and to disturb conduction and transmission of impulses.

The *quality of the nerve plexus* of the ganglion closely depends on the origin of the nerve stems entering into and emerging from the ganglia, as well as on the development of the process of the nerve cells forming the ganglia. As the roots of the ciliary ganglia are not uniform in the different species of animals, we began to pay attention to the quality of the fibers involved in the nerve plexus of the ganglion. From the morphological viewpoint three types of fibers may be distinguished in this plexus.

The preganglionary fibers provided with myelin sheaths appearing in the root of the oculomotorius may be classified to the first type. After entering into the ganglia, these fibers gradually lose their sheaths. Among them thinner and some thicker ones taking an undulating course and staining quite dark may be found (Fig. 1e). In our opinion the latter do not emerge peripherically, but centrally in the nuclei of the cerebral nerve. Even where several nerve roots arrive to the ciliary ganglion, and— owing to anatomical connections—the trigeminal origin of one of these is to be surmised, no difference can be observed, as such a nerve root also consists of both thick and thin myelinated preganglionic fibers. In not a single case did we find a stem which contained thick fibers of completely identical size at its enterance, as could be expected properly from the trigeminal root. We wish to emphasize that we have not seen such stems either as might have passed straightly through the ganglion, without forming a filamentous system and cellular connexions.

The second type is represented by thin, seemingly bare fibers, resp. by such ones as are provided with neurilemmas taking a straight course. These fibers emerge from the ganglion and run in the postganglionary nerves (Fig. 1f). In these nerves the fibers show no difference in size. Thick fibers with myelin sheath were never observed in the postganglionic nerve.

To the third type we may classify uniformly thin fibers of undulating course, which always have strikingly long Schwann nuclei and always accompany the blood vessels (Fig. 4a). We believe these fibers are sympathetic fibers originating from the carotic plexus, which in our opinion

—maintaining all what has been said about their original independence— probably enter the ganglion by the way of the vessels, i.e. having only connexions to the vessels.

The types of ganglion cells and the number of the processes help to determine the ganglionic fiber system. Where the ganglion is built up of multipolar cells with long processes (e. g. *Felis)*, the fibrous network is much denser than in the cells of the fenestrated type (e. g. *Sus)*. Apart from this general statement, we wish, however, to emphasize that the appearance of the fibrous plexus is also dependent on the synapses occurring in the ganglion.

CHARACTER, FORM AND OCCURENCE OF THE SYNAPSES

Apart from the macroscopical and microscopical differences in the structure of the ciliary ganglion of mammals, the most remarkable varieties may be observed in the character and form of the nerve endings.

As regards the character of the endings, both the motor and sensory end type may occur in the ciliary ganglion. The form and rate of occurrence is rather variable in the species examined.

Motor synapses. The endings of the ciliary ganglion are mostly those of the preganglionic oculomotor fibers which in the different species appear in the form of pericellular baskets, end knobs, resp. end rings and end bulbs.

The most striking form of ending is the *pericellular basket* (Fig. 5). It develops in the way described in the ciliary ganglion of most birds (Ábrahám and Stammer 1954) from the rich filamentous system of the preganglionary fiber, on the surface of the ganglion cells. Giving off several side branches, the terminal fiber builds up a pericellular basket of densely interwoven filaments. In these side branches neurofibrillar slackenings, twists and endings in terminal knobs occur rather frequently (Fig. 5*d*, *e*). The various terminal knobs of the pericellular basket account for the fact that the preganglionic fiber establishes synaptic contact at many places with the ganglion cell body and its processes.

Pericellular baskets were most frequently observed in the ciliary ganglion of the dog, where this form of ending occurs practically on every cell. The pericellular basket either extends over the whole surface, even over the cellular processes and the fenestrated system, or is confined to the region where the preganglionic fiber approaches the cell. The pericellular form of ending was found in considerable number not only in the ciliary ganglia of the dog, but also in those of the fox and the sheep, and were much fewer with the rabbit, guinea pig and deer.

The small *end knobs* or *end rings*, which are the most general forms of endings (Ábrahám 1956), occur also on the cell of the ciliary ganglion, where their number, however, is not considerable. It is most probably due to their extremely small size that we succeeded but very rarely in observing these endings. It is a characteristic feature that several of them may appear on one cell, some in the form of a solid knob, others in that of a ring, most frequently around the ganglion cells of *Erinaceus*, *Cavia*, *Felis* (Fig. 4*b*) generally on the surface of the multipolar cells with

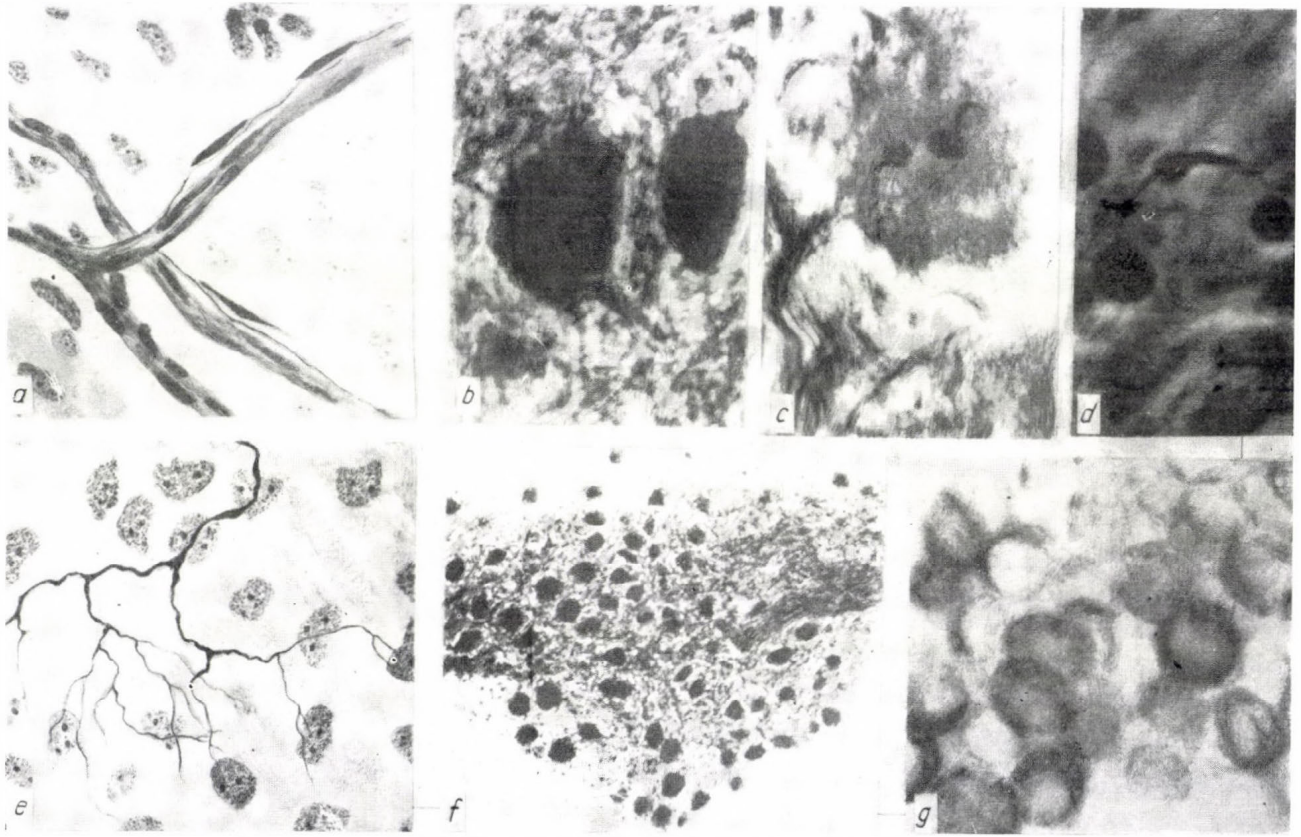

Fig. 4. – *a* = *Canis familiaris*, ciliary ganglion. Vessel and nerve in the connective tissue sheath of the ganglion, *b* = *Felis domestica*, ciliary ganglion. Endknobs on the cell body, *c*=*Bos taurus*, end bulbs on the ciliary ganglion, *d* = *Equus caballus*, endbulb in the connective tissue capsule of the ciliaryganglion, *e*=*Ovis aries*, ciliary ganglion. Tree-like ramification in the connective tissue capsule, *f* = *Felis domestica*, cholinesterase activity in the ciliary ganglion, *g*=*Canis familiaris*, cholinesterase activity in the ciliary ganglion

long processes. Similar end knobs, but of larger size, single or double rings were also observed in the intercellular spaces, in the connective tissue sheaths, resp. septa of the ganglia. Such endings were found in a remarkably large number in the connective tissue sheaths of *Canis*, *Ovis*, *Bos* (Fig. 6).*

Rather large-sized endings (40—50 μ), so-called *end bulbs* are especially characteristic for the ciliary ganglia of horses and cattle, but may be also found in species in which the pericellular baskets are the prevailing forms of endings *(Ovis, Canis, Felis)*. These endings may be found on the ganglion cells, or in the intercellular connective tissue, or occasionally in the connective tissue sheaths of the ganglion (Fig. 4*c,d*). Well marked by their dark staining, they may be considered as either preganglionic motor endings, or vasomotor endings, or pathological changes. The first assumption is supported by the fact that the preterminal fiber giving rise to these endings in some cases can be traced from the entering oculomotorius trunk. If connected with thick fibers, they account for the second surmise, whereas the fact is that such endings (Ábrahám 1953) having been demonstrated by several authors, and also very frequently observed by ourselves in older animals, might be considered in favour of the last-mentioned possibility.

It has to be mentioned, however, that these endings cannot be separated rigorously into these three groups, as we occasionally found sections in which the three forms of endings could be observed close to each other (Fig. 6), while in others not a single ending could be found.

Sensory endings. Sensory nerve terminals encountered occasionally in the ganglia of the vegetative nervous system—a much discussed problem—are worthy of examination also with respect to the ciliary ganglion. They are the more so, since this question has not been dealt with yet. According to our own observations, there are no sensory endings; apart from tree-like ramifications and glomerular endings of the ganglion capsule, no sensory apparatuses exist in the interior of the ganglion and between the cells. Our research on the sensory endings, published in a previous paper (Stammer 1956), dealt with the extended ending system (with end plates or end bulbs) to be found in the connective tissue capsule and its larger septa. Apart from these, simple tree-like ramifications—similar to the endings of the meninges—(Fig, 4*e*), or glomerular structures may be observed quite often. Such formations are, most probably, the endings of trigeminal fibers. Apart from the dark staining end bulbs of dubious origin we never found any formations inside the ganglia that could have been considered as sensory endings.

The appearance of free glomerular formations was also observed rather often. On thorough examination, however, these formations always proved to be either the remnants of pericellular baskets or fenestrated cells cut tangentially.

* The figures were drawn by Elizabeth Dános, designer in our Department.

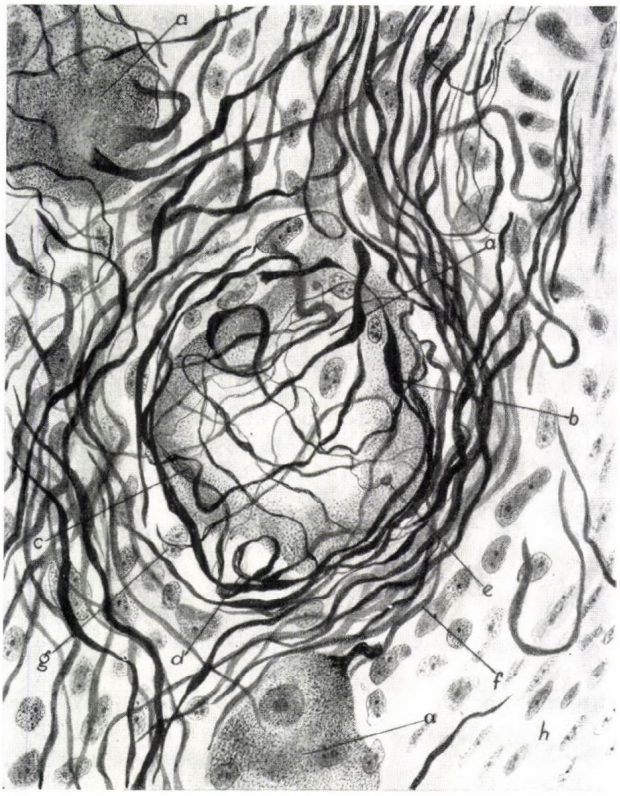

Fig. 5.—*Ovis aries*, ciliary ganglion. Pericellular basket on the surface of the ganglion cell. *a*=nerve cell, *b*=preganglionic fiber, *c*=neurofibrillar slackening, *d*=twisting, *e*=end knob, *f*=nerve fiber, *g*=pericyte (glia) nucleus, *h*=connective tissue septum. Bielschowsky—Ábrahám method, × 800

CHOLINESTERASE ACTIVITY IN THE GANGLION

The silver staining methods reveal the differences between cellular forms and synaptic structures of the ciliary ganglion in the various classes and even species of the vertebrates. The question now arises whether or not these differences have their counterparts in the chemoarchitecture of the ganglia. The most generally used histochemical procedure for the examination of vegetative ganglia is the Koelle–Friedenwald method modified by Gerebtzoff, which seems to be especially suitable for the study of this ganglion. There are but few ganglia in the organism in which the endings of the preganglionary fibers appear so unmistakably as they do in the ciliary ganglion. It was therefore not by chance that the question of the cholinesterase in vegetative ganglia was solved a few years ago by Szentágothai and co-workers (1955) just in the ciliary ganglion of birds. We are informed of others having also examined cholinesterase in certain

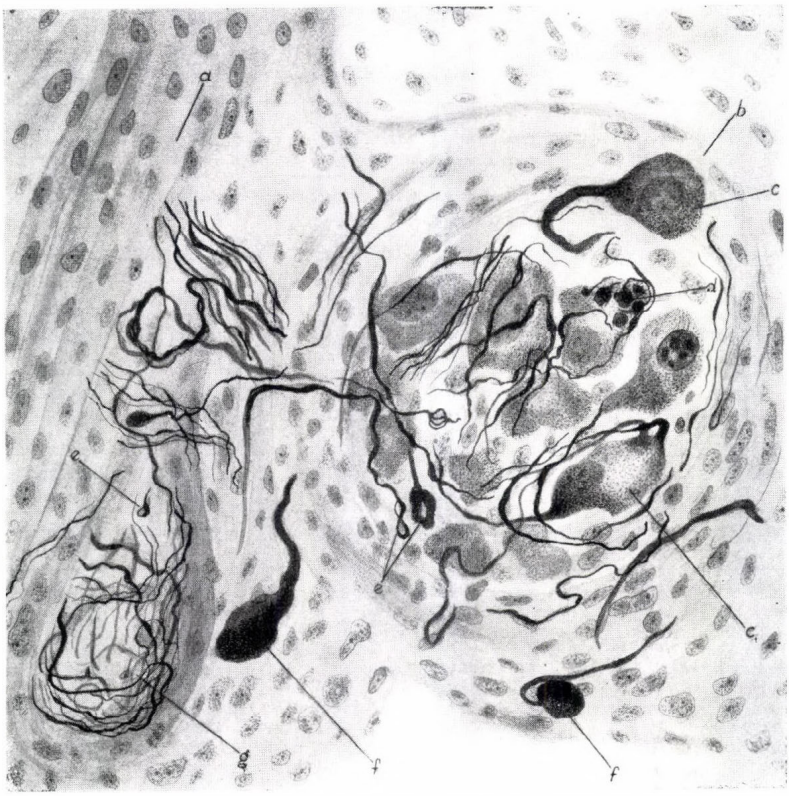

Fig. 6.—*Canis familiaris*, ciliary ganglion. Nerve endings in the ganglion cell group situated directly beneath the connective tissue sheath. *a*=connective tissue capsule, *b*=septum, *c* = nerve cell, *d* = end knob, *e* = end ring, *f* = end bulb, *g* = pericellular basket cut through. Bielschowsky—Ábrahám method, × 600

species of mammals, comparative data, however, are still lacking. Gross anatomic and microscopic examinations of the ciliary ganglion in different species of mammals have been completed therefore with the cholinesterase methods. Specific cholinesterase was examined in the easily available species of mammals. We wanted to get answers to the following three questions: (1) is there any difference in the cholinesterase activity in the ciliary ganglion of the different species of mammals, (2) is there any difference in the localization of cholinesterase in the morphologically different preganglionary endings,(3) may the motor and sensory endings be differentiated by histochemical methods?

The answer to the first question is in the affirmative. In the animals examined, differences were observed in the incubation time needed for the appearance of the reaction, in the strength of the colour reaction, and in the tendency for diffusion. These differences may be attributed to morphological causes. When the multipolar cells with long dendrites prevail, incubation time is shorter (2 or 3 hours), the colour reaction stronger,

TABLE I

Species	Prevailing cell type	Found motor form of endings
Erinaceus europeus	multipolar with long processes	end knob
Myotis myotis	multipolar with long processes	end knob
Felis domestica	multipolar with long processes	end knob end bulbs
Canis familiaris	multipolar fenestrated	pericellular basket, end bulb
Vulpes vulpes	fenestrated multipolar (many transitions)	pericellular basket, end bulb
Lepus europeus	multipolar with long processes	pericellular basket, end bulb
Cavia cobaya	multipolar with long processes	pericellular basket
Sus scrofa domestica	multipolar with long processes (many transitions)	end bulb
Capreolus capreolus	fenestrated multipolar	pericellular basket
Cervus elaphus	fenestrated multipolar	pericellular basket, end bulb
Ovis aries	fenestrated multipolar	pericellular basket, end bulb
Bos taurus	fenestrated multipolar	end bulb
Equus caballus	multipolar with long processes	end knob end bulb

and the tendency for diffusion is greater. Incubation time is about 4 or 5 hours, the colour reaction weaker, and the tendency for diffusion is lesser when the ganglion is built up of fenestrated cells. This difference is especially striking in the cholinesterase activity of the ganglia of *Felis* and *Canis*. In the ganglion of the cat the reaction extends over the whole cellular surface, even on the initial part of the processes. We get almost the same picture as with impregnation, except for the nervefiber plexus of the ganglion that does not stain (Fig. 4*f*). The colouring to be observed also at the endings of the preganglionary fibers may be, however, not easily differentiated, because of the strong activity of the cellular body. In the ganglion of the dog the cellular surface scarcely stains, while—

Quantity of the motor endings	Sensory form of endings in the connective tissue	Quantity of the sensory endings
few		
rather few		
few (more on one cell) few (intracellular)	end system granule-like	few quite many
a good many quite many	end system tree-like ramification	quite many many
many few	end system tree-like ramification	few few
few few		
few	granule-like	few
few	end system	quite many
quite many	tree-like ramification	quite many
quite many quite many	tree-like ramification	many
many few	tree-like ramification granule-like	many quite many
quite many	end system tree-like ramification	quite many many
quite many (more on one cell) quite many (on the cell, resp. between the cells)	end system granule-like	few many

owing to the strong cholinesterase activity of the preganglionic fibers — rings appear on the cellular surface (Fig. 4*g*), perfectly verifying the negative localization picture described by Couteaux and Szentágothai and co-workers.

Our answer is negative to the second question. With the histochemical methods used the fine structures of the endings could not be demonstrated directly.

The third question, on the other hand, may be answered positively, as this histochemical method helps to differentiate both the motor and sensory endings. Cholinesterase is found not only in the synaptic terminals of preganglionic fibers and in ganglion cell bodies, but occurs also in the sensory endings within the connective tissue capsule of the ganglion.

However, the cholinesterase of the sensory endings becomes manifest only after longer periods of incubation when diffusion has already distorted the picture to a considerable degree.

SUMMARY

Comparative examination of the ciliary ganglia in 13 species of mammals led to the following conclusions.

(1) On the basis of the macroscopical structure two types of ganglia may be distinguished: one oviform connected exclusively with the oculomotor nerve and continuous with the post-ganglionic nerve, the other stellate having several pre- and postganglionic nerve branches.

(2) The size of the ganglion is directly proportional to the body size and the development of the vegetative nervous system.

(3) The microscopic structure reveals a minimal change in the size and number of cells; two kinds of cells (multipolar with long processes and fenestrated multipolar) and three types of nerve fibers (preganglionic, postganglionic and vasomotor) can be differentiated.

(4) The character, form and occurrence of the synapses show remarkable varieties.

(5) The overwhelming majority of the nerve endings are of preganglionic type and terminate in the form of pericellular baskets, terminal knobs, end rings or end bulbs.

(6) Although there are several kinds of preganglionic terminals to be found in the ciliary ganglion in a given species, generally, however, one of the three forms prevails.

(7) Sensory endings in the form of extended end systems: tree-like ramifications or glomerular formations are only in the connective tissue capsule of the ganglia or their septa. The presence of sensory endings between the ganglioncells seems to be rather contestable.

(8) Cholinesterase-activity shows a different appearance in the ciliary ganglion cells of the dog and of the cat, owing probably to structural differences. Specific cholinesterase could be demonstrated in the preganglionic fibers and—though less intensively—also in the sensory endings of the connective tissue capsule.

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ELEKTRONENMIKROSKOPISCHE UNTERSUCHUNGEN ÜBER DIE FEINSTRUKTUR VEGETATIVER SYNAPSEN AN BLUTGEFÄSSEN

von

H. Brettschneider

ELEKTRONENMIKROSKOPISCHE ABTEILUNG DES ANATOMISCHEN INSTITUTES DER UNIVERSITÄT MÜNSTER
DEUTSCHE BUNDESREPUBLIK

Unsere Kenntnisse über die Feinstruktur peripherer vegetativer Nervenfasern haben sich durch Einführung elektronenmikroskopischer Untersuchungsmethoden in starkem Maße vermehrt. Das einzelne Bauelement der vegetativen Peripherie wurde vor noch nicht allzulanger Zeit von vielen Untersuchern für eine fibrilläre Struktur gehalten. Wir wissen heute, daß es sich auch in der äußersten Peripherie des vegetativen Nervensystems nicht um eine Vernetzung fibrillärer Elemente handelt, sondern daß die Zellindividualität der vegetativen Ganglienzelle in dieser äußersten Peripherie repräsentativ durch cytoplasmatische Einheiten, die Axone, vertreten ist. Wir haben nicht nur genaue Kenntnisse über den Aufbau des peripheren Axoplasmas und seiner Cytoplasmamembran, dem Axolemm, sondern wir haben bereits gewisse Vorstellungen über die Beziehungen der Axone zum Schwannschen Leitgewebe sowie über Beziehungen vegetativer Nervenfasern zum Effektorgewebe. Zu diesen beiden ebengenannten Problemen bleiben noch viele Fragen offen, und die Neuromorphologie bleibt aufgerufen, gerade hier mit großer Intensität weiter zu arbeiten. Die Beziehungen markloser vegetativer Nervenfasern zum Leitgewebe konnte Betty Geren-Uzman (1954) vor vielen Jahren klären, so daß heute ganz konkrete Vorstellungen über diese Beziehungen bestehen. Es ist mir ein besonderes Bedürfnis hier an diesem Ort auf lichtmikroskopische Untersuchungen aufmerksam zu machen, die von einem der Veranstalter dieser Tagung, Herrn Professor Dr. Szentágothai, vor vielen Jahren veröffentlicht wurden. Ich habe diese Untersuchungen schon in meinen Arbeiten erwähnt. Mir sind keine lichtmikroskopischen Untersuchungen bekannt, die in solch exakter Weise die Beziehungen zwischen Leitgewebe und Axonen aufzeigen konnten, wie die Untersuchungen von Szentágothai (1957). Im Laufe der letzten Jahre hat es sich nun herausgestellt, daß auch in der äußersten Peripherie die Axone in gleicher Weise in das Leitgewebe eingefaltet sind, wie das Elfvin (1958) am Milznerv beschreiben konnte. Diejenigen nervösen Strukturen, die uns als fibrilläre Elemente des Terminalretikulums vor Augen geführt wurden, sind, wie es uns das Elektronenmikroskop gezeigt hat, in Leitgewebe eingefaltete Axone. Das Schwannsche Leitgewebe läßt sich entlang der Axone bis zur vegetativen Synapse verfolgen. Nur hier, im Bereiche der Synapse ist in der Regel eine Ausfaltung des einzelnen Axons aus der Umhüllung durch die Schwannsche Zelle zu beobachten. Die Abb. 1 zeigt Ihnen den Querschnitt einer vegetativen präterminalen Nervenfaser aus der Adventitia einer Muskelarterie der weißen Ratte. Die Beziehungen der Axone zum Leitgewebe sind deutlich zu sehen und

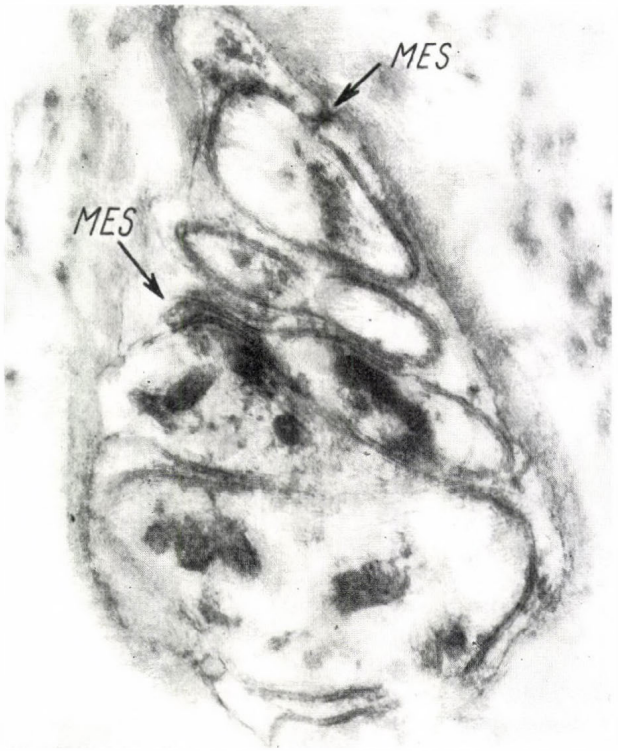

Abb. 1. — Schnitt durch ein vegetatives Axonbündel aus der Adventitia einer Oberschenkelarterie der weißen Ratte. Die Axone sind in die Schwannsche Zelle ein gefaltet. Mesaxone (*MES*) sind zu erkennen. Das Cytoplasma der Schwannschen Zelle ist sehr struktur- und kontrast-tarm. Vergr. 52 000mal

sind immer doch wieder die gleichen, wo auch immer man untersucht. Wenn sich somit die Zusammensetzung und Struktur einer vegetativen Nervenfaser bis in den präsynaptischen Bereich im Prinzip nicht ändern, so sind doch gelegentliche Unterschiede aufzuzeigen, die, die Masse des Schwannschen Cytoplasmas betreffend, immer wieder, aber besonders an der Gefäßwandung, beobachtet werden. So trifft man im präterminalen Bereich auf vegetative Nervenfasern, wie sie die Abb. 1 vor Augen führt, mit einer relativ großen Menge Schwannschen Cytoplasmas zwischen den Axonen. Andererseits werden solche Nervenfasern gefunden, bei denen die Menge des Schwannschen Cytoplasmas auf einen schmalen, dann aber sehr kontrastreichen Zellsaum um die Axone beschränkt ist (Abb. 2). Welche Bedeutung diese unterschiedliche Verhaltensweise des Leitgewebes hat, kann noch nicht gesagt werden, da systematische Untersuchungen hierüber noch ausstehen. Soweit bislang beobachtet werden konnte, unterscheidet sich die Feinstruktur des Axoplasmas der einen und der anderen Faser nicht voneinander. Morphologische Unterschiede dieser Art im präterminalen Bereich der vegetativen Faserstrecke können im terminalen

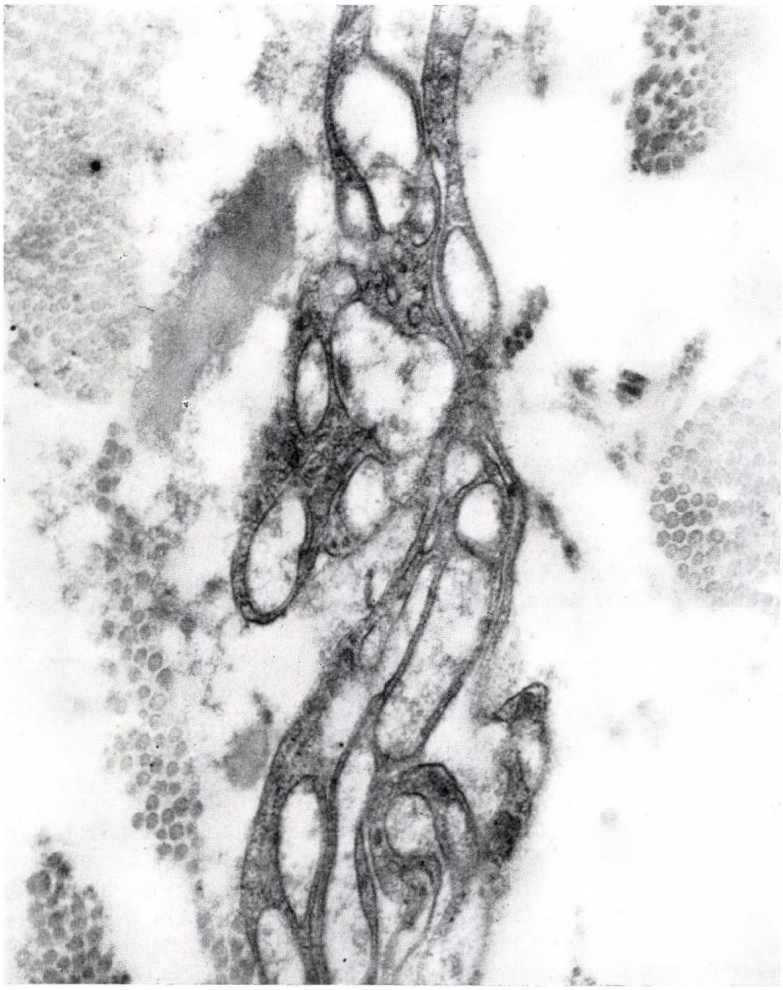

Abb. 2. — Ein Bündel vegetativer Axone aus der Adventitia einer Oberschenkelarterie der weißen Ratte. Der schmale Saum des Leitgewebes dieses Bündels ist kontrastreich und unterscheidet sich damit deutlich von der Struktur des Leitgewebes der Abb. 1. Vergr. 30 000mal

Bereich nicht beobachtet werden. Hier ist eher eine größere Menge und auch eine stärkere Differenzierung des Cytoplasmas der Schwannschen Zellen zu beobachten. Wie schon angedeutet, ändert sich im Aufbau der vegetativen Struktur hier nichts. Keine andere Zellform als die Zellen des Leitgewebes hat eine innigere Beziehung zu den Axonen. Es besteht daher keine Veranlassung hier in der äußersten Peripherie interstitielle Zellen von den Zellen des Leitgewebes zu unterscheiden, wie das gelegentlich getan wurde. Auch in der äußersten Peripherie sind Strukturelemente der vegetativen Nervenfasern, die aus der Einfaltung des Axons in die Schwannsche Zelle entstehen, wie beispielsweise das Mesaxon, noch zu beobachten. In diesen periterminalen Bereichen ist also die Individualität des einzelnen

Axons immer wieder nachweisbar. Auch das Leitgewebe besteht aus einzelnen Zellen, wie uns darstellbare Zellgrenzen zwischen diesen beweisen. Wenn Boeke (1933) die Individualität der Neurofibrillen seines sympathischen Grundplexus betont, so trifft er mit dieser Aussage sicherlich diesen präterminalen Bereich der vegetativen Faser und unterscheidet sich mit dieser Aussage keineswegs von den modernen elektronenmikroskopischen Befunden. Das periterminale Netzwerk Boekes jedoch, das den synaptischen Bereich vegetativer Nerven darstellt, ist elektronenmikroskopisch genau so wenig nachzuweisen wie das Terminalretikulum der Stöhrschen Schule. Auch im synaptischen Bereich bleibt die Zellindividualität gewahrt. Bei den meisten bisher untersuchten vegetativen Synapsen handelt es sich um komplexe Organe, die sich aus mehreren Axonen und dem Leitgewebe aufbauen. Monoterminale Innervation wurde auch beobachtet, jedoch überwiegt zahlenmäßig die multiterminale Synapse. Es stellt sich bei der Beobachtung solcher multiterminaler Synapsen die Frage, ob wir es hier mit vegetativen Endorganen zu tun haben, ob also die Synapse vom Axonende oder von jeder anderen beliebigen Stelle des Axolemms dargestellt werden kann. Zur Klärung dieser und auch noch anderer offenen Fragen nach der Existenz eines vegetativen Endorganes ziehen wir am besten die Gefäßwand von Muskelarterien heran. Es hat sich nämlich während zahlreicher elektronenmikroskopischer Untersuchungen gezeigt, daß im Bereiche der Media und darüber hinaus bis zur Intima von Muskelarterien vegetative Axone nicht gefunden werden. Wenn wir also (Abb. 3) Nervenfasern beobachten, die von dem vegetativen Fasergeflecht der äußeren Adventitiazone sich abzweigen, in Richtung auf die Media vordringen, so können diese Fasern über die Elastica externa hinaus, d. h. in die Intercellularräume der Mediamuskulatur hinein, nicht verfolgt werden. Bei diesen einzelnen dünnen, häufig nicht mehr als ein μ breiten Fasern handelt es sich um komplexe Gebilde aus Axonen und Leitgewebe. Ich habe diese Gebilde in früheren Veröffentlichungen 'Endorgane' genannt und war immer bemüht, im Bereiche dieser Organe wirkliche Endigungen der Axone und auch des Leitgewebes nachzuweisen. Die Abb. 4 stellt ein solches vegetatives Endorgan dar, das hier in unmittelbare Nähe eines Zellfortsatzes einer peripheren Mediamuskulatur gerät. Dieser Zellfortsatz hat die Elastica externa durchbrochen und liegt im Bereich der inneren Adventitiazone. Solche Zellfortsätze der Mediamuskulatur werden selten in der Nähe der Endorgane vermißt. Viele Axone eines solchen Endorganes haben sich, wie man an den freien Oberflächen des Axolemms erkennt, aus der Umhüllung der Schwannschen Zelle ausgefaltet und grenzen mit einer freien Oberfläche an das Bindegewebe des Interstitiums. Ein Axonende, das mehr als ein μ aus der Umfassung durch die Schwannsche Zelle hervorsieht, zeigt die Abb 5. So dünne gegen das Effektorgewebe auslaufende Axonenden habe ich erst vor kurzem bei einem Vortrag in München demonstrieren können. Ich möchte mich heute nicht wiederholen und zeige Ihnen daher noch auf der folgenden Aufnahme (Abb. 6) eine ähnliche Situation zwischen Effektorgewebe und Axonende wie auf der Abb. 5. Das Axolemm als präsynaptische Membran kommt dabei der Cytoplasmamembran des Muskelzellfortsatzes auf etwa 500—1,000 Å nahe. Diese intersynaptischen Räume sind oftmals noch weiter. Sie sind jedenfalls nicht zu vergleichen mit dem intersynaptischen Spalt zentraler inter-

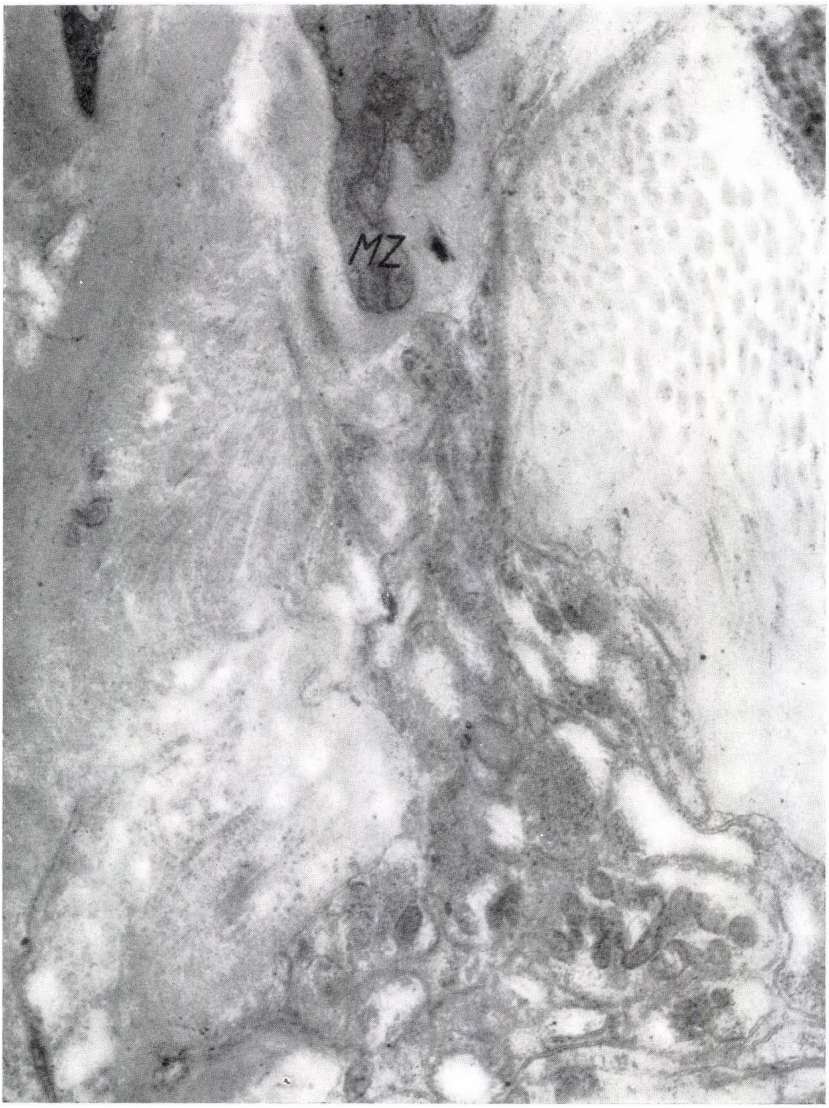

Abb. 3.—Von einem Fasergeflecht aus dem Bereich der äußeren Adventitiazone einer Arterie muskulösen Typs zweigen sich einzelne Axone ab und dringen gegen die Gefäßwand vor. Diesem vegetativen Axonbündel kommt ein Fortsatz einer Muskelzelle der Arterienmedia entgegen. Dieser Fortsatz (*MZ*) hat die Elastica externa durchbrochen Vergr. 30 000mal

neuraler Synapsen, der bekanntlich nur 150—200 Å mißt. Daß es sich bei den hier gezeigten Axonen der vegetativen Endorgane um efferente Strukturen handelt, möchte ich aus dem Vorhandensein zahlreicher synaptischer Bläschen des Axoplasmas schließen. Die Abb. 7 zeigt uns eine Teilansicht eines vegetativen Endorganes bei stärkerer Vergrößerung. Außer

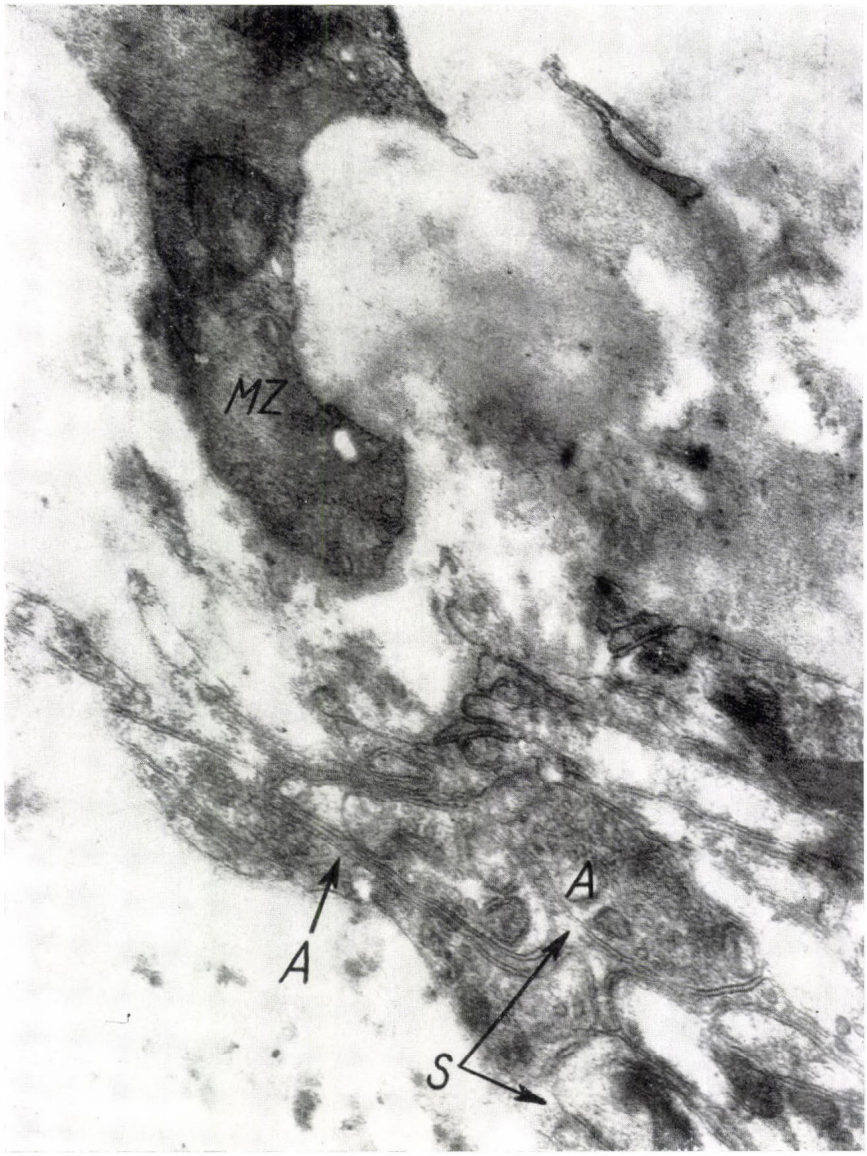

Abb. 4.—Vegetatives Endorgan an dem Fortsatz (*MZ*) einer glatten Muskelzelle der Arterienmedia. Das Endorgan besteht aus Axonen (*A*) und Leitgewebe (*S*). Die Axonen sind zu langen, dünnen Zipfeln ausgezogen. Vergr. 63 000mal

wenigen fädigen Elementen sind es vor allen Dingen die synaptischen Bläschen in unmittelbarer Nähe von Mitochondrienansammlungen, die die Struktur des Axoplasmas hier bestimmen. Die relative Weite des intersynaptischen Raumes wie die vorgewiesene Differenzierung des terminalen Axoplasmas sprechen für eine humorale Erregungsübertragung. Gelegentlich

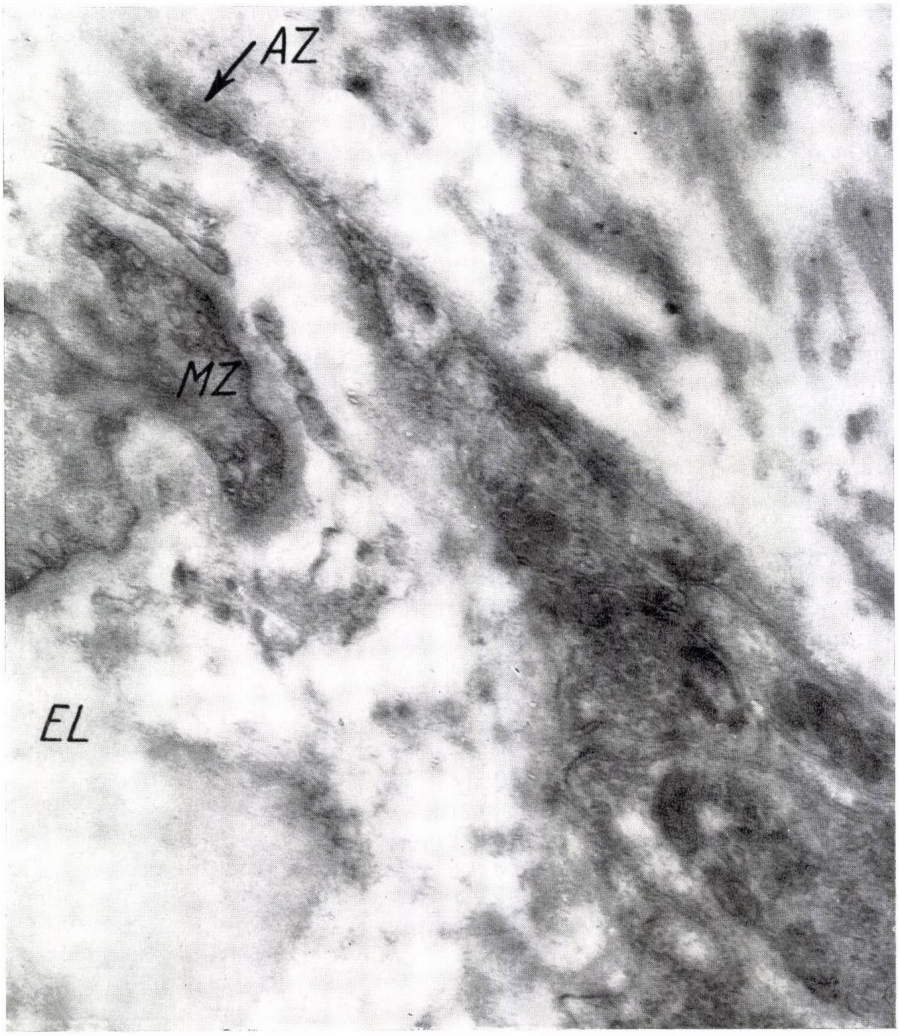

Abb. 5.—Zipflig ausgezogene Endigung eines Axons eines vegetativen Endorgans an der Arterienmedia einer Muskelarterie. *MZ* = Muskelzelle; *EL* = Elastica externa; *AZ* = zipflig ausgezogenes Axonende. Vergr. 63 000mal

beobachtet man jedoch, daß das Axonende der Effektorstruktur so nahe kommt, wie das von den Anhängern der elektrischen Erregungsübertragung gefordert werden muß und wie sie auch als interneurale oder neurosomatische Synapse in den Zentralorganen beobachtet werden. Die Abb. 8 ist ein Beispiel für einen derartig engen intersynaptischen Spalt. Befunde dieser Art sind jedoch nicht häufig genug, als daß wir einen so engen intersynaptischen Spalt von etwa 200 Å als Strukturmerkmal peripherer vegetativer Synapsen annehmen können. Ein relativ weiter intersynaptischer Spalt, die Ausfaltung des Axons aus der Schwannschen Zelle, das Vorkommen von synaptischen Bläschen und Mitochondrien im terminalen

Axoplasma sind eher Merkmale einer vegetativen multiterminalen Synapse, Merkmale, die auch an anderen Orten gefunden werden. Zur Illustration dieser Aussage möchte ich Ihnen einige Befunde über Gefäßinnervation innerer Organe und des Herzens vorweisen, die von meinen Doktoranden und mir in unserem Laboratorium erhoben werden konnten. So findet Stahl an einer Pankreasarteriole eine multiterminale Synapse, die Ausfaltung der Axone aus dem Schwannschen Cytoplasma und die relative Weite des intersynaptischen Spaltes sind kennzeichnend für periphere vegetative Synapsen. Diese Weite des intersynaptischen Spaltes wird noch eindrucksvoller durch den Befund der Abb. 10 demonstriert. Auch hier bildet Stahl die Innervation einer Pankreasarteriole ab. Die einzelnen Axone haben sich partiell aus der Umhüllung durch die Schwannsche Zelle ausgefaltet. Gegen das Drüsengewebe ist allerdings die Doppelmembran, nämlich Schwannsche Zellmembran und Axolemm, deutlich zu erkennen. Der intersynaptische Spalt ist nicht leer. In ihm ist entlang der Cytoplasmamembran der Arteriolenmuskulatur der Saum einer Basalmembran gut zu erkennen. Die postsynaptische Zellmembran der glatten Muskelzellen zeigt zahlreiche Invaginationen, die auf Stoffwechselvorgänge hinweisen. Diese Invaginationen sind allerdings nicht nur dort zu finden, wo vegetative Axone den Muskelzellen anliegen, sondern auch in den übrigen Bereichen der Cytoplasmamembran. An Arteriolen von Drüsenorganen werden vegetative Axonbündel besonders zahlreich gefunden. Eine Abbildung von der Wandung einer Schilddrüsenarteriole (Abb. 11) läßt ein relativ dickes präterminales vegetatives Faserbündel erkennen. Darüber hinaus ist ein kleines vegetatives Endorgan angeschnitten (Pfeil). Auf dieser Abbildung möchte ich aber besonders auf zwei starkvakuolisierte Cytoplasmaausläufer von Bindegewebszellen hinweisen, die sehr häufig in unmittelbarer Nachbarschaft zu vegetativen Nervenfasern gefunden werden. An Arterien und Arteriolen grenzen diese Bindegewebszellen den synaptischen Bereich der inneren Adventitiazone ab. Auch an Arteriolen der Herzmuskulatur werden Bindegewebszellen dieser Art selten vermißt. Die Abb. 12 zeigt die Wandung einer Herzmuskelarteriole, die aus einer einschichtigen Lage glatter Muskulatur und dem Endothel besteht. Zwischen der glatten Muskelzelle und der auch hier vorhandenen Bindegewebszelle der Gefäßwandung liegt ein präterminales vegetatives Faserbündel. Im Vergleich zu Drüsenarteriolen sind an Arteriolen der Herzmuskulatur nur wenige und sehr dünne vegetative Nervenfasern nachweisbar. Die Axonquerschnitte dieses hier gezeigten Bündels sind allenorts von Schwannschem Cytoplasma umgeben. Auch hier im präterminalen Bereich sind Mesaxone (Pfeile) deutlich zu sehen. Das Schwannsche Cytoplasma ist sehr strukturarm. Eine noch feinere vegetative Nervenfaser an einer Muskelzelle einer Herzmuskelarteriole zeigt die Abb. 13. Ein Axon dieser Faser hat sich teilweise aus der Umhüllung der Schwannschen Zelle ausgefaltet und liegt mit seinem Axolemm der Cytoplasmamembran der benachbarten Muskelzelle gegenüber. Ich möchte auch diesen Befund als vegetative Synapse ansprechen. Auch hier liegt die zarte vegetative Nervenfaser zwischen fadenförmigen Ausläufern von Bindegewebszellen und der Muskulatur der Herzmuskelarteriole. Einen ähnlichen Befund zeigt die Abb. 14. Ein sehr dünnes Bündel vegetativer Fasern liegt im gleichen Raum zwischen Muskelzelle und Bindegewebsausläufer wie auf der vorherigen Abbildung. Der intersynaptische Spalt ist

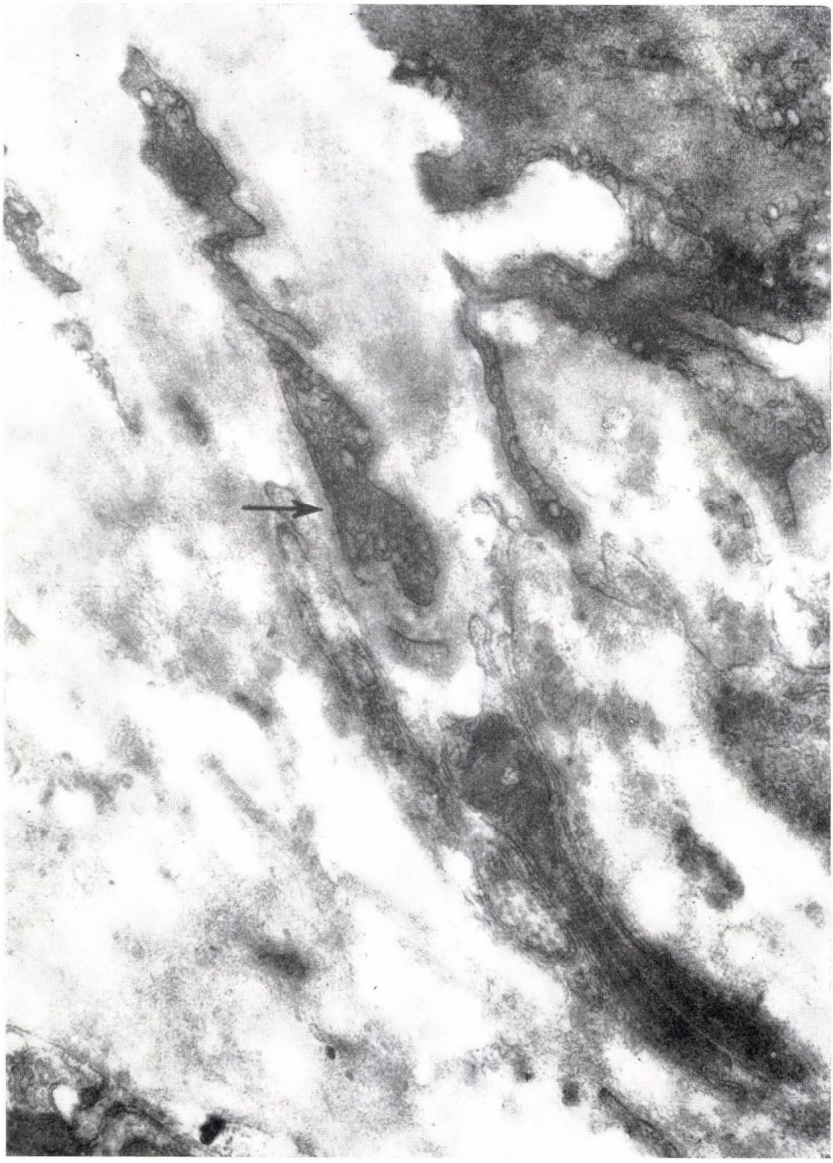

Abb. 6—Zipflig ausgezogene Endigungen von Axonen eines vegetativen Endorganes. Der intersynaptische Spalt (Pfeil) zwischen Axolemm und Cytoplasmamembran des Muskelzellfortsatzes ist hier etwa 600—800 Å weit. Vergr. 63 000mal

hier besonders weit und von einer Substanz mittleren Elektronenkontrastes erfüllt. Es ist doch sehr auffällig, hier an Herzmuskelarteriolen der weißen Ratte nur wenige und sehr zarte vegetative Fasern zu finden. Stärkere vegetative Fasern, wie sie die Abb. 15 zeigt, sind an Arteriolen sehr selten.

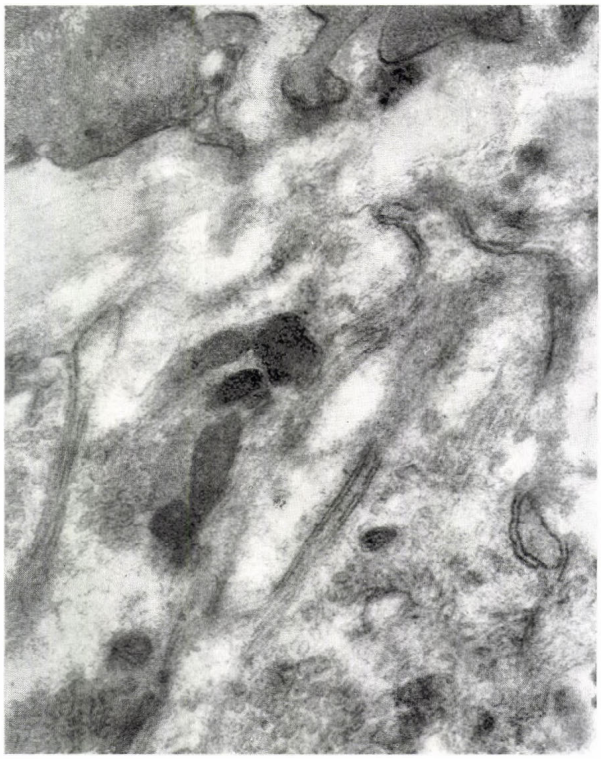

Abb. 7. — Differenzierung des terminalen Axoplasmas. In den relativ weiten Axonenden sind vor allem synaptische Bläschen und Mitochondrien zu beobachten, die in geschlossenen Ansammlungen beieinander liegen. Vergr. 64 000mal

Das Axon A_1 dieser Abbildung hat sich partiell aus der Umhüllung der Schwannschen Zelle ausgefaltet und liegt der Cytoplasmamembran der Muskelzelle sehr nahe. Sowohl die präsynaptische Membran des Axons als auch die postsynaptische Membran der glatten Muskelzelle zeigen Invaginationen. Das Axonende selbst ist angefüllt von synaptischen Bläschen und einigen Mitochondrien. Im Prinzip unterscheiden sich diese aus nur wenigen und kleinen Axonen bestehenden Endorgane an den Herzmuskelarteriolen nicht von den umfangreicheren an Muskelarterien der Oberschenkelmuskulatur. Auch hier die Ausfaltung aus der Schwannschen Zelle, der relativ weite intersynaptische Spalt, die Auffüllung des Axoplasmas mit synaptischen Bläschen und das Auftreten meistens mehrerer Axone. Wenn somit das Aufsuchen vegetativer Nervenfasern an Arteriolen der Herzmuskulatur einige Mühe macht, sind sie an bestimmten Kapillaren eher zu beobachten. Es handelt sich dabei nicht um solche Kapillaren, die den Herzmuskelzellen dicht anliegen, sondern um Kapillaren im Bereich des bindegewebigen Interstitiums. Sie sind vielfach in Begleitung stärkerer vegetativer Nervenfasern, wie das die Abb. 16 zeigt. Einige Axone eines

Abb. 8. — Einem langgestielten Fortsatz einer Mediamuskelzelle (*MZ*), der durch Öffnung der Elastica externa gerade die innere Adventitiazone erreicht, legt sich das spitzauslaufende Axonende eines Endorganes so dicht an, daß der intersynaptische Spalt hier ungewöhnlich eng ist. Vergr. 84 000mal

solchen Bündels falten sich aus der Schwannschen Zelle aus und liegen dann der Cytoplasmamembran des Endothels sehr nahe. Im allgemeinen ist eine Kapillarinnervation im elektronenmikroskopischen Dünnschnitt nur selten zu finden, doch kommen vegetative Nervenfasern an Kapillaren dieses Typs im Bereiche des bindegewebigen Interstitiums des Rattenmyokards häufiger vor. Auch an Organkapillaren sind vegetative Nervenfasern zu sehen. Ich darf Ihnen auf den Abbildungen 17 und 18 Befunde aus der Bauchspeicheldrüse demonstrieren, die Herr Stahl (1964) in meinem Laboratorium angefertigt hat. Es handelt sich jeweils um vegetative Axonbündel, von denen sich einzelne Axone ausfalten und mit ihrem Axolemm der Cytoplasmamembran des Endothels gegenüberliegen. Auch hier ist wie bei anderen vegetativen Synapsen der intersynaptische Spalt relativ weit und substanzerfüllt, und damit unterscheiden sich die Befunde der Kapillarinnervation ebenfalls nicht von denen größerer Gefäße. Ob es sich bei diesen Kapillarnerven um efferente oder um afferente Axone handelt,

Abb. 9.—Multiterminale Synapse an einer Arteriole der Bauchspeicheldrüse. Ein Teil der Axone hat sich aus der Umhüllung durch die Schwannsche Zelle ausgefaltet und liegt der Cytoplasmamembran der Muskelzelle (*MZ*) mit einer freien Oberfläche gegenüber. *A* = Axone. Aufnahme Stahl. Vergr. 30 000mal

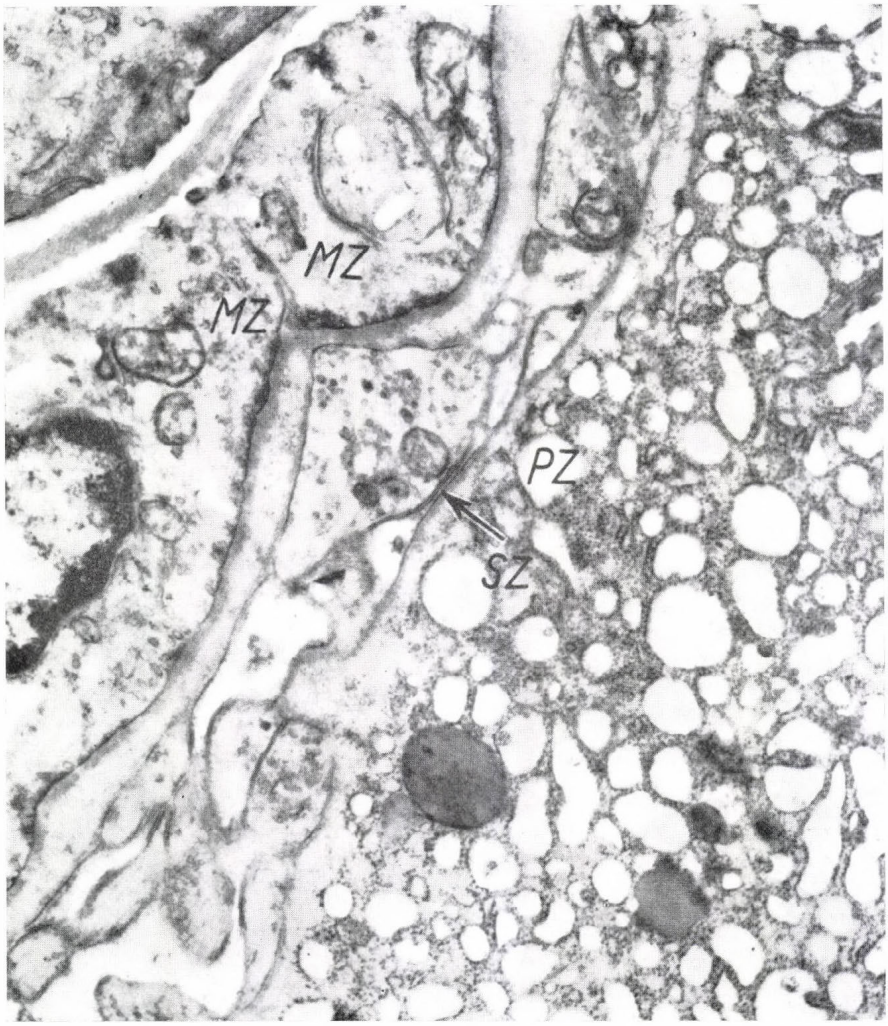

Abb. 10. Innervation einer Pankreasarteriole. Die Axone sind von den Muskelzellen (*MZ*) durch einen relativ weiten und substanzerfüllten intersynaptischen Spalt getrennt. Von der exokrinen Pankreaszelle (*PZ*) sind diese das Gefäß innervierenden Axone durch die Cytoplasmamembran des Leitgewebes (*SZ*) getrennt. Der Pfeil weist auf Axone, die der Cytoplasmamembran der Pankreaszelle unmittelbar anliegen. Aufnahme Stahl. Vergr. 30 000mal

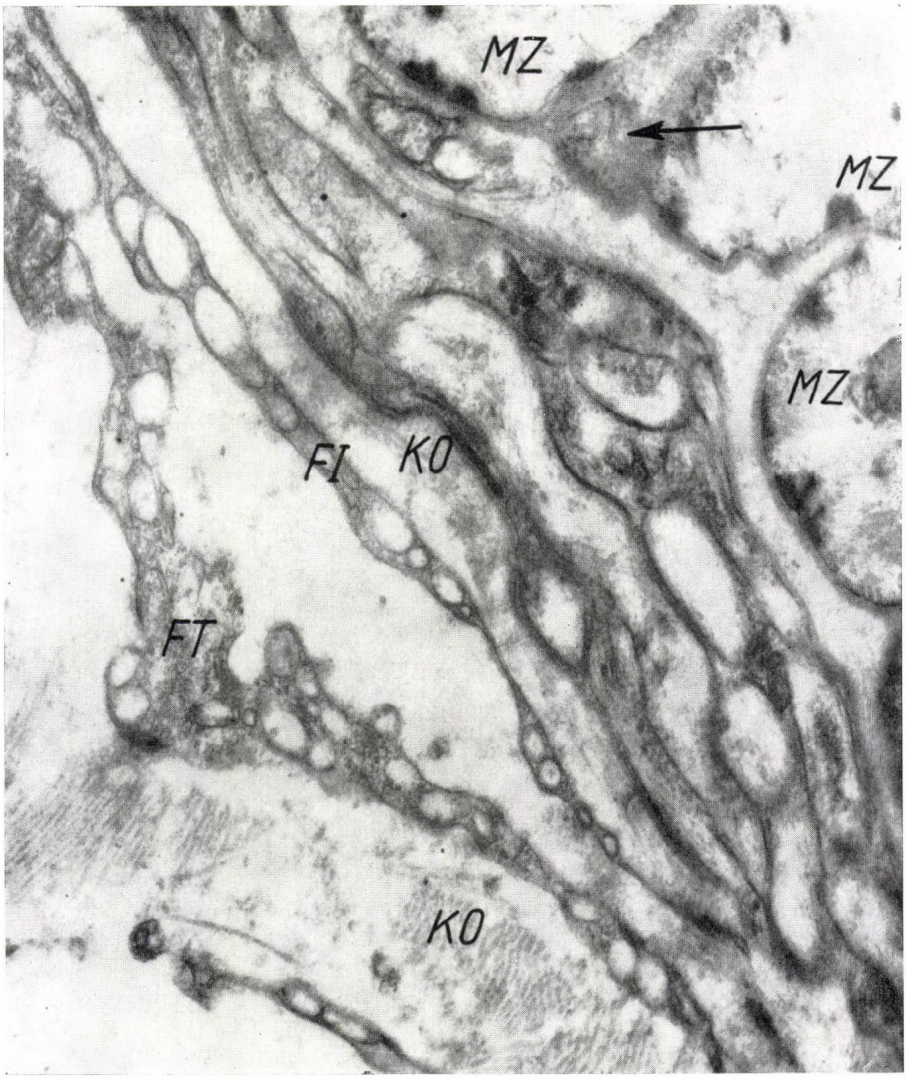

Abb. 11.—Präterminales vegetatives Axonbündel an einer Schilddrüsenarteriole. Von dem Interstitium ist dieses Bündel durch vakuolisierte Zellausläufer von Bindegewebszellen getrennt (*FI*). Der Pfeil weist auf einen Intercellularraum der Mediamuskulatur, in dem sich ein einzelnes Axon aus der S hwannschen Zelle ausgefaltet hat, da ist offensichtlich ein Teil eines vegetativen Endorganes angeschnitten. *MZ* = Muskelzellen, *KO* = Faserkollagen des Interstitiums. Vergr. 25 000mal

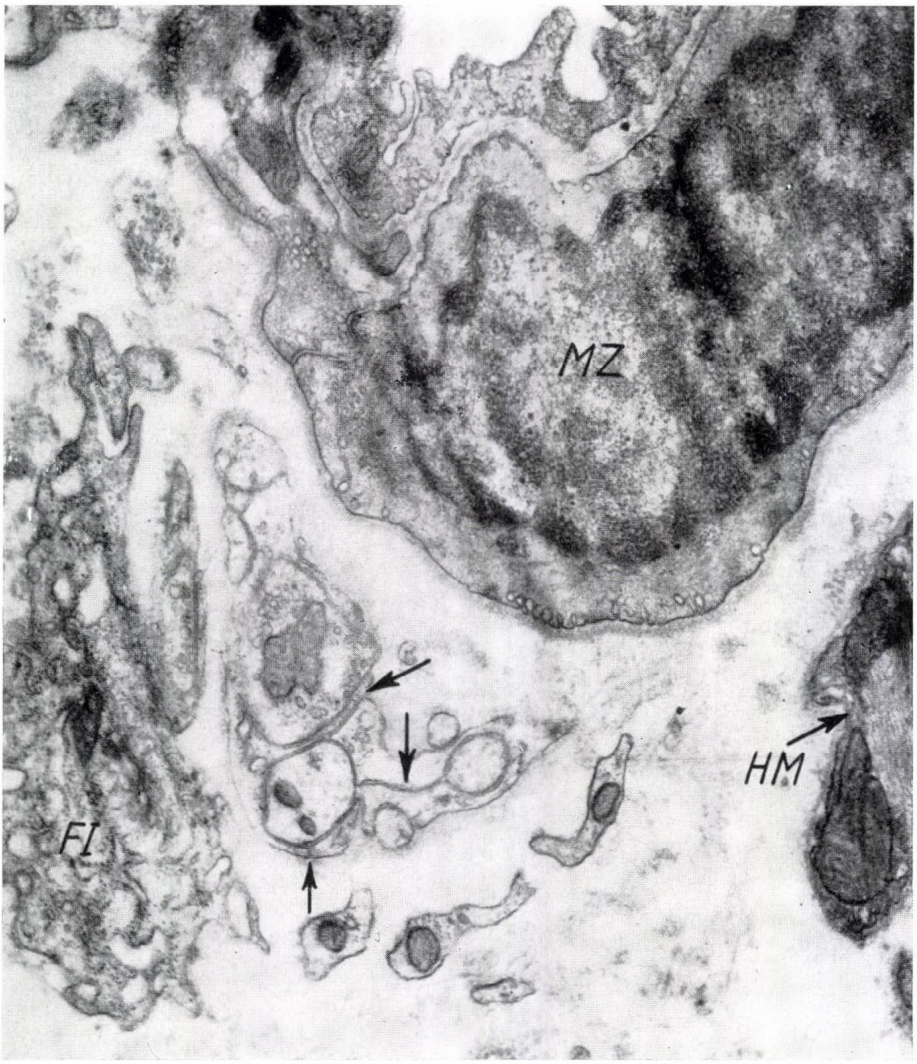

Abb. 12. — Präterminales Axonbündel an einer glatten Muskelzelle einer Herzmuskelarteriole. *HM* = Herzmuskulatur; *MZ* = Muskelzelle der Gefäßwand, *FI* = Bindegewebszelle. Die Pfeile weisen auf Mesaxone des präterminalen Bündels. Vergr. 30 000mal

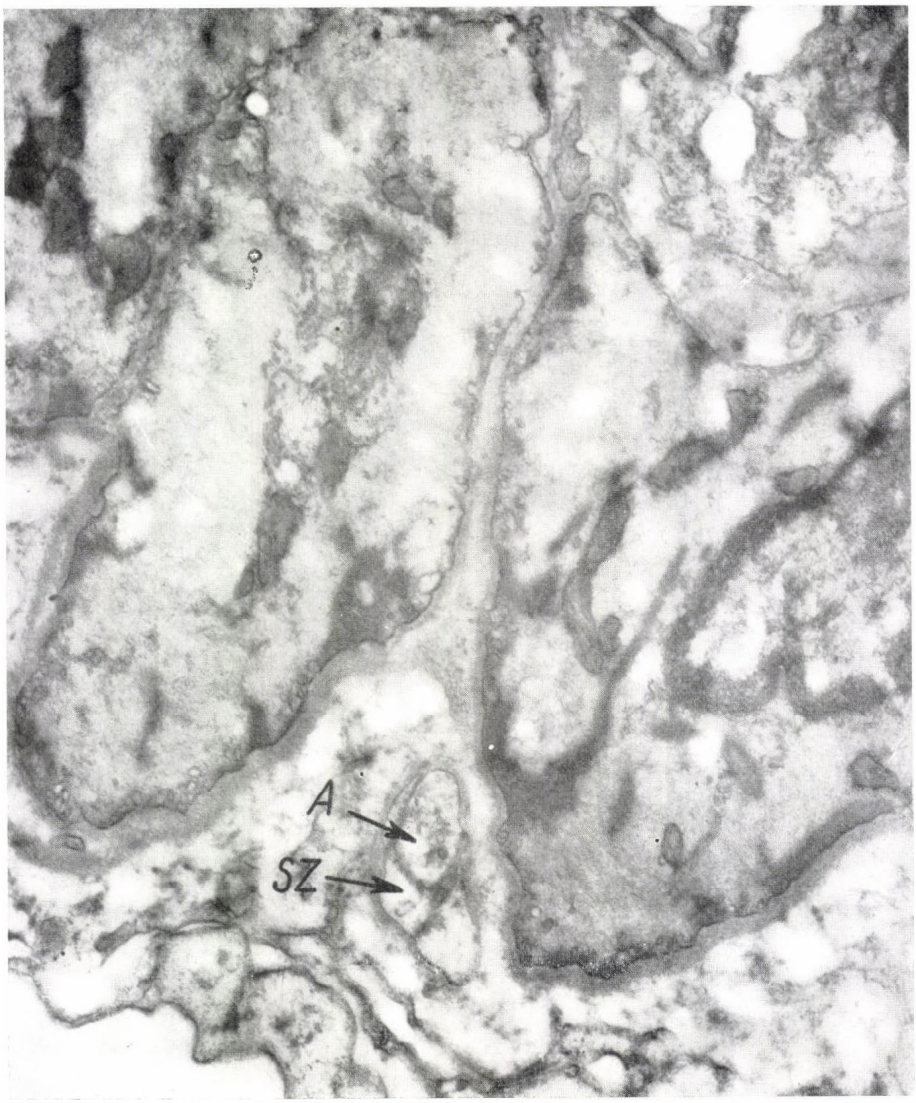

Abb. 13. — Kleine vegetative Synapse an der Muskelzelle einer Arteriole der Herzmuskulatur. Das Axon *A* hat sich teilweise aus der Umhüllung durch die Schwannsche Zelle (*SZ*) ausgefaltet und liegt der Cytoplasmamembran der Muskelzelle gegenüber. Der sehr weite intersynaptische Spalt dieser vegetativen Synapse enthält die Fortsetzung der beiderseitigen Basalmembranen. Vergr. 30 000mal

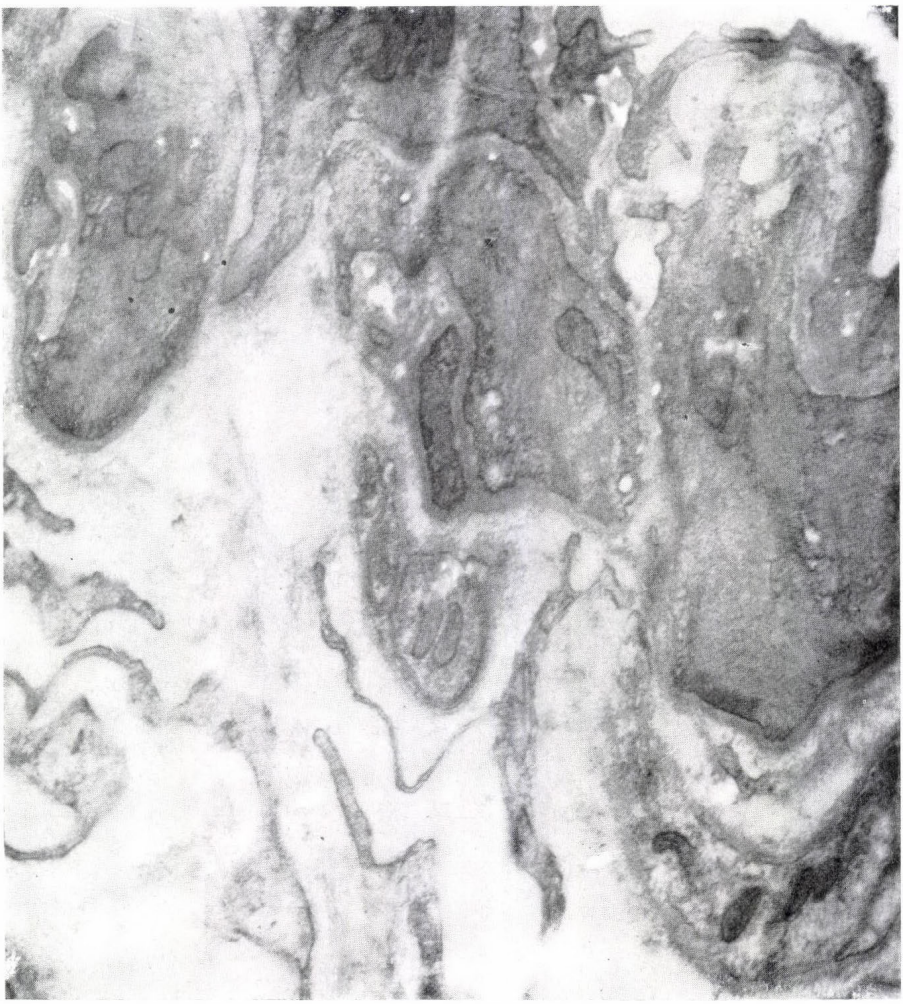

Abb. 14.—Ein kleines, aus wenigen Axonen bestehendes vegetatives Endorgan an der Muskulatur einer Herzmuskelarteriole. Das Endorgan ist gegen das Interstitium durch fadenförmige Ausläufer von Bindegewebszellen abgegrenzt. Auch hier ist die Weite des intersynaptischen Spaltes beachtlich groß. Vergr. 30 000mal

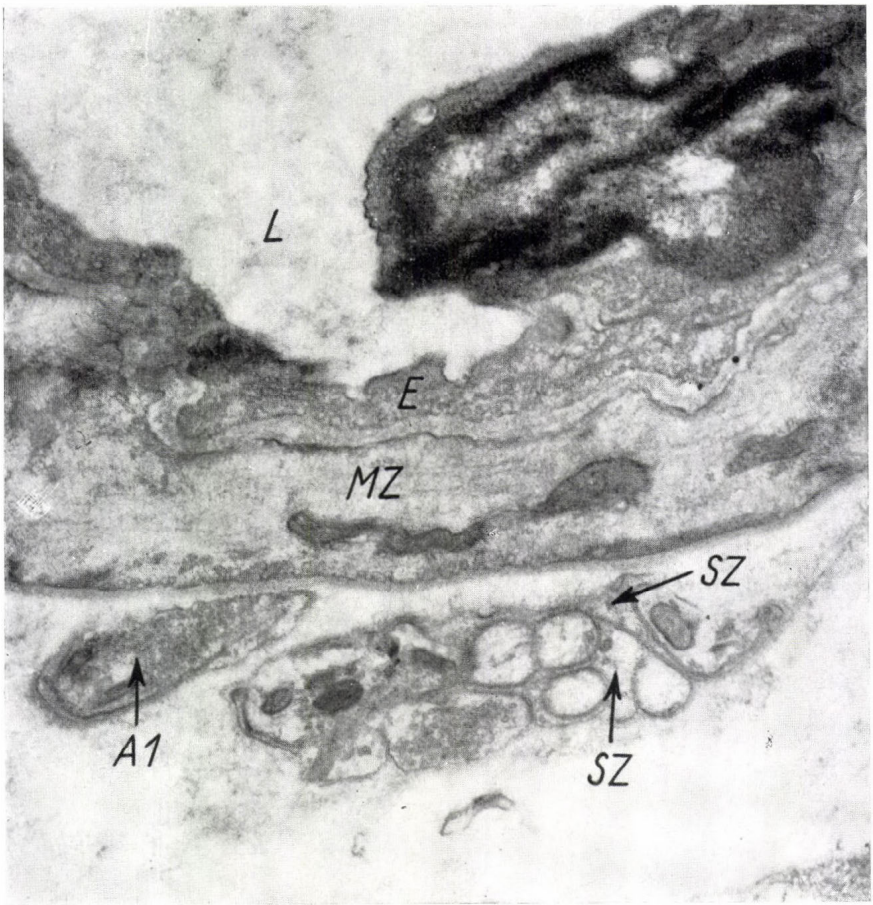

Abb. 15.—Eine stärkere präterminale vegetative Nervenfaser an einer Herzmuskelarteriole. Ein einzelnes Axon hat offenbar synaptischen Kontakt zu glatten Muskelzellen der Arteriolenwandung. *MZ* = Muskelzelle, *E* = Endothel, L = Lichtung der Arteriole, *SZ* = Schwannsches Cytoplasma. Das spitzzulaufende Axonende (A_1) ist von synaptischen Bläschen und Mitochondrien erfüllt. Das der Muskelzelle gegenüberliegende präsynaptische Axolemm zeigt zahlreich kleinere Invaginationen. Vergr. 30 000mal

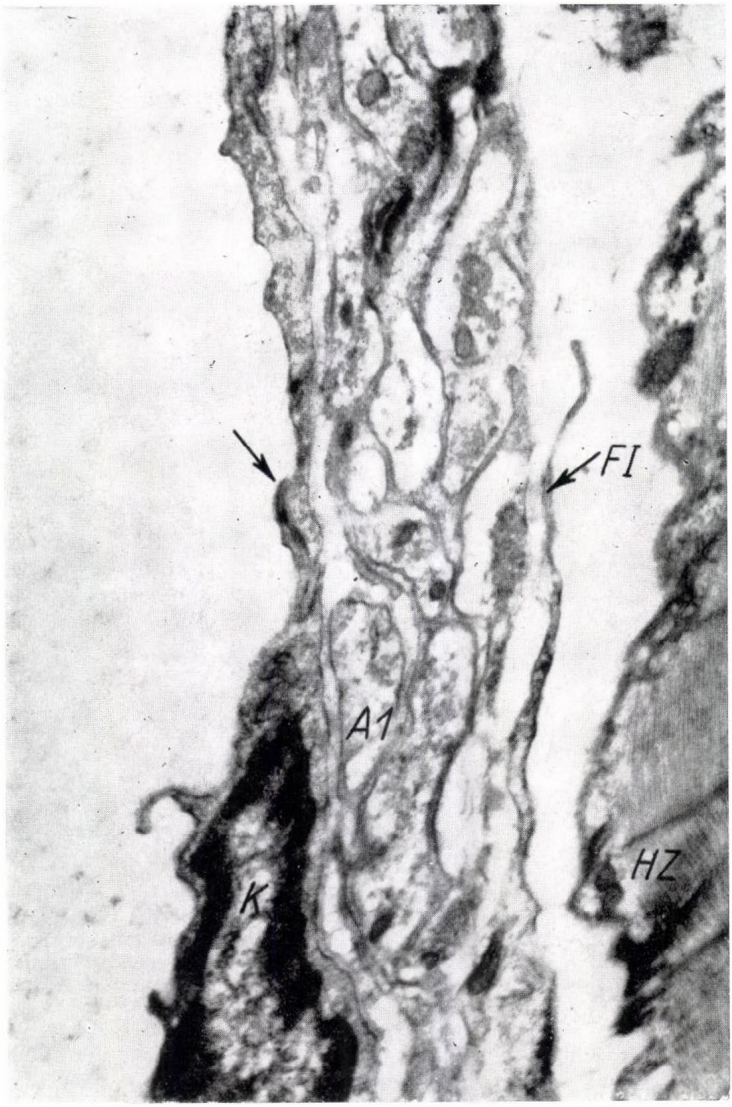

Abb. 16.—Vegetative Nervenfaser an einer Kapillare der Herzmuskulatur. *HZ* = Herzmuskelzelle, *K* = Kern des Kapillarendothels. Der Pfeil weist auf eine Zellgrenze zwischen zwei Endothelzellen hin. Der Ausläufer einer Bindegewebszelle des Interstitiums (*FI*) trennt dieses Bündel von der Herzmuskelzelle. Das Axon A_1 liegt mit seiner freien Zelloberfläche der Cytoplasmamembran der Endothelzelle dicht an. Vergr. 25 000mal

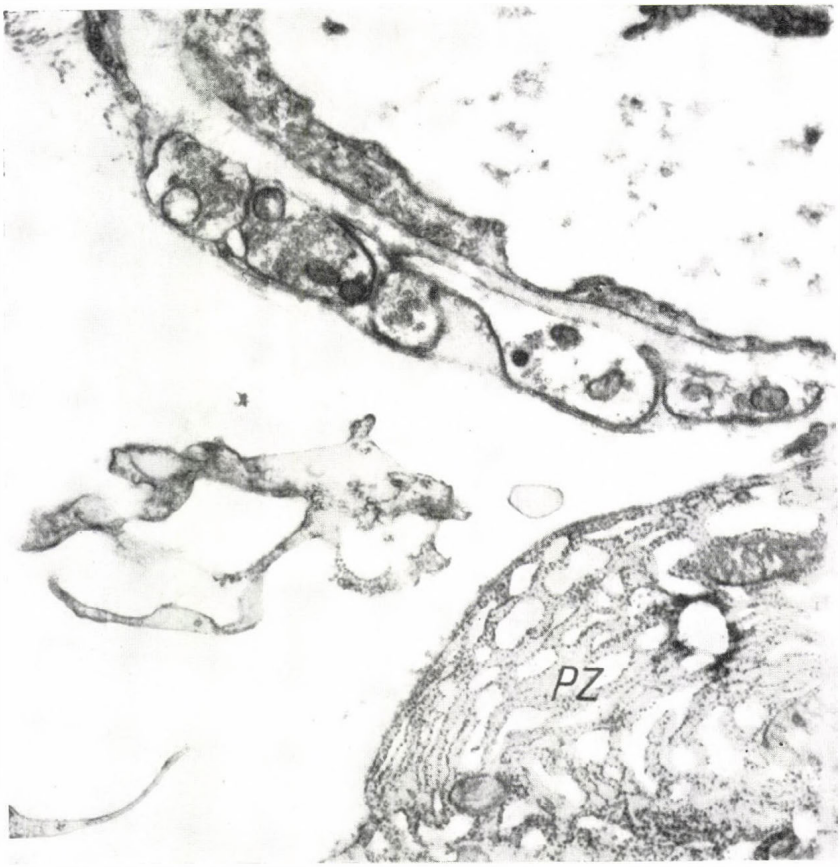

Abb. 17. — Ein zartes vegetatives Axonbündel am Kapillarendothel einer Pankreaskapillare. Auch hier haben sich die Axone aus der Schwannschen Zelle ausgefaltet, und das Axolemm liegt dem Effektorgewebe direkt gegenüber. Auf der anderen Seite des Bündels ist die Schwannsche Cytoplasmamembran deutlich zu erkennen. *PZ* = exokrine Pankreaszelle. Aufnahme Stahl, Vergr. 30 000mal

Abb. 18.—Vegetatives Faserbündel an dem Endothel einer Pankreaskapillare. Durch Ausfaltung aus der Schwannschen Zelle entsteht auch hier ein unmittelbarer Kontakt zwischen Effektorgewebe und Nervengewebe. Es handelt sich hier um ein kleines vegetatives Endorgan an einer Kapillare. *K* = Zellkern des Kapillarendothels; *PZ* = exokrine Pankreaszelle. Aufnahme Stahl, Vergr. 30 000mal

kann ich der Feinstruktur der vegetativen Nervenfasern nicht entnehmen. Da einzelne Axone immer wieder synaptische Bläschen aufweisen, ist auch an eine efferente Innervation zu denken. Andere wiederum, wie die Axone der Abb. 18, sind außerordentlich arm an synaptischen Bläschen, so daß hier möglicherweise auch ein afferentes System vegetativer Fasern vorliegt. Besonders auffällig ist die relative Enge des intersynaptischen Spaltes, doch ist auch er weiter als bei den interneuralen Synapsen und ist ebenfalls substanzerfüllt. Aus der Differenzierung des Axoplasmas allein kann auf die Leitungsrichtung nicht geschlossen werden. Mit einem Blick auf die Kapillarinnervation möchte ich meinen Bericht abschließen und meine Befunde wie folgt zusammenfassen.

ZUSAMMENFASSUNG

Die periphere vegetative Nervenfaser zeigt auch im präterminalen Bereich noch den gleichen Aufbau wie ein stärkerer vegetativer Nerv. Bis zur Synapse ist die Einfaltung der vegetativen Axone in das Leitgewebe zu beobachten. Auf Grund der unterschiedlichen Cytoplasmastruktur der Schwannschen Zellen können zwei Fasertypen vegetativer Nervenfasern im Bereiche der Adventitia von Muskelarterien beschrieben werden. Einmal handelt es sich um solche Fasern, die relativ viel Schwannsches Cytoplasma von geringer Elektronendichte aufweisen, und das andere Mal um solche, bei denen das Leitgewebe nur in Form schmaler und sehr kontrastreicher Säume um die Axone zu beobachten ist. Beobachtungen dieser Art können nur im Bereiche der äußeren Adventitiazone gemacht werden. Die in der inneren Adventitiazone zu beobachtenden präsynaptischen Faserstrecken weisen diese Unterschiede nicht auf. Das sehr strukturarme Cytoplasma der Schwannschen Zelle dieses Bereiches unterscheidet sich damit von dem durch zahlreiche Bläschen und Mitochondrien angereicherten Axoplasma. Beobachtungen an Arterien der Skelettmuskulatur, an Arteriolen parenchymatöser Organe und an Gefäßen des Myokards ergeben, daß der Ort der Reizübertragung als eine multiterminale Synapse angesprochen werden muß, die aus Axonen und Leitgewebe besteht. Eine derartige multiterminale Synapse wird auch 'vegetatives Endorgan' genannt, weil es sich offenbar hier um echte Axonenden handelt, die sich aus der Umhüllung durch die Schwannsche Zelle ganz oder teilweise ausgefaltet haben. Wie die Beobachtungen an allen bislang untersuchten Gefäßen zeigen, laufen diese Axonenden zipflig oder microvillusartig gegen das Effektorgewebe aus und sind schon nach mehreren Schnitten einer Dünnschnittserie nicht mehr zu sehen. Der Abstand der nervösen Struktur vom Effektorgewebe ist bei solchen vegetativen Synapsen ein relativ weiter. Gelegentlich können intersynaptische Spalten von 150—200 Å beobachtet werden, doch beträgt in der Regel die Tiefe des intersynaptischen Spaltes mehr als 800 Å. Der intersynaptische Spalt ist nicht leer, sondern von einer Substanz mittleren Elektronenkontrastes gefüllt. Diese Substanz erweist sich in den meisten Fällen als Fortsetzung von Basalmembranen. Während somit prinzipielle Unterschiede in der Struktur vegetativer Synapsen an Blutgefäßen nicht gefunden werden können, sind solche jedoch in der Verteilung und Größe der vegetativen Endorgane festzustellen. An Arteriolen parenchymatöser

Organe, aber auch an Arterien der Skelettmuskulatur, sind vegetative Endorgane nicht nur sehr häufig zu finden, sondern sie sind hier wesentlich umfangreicher als bei den Gefäßen der Herzmuskulatur. Die an den Arteriolen der Muskulatur des Rattenherzens beschriebenen vegetativen Endorgane bestehen nur aus einigen wenigen Axonen und dem dazugehörigen Leitgewebe. Kapillarinnervation wird an Beispielen des exokrinen Teiles der Bauchspeicheldrüse wie auch an Kapillaren aus dem bindegewebigen Interstitium des Rattenherzens beschrieben. Aus den Beobachtungen wird der Schluß gezogen, daß das periphere vegetative Nervensystem neuronal gebaut ist und daß die an den Gefäßen zu beobachtenden efferenten vegetativen Synapsen durch Abgabe von Stoffen den Reiz auf das Effektorgewebe übertragen.

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NEUERE BEFUNDE ÜBER DIE NERVENVERSORGUNG DER IRISGEFÄSSE DES KANINCHENS

VON

G. LASSMANN

NEUROLOGISCHES INSTITUT DER UNIVERSITÄT
WIEN, ÖSTERREICH

Die Darstellung nervöser Formationen in den inneren Gefäßwandschichten stößt erfahrungsgemäß auf große technische Schwierigkeiten. Ein eindeutiger Nachweis solcher Formationen ist bisher nur bei Venen gelungen, wo, wie aus der Abbildung ersichtlich, ein reiches Geflecht feiner Nervenfasern in unmittelbarer Beziehung zu den Intimazellen sichtbar gemacht werden kann (Abb. 1). Das Vorliegen gleicher Verhältnisse wurde zwar für arterielle Gefäße als gegeben angenommen, jedoch bisher niemals eindeutig nachgewiesen. Die erwähnten technischen Schwierigkeiten können teilweise durch die Anwendung der Osmium-Zinkjodidmethode von Maillet, bei welcher die Fixation und Imprägnation gleichzeitig erfolgt, überwunden werden. Diese Methode hat sich als wertvolle Ergänzung der bisher verwendeten Versilberungsmethoden erwiesen. Die Darstellung der nervösen Elemente ist dabei ähnlich jener, wie sie bei Verwendung der technisch komplizierteren Methylenblaumethoden zustande kommt.

Besondere Fragestellungen der Nervenversorgung im Bereich des peripheren Nervensystems lassen sich nur an bestimmten Orten einer weiteren Klärung zuführen. Für die Untersuchung einer bis zur Intima vordringenden Nervenversorgung haben sich die Gefäße der Iris wegen ihres besonderen Baues als günstig erwiesen. Diese Gefäße besitzen einen Mantel aus kollagenen Fibrillen, welcher die äußeren von den inneren Gefäßwandschichten trennt. An der Außenseite dieses Fasermantels bilden die ortständigen Pigmentzellen mit ihren zahlreichen Ausläufern ein dichtes Zellgeflecht. Zwischen diesem und dem darunter liegenden Fasermantel liegt, bei größeren Gefäßen sehr ausgeprägt, bei kleineren Gefäßen ihrem Kaliber entsprechend, ein weniger dichtes Nervengeflecht in der Form und Anordnung des adventitiellen Plexus, wie es in unterschiedlichem Ausmaß allen Gefäßen des Organismus eigen ist. Seine Darstellung mit Färbe- und Imprägnationsmethoden stößt auf keine Schwierigkeiten, daher sind sein Aufbau und seine strukturellen Besonderheiten in vielen Arbeiten eingehend beschrieben. Vielfach wurde diese Formation aus oben angeführten technischen Schwierigkeiten als einzige an den Gefäßen vorhandene nervöse Einrichtung gefunden. Die Darstellung der diesen Plexus bildenden Nervenfasern ist abhängig von der angewandten Methode. Mit Hilfe von Versilberungsmethoden wird vordringlich das kernhältige Protoplasma der neuralen Formationen, bei Verwendung der Osmium-Zinkjodidmethode seine fibrilläre Struktur zur Ansicht gebracht (Abb. 2, A, B).

Eine Darstellung nervöser Elemente innerhalb des Kollagenfasermantels ist mit Versilberungsmethoden nicht gelungen (Abb. 3). Hingegen konn-

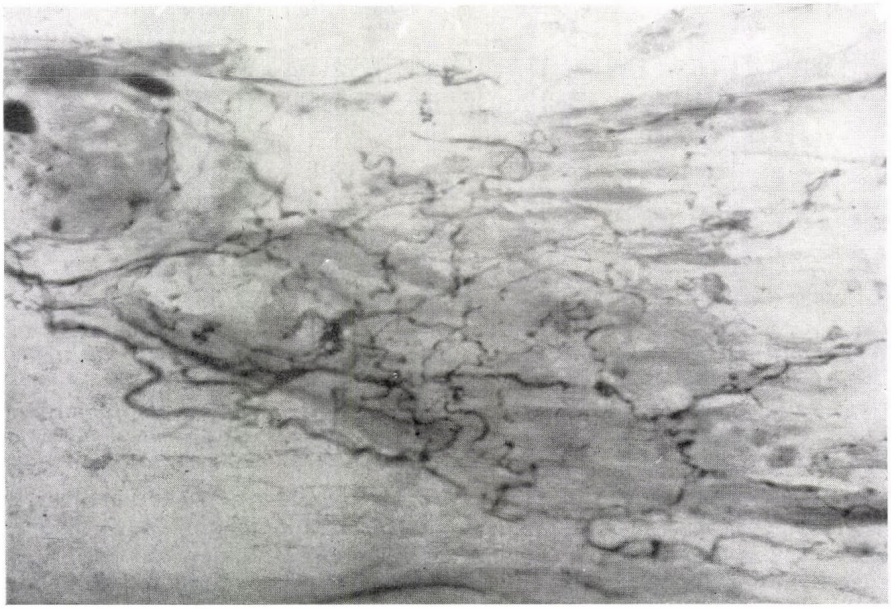

Abb. 1.—Harnblase, Kaninchen. Kleine Venen in den äußeren Wandschichten der Harnblase mit zahlreichen an die Intimazellen reichenden Nervenfasern. Osmium-Zinkjodidmethode, Reichert Biozet, 8 × 40 : 1

ten mit Hilfe der Osmium—Zinkjodidmethode Neurobfibrillen dargestellt werden, welche einzeln offenbar in einem durch die Anordnung der kollagenen Fibrillen bedingten spiralförmigen Verlauf durch diesen Mantel hindurchziehen (Abb. 4). Diese Nervenfasern bilden in größeren Gefäßen unmittelbar an der Basis der Intimazellen ein an dendritischen Endaufzweigungen reiches Geflecht (Abb. 5). Diese terminalen Aufzweigungen besitzen meist keine fibrilläre Struktur mehr, sondern erscheinen als zahlreiche einzelne Granula, die den ursprünglichen Fasernlauf fortsetzen. Am Ende solcher perlschnurartig angeordneter Granula liegt oft ein größeres stärker tingiertes derartiges Element. Man darf annehmen, daß dieses Gebilde das tatsächliche Ende der Fasern darstellt, doch können auf Grund lichtoptischer Untersuchungen bindende Aussagen hierüber nicht gemacht werden. Ebenso ist es nicht möglich, Aussagen über die Beziehungen dieser Endigungen zum Plasma der Intimazellen zu machen.

Der spiralförmige Verlauf der feinen Einzelfibrillen ist auch dann noch erhalten, wenn solche an kleinen Gefäßen über längere Strecken verfolgt werden können (Abb. 6). Selbst in unmittelbarer Nachbarschaft der Intimazellen, bzw. Endothelzellen, umkreisen die mit kleinen knopfförmigen Auftreibungen versehenen Nervenfasern in immer enger werdenden Windungen das Gefäßrohr.

Die Frage, ob diese Nervenfasern Begleitkerne besitzen oder über lange Strecken kernlos verlaufen, konnte bisher nicht eindeutig geklärt werden. Mit Hilfe der Osmium-Zinkjodidmethode kommen keine Kerne zur Darstellung. Eine Zuordnung der in geringer Zahl innerhalb des Kollagenfaser-

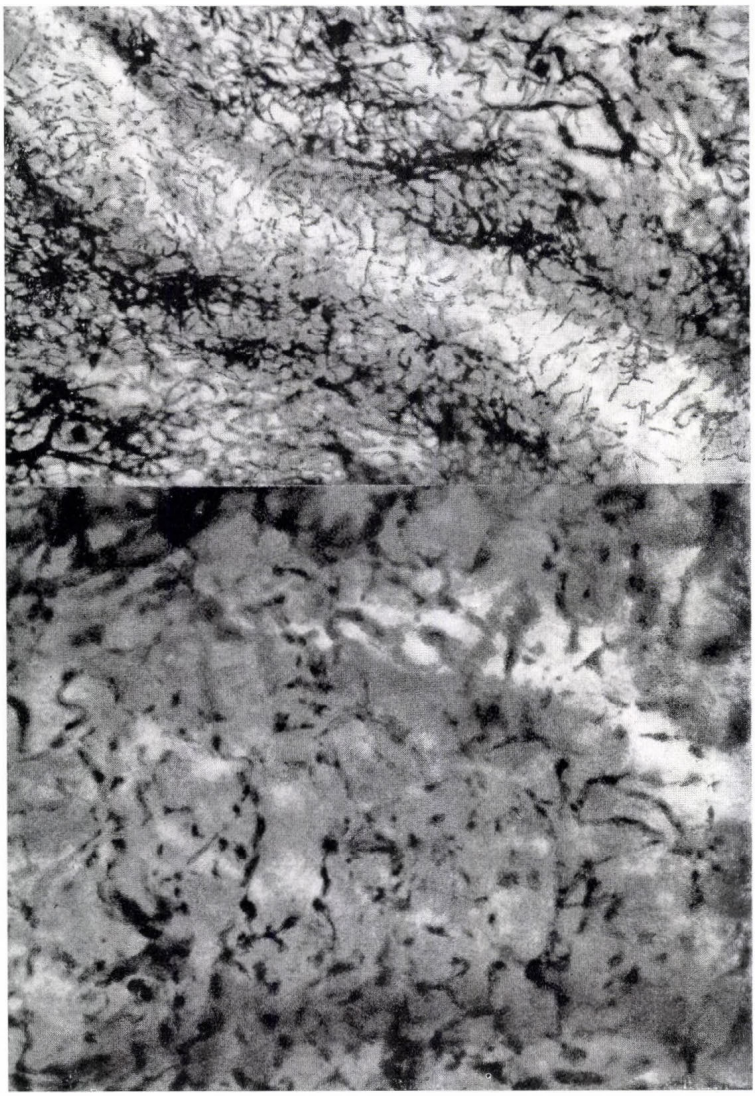

Abb. 2.—Iris, Kaninchen. *A* = Kleine Arterie an der Basis der Iris. *B* = Detail aus *A*. Osmium-Zinkjodimethode, Reichert Zetopan, *A* = 8×10 : 1 B = 8×40 : 1

mantels liegenden dreieckigen oder spindelförmigen Kerne, wie sie bei entsprechenden Gegenfärbungen zur Darstellung kommen, zu den Nervenformationen ist nicht eindeutig möglich. Wir nehmen an, daß diese Nerven ähnlich wie in der Hornhaut oder wie intraepithelial gelegene Fasern über lange Strecken ohne Begleitkerne verlaufen.

Eine Aussage über die Funktion dieser Nerven ist auf Grund der morphologischen Darstellung nicht möglich. Es ist aber anzunehmen, daß es sich

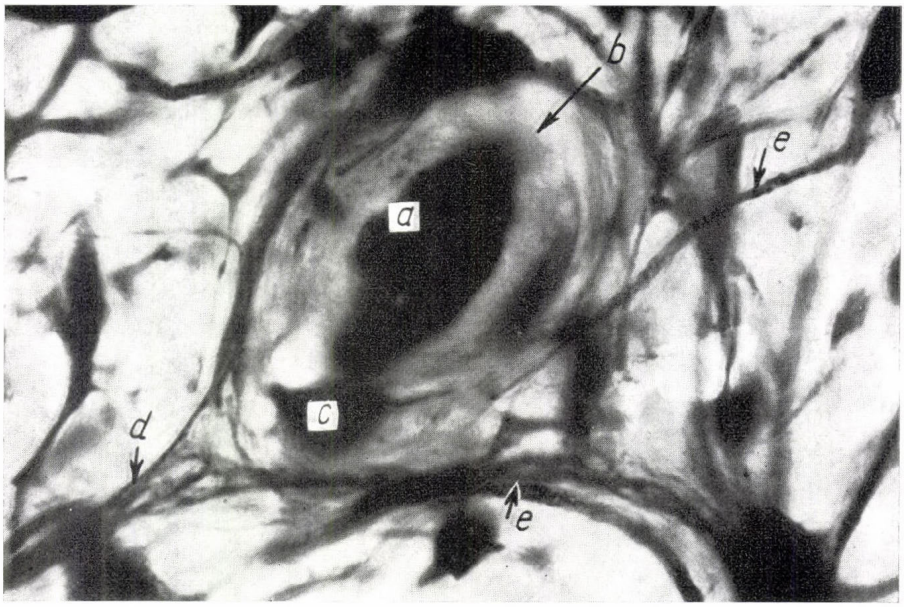

Abb. 3.—Iris, Kaninchen. Kleine Kapillare im mittleren Stromablatt. *a* = Gefäßendothelrohr mit einzelnen Kernen, *b* = Kollagenfasermantel, *c* = Kerne innerhalb des Fasermantels, *d* = an das Gefäß herantretende terminale neurale Formation, *e* = = Pigmentzellfortsätze. Versilberung nach Jabonero, Reichert Zetopan, 8×100 : 1

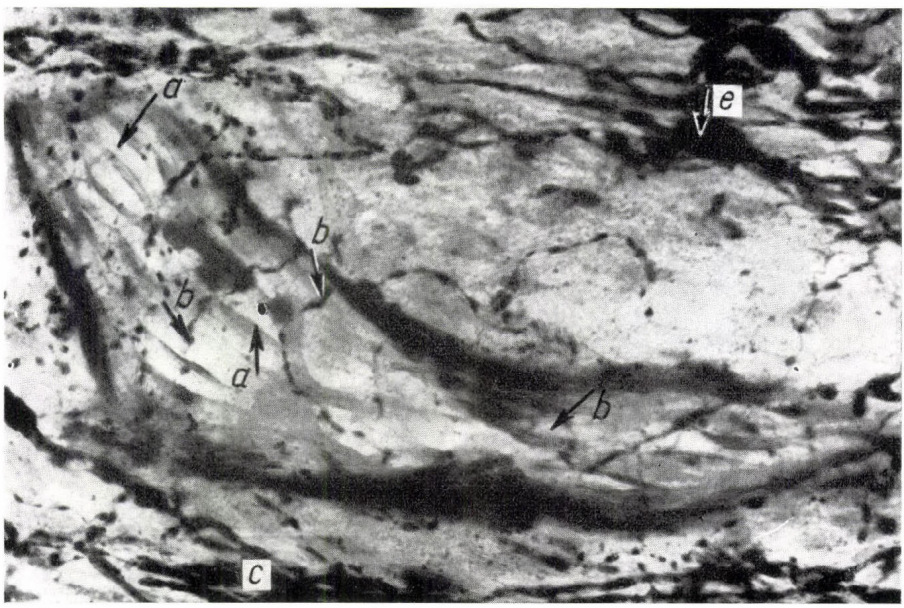

Abb. 4.—Iris, Kaninchen. In der Längsrichtung angeschnittenes Gefäß im Iristroma. *a* = Intimazellen, *b* = zahlreiche in spiralem Verlauf den Kollagenfasermantel durchstoßende Nervenfasern, *c* = Pigment-zellen, Osmium-Zinkjodidmethode, Reichert Biozet, 8×40 : 1

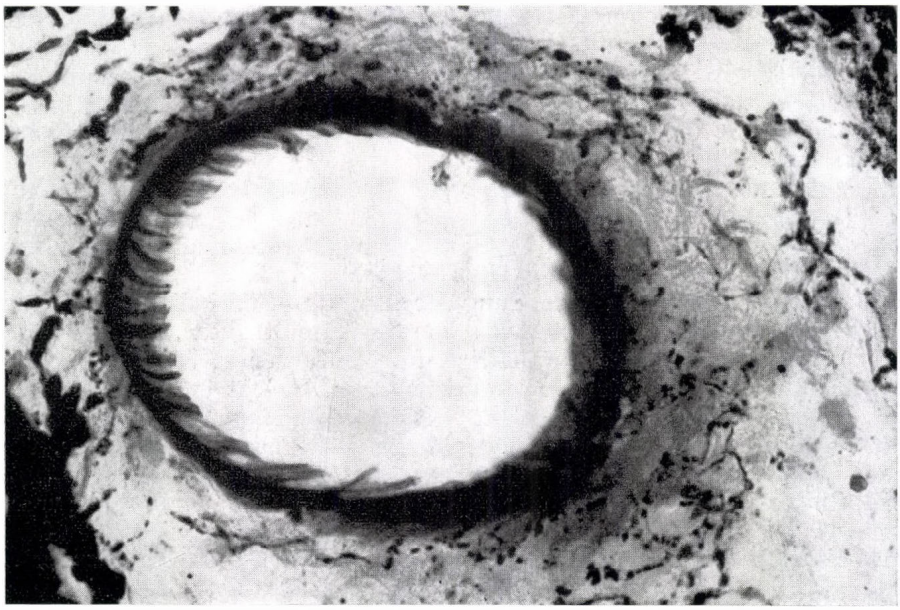

Abb. 5.—Iris, Kaninchen. Größeres, etwas schräg getroffenes Gefäßrohr, umgeben von einem dichter gelegenen Nervengeflecht. Osmium-Zinkjodidmethode, Reichert Biozet, $8 \times 40 : 1$

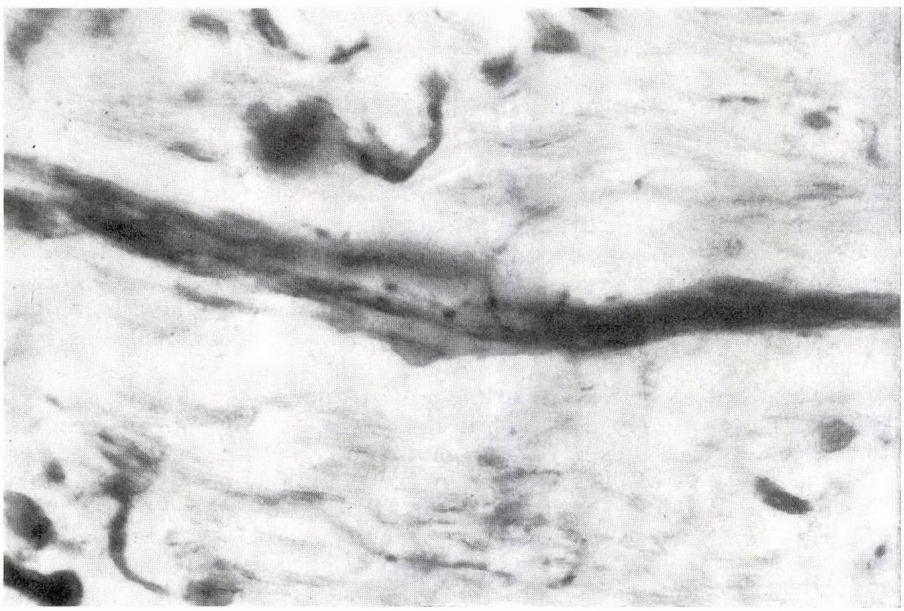

Abb. 6.—Iris, Kaninchen. Kleine Kapillare längsgetroffen mit einer das Endothelrohr umschlingenden, knopfförmige Auftreibungen enthaltenden Nervenfaser. Osmium-Zinkjodidmethode, Reichert Biozet, $8 \times 60 : 1$

um afferente, sensible Formationen handelt, wofür unter anderem die besenreisartige Aufsplitterung an ihren Enden sprechen würde, eine Endigungsform, die Fasern dieser Qualität auch in anderen Körperregionen eigen ist. Ihnen eine Funktion im Sinne einer sympathisch-motorischen Funktion zuzusprechen, ist in Ermangelung der Darstellung eindeutiger Endigungen an den Perizyten nicht möglich.

Zusammenfassend kann festgestellt werden, daß die Irisgefäße, wie Gefäße in allen übrigen Körperregionen, über einen dem Gefäßkaliber entsprechenden adventitiellen Plexus verfügen, dessen Darstellung hier wie überall auf keine technischen Schwierigkeiten stößt. Die ihn formierenden, nervösen Formationen zeigen bei Verwendung der Osmium—Zinkjodidmethode ähnlich wie bei Methylenblaufärbungen eine fibrilläre Struktur, in Versilberungen hingegen ist meist das kernhältige, fibrillenführende, bandförmig angeordnete Protoplasma dieser Formationen zur Ansicht gebracht.

Der besondere Bau der Irisgefäße mit der räumlichen Trennung der einzelnen Gefäßwandschichten ermöglicht die Sichtbarmachung feiner, über lange Strecken kernlos verlaufender Nervenfasern welche bis an die Gefäßintima heranziehen und dort in Form eines mitunter dichten Maschenwerkes endigen. Die Fasern spalten sich an ihren Enden besenreisartig auf und zerfallen im terminalen Bereich in einzelne fadenförmig aneinandergereihte Granula. Das Vorhandensein einzelner, die granuläre Reihe beschließender, größerer, stärker tingierter Elemente läßt darauf schließen, daß es sich dabei um wirkliche Endigungen dieser Fasern handelt. Die Beziehungen dieser Endigungen zu den Intimazellen konnten noch nicht eindeutig klargestellt werden. Diese Formationen werden als afferente Nervenelemente gedeutet.

BESPRECHUNG DER BEITRÄGE VON DR. BRETTSCHNEIDER UND DR. LASSMANN

Röhlich: 1. Gibt es gehäufte Invaginationen der Zellmembran in der synaptischen Region der glatten Muskelzellen? 2. Ist die flache, das synaptische Gebiet von außen begrenzende Zelle nicht der Fortsatz des Perineuriums? 3. Die in den auf der Endothelzelle wahrnehmbaren Nervenendigungen befindlichen synaptischen Bläschen sprechen nicht unbedingt für die efferente Struktur. Synaptische Vesikel sind auch in sensorischen Endigungen zu finden.

Molnár: Gibt es eine Kapillarinnervation auch im Gehirn?

Walberg: As an answer to the question asked by Dr. Molnár I can say that terminal nerve fibers have been shown to be in contact with the basement membrane of capillaries only in the neurohypophysis. Here Palay in one picture has shown that the nerve fibers are in direct contact with vessel wall, but a layer of connective tissue separates an outer basement membrane from an inner. The situation is therefore here very special.

Lassmann: Auf Grund der lichtoptisch mit unterschiedlichen Imprägnationsmethoden erhobenen Befunde wird heute der Standpunkt vertreten, daß der größte Teil der Kapillaren über keine eigene Innervation verfügt. Sowohl die von Zweifach und Nelemans als auch die von Grigorewa vertre-

tenen Thesen nehmen diesen Standpunkt ein. Bei Anwendung färberischer Methoden wie der von Feyrter angegebenen Einschlußfärbung mit Ehrlichs saurem Hämatoxylin am nativen Gefrierschnitt werden jedoch viele Kapillaren von fädigen Elementen begleitet, welche man als neurale Elemente ansprechen muß (mündliche Mitteilung von Feyerter). Aus diesen divergierenden Befunden geht unseres Erachtens hervor, daß mit Hilfe der üblichen Imprägnationsmethoden nicht unbedingt alle nervösen Elemente zur Ansicht gebracht werden und dadurch derartig divergierende Auffassungen zustande kommen. Wie aus unserem letzten hier gezeigten Bild zu ersehen ist, finden sich auch in der Iris des Kaninchens Kapillaren, welche über lange Strecken in der für die Irisgefäße typischen spiraligen Verlaufsform von einer Nervenfaser begleitet werden. Hier kann man nicht mehr von einem zufälligen gemeinsamen Verlauf beider Elemente sprechen, sondern darf auf Grund des Verhaltens beider Elemente zueinander eine funktionelle Zusammengehörigkeit annehmen. Wie an anderen Orten haben wir, wie auch andere Untersucher, bisher keinen eindeutigen Beweis für die Innervation von Perizyten erheben können, wodurch solche Begleitfasern in gewisser Beziehung als motorische Innervation gedeutet werden könnten. Inwieweit sie als afferente Formationen anzusehen sind, wie man vielleicht per exclusionem anzunehmen geneigt ist, kann ebenfalls heute noch nicht festgestellt werden.

Die Schilddrüsengefäße besitzen, wie ich auf Grund eigener Untersuchungen feststellen kann, einen außerordentlich reichen adventitiellen Nervenplexus. In keinem anderen Organ des Körpers habe ich bisher eine derartig dichte Anordnung und Häufung nervöser Elemente in diesen Gefäßanteilen gefunden. Ich möchte diesen Umstand damit erklären, daß die Schilddrüsengefäße aller Kaliber entgegen der oft vertretenen Ansicht außerordentlich reichlich mit Rezeptoren ausgestattet sind, deren zuführende Fasern natürlich auch innerhalb des adventitiellen Geflechtes verlaufen und damit dessen außerordentliche Dichte bedingen.

Szentágothai: Es freute mich besonders, die schönen elektronenmikroskopischen Befunde von Herren Brettschneider zu sehen, die bekanntlich wesentlich dazu beitragen, den alten Streit um die vegetativen nervösen Endorgane im Sinne der Neuronenlehre zu entscheiden. — Bezüglich einer Innervation der Kapillaren war ich auf Grund lichtmikroskopischer Erfahrungen auch recht skeptisch. Heute müssen wir jedoch die Entscheidung dieser Frage der Elektronenmikroskopie überlassen.

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G. Lassmann (dzt. im Druck) Beitrag zur Innervation der Blutgefäße

SECTION THREE

NEUROMUSCULAR JUNCTIONS

Symp. Biol. Hung., **5**, 143—147 (1965)

ÜBER DIE QUANTITATIVEN VERÄNDERUNGEN DER CHOLINESTERASE-AKTIVITÄT DER MYONEURALEN VERBINDUNG IN PATHOLOGISCHEN ZUSTÄNDEN

von

B. Horányi und G. Bozsik

NEUROLOGISCHE UNIVERSITÄTSKLINIK
BUDAPEST, UNGARN

Zum Nachweis der erhöhten Cholinesterase-Aktivität der myoneuralen Junktion (Marney und Nachmansohn, 1938) stehen bewährte Methoden der Enzymhistochemie zur Verfügung. Besonders nach der Einführung der Thiocholin-Methode befaßten sich bedeutende Arbeiten (Koelle und Friedenwald 1949, Couteaux und Taxi 1952, Holt 1956, Coërs 1959, Gerebtzoff 1959, Csillik und Sávay 1955) mit der Cholinesterase-Aktivität der motorischen Endplatte unter normalen Bedingungen sowie mit ihren Veränderungen in pathologischen Zuständen, vor allem nach Denervation, und gelangten zu wichtigen—im wesentlichen übereinstimmenden—Feststellungen. Um so widersprechender sind die diesbezüglichen biochemischen Angaben, die bald über eine erhebliche Abnahme (Martini und Torda 1937), bald über eine bedeutende Zunahme (Marney und Nachmansohn, 1937) oder über eine unveränderte Aktivität (Brooks und Myers 1952) nach der Denervation berichten. Da die motorische Endplatte infolge ihrer elektiv hohen Aktivität auch als Modell zur Untersuchung der Cholinesterase-Aktivität sehr geeignet ist (Holt 1956), versuchten wir, den Veränderungen mit der mikroskop-photometrischen Methode, an histologischen Schnitten quantitativ näherzukommen.

Untersuchungsmaterial und Methode. Zu unseren Untersuchungen verwendeten wir voll entwickelte, 2000—2500 g schwere Kaninchen — insgesamt 15 Tiere. Bei 6 Kaninchen schnitten wir in Evipan-Narkose nach hoher Glutäal-Freilegung einen 1 cm langen Teil vom rechtsseitigen N. ischiadicus aus und töteten je 2 Tiere nach 1,7 und 15 Tagen. Anderen 6 Tieren verabreichten wir 120 mg Triorthokresylphosphat (TOCP) je kg Körpergewicht intraperitoneal, und töteten je 2 Tiere nach 2, 6 bzw. 12 Stunden. Zur Kontrolle dienten 3 Tiere. Unmittelbar nach der Tötung der Tiere wurde der M. soleus auf beiden Seiten freipräpariert und in einem Kühlschrank in 6%igem neutralem Formalin bei 4 °C fixiert. Dann werden Gefrierschnitte von 60 μ hergestellt. Nach einer vier stündigen Standardfixierung wandten wir die von Gerebtzoff (1959) modifizierte Azetylthiocholin- und Butyrylthiocholin-Methode an, mit einer Standard-Inkubationszeit (10 Minuten bei 37 °C). Zor Blockierung verwendeten wir eine di-Isopropylfluorophosphat-Lösung in einer Konzentration von 10^{-7} Mol. Wir entwässerten die Schnitte in einer aszendierenden Alkohol-Serie, und nach Bedecken mit Xylol bzw. Kanadabalsam bestimmten wir die Cholinesterase-Aktivität der Endplatten photometrisch. Die Messungen wurden mit einem Mikroskop-Photometer Typ Lison vorgenommen, dessen genaue Beschreibung in einer früheren Arbeit (Horányi und Bozsik 1962) zu finden ist.

Die Immersionsvergrößerung haben wir auf das 3000fache ausprojiziert, in einer motorischen Endplatte im allgemeinen 10 (bei großer Streuung 20) Punkte mit einer 4-mm-Meßblende (1,396 μ Quadratgebiet) abgemessen und den Mittelwert bestimmt. Die Auswahl der Meßpunkte erfolgte nach Möglichkeit der statistischen Verteilung entsprechend. In den einzelnen Tiergruppen haben wir wahllos im Durchschnitt 50 motorische Endplatten abgemessen und aus den erhaltenen Werten den Mittelwert sowie den Standard-Fehler bestimmt. Als Arbeitseinheit diente das Multiplikationsprodukt der Gebietseinheit und der Extinktion, das mit 10 multipliziert wurde. Die Messungen erfolgten auf 517 nm, mit einem Zeissschen Metallinterferenzfilter (Halbwertbreite 7,0 nm). Zur Bestimmung der Signifikanz zwischen den einzelnen Gruppen nahmen wir auf Grund der Studentschen Formel einen *t*-Test vor. Jede Gruppe bestand aus wenigstens 2 Tieren, und die Messungen wurden an den von beiden Tieren stammenden Präparaten teilweise durchgeführt. Die Dicke der Schnitte wurde mit einem Suhlschen Mikrometer (Fehlergrenze $\pm$ 2 μ) kontrolliert, und da es sich um Abweichungen von weniger als 10 μ handelte, wurden keine Korrektionen in den Berechnungen vorgenommen.

Ergebnisse. Unter normalen Bedingungen sind zwischen der Azetyl- und-Butyryl-Thiocholin-Methode und der Azetyl-Thiocholin-Methode nach DFP-Blockierung die folgenden Abweichungen nach einer Inkubation von 10 Minuten zu sehen (Abb. 1). Auf Grund der Messungen entspricht dies in Arbeitseinheiten: Azetylthiocholin 7,03 $\pm$ 0,16, Butyrylthiocholin 2,13 $\pm$ $\pm$ 0,06, nach DFP-Hemmung Azetylthiocholin 4,07 $\pm$ 0,14.

Aus diesen Angaben geht hervor, daß im M. soleus des normalen Kaninchens der mit der Butyrylthiocholin-Methode erhaltene Wert nur 30% der mit der Azetylthiocholin-Methode gemessenen Aktivität entspricht, was damit gleichbedeutend ist, daß ein ziemlich hoher Pseudocholinesterase-Gehalt in der motorischen Endplatte zu finden ist. Nach DFP-Hemmung (wodurch in erster Linie die Pseudocholinesterase gehemmt wird) entspricht die verbleibende Aktivität 58%, also neben dem fehlenden Pseudocholinesterase-Wert ist zu 12% entweder auch die spezifische Cholinesterase gehemmt, oder eine andere, auf DFP nicht reagierende Esterase ist anwesend. Diese Daten stimmen im allgemeinen mit den Angaben des Schrifttums überein. Holmstedt (1957) hat bei einer DFP-Blockierung von dieser Konzentration gefunden, daß außer der völligen Hemmung der Pseudocholinesterase auch die Menge der spezifischen Cholinesterase zu etwa 5% gehemmt ist.

Die mit der Azetylcholin-Methode bestimmte Aktivität entspricht nach ein tägiger Denervation 5,93 $\pm$ 0,16, nach 7tägiger Denervation 5,36 $\pm$ 0,19, und nach einer 15tägigen Denervation hat sich dieser Wert auf 3,62 $\pm$ 0,15 vermindert (Abb. 2).

Die Ergebnisse der Denervation können folgendermaßen ausgewertet werden: Die Verminderung der Cholinesterase-Aktivität ist bereits nach 1 Tag als signifikant zu bezeichnen ($P < 0{,}01$), und sie setzt sich kontinuierlich fort; nach 15 Tagen enstpricht die Verminderung bereits 51% des ursprünglichen Wertes. Es sei erwähnt, daß sich in diesem letzten Stadium bereits feinere morphologische Veränderungen an den motorischen Endplatten unterscheiden lassen: ihre Form ist unregelmäßiger, die Reaktion in den Lamellen ungleichmäßiger und ihre auffallende

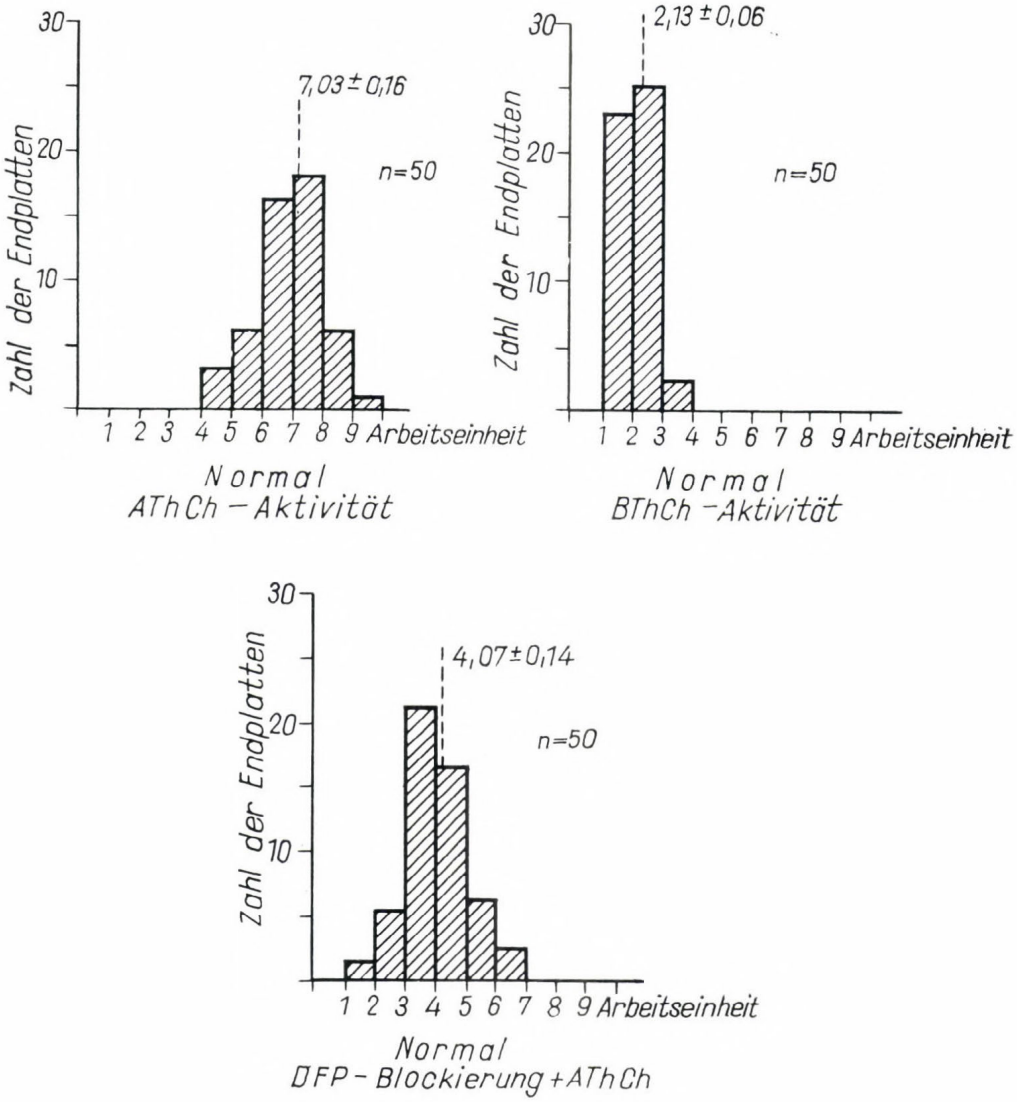

Fig. 1.

Verblassung bereits mit bloßem Auge sichtbar. Wenn wir unsere Ergebnisse mit den Literaturangaben vergleichen, so kann festgestellt werden, daß sie der mit den histochemischen Methoden im allgemeinen beobachteten langsamen, jedoch kontinuierlichen Verminderung entsprechen [Couteaux und Taxi (1952), Coërs (1959), Gerebtzoff (1959), Csillik und Sávay (1955)]. Die Verminderung der Aktivität ist kontinuierlich und signifikant, und am 15. Tag enstpricht sie, was ihren Grad anbelangt, etwa den biochemischen Angaben von Couteaux und Nachmansohn (1942).

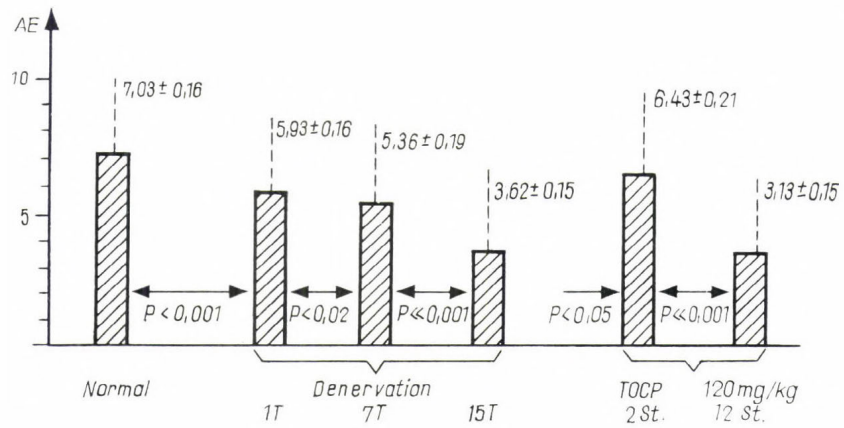

Fig. 2.

Nach der intraperitonealen Verabreichung von 120 mg TOCP/kg Körpergewicht wurde beim 2 Stunden später getöteten Tier mit der Azetylthiocholin-Methode ein Wert von 6,43 $\pm$ 0,21, und 12 Stunden nach der Verabreichung ein Wert von 3,13 $\pm$ 0,15 erhalten (Abb. 2). Zwei Stunden nach der TOCP-Gabe ist die Verminderung zwar noch geringfügig, jedoch signifikant ($P < 0{,}05$). Wahrscheinlich sind dafür die Resorptions- und Transformationsprozesse verantwortlich; schwerere toxische Symptome ließen sich bei diesen Tieren nicht einmal klinisch nachweisen. Bei den 12 Stunden überlebenden Tieren ist die Verminderung bereits erheblich: 44% der ursprünglichen Aktivität. Dies entspricht etwa den auf das Parathion bezüglichen Literaturangaben; immerhin ist bekannt, daß die Aktivität der einzelnen Organe bei den organischen Phosphatvergiftungen nicht in der gleichen Weise betroffen ist.

Zusammenfassung. Im *M. soleus* des Kaninchens wurde die Cholinesterase-Aktivität mit der mikroskop-photometrischen Methode quantitativ untersucht. Unter normalen Bedingungen wurden bei Anwendung der Azetylthiocholin-Methode 7,03 $\pm$ 0,16 Arbeitseinheiten, bei der Butyrylthiocholin-Methode 2,13 $\pm$ 0,06 Arbeitseinheiten und nach Hemmung mit DFP mit der Azetylthiocholin-Methode ein Wert von 4,07 $\pm$ 0,14 Arbeitseinheiten erhalten. Auf diese Grundwerte bezogen haben wir bei denervierten Tieren bereits vom ersten Tag an eine langsame, jedoch signifikante Verminderung beobachtet, am 15. Tag entsprach der Wert lediglich 51% des ursprünglichen Wertes. Eine ähnliche Veränderung fanden wir auch nach TOCP-Vergiftungen.

DISKUSSION

Mária Wollemann: Worauf wurde die Arbeitseinheit bezogen? Was für eine DFP Konzentration wurde verwendet? Wurde das DFP in vitro oder in vivo gegeben? Welchen Vorteil hat die Methode gegenüber dem mikrogasometrischen Verfahren?

Kadanoff: An die Herren Kollegen Bozsik und Horányi möchte ich die Frage stellen, ob die Veränderungen und was für welche in den segmentierten, d. h. aus Läppchen bestehenden Endplatten festgestellt haben und ob sie zwei oder mehrere motorische Endplatten an einer und derselben Muskelfaser beobachtet haben und wie oft. Untersuchungen, die am Anat. Institut Sofia unter Anwendung histochemischer Methoden durchgeführt wurden, haben gezeigt, daß unter den motorischen Endplatten bei den verschiedenen Skelettmuskeln Unterschiede in bezug auf Form, Größe und Aufbau bestehen und daß ca. 12—15% der Muskelfasern mehr als eine motorische Endplatte (gewöhnlich zwei) besitzen.

Bozsik: Als Arbeitseinheit diente das Multiplikationsprodukt der Gebietseinheit und der Extinktion, das mit 10 multipliziert wurde. Gebietseinheit war bei jeder Messung dieselbe (1,396 μ Quadratgebiet). Wir verwendeten das DFP in vitro, in einer Konzentration von 10^{-7} Mol. Die Ergebnisse der Methode stehen gleich dem mikrogasometrischen Verfahren und sind anwendbar auch im Schnittpräparat.

Horányi: Unsere Erfahrungen beziehen sich in jedem Falle auf den M. soleus des Kaninchens. Die zahlenmäßigen Verhältnisse haben wir zwischen den Muskelfasern und den motorischen Endplatten außer acht gelassen, weil wegen mikrotechnischer Ursachen der M. soleus in mehrere Blöcke zerschnitten wurde.

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ACETYLCHOLINE-INDUCED CALCIUM RELEASE IN THE POST-JUNCTIONAL SARCOPLASM*

by

G. SÁVAY

DEPARTMENT OF ANATOMY, UNIVERSITY MEDICAL SCHOOL
SZEGED, HUNGARY

B. CSILLIK**

DEPARTMENT OF PHARMACOLOGY, UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA, PA., USA

Cross-striated muscles are known to contain a considerable amount of calcium (Brink 1954). As shown by Heilbrunn and Wierzinsky (1947) calcium is the only physiological ion which, if injected at low concentrations into muscle fibers, causes shortening. Direct action of calcium on myofibrils has also been reported (Weber 1962). It has been suggested that release of calcium, bound normally to the sarcoplasmic reticulum, might be the link between membrane depolarization and myofibrillar contraction (Bianchi 1961).

In spite of the physiological evidence, the microscopic localization of this activity-induced calcium release is unknown. According to the fact that in resting muscles calcium is present in a 'bound' form (Shanes 1959), it cannot be detected histochemically [except by microincineration technique (Scott 1932), which visualizes both bound and free calcium]. On the other hand, no attempt has been performed to localize calcium in experimentally stimulated muscles.

Several years ago, Koshtoyants and co-workers (1951) have shown that stimulation of the cardiac results in the appearance of histochemically detectable calcium at cell boundaries. This study began from the premise that stimulation might result in a similar effect in skeletal muscle, too. It was found, however, that in mammalian skeletal muscle both electrical and chemical stimulation resulted in histochemically demonstrable calcium localized strictly to the post-junctional sarcoplasm (sole-plate) right underlying the motor nerve terminal, as reported briefly in a previous paper (Csillik and Sávay 1963). It will be shown in this publication that this calcium liberation is a direct consequence of the action of acetylcholine on the post-junctional membrane. It will be shown, furthermore, that by means of staining this liberated calcium it is possible to localize microscopically the acetylcholine-affected sites both in normal and in denervated muscle. The extension of the acetylcholine receptor can thus be estimated in histochemical sections.

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** Present address: Department of Anatomy, University Medical School, Szeged.

METHODS

72 albino rats (150—200 g) of both sexes were used. The animals were killed by decapitation, the diaphragm and/or the deep plantar muscle (m. quadratus plantae) removed and cut on the cryostat (10—15 μ sections). The sections were transferred immediately into acetone (or other suitable fixative) for 2 minutes and floated in 2% sodium barbitale for 2 minutes.

Instead of acetone, aethylalcohol (70%), methylalcohol, xylene, benzene, ether, petrol, carbon tetrychloride, or 10% formalin saturated with NaCl can be used. If a cryostat is not available, the fresh muscles fixed in any of the aforementioned fixatives may be cut on the freezing microtome provided that the samples have been floated in pH = 8 distilled water for 10 minutes after fixation.

Staining of calcium has been performed by one of the following procedures:

(1) Staining with alizarin red (1 per cent. aqueous solution for 10 minutes). Wash in distilled water, mount in glycerol. Photomicrographs must be taken immediately, as the red color fades very rapidly.

(2) Staining with aqueous haematoxylin (10 minutes). Wash in distilled water, mount in glycerol. The blue color is more stable than that of alizarin, however, even these preparations undergo fading in 2 or 3 weeks.

(3) Permanent sections, demonstrating virtually the same localization of calcium as the alizarin red or haematoxylin treated ones, can be obtained by means of an aspecific exchange reaction between Ca and heavy metals. This reaction is essentially identical with the final step of the Gomori phosphatase reaction. The sections are treated with a 5 per cent aqueous solution of $Co(NO_3)_2$ or $Pb(NO_3)_2$, rinsed in distilled water and developed in a 2 per cent aqueous solution of Na_2S or $(NH_4)_2S_2$. Sections may be mounted in glycerol-jelly, in glue or—after dehydration—in Permount. The black color is indefinitely stable.

Stimulation of the muscles has been performed either chemically or electrically. Chemical stimulation has been achieved by injecting intraperitoneally neostigmine (Prostigmin 'Roche' or Stigmosan 'Chinoin') 1 mg/kg. Electrical stimulation has been performed by means of a square wave generator (1.2 V, 50 Hz, 45 minutes), either directly or indirectly.

All pictures are longitudial sections of the rat diaphram. Except Fig 1. the sections were with one of the heavy-metal reactions (either cobalt nitrate or lead nitrate).

Fig. 1.—Calcium release in the motor end plattes in the diaphragm of the rat injected with 1 mg/kg neostigmine, 8 minutes prior to i.p. decapitation. Alizarin red. Arrow points to one of the end plates. *N* *N* = nerve trunk × 160

Fig. 2. — Localization of the liberated calcium in the sole plasm (Doyère-cytoplasm) of the motor end plates, 8 minutes after i.p. injection of mg/kg neostigmine. *N* = contour of nerve fibers (unstained), *a*, *b* and *c* = sole nuclei, × 1200

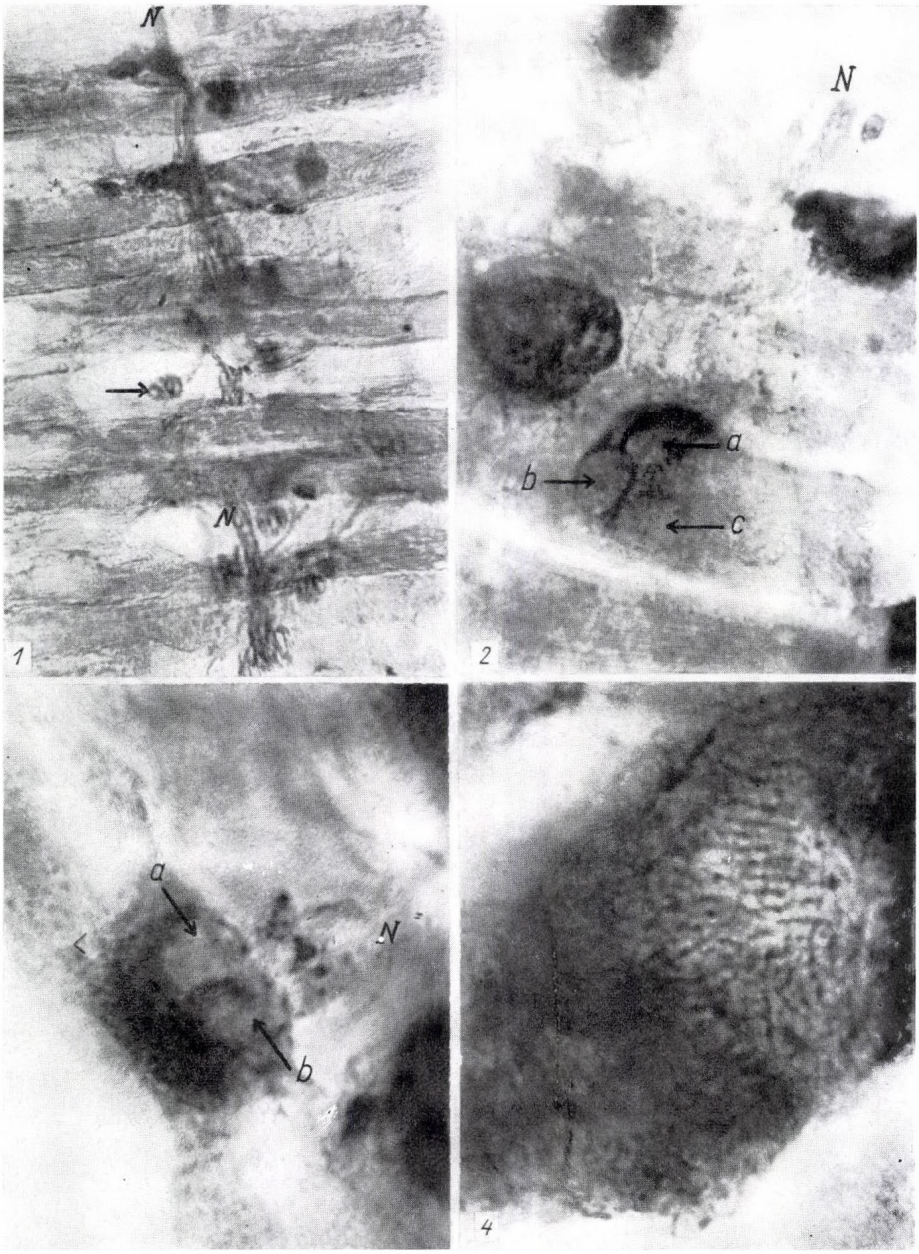
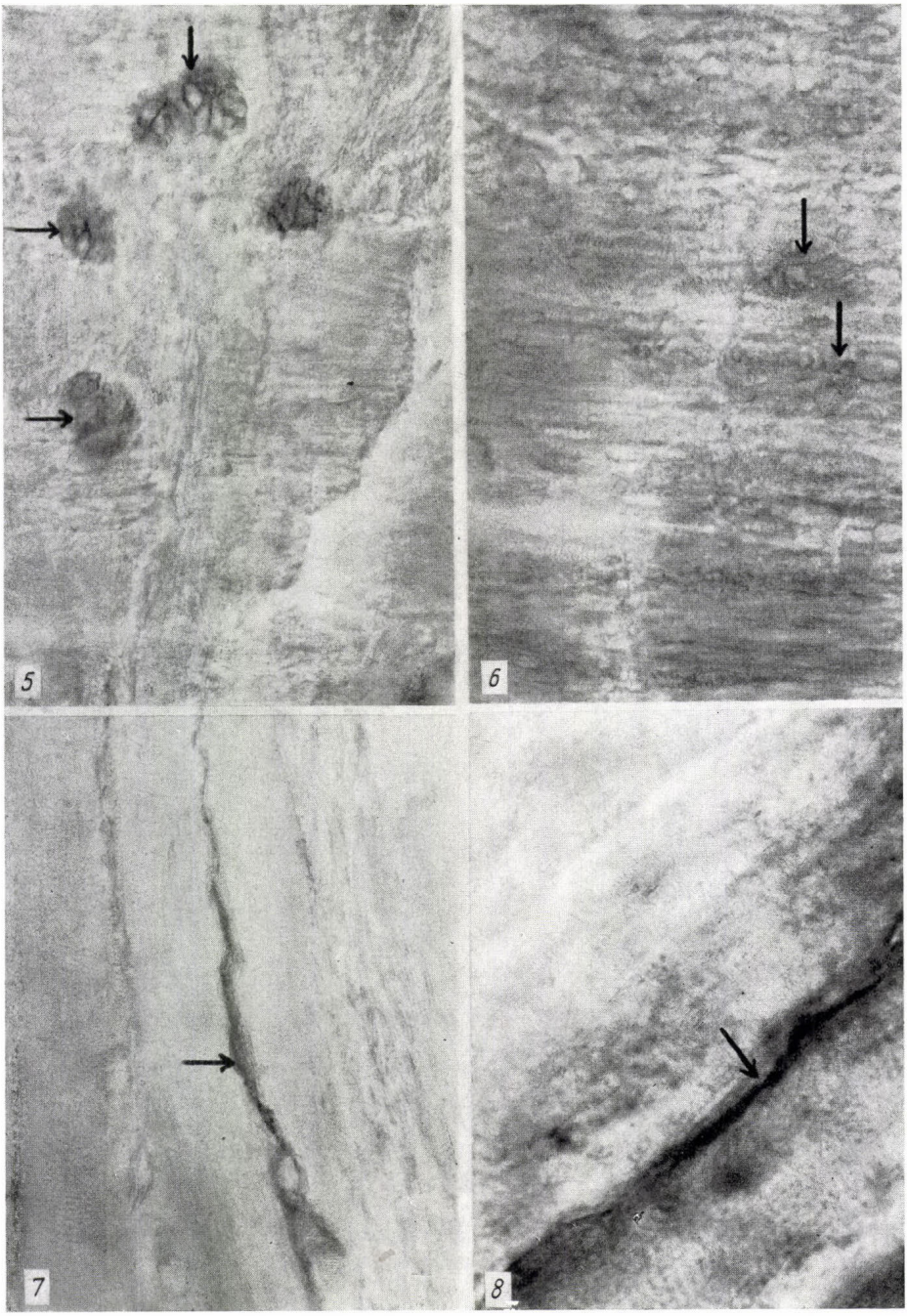


Fig. 3.—Structural details of the localization of the liberated calcium in the sole plasm of the motor end plate, 8 minutes after i.p. injection of 1 mg/kg neostigmine. N = nerve-fiber entering the end plate, a and b = sole nuclei. Note the granular localization of the liberated calcium in the sole plasm, × 2000

Fig. 4.—Virtually cross-section of a muscle fiber right below the plane of a motor end plate, 8 minutes after i.p. injection of 1 mg/kg neostigmine. Note the localization of the liberated calcium in the sarcoplasmic reticulum. This pattern should be compared with Abb. 7 in P. Krüger and P. G. Günther's publication *(Acta Anatomica 28*, 135—149, 1965), × 2000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RESULTS

(1) In normal *resting muscles*, as expected, no calcium could be demonstrated histochemically. Only mast cell granules showed occasionally a faint staining.

Three minutes after i.p. administration of *neostigmine* (1 mg/kg), a slight staining of the myoneural junctions occurred, which became considerably stronger if the fasciculation of the muscle, evoked by this drug, lasted 5 minutes or longer (Fig. 1). Examination of the sections under high power revealed that the staining took place almost exclusively in the protoplasm of the 'Doyère hillock', that is in the post-synaptic sarcoplasm underlying the telodendrial nerve fibre (Fig. 2). The reaction was confined to granules of the size of 1—2 μ or less, located in the cytoplasm of the large 'fundamental cells' of the sole-plate. Although the fundamental nuclei themselves did not exhibit any reaction, their outlines were clearly marked by the reaction product (Fig. 3). 8—10 minutes after the injection of neostigmine, the reaction of the junctions was sometimes accompanied by a more or less marked staining of the neighboring sarcoplasm; here the reaction product was located among the myofibrils, suggesting the localization of liberated calcium in the sarcotubular system (Fig. 4).

Electrical stimulation of the sciatic nerve resulted in similar calcium-liberation in the deep plantar muscle. *Direct electrical stimulation* of the muscle yielded the same result. *d-tubocurarine*, was injected intraperitoneally in a dose of 60 μg/kg 15 minutes prior to direct or indirect electrical stimulation, it completely prevented the calcium-release: the histological pattern obtained was identical with that of a resting muscle.

Acetylcholine (1—5 mg/kg) or *acetyl-beta-methylcholine* (Mecholil, 1—5 mg/kg) failed to produce any calcium liberation. Yet *carbaminoylcholine* (Carbachol, 1 mg/kg) resulted in similar calcium release in the sole plates of myoneural junctions like neostigmine or electrical stimulation.

Cyanide, *iodoacetate* and *sodium fluoride* did not evoke histochemically detectable calcium liberation. *Dinitro-phenol*, a discoupling agent, however, if injected intraperitoneally in three, daily repeated doses of 20 mg/kg, resulted in histological patterns similar to neostigmine, Carbachol or electrical stimulation.

(2) The effect of *denervation* on the calcium liberation was studied on partially denervated hemidiaphragms. The ventral branch of the phrenic nerve has been transected by means of an intramuscular incision, performed through an abdominal opening. Accordingly, in the ventral half of the

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Fig. 5.—Calcium release in the end plates 24 hours after intramuscular transection of the phrenic nerve and 7 minutes after i.p. injection of 1 mg/kg carbaminoylcholine. Arrows point to the end plates, × 400

Fig. 6.—Absence of calcium release in the end plates 5 days after intramuscular transection of the phrenic nerve and 7 minutes after i.p. injection of 1 mg/kg carbaminoylcholine. Arrows point to the sites of the late end plates, × 400

Fig. 7.—Calcium release in a linear structure (arrow) on the surface of a muscle fiber, 15 days after intramuscular transection of the phrenic nerve and 7 minutes after i.p. injection of 1 mg/kg carbaminoylcholine, × 400

Fig. 8.—Calcium release in a linear structure (arrow) on the surface of a muscle fiber of a 3 day old rat baby, 6 minutes after i.p. injection of 15 mg/kg neostigmine, × 800

hemidiaphragm nerve fibers underwent degeneration, while those in the dorsal portion remained intact. As a result, ventral muscle fibers were denervated, while the dorsal muscle fibers of the same muscle, with an intact innervation, could be used as controls.

Neostigmine, if injected i.p. during the first postoperative days, resulted in the usual pattern of calcium liberation both in denervated and in normal muscle fibers. (Fig. 5). From the third postoperative day on, the amount of freed calcium diminished rapidly in denervated muscle fibers and from the fifth postoperative day on, no calcium liberation could be observed in denervated muscle fibers after injection of neostigmine. (Fig. 6). On the other hand, 8 and 15 days after transection of the ventral branch of the phrenic nerve, i.p. injection of carbaminoylcholine resulted in calcium liberation, confined to thin linear structures amongst nuclei on the surface of the muscle fibers. The length of this linear structure was 50—70 microns 8 days after transection of the nerve and extended to 100—200 micra 15 days postoperatively. (Fig. 7). The center of these linear structures was at the site of the late end-plate, as it could be judged by the presence of myelin debris.

(3) *Ontogenetic aspects* of calcium liberation was studied on 18 day old rat embryos and on 1—14 day old rat babies. In the diaphragm of 18—day old embryos a light calcium staining could be achieved, confined to 50—100 micra long linear structures at the surface of muscle fibers, 8 minutes after 1 mg/kg neostigmine has been injected intraperitoneally to the pregnant rat. The reaction was similar, although markedly stronger in new-born and 1—5 day old rat babies, injected intraperitoneally with neostigmine. (Fig. 8). From the sixth postnatal day on, the reactive area gradually decreased, with a simultaneous enhancement of the reaction. It reached its normal extension and shape on the tenth postnatal day.

CONCLUSIONS

There is little doubt about the fact that the substance demonstrated in these experiments is ionized calcium. Although alizarin red, haematoxyline and the even more aspecific heavy metal exchange reactions give positive staining with other cations (e.g., magnesium), too, none of these is present in sufficient amount to account for the staining. The fact that 'regular' histological fixatives (neutral formalin, osmium tetroxide, etc.) failed to preserve freed calcium may be a consequence of the extreme solubility of the ionized form of this cation. Some rough precipitating agent seems to be necessary to keep the free calcium at the very site of its liberation. Acetone, alcohol, carbon tetrachloride, etc., as 'rough' histological fixatives, destroy the fine submicroscopical coacervate structure of the protoplasm, thus keeping free calcium in the precipitate. It could be argued that the lipid solvent character of these agents plays a major role in this respect; this will be ruled out, however, by the fact that NaCl-saturated formalin has the same effect. Still exists, however, the possibility, that calcium liberation is not restricted to the cytoplasm of the Doyère eminence and the neighboring parts of the sarcoplasmic reticulum but it extends to more remote areas, too, which are not preserved by the fixative. The histological

pictures clearly point to an enhanced sensitivity of the cytoplasmatic area underlying the zone of innervation, or, in other words, the Doyère cytoplasm seems to be the most vulnerable part of sarcoplasmatic calcium stores. Electron microscopical investigations suggest a direct contact between sarcotubular system and structures in the Doyère cytoplasm. It would be of particular interest to check electron microscopically the fine structural localization of freed calcium.

What is the cause of the calcium liberation? Our experiments suggest the role of acetylcholine. Electrical stimulation of both the nerve and the muscle results in acetylcholine release; neostigmine preserves the small amounts of acetylcholine spontaneously released by the miniature end-plate potentials (Liley 1956); carbaminoylcholine, an acetylcholine-like ester, which is not hydrolyzed by acetylcholinesterase, mimics the effect of a prolonged acetylcholine action. It seems likely, therefore, that the cytochemical effects observed in these experiments are evoked either by acetylcholine (or a related compound) itself, or by the action of the ester on the post-junctional membrane. The fact that pre-treatment of the animal with *d*-tubocurarine (which is supposed to interact with acetylcholine at the receptor sites) prevented the effect of nerve stimulation to produce histochemical alterations, suggests that the calcium release in the post-junctional sarcoplasm is a consequence of the depolarization of the post-junctional membrane by the ester. Thus, with some simplification, the calcium release can be looked upon as a morphological sign of the action of acetylcholine upon the receptor.

The failure of neostigmine to produce calcium release five days after degeneration of the terminals is in good accordance with the above assumption, since the degeneration of the nerve fibers has destroyed the source of acetylcholine, which could have been 'preserved' by the enzyme inhibitor. It is a well-known fact that the enzyme acetylcholinesterase is still present at the post-synaptic site for a long period after the nerve fibers have undergone degeneration (Couteaux 1958; Sávay and Csillik 1956). A striking fact is, however, that even carbaminoylcholine failed to produce the normal calcium release in the sole plates of the denervated muscle. This suggests that not only have the acetylcholine-stores of the terminals been depleted as a result of degeneration, but also some serious alterations took place during these five days in the structure and reactivity of the post-junctional membrane and/or of the post-junctional cytoplasm. Electron microscopically no major alterations have been seen in the structure of the post-junctional membrane after denervation, neither in mammals (Reger 1957) nor in amphibia (Birks et al 1960). It may well be, however, that the degenerative 'alterations' of the membrane are at the molecular level, which could not be resolved by the electron microscope. Indeed, one of us (Csillik) showed marked molecular rearrangement in the post-junctional membrane 10 days after the motor nerve was sectioned. It has been suggested that this molecular rearrangement might result in a changed reactivity of the membrane to acetylcholine (Csillik 1963). It seems that these molecular alterations of the membrane structure, (possibly accompanied by unknown alterations in the structure of the post-junctional cytoplasm) are responsible for the failure of ester-induced calcium liberation after motor nerve section.

Nerve degeneration, however, not only decreases the reactivity of the post-junctional calcium-stores, but also gives rise to the appearance of an entirely different form of calcium liberation. The appearance of calcium in linear structures on the surface of denervated muscle fibers points to the fact that sites with a capacity of calcium release (and with a capacity to react with acetylcholine) begin to spread over a more extended area after nerve degeneration. Since similar patterns were observed in foetal and new-born muscles, which are characterized by an incomplete innervation apparatus (Couteaux 1941; Zelena and Szentágothai 1957; Csillik 1960), it seems like that a normal and well-developed innervation structure is required for the normal calcium liberation in the post-junctional sarcoplasm. It is tempting to correlate these linear structures with the findings of Ginetzinsky and Shamarina (1942) and with those of Axelsson and Thesleff (1959) and Miledi (1960, 1962). The spreading of the acetylcholine sensitive area over the surface of foetal and denervated muscle fibers, shown by these authors by means of micro-application of acetylcholine, might well be based upon the same cytological alterations of the muscle surface membrane which result in a 'spreading' of the calcium-liberating zone.

There are, however, serious discrepancies between the micro-application experiments and our results with respect to the size of the reactive areas. In our experiments, calcium release never extended over 200 micra, while the values published by the aforementioned authors range to several millimeters. The same applies, however, to the size of the acetylcholine sensitive area in normal muscles too. According to Miledi, the acetylcholine sensitive area averages 520 micra in the rat diaphragm, whereas the size of the motor end-plates (and also of the calcium-releasing area) is 19—28 in the same muscle (Cole 1955). The acetylcholine-sensitive area is thus about twenty times larger than the actual size of the end-plate. Diffusion of the drug from the micropipette in these experiments might possibly be responsible for the many times larger size of the area found sensitive for acetylcholine; on the other hand, it may well be that calcium release is more restricted spatially than acetylcholine sensitivity.

Anyway, if one takes the factor of 20 into consideration, it would appear that a calcium-responsive zone 200 micra long in denervated muscle corresponds to a 4 mm long acetylcholine sensitive area. This value agrees fairly well with the figures obtained by the English group.

The histochemical patterns obtained in this study show the result of a drastically enhanced activity—indispensable to evoke those major structural changes which can be visualized also by these relatively insensitive histological methods. They enable us, however, to trace back the sequence of events under physiological conditions. Accordingly, the following scheme may be suggested:

Depolarization of the post-synaptic end-plate membrane by acetylcholine evokes an end-plate potential which in turn generates a muscle action potential. The current liberates bound calcium both in the Doyère cytoplasm and in other areas of the sarcoplasmic reticulum. Liberated calcium evokes contraction of myofibrils and, after contraction, will be rebound again by the sarcotubular system. Liberation and rebinding of calcium is so rapid under normal conditions that it cannot be traced by microscopical methods. A massive series of impulses, or a large amount of acetylcholine

or related compounds, however, results in such an amount of free calcium, that the sarcotubular system is not capable of rebinding all of it. Therefore, the sites of maximal liberation (or, minimal rebinding) will show up in the histological pattern: that is the Doyère cytoplasm and the neighboring parts of the sarcoplasmic reticulum.

Rebinding of calcium is the physiological function of the 'relaxing factor' described first by Marsh (1952) and by Bendall (1958) and identified as the sarcotubular system by Ebashi and Lipmann (1962) and others. The fact that DNP treatment in our experiments resulted in the appearance of histochemically detectable calcium proves the energy requirements of this rebinding process.

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INVESTIGATIONS INTO THE ULTRASTRUCTURE OF MYONEURAL JUNCTIONS IN INSECTS

by

J. HÁMORI

DEPARTMENT OF ANATOMY, UNIVERSITY MEDICAL SCHOOL
PÉCS, HUNGARY*

As known from electrophysiological experiments (Hoyle, 1957) the initiation of a local depolarization area at each myoneural junction is highly characteristic for the muscles of insects, whereas in vertebrates—with the only exception of the slow system of the frog—a propagated change of the membrane potential occurs. Correspondingly—as known already from light microscope studies—the myoneural junctions usually referred to as multiterminal innervations, are established in insects at several points of each fiber, while in the cross-striated muscle fibers of most vertebrates only a single end plate is present. Another important feature of some insects' muscles is the 'polyneural' innervation, i. e. the innervation of a muscle fiber by several functionally different axons. A beautiful example of polyneural innervation may be found in the jumping muscle of locusts (Hoyle 1955; Hámori 1961b) where the muscle fiber is innervated by one fast and several slow axons. In the fibrillar flight muscle another kind of specialization is required by the discrepancy between very high—up to 1000/sec (Sotavolta 1947)—contraction frequency and the much lower number of motor impulses. Other types of less specialized myoneural structures in Coleoptera and Hymenoptera leg muscles, with mono- or bineural supply have also been investigated with the light microscope (Auber 1960; Hámori 1961b), as well as with the electron microscope (Edwards 1958). Unfortunately, however, relatively little progress has been made to reveal the correlation between the morphological and physiological properties of the muscle innervation of insects. In this paper investigations into the ultrastructure of three distinct types of myoneural junctions will be presented.

MATERIAL AND METHODS

Femoral muscles of *Apis mellifica* (Hymenoptera), *Geotrupes vernalis* (Coleoptera), as well as the extensor tibialis muscle of the metathoracic leg of *Locusta viridissima*, *Chorthippus declivus* (Orthoptera) and *Dytiscus marginalis* (Coleoptera), and indirect flight muscles of *Lucanus cervus*, *Cetonia aurata* (Coleoptera) have been used. Preparatory technic for electron microscopy has already been described elsewhere (Hámori 1963). Obser-

* Present address: Department of Anatomy, University Medical School Budapest, Hungary

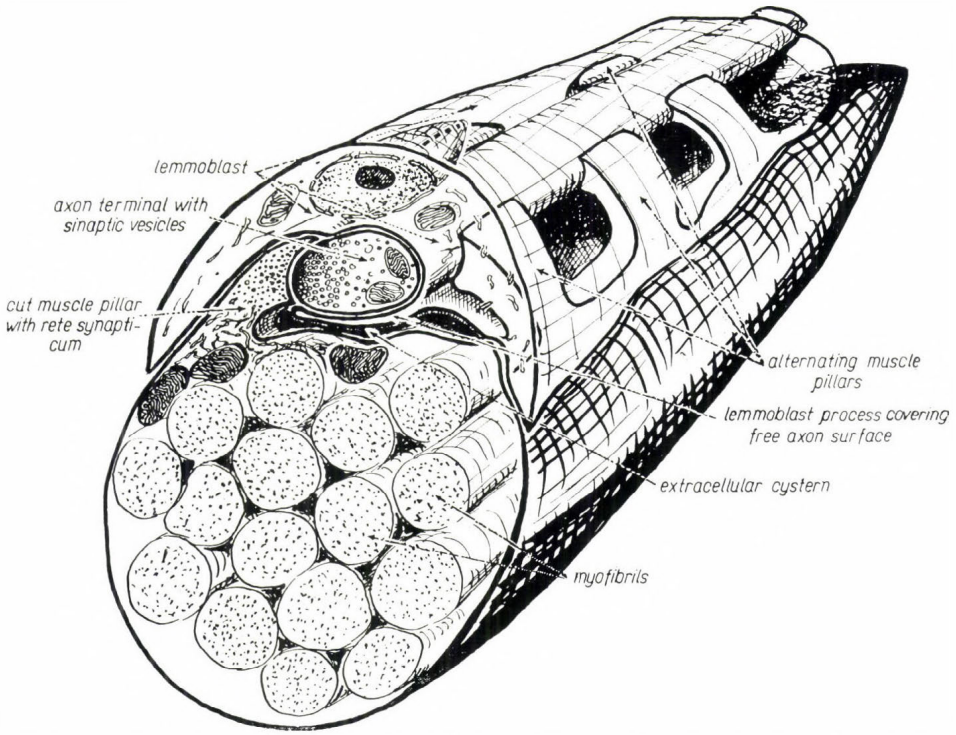

Fig. 1.—Stereoscopic diagram of end plate in *Apis mellifica*, *Cetonia aurata* and *Geotrupes vernalis*, showing relationship between axon terminal, muscle cell, muscle pillars and lemmoblast. Extracellular cistern around pillars and particularly between the nerve fibre and the surface of the muscle cell situated between the origin of the pillars is also illustrated (Hámori 1963)

vations were made with Zeiss (Jena) El-Mi II and the 'Tesla' (Brno) electron microscopes. For additional light microscopic investigation Lövit — Fischer's gold chloride method has been employed.

RESULTS

(a) *The simplest type of leg muscle innervation*, one even might venture to say the archetype of muscle innervation of insects, very common in the leg muscles of Hymenoptera (e. g. *Apis m.)*, Coleoptera *(Geotrupes v., Cerambys c.)*, is demonstrated first by means of highly schematized stereoscopic diagram (Fig. 1) showing that the single terminal nerve fiber is running for a short distance parallel along the surface of the muscle fiber in the region of the end plate, where the fiber establishes contact with processes of the muscle fibre, characteristically arranged as alternating pillars, looking as if they would 'support' the nerve terminal. These pillars are filled with mitochondria and a rather dense postsynaptic reticulum. The whole apparatus is covered and isolated from the environment by the lemmoblast cells; there remain, however, extracellular cisterns around the pillars, particularly between the nerve fiber and the surface of the muscle

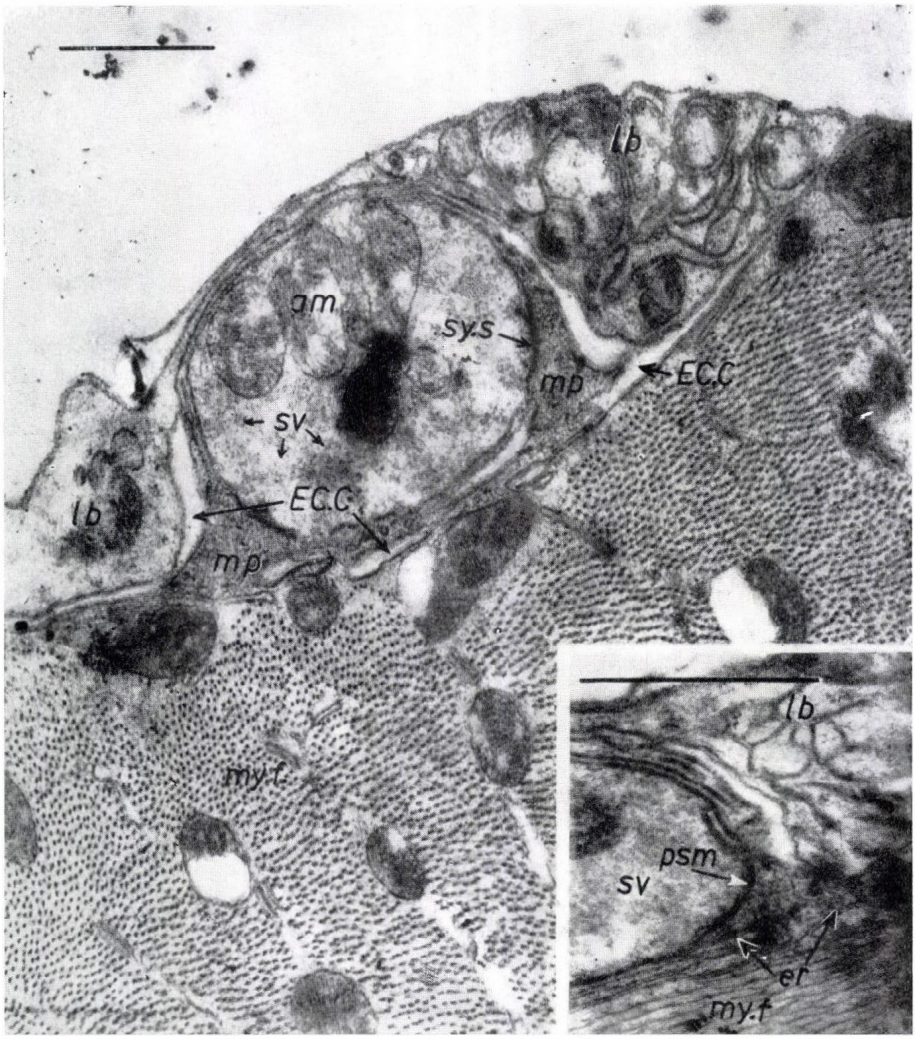

Fig. 2.—Slightly oblique cross-section of an end plate, showing axon terminal muscle pillars *(mp)*, lemmoblast *(lb)* and extracellular cistern (EC.C); *am*=axon mitochondria, *my.f.* = myofilaments, *sv* = synaptic vesicles. *Apis mellifica*, ×21.000. Inset = higher power electron micrograph, illustrating thickening of the postsynaptic membrane *(psm.)* at the region of synapse. Note postsynaptic reticulum *(er)* connecting the base of muscle pillar with the surface of myofibril *(my.f.)*. *Apis mellifica*, ×32,000 (Hámori, 1963)

cell. In a transverse section (Fig. 2)—with one fiber and pillars on both sides that came into the section because of its slightly oblique plane—it can be seen that the synapsing inner portion of the axon is free of its lemmoblast sheath. The axon contains mitochondria and synaptic vesicles. The plasma membranes of the axon and muscle fiber get in close apposition only in the contact region between the pillars and the terminal axon.

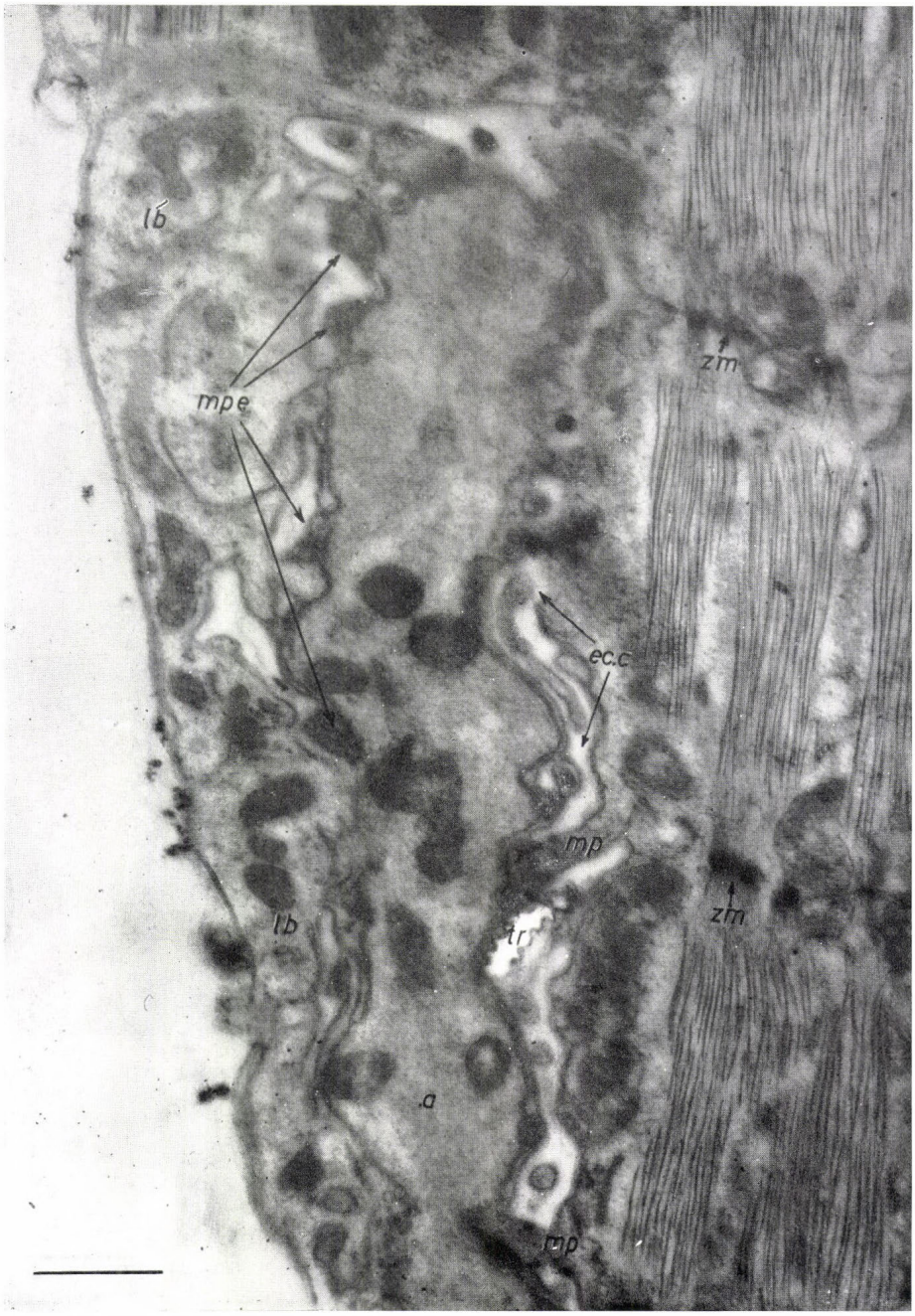

Fig. 3.—Longitudinal, somewhat tangential section of an end plate, showing on one side the muscle pillars *(mp)*, the surrounding cistern *(ec.c)* and on the opposite side some of the ends of the alternating opposite pillars *(mpe)*; *a* = axon, *lb* = lemmoblast, *tr* = tracheole, *zm* = *Z* membrane. *Apis mellifica*, ×21,000 (Hámori 1963)

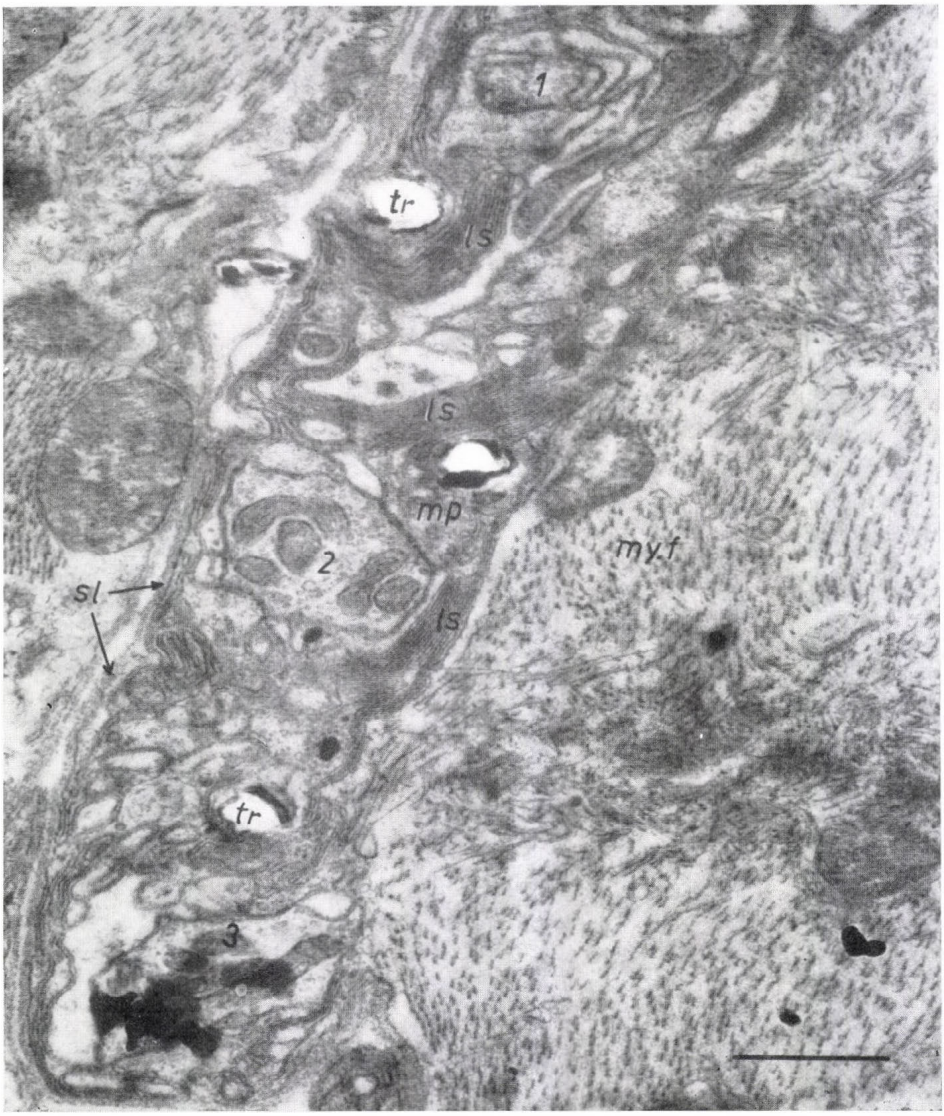

Fig. 4. — Cross-section of end plate, with three (1, 2, 3) axons, muscle pillars *(mp)* and lamellar system *(ls)* corresponding to the foldings of the muscle plasma membrane. *tr* = tracheole, *my.f* = myofilaments, *sl* = outer layer of the sarcolemma, *Lucanus cervus*, leg muscle, ×21,000

The synaptic interspace is only of about 150 Å width. Lack of foldings of the synaptic membranes is also characteristic for all myoneural junctions of insects investigated so far. The postsynaptic membrane exhibits a definite thickening at this point (see especially inset of Fig. 2). The postsynaptic area is highly specialized as compared with other regions of

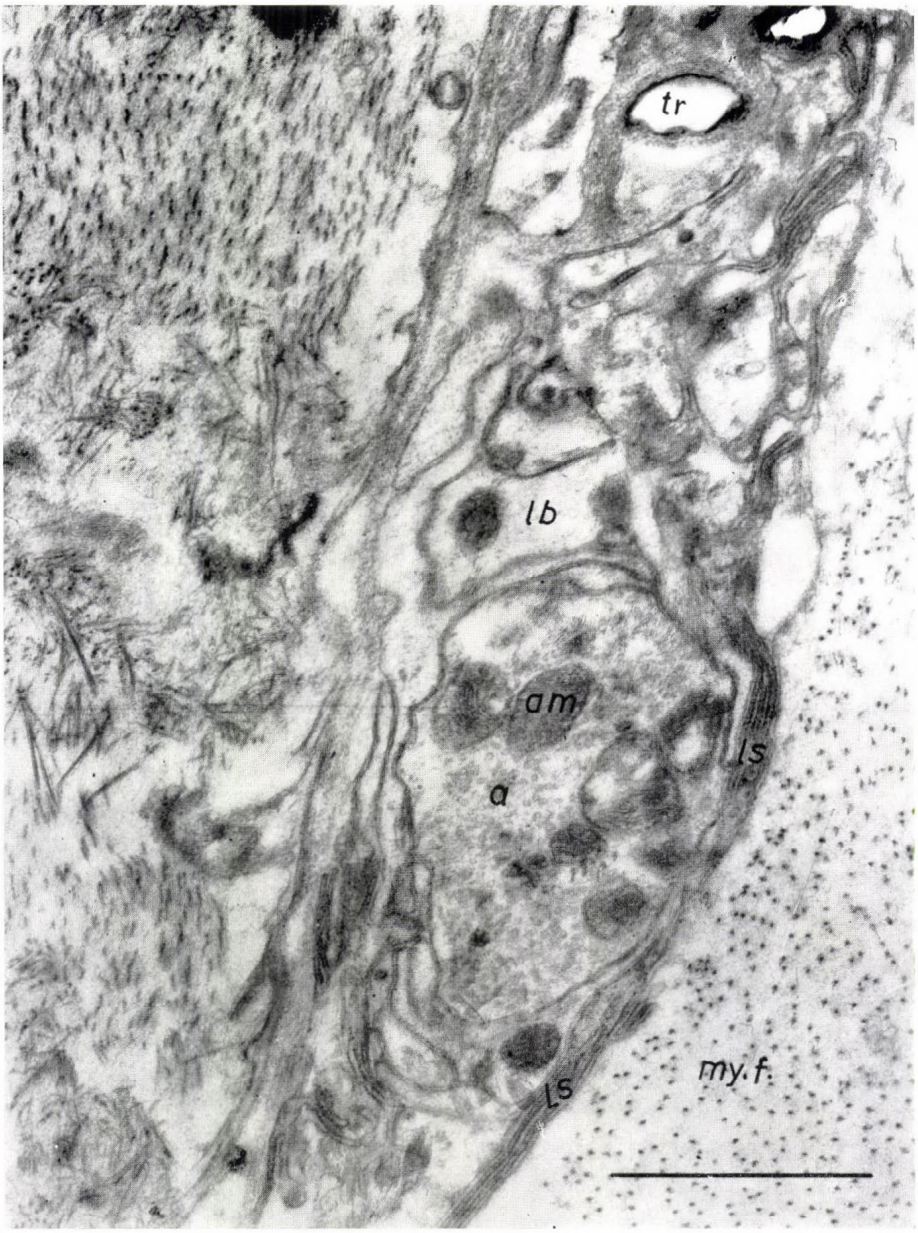

Fig. 5. — Part of end plate in *Lucanus cervus* leg muscle. *a* = axon, *ls* = lamellar system, *am* = axon mitochondria, *my.f.* = myofilaments, *tr* = tracheole, *lb* = lemmoblast, ×32,000

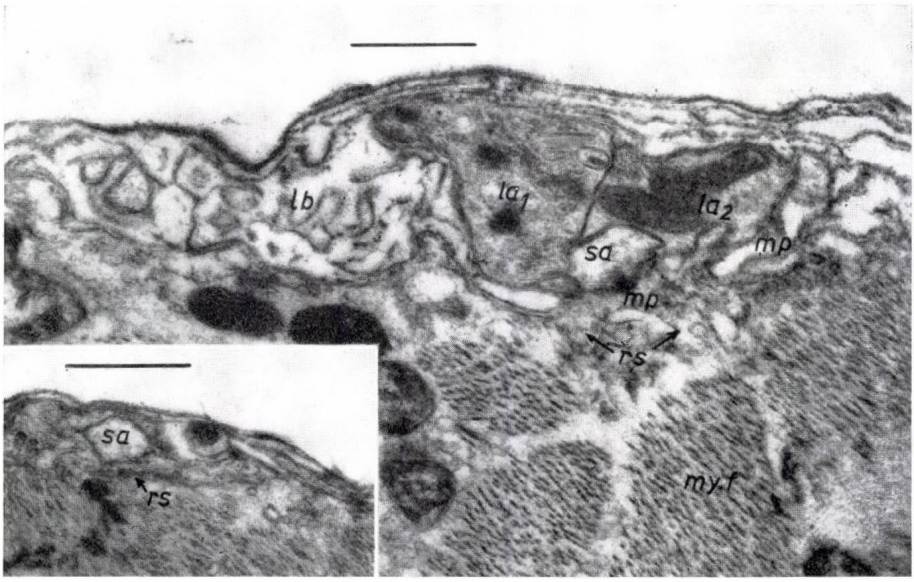

Fig. 6.—Polyneural junction in the jumping muscle of locust with two larger (la_1, la_2) axons and a small empty one (*sa*). *mp*-muscle pillars, *my.f* = myofilaments, *lb* = = lemmoblast, *rs* = rete synapticum, ×20,000

muscle sarcoplasm, it is characterized by strongly osmiophilic granulae and an augmented endoplasmic reticulum which appears to surround very closely the neighbouring superficial myofibrils. Fig. 3 represents a nearly longitudinal—somewhat tangential—section, showing on one side the pillars and the surrounding cisterns, and some of the ends of the alternating opposite pillars on the other.

A more complicated type of end plate is found in the leg muscles of *Lucanus cervus*, with essentially the same pillars, much less regular though and also difficult to distinguish because of 3 or 4 fibers terminating in the same end plate. In the close neighbourhood of the pillars, having immediate contact with the muscle membrane, the axon seems to be blocked from the contractile material by a strange lamellar system (Figs 4 and 5), made up probably of foldings of the muscle plasma membrane, which are very similar to the loose myelin formation in vegetative (own unpublished observation) and certain sensory ganglia (Rosenbluth and Palay 1961). Another explanation of this strange structure might be in the reverse, in that it is a lamellar system directly concerned with the impulse transmission, perhaps even some analogue of the membrane system in photoreceptors, i. e. some transduction device.

(b) From the '*non-end plate*' *types* of nerve muscle junction the jumping muscle of the locust might be taken as a first example. Here one muscle fiber is supplied by the terminals of two different axons, one densely filled with vesicles (Fig. 6), and a second type (Fig. 6, inset) which has been very frequently found in the jumping muscle and so is much more characteristic for it. It is smaller and looks empty as compared with the

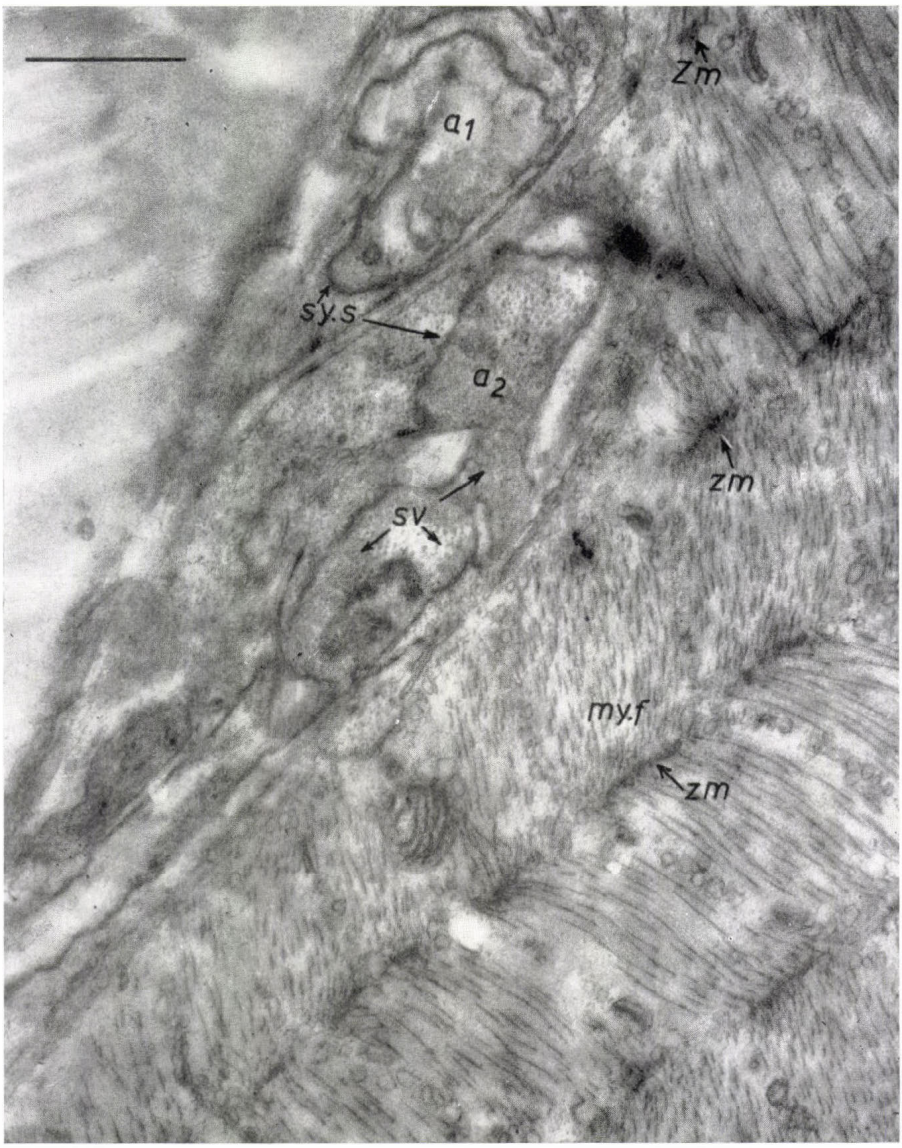

Fig. 7.—A tangential, somewhat longitudinal section of the third metathoracic leg muscle in *Dytiscus marginalis*. Axon terminals are spaced regularly between two Z-membranes. a_1, a_2 = axon terminals, *sv* = synaptic vesicles, *sy*, *s* = synaptic surface, *zm* = Z-membranes, *my.f* = myofilaments, ×20,000

larger ones. This arrangement is strange as on the basis of its general occurrence this second type ought to be considered as 'fast' type ending, while the first type, which occurs rather rarely, might be regarded as 'slow' junction. In the 'swimming' muscle—in the femur of the posterior

Fig. 8.—Bineural junction in *Dytiscus marginalis* swimming muscle. One of the axons *(a_1)* contains synaptic vesicles *(sv)* of usual size (3—400 Å), the respective postsynaptic area having a very rich rete synapticum *(rs)*; in the second axon *(a_2)* the vesicles are larger and only a very poor rete synapticum can be seen. *ln* = lemmoblast nucleus, *pn* = preterminal nerve fiber, *tr* = tracheole, *my.f.* = myofilaments, ×20,000

legs—of *Dytiscus* a very similar type of innervation is found. In this muscle the fine terminal axon branches run in circular direction, generally between two Z-membranes (Hámori 1959). This arrangement can be seen in Fig. 7, where in the somewhat tangentially longitudinal section the terminal axons

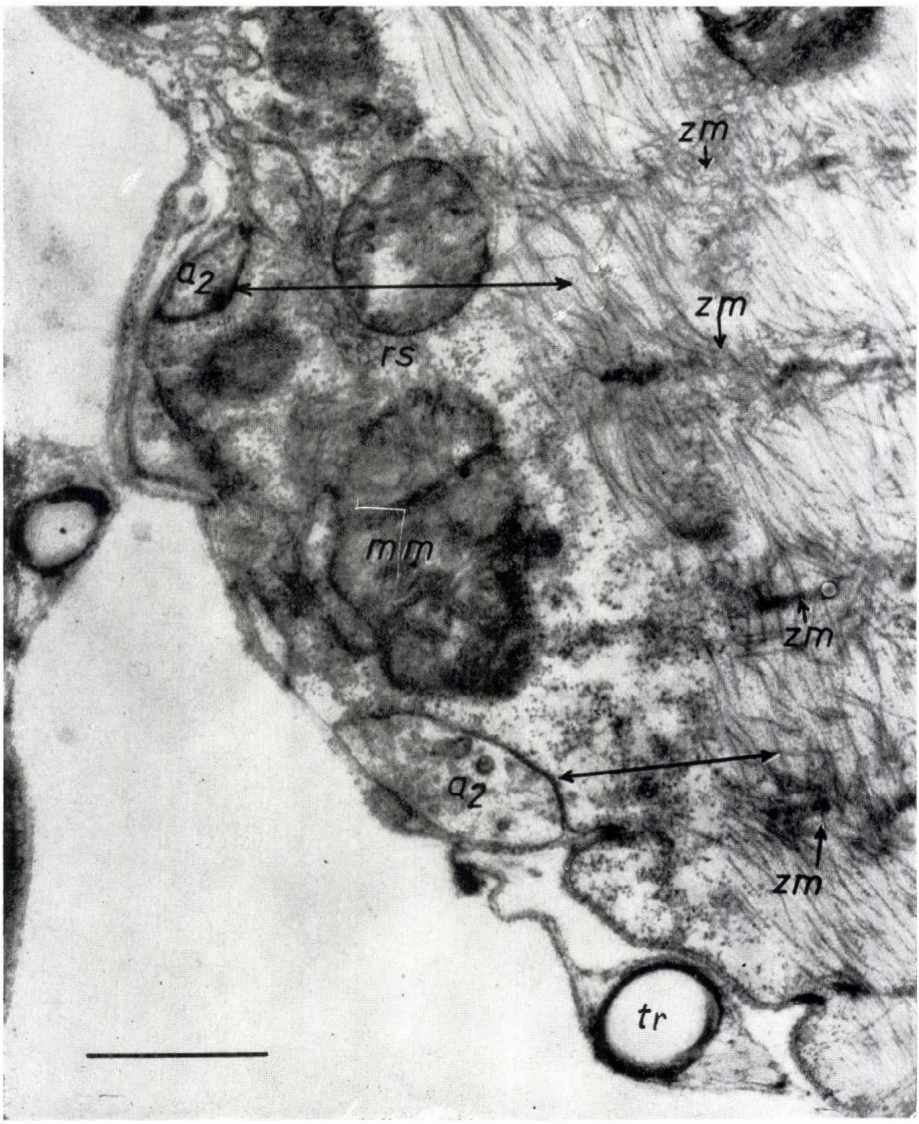

Fig. 9. — Large vesicle endings (a_2) in swimming muscle of *Dytiscus marginalis*. A very poor 'rete synapticum' *(rs)* is seen only. Note the 'inter-Z'-localization of the axons (arrows). *tr* = tracheole, *mm* = muscle mitochondria, *zm* = Z-membranes, ×20,000

are found between two Z-membranes. As in the jumping muscle of the locust, two axons, different in shape, and internal organization may establish contact with the same muscle fiber (Fig. 8). One of the axons is filled with synaptic vesicles of usual size (3—400 Å diameter). The respective post-synaptic area is very rich in sarcoplasmic reticulum (see also Fig. 10)

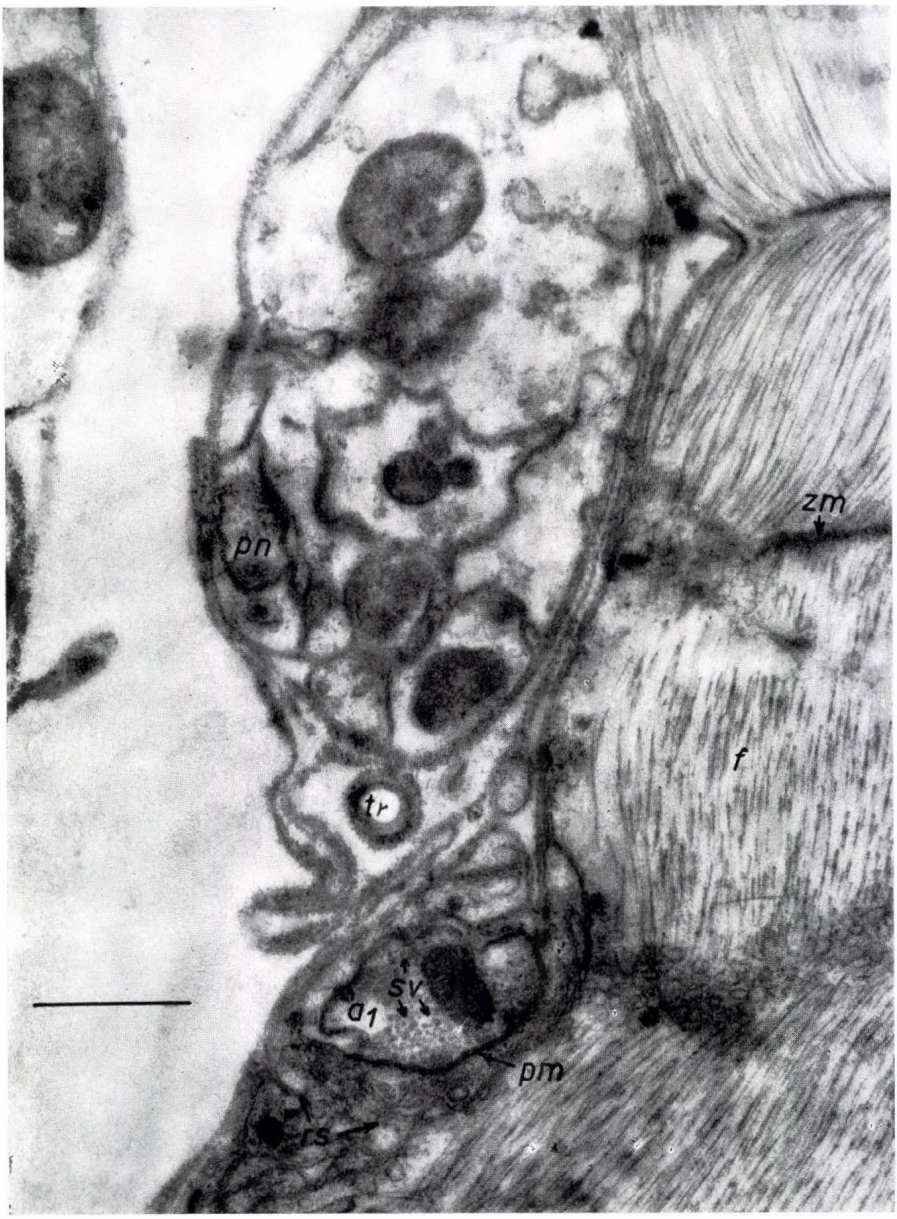

Fig. 10.—Normal vesicle ending (a_1) in swimming muscle of *Dytiscus marginalis*. A rich rete synapticum *(rs)* connects postsynaptic membrane *(pm)* and myofibril *(f)*. *tr* = tracheole, *zm* = Z-membrane, *pn* = preterminal nerve fiber, *sv* = synaptic vesicles, ×21,000

Fig. 11. — Myoneural junction of flight muscle in *Lucanus cervus*. Inset = Terminal nerve fiber, gold impregnation. Aurophilic thickenigs appear between each myofibril (myofibrils are seen as broad light bands). On electron microscopic picture of the terminal fiber is well seen that only axon thickenings *(at)* have true synaptic surface, other parts of the axon being separated from myofibrils by the lemmoblastic envelope *(lb)*; *rs* = rete synapticum, *tr* = tracheole, *f* = myofibrils, *sv* = synaptic vesicles, *mp* = muscle pillars, ×21,000

Fig. 12—Cross-section of terminal (and preterminal) axons in the flight muscle in *Lucanus cervus*. *a* = axon, *rs* = rete synapticum, *my.f* = myofilaments, *pa* = preterminal axon, *lb* = lemmoblast, *mm* = muscle mitochondria, ×20,000. Inset: *sv* = synaptic vesicles, *pm* = postsynaptic membrane, *lb* = lemmoblast, *my.f* = myofilaments, *rs* = rete synapticum, ×32,000

and apostsynaptic vesicles, which in many cases seem to be in direct connexion with the outer tubular part of the sarcoplasmic reticulum encircling the outer margins of the Z-membranes, while in the second type of endings the synaptic vesicles are larger and only a very poor 'rete synapticum' (Fig. 8 and 9) is seen in the corresponding postsynaptic area. There are

no pillars in these endings, the axon being simply embedded in a circular groove of the muscle fiber.

(c) *The indirect flight muscle* of insects has also a diffuse non-end plate type of innervation, also with predominantly circular terminal branches. The circular branches of the same muscle fiber are not more than 20 μ apart from each other. Characteristic for the terminal axon (Fig. 11, inset) are the gold-stained thickenings spaced in a regular pattern, corresponding to the furrows between myofibrils. Only the thickenings have direct junctional contacts (Fig. 11) while other portions of the axon are separated from the muscle plasma membrane by the lemmoblast sheath. A very rich rete synapticum, connecting the postsynaptic membrane with the surface of neighbouring myofibrils, can be recognized in Fig. 12. The sarcoplasmic regions that effect the junction with the axon thickenings resemble somewhat the 'pillars' in the simple leg muscle nerve endings, their postjunctional surfaces arranged in a flat 'V'-shaped furrow, in which the axon thickening is embedded. The synaptic interspace (see inset of Fig. 12) is only about 120 Å.

CONCLUSION

In attempting to interpret the structural features here presented, concerning neuromuscular transmission the following propositions might be considered.

(a) The highly specialized rete synapticum of the postsynaptic area, first described by Edwards et al. (1958) in cicada flight muscle, is characteristic for most myoneural junctions in insects. It might be the histological substrate of the mechanism, coupling membrane changes to contraction, that with respect to topography and structure might well be suited to transfer membrane potentials. As regards transfer of excitation from the nerve-muscle junction to the contractile material, it is noteworthy how small a fraction of the surface of the terminal axon is in direct contact with the muscle membrane. This is especially clear in the simple leg muscle junctions, where, apart from the contact with the small junctional surfaces with the pillar, the axon surface is surrounded by a lemmoblast cell envelope and is additionally separated from the muscle membrane by the extracellular 'cistern' system (Fig. 3). It is difficult to imagine that such a highly complex structural arrangement has no peculiar functional significance. The intricate membrane systems of the more complex leg-muscle endings are even more difficult to understand.

(b) The observation of structurally and topographically different nerve endings in the muscles especially of locusts is well in accord with their polyneural innervation by functionally different components. As inferred from physiological reasons (Hoyle 1955), the fast nerve fibers must be more numerous than the slow ones. The "fast" endings can with some plausibility be identified with the light watery terminals. However, further investigations are required to clarify this problem.

(c) The presence of synaptic vesicles within the axon might suggest a chemical mediation process similar to that in the neuromuscular junction of vertebrates. From pharmacological investigations, however, it is known that the myoneural junction of insects is insensitive to directly

applied chemicals, for example acetylcholine, adrenaline, serotonine, etc. Thus the role played in impulse transmission by the cholinesterase found in these junctions by histochemical methods (Hámori 1961a) cannot be taken for granted.

SUMMARY

Ultrastructure of three different types of myoneural junctions of insects has been described. Some general features: as pillar-like organization of the postsynaptic sarcoplasm, the occurrence of a highly specialized postsynaptic rete synapticum, and observations concerning ultrastructure of polyneural innervation have been briefly discussed from the functional point of view.

DISCUSSION

Röhlich : Is there any structural connexion between the 'rete synapticum' and the Z-lines? If so, what do you believe its functional importance is?

Hámori : Direct connexion between the rete synapticum and the Z-lines is evident only in Dystiscus leg muscle and Coleoptera flight muscle. There are, however, in most cases fairly close relations between the rete and the outer membranes of myofibrils situated in close neighbourhood of the nerve terminal. As mentioned, they might serve as the histological basis of impulse transmission from the muscle-cell membrane to the fibrillar membrane.

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SECTION FOUR

CENTRAL NERVOUS SYSTEM

Symp. Biol. Hung., **5**, 177—217 (1965)

CONNECTIONS OF THE CEREBRAL CORTEX OF THE MONKEY

by

W. J. S. KRIEG

NORTHWESTERN UNIVERSITY MEDICAL SCHOOL
CHICAGO, ILLINOIS, USA

Knowledge of the mechanisms and pathways of the cerebral cortex is fundamental to an understanding of the functioning of the nervous system, to interpretation and localization of damage to the cerebrum, and to analysis of mental processes. Yet the study of the connections of the cerebral cortex has been the least explored of all the central problems of medical science. True, the subject has been approached from above through experimental psychology, empirical psychiatry, and psychoanalysis. The objective and subjective effects of local stimulation of the brains of animals and men have been analysed. Local activity has been visualized after distant stimulation, after local strychninization, and after injury, using oscilloscope and EEG. Recently, even single cell activity has been studied in various cortical layers. On the anatomical side, the local variations in pattern of layering of the cortex were standardized soon after the turn of the present century. However, only the most scattered attempts have been made to unravel the fiber tangle below the cortex whereby points on the cortex intercommunicate, and which is the structural expression of the higher neural and mental processes.

Yet since 1886 neuroanatomists have been in possession of a method which can show the efferent connections and courses of the fibers from any point in the brain. They have stereotaxic apparatus which permits them to select any point at will. This method, the Marchi method, writes a permanent record in serial sections which can be examined in detail, reconstructed and compared. All this time it has been understood that the cerebral cortex may be divided into some fifty units, the cortical areas, which show differing structures and differing functions, yet the pathways from them or between them have been almost unknown, except for the projection tracts from the motor region.

Such a vacuum should long ago have been filled, and the writer confesses that from early years the tracing and visualization of the pathways of the brain has seemed the most fascinating subject with which one could occupy oneself for a protracted period.

However, on surveying the field, it is apparent why the study of the cortical connections has lagged behind the study of brain stem. The mass of the cerebrum exceeds that of the brain stem by a factor of five or more, so that to convert a monkey's whole brain into serial sections is a considerable task. The interpretation of the convolutional pattern in sections is not easy. The fibrous medullary center is an undifferentiated mass, and the cortical areas must be identified on the basis of subtle distinctions. Con-

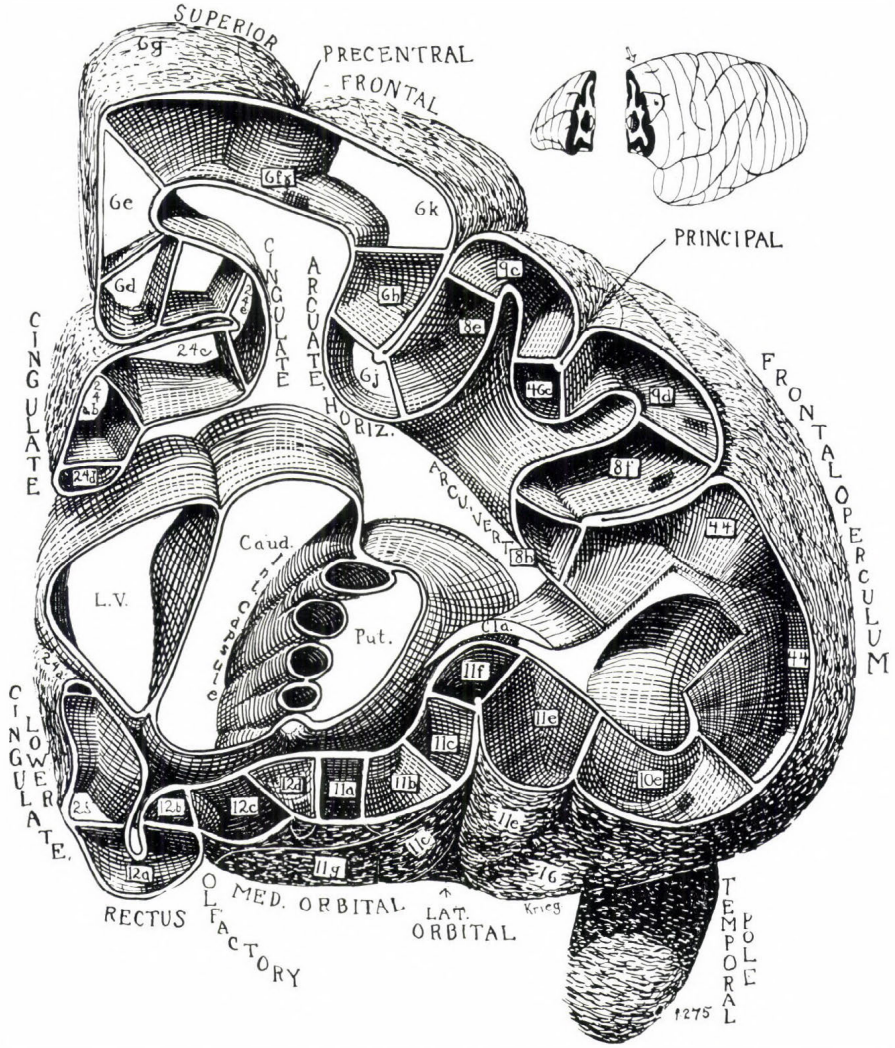

Fig. 1.—Slice reconstruction of macaque cerebrum

nections cannot be followed in normal sections, so lesions must be made and degenerated fibers traced in separate brain series.

In order that the osmic acid may penetrate, the fixed brain must be divided into 4 mm slices. By making a mold and slicing guide, these 4 mm slices have been standardized and serve as the basic units for reconstructing the degenerated fiber tracts in all the series. The distribution of the areas through the slices was worked out and added to the slices, making a standard labelled three-dimensional outline of the configuration of the cortical units as a basis for the reconstruction of all connections (Fig. 1).

In practice, the lesion is located on the slices and reconstructed as a charred pit. The granules emanating from the lesion are traced and their

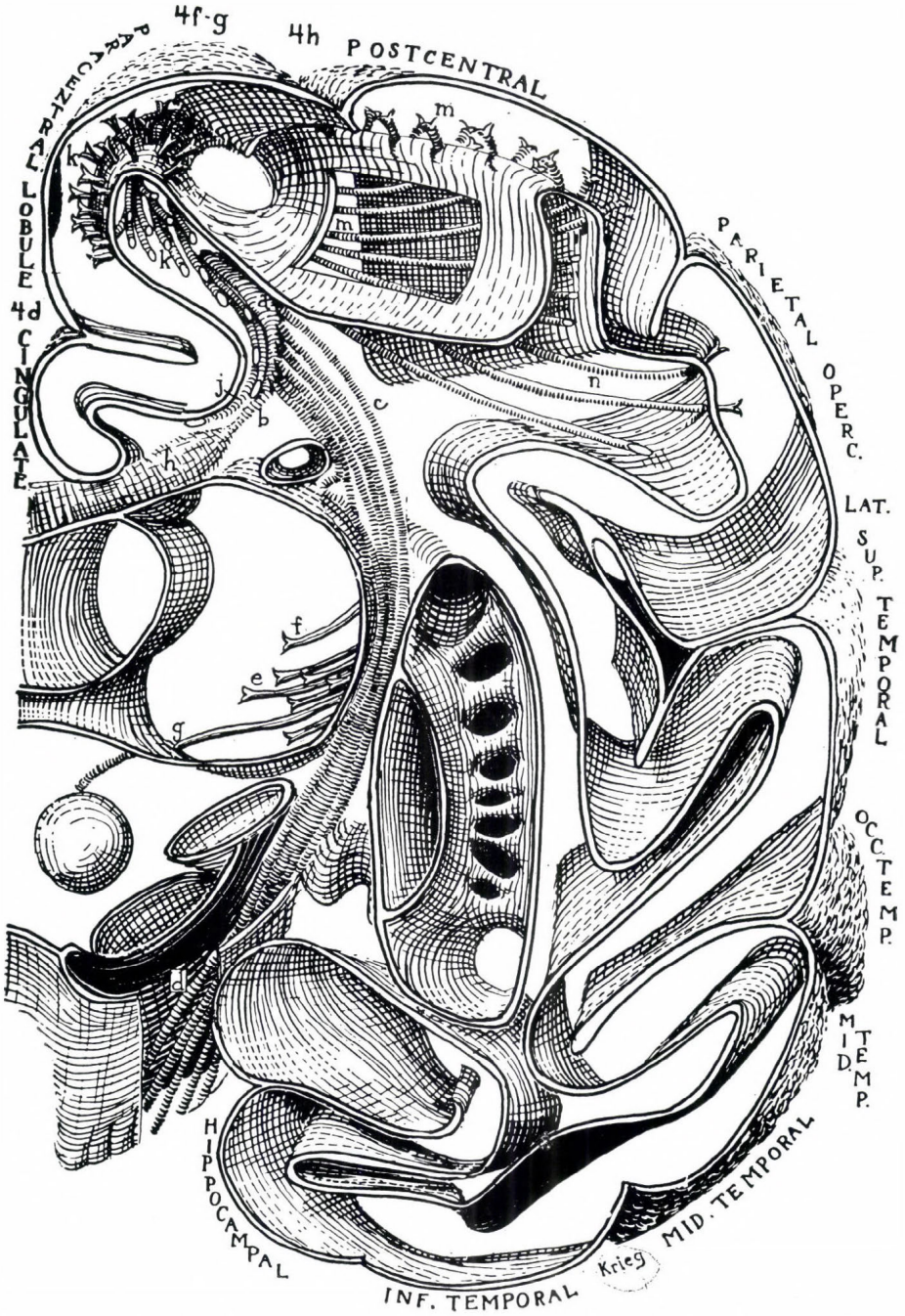

Fig. 2. — Tracing of degenerated tracts in slice reconstruction of macaque cerebrum

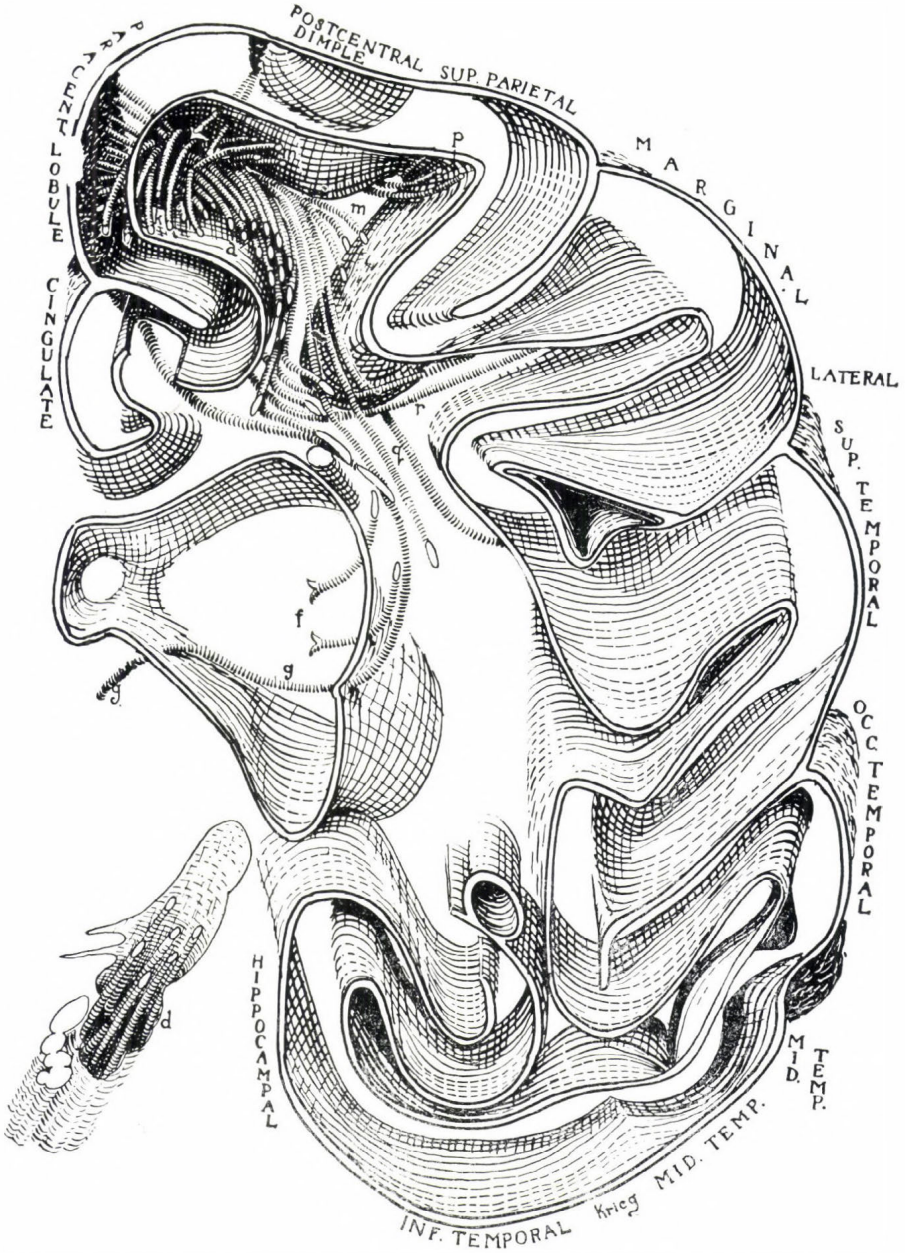

Fig. 3. — Tracing of degenerated tracts in slice reconstruction of macaque cerebrum

trails are reconstructed as a cable and continued from slice to slice, furnishing a permanent record which may be compared with any other lesion (Figs 2 and 3). Having reconstructed 191 lesions in this manner over 15 years,

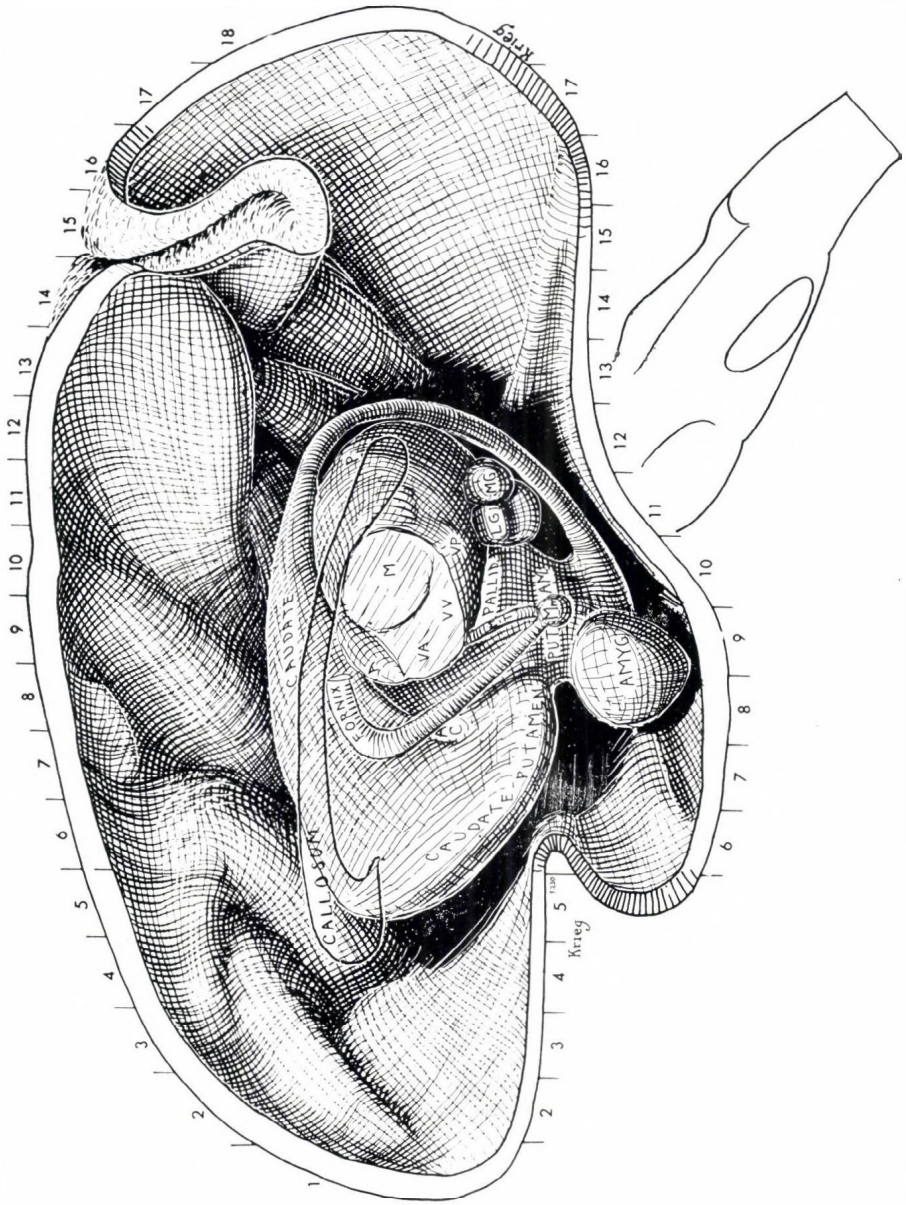

Fig. 4. — Thalamus and basal ganglia laid on the half-shell. Macaque

we now have a large library of information which may be compared or combined. Thus, the provenance of all fibers which traverse any cubic millimeter may be assessed. The use which may be made in future experimental probings of the brain is incalculable. We now know the connec-

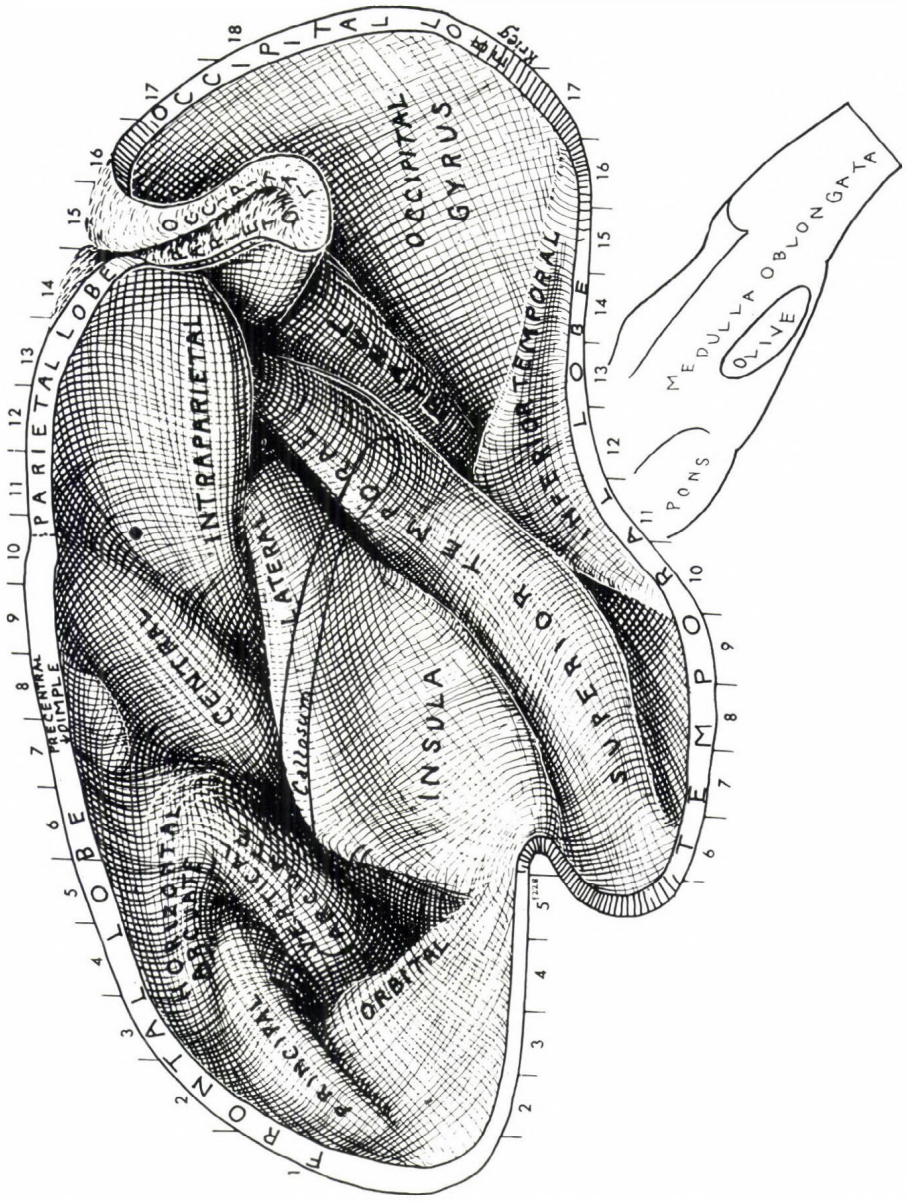

Fig. 5. — Inner surface configuration of lateral cerebral cortex of the macaque. The half shell. The gyri are labelled

tions of virtually all points of the cerebral cortex of the monkey. It remains for the future to discover the activity of each connection. From the combined information a working model of the cerebrum may some day be constructed.

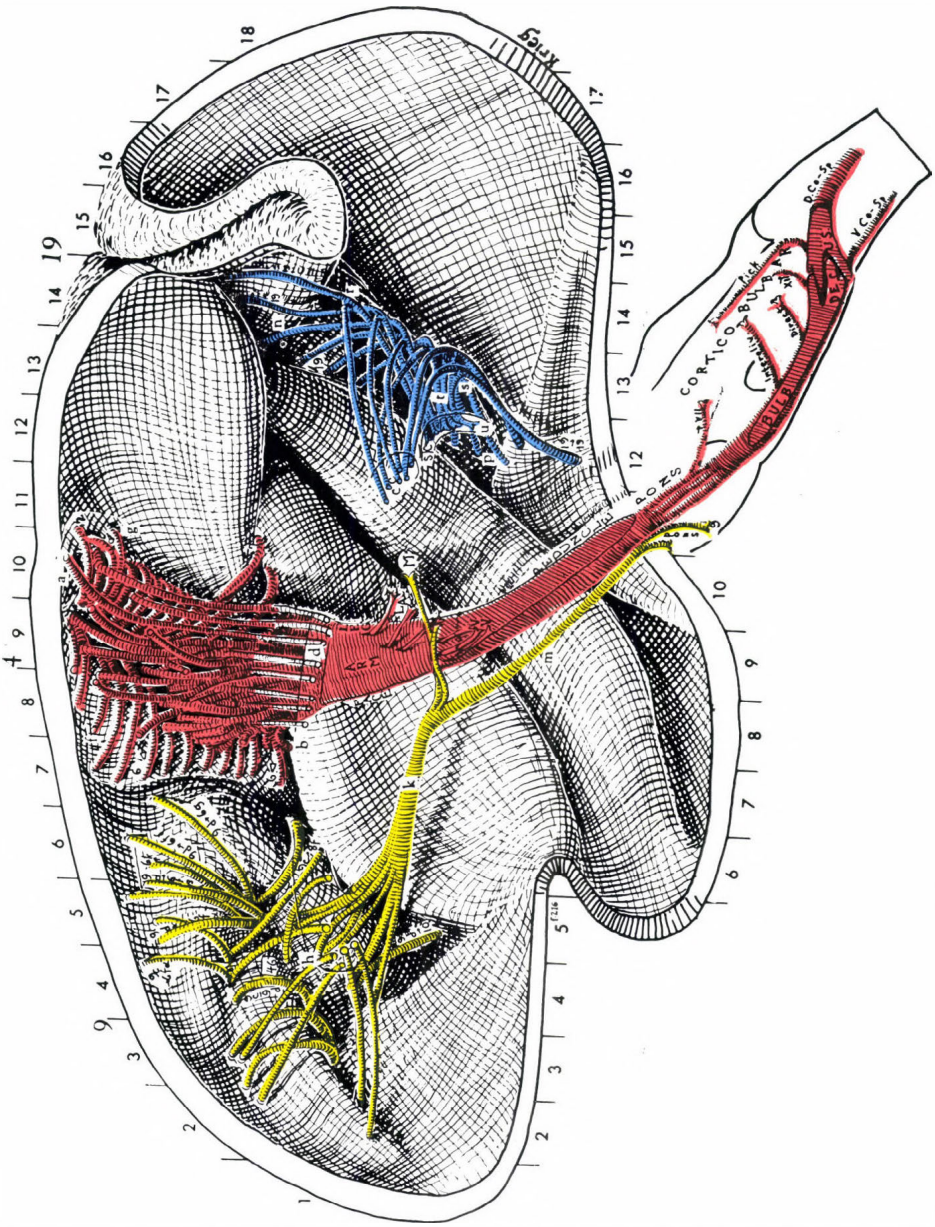

Fig. 6. — Corticifugal connections of Areas 4 (red), 9 (yellow), and 19 (blue) in the macaque

The principal findings of this long project are embalmed in two books, one of which was published in 1954 (cf. References) and the other to appear this fall (1963), together with several long papers. Hence to attempt to de-

tail the findings as shown in the slice reconstructions would require many hours. Yet, this is the essential product of the work, and the principal object. In this presentation we shall avoid the slice reconstructions as they are records of individual experiments, although they do form the principal body of record, and the illustrations of the two books. They number in hundreds.

Instead, we shall present summaries of the principal connections by laying them on a half-shell of the inner surface of the cerebral cortex (Fig. 4). We have devised a method of making a latex cast of the cerebral cortex alone, so that its inner surface may be viewed. Here the sulci become elevations, the gyri are valleys or clefts, and the hollow of the shell represents the medullary center of fibers serving the cerebrum, except that in the middle are nested the corpus striatum and thalamus. All remaining space in this drawing is composed of fibers.

Identification of the sulci is not difficult in the monkey (Fig. 5). The insula, of course, is quite large, it trails away behind as the lateral or Sylvian sulcus. The central sulcus divides off frontal lobe and is flanked by valleys representing precentral and post-central gyri. In the frontal lobe are arcuate and principal sulci, actually not very deep. The intraparietal sulcus is a deep one, and the superior temporal sulcus is particularly deep and long in the monkey. The parieto-occipital, with the lunate, nearly pinch off the occipital lobe.

We shall now briefly summarize the connections of the principal cortical areas as seen in the half shells, and will begin, of course, with Area 4, the pyramidal or motor area (Fig. 6, red). The connections of this area, as an exception, have been closely studied by others. It has the largest and longest projection tract of any area, forming the massive corticospinal tract. Distinct corticobulbar components are confirmed, and these pass to the primary motor nuclei, principally facial and hypoglossal, and not to reticular substance. The order of bodily segments is farily well followed in the internal capsule, but all order is lost in the bulbar pyramid.

As the thalamus is passed, corticothalamics filter in to terminate in ventralis lateralis and lateralis anterior. A few reach a restricted sector of paracentralis. These are nuclei which are known to send to Area 4; ventralis lateralis relaying from brachium conjunctivum and paracentralis continuing the ascending activator system.

Associations are strong within the area, particularly within the region, as arm, leg, and also strong to the areal strips immediately adjacent to the postcentral somesthetic receptive areas and to the precentral motor Area 6; but not to more distant areas. These connections are of the nature of feedback, as they are richer in the opposite direction. Apparently it is not the business of the motor area to report to other areas what it is doing, and the lack of a connection to cerebellum through pons is an exceptional circumstance.

Similarly, the callosal connection is very weak, except for the face region. Most discrete motor acts of the extremities are much more unilateral than are others.

As exemplified by the motor area, the corticifugal connections of all cortical areas may be categorized into projectional, callosal, thalamic and associational. This systemization will be followed for the other areas.

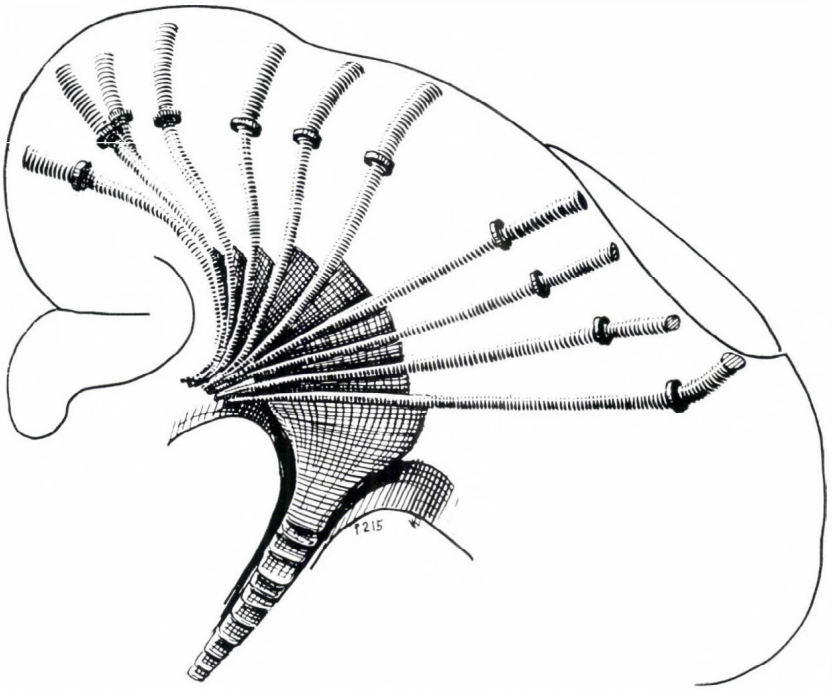

Fig. 7.—Diagram to show how each albal stalk drops fringes of capsular fibers

Except for associations, the fibers from any station on the cortex pass down the gyral core as a discrete stalk (Fig. 7). At the corona radiata this stalk bifurcates into callosal and projectional divisions, and it is the fibers themselves which do so. Rather, the callosal fiber continues in the same curvature as the main stalk, though it may be the smaller, while the projectional fiber branches off at a right angle or as nearly so as local conditions permit, indicating that it is a true collateral. This is paradoxical, but has been universally confirmed over the cerebrum.

So we have one unifying principle in interpreting the architecture of the cerebral white matter: a stalk which takes a direct course toward the corpus callosum, as though it had nothing else to do, and a projectional fiber which drops off into the internal capsule at an approach to a right angle, as a collateral. Accordingly, we see, as in this diagram, a series of converging stalks toward the corpus callosum, and from these fringes of capsular fibers which drop into the capsule in planes, like curtains, which they further resemble by being gathered back, as it were, where conditions are crowded. Thalamic fibers drop off the capsular component as it passes the thalamus. Association fibers go their own way, only exceptionally branching off the main stalk.

The premotor Area, 6, one of the most spacious of the entire cerebrum, is not an indivisible unit by any means. Anyone who has studied it closely (Vogt, Förster) has broken it into at least four subareas. On architectonic grounds we have found twelve subareas, and later, in doing the Marchi studies, have found that hodology respects architectonics.

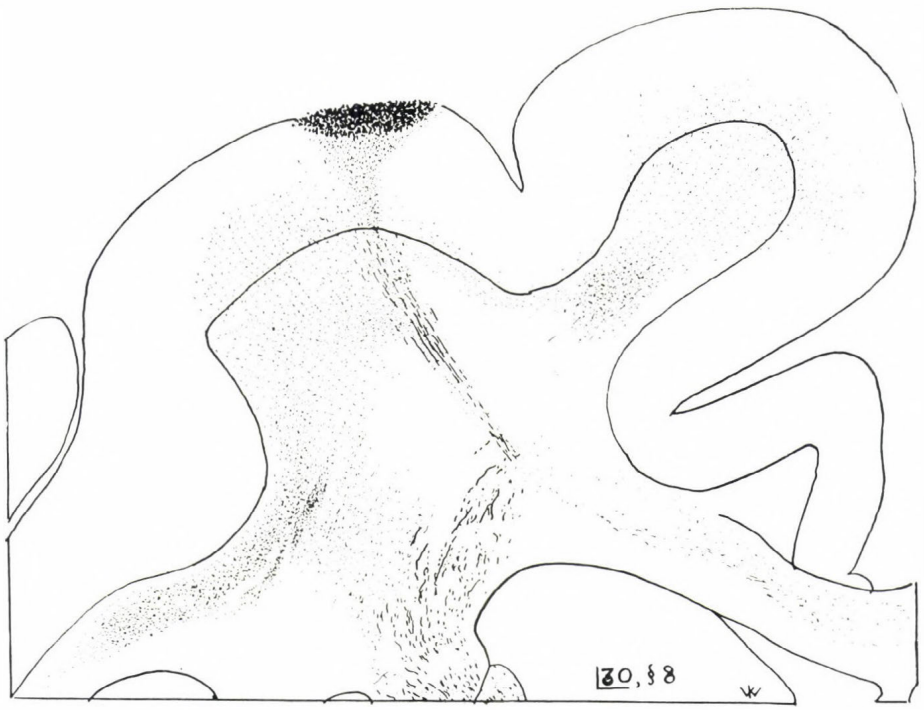

Fig. 8. — Degeneration after lesion in premotor area

Figure 8 shows degeneration after a lesion in the premotor area. Stalk, projection and callosal fibers are fairly coarse and follow the usual pattern. The association fibers are fine granules. They end liberally in the cortex immediately lateral, part of Area 6k, but skip the subareas medially, but end in the dorsomedial part of Area 6. A liberal component glides laterally, but avoids the cortex above it. This is a single example of a repeated observation that connections are specific not only for areas, but for subareas.

Now, those subareas which did not receive associations are sulcal, even though the precentral sulcus is only a dimple, while those which did are gyral. This is an expression of another general principle that the portion of an area walling a sulcus is of distinguishable architectural type from that of the gyral crest, while that the sulcal cortex receives and sends fewer connections in most cases, and hence that sulcal type cortex is submerged because its paucity of connections permits it to collapse, while gyral cortex, richer in connections of all sorts, is supported and elevated. We shall see that some sulcal cortex is merely folding of a stretch of cortex that is served by relatively few fibers throughout, as in visual cortex.

The proportion among the four connection categories differs widely in the several subareas of Area 6 (Fig. 9, red). The posterior part has the most abundant projections, the forward part very few. They all converge rapidly into the anterior limb of the internal capsule. They do not send myelinated

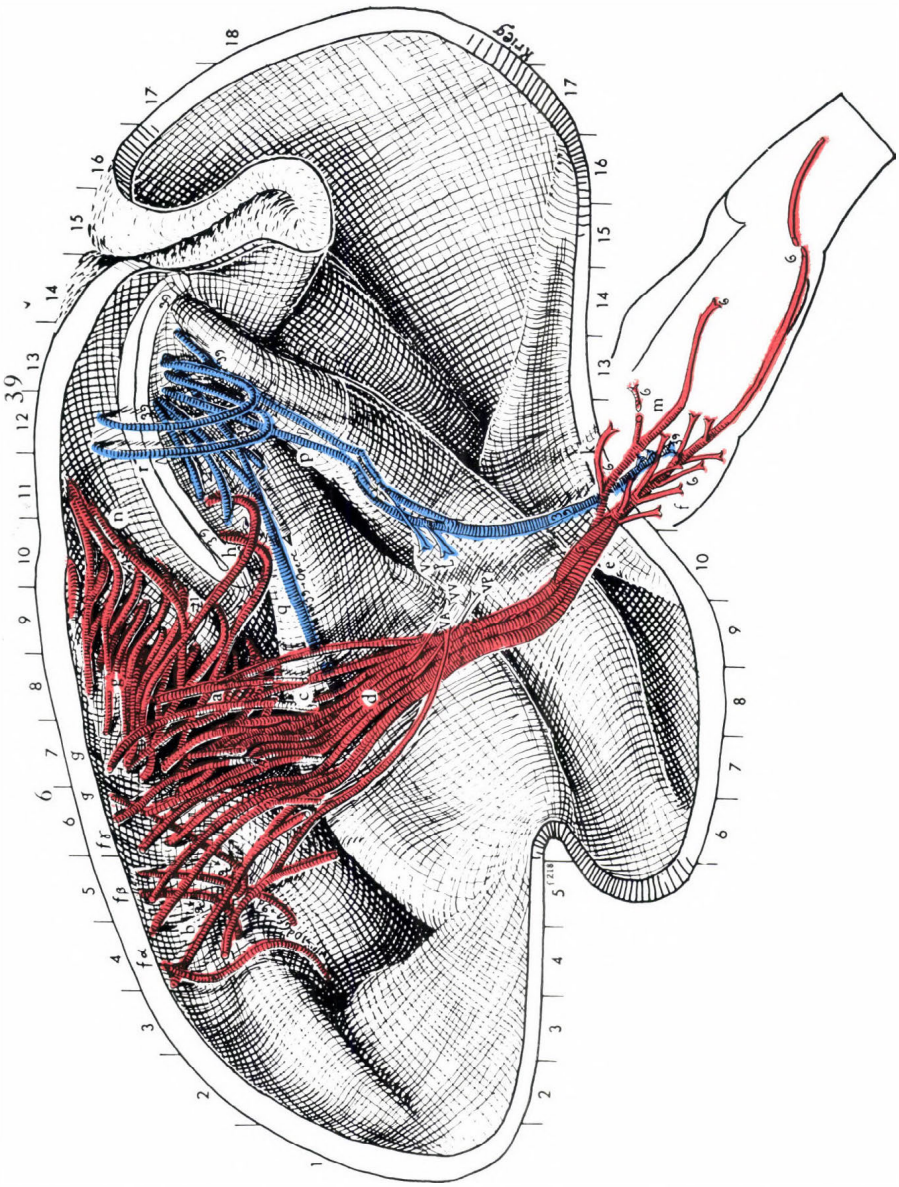

Fig. 9. — Corticifugal connections of Areas 6 (red) and 39 (blue) in the macaque. The salience of the intraparietal sulcus is amputated

fibers to any part of the striatal complex, for that matter we may say once and for all, we have never seen connections from any part of the cerebral cortex to the striatum in any of our series. The possibility of unmyelinated connections is not denied. Nor does Area 6 send any number of connections to hypothalamus, subthalamus or substantia nigra. Many

continue through cerebral peduncle and terminate profusely among the cells of the medial part of the pons. Only a few run the gamut and course through the bulbar pyramid. However, many diverge from the peduncle at midbrain levels and run downward in the medial lemniscus as aberrant corticobulbars, to end in the tegmental and reticular nuclei, rather than in the primary motor nuclei.

Thalamic nuclei receiving fibers are chiefly of the ventral group: ventralis lateralis, ventralis anterior and ventralis posterior inferior.

Virtually none of the many association pass to the prefrontal region, but a great number pass back to the motor Area 4; and many continue below central sulcus to somesthetic areas. These long associations are from the posterior subareas. An important observation is that a chain of short connections passes backward by stages within the premotor area, permitting a processing of inclinations to movement into specific contractions in sequence.

The entire picture of the connections of the premotor area confirms that it is not a prime mover, except for postural adjustments of massive motions; but instead it converts motor intents to actualities which are effected by Area 4, and that it controls the reticular motor system and informs the cerebellum.

Area 8, the area for conjugate deviation, is presumably much more than that in actuality. Some have regarded it as the frontal suppressor strip. It is the most posterior of the prefrontal areas to contain a granular layer iv. We regard it as essentially sulcal in type, which it is in actuality in the arcuate sulcus, but it enlarges above and below where, for exigencies of space, sulci could not exist. It is characterized by having no projections into the cerebral peduncle (Fig. 10, red), so its influence must be on the cortex, specifically premotor cortex, in its forward parts—a more forward link in the fronto-motor concatenation which we have come to regard as important. It connects strongly with the medialis nucleus of the thalamus, and receives from it, too, but it projects to ventralis anterior although it does not receive from it. Area 8 receives few connections from any part of the frontal lobe either before or behind. We shall see later that it receives strongly from superior temporal and insular regions, in common with other prefrontal areas.

Area 9, the principal frontal granular area, occupies the middle and inferior frontal gyri. All prefrontal areas send out only few projectional or associational connections (Fig. 6, yellow). Perhaps this observation is more indicative of their functional nature than a recital of their connections. The callosal connection is well developed; a capsular component begins fairly strong but most of its fibers enter the rostral pole of the thalamus; all of those which continue end among the medial pontile cells. Apparently Area 9 informs the cerebellum what actions are contemplated but it does not itself effect any action. A fair number of associations pass to various parts of Area 6, initiating activity.

While Area 9 is considered as the major region of contemplation, planning and willed activity, Area 46, the sulcal counterpart of Area 9, (Fig. 11, yellow) and similar to it, but must be one level higher, or at least more remote, for it has fewer connections of any sort, except to Area 9 and Area 8—the real ivory tower of the monkey's intellect. It is destined to increase

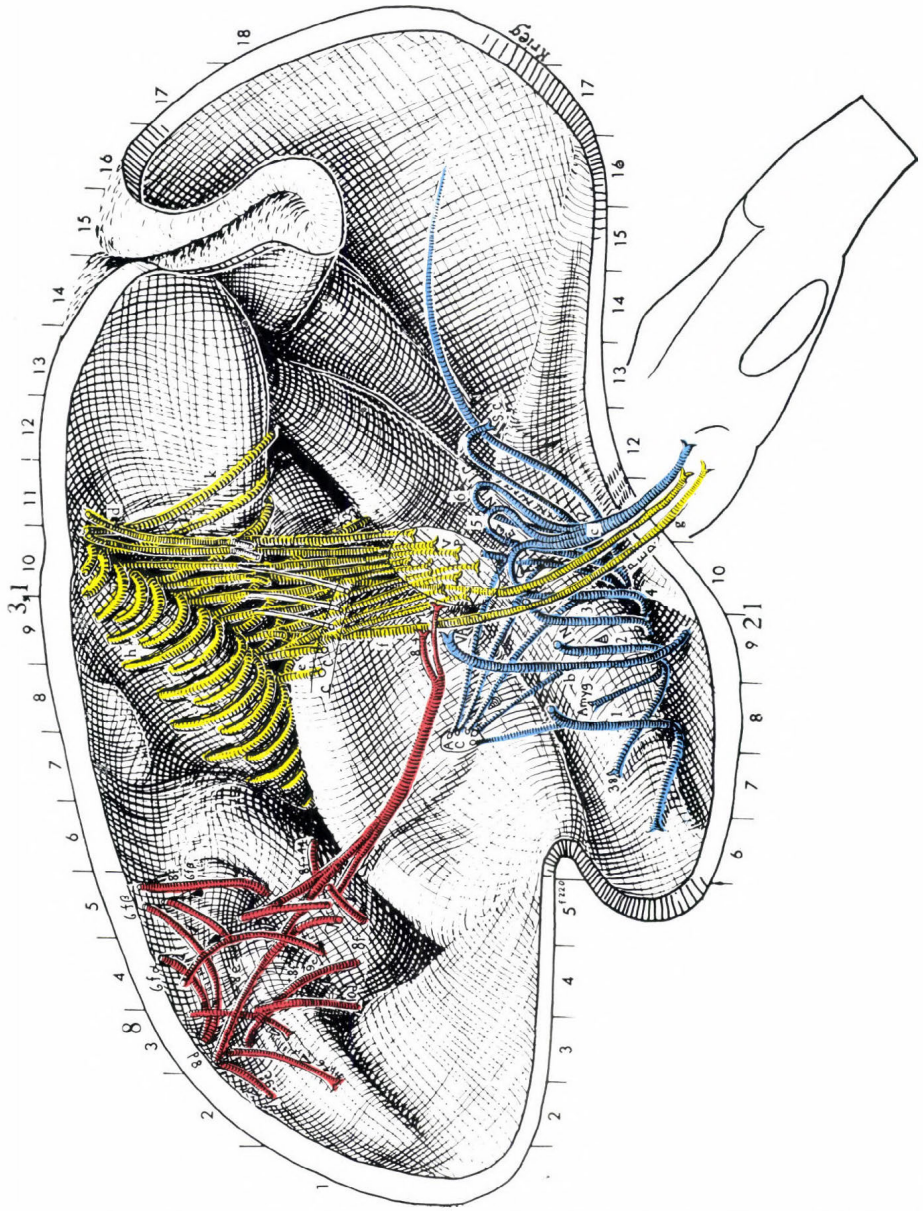

Fig. 10. — Cortifugal connections of Areas 8 (red), 3, 1 (yellow) and 21 (blue) in the macaque. AC = anterior commissure; CC = corpus callosum; PED = cerebral peduncle; SC = superior colliculus; VS = ventralis anterior; VP = ventralis posterior; 1—6 = stalks from Area 21

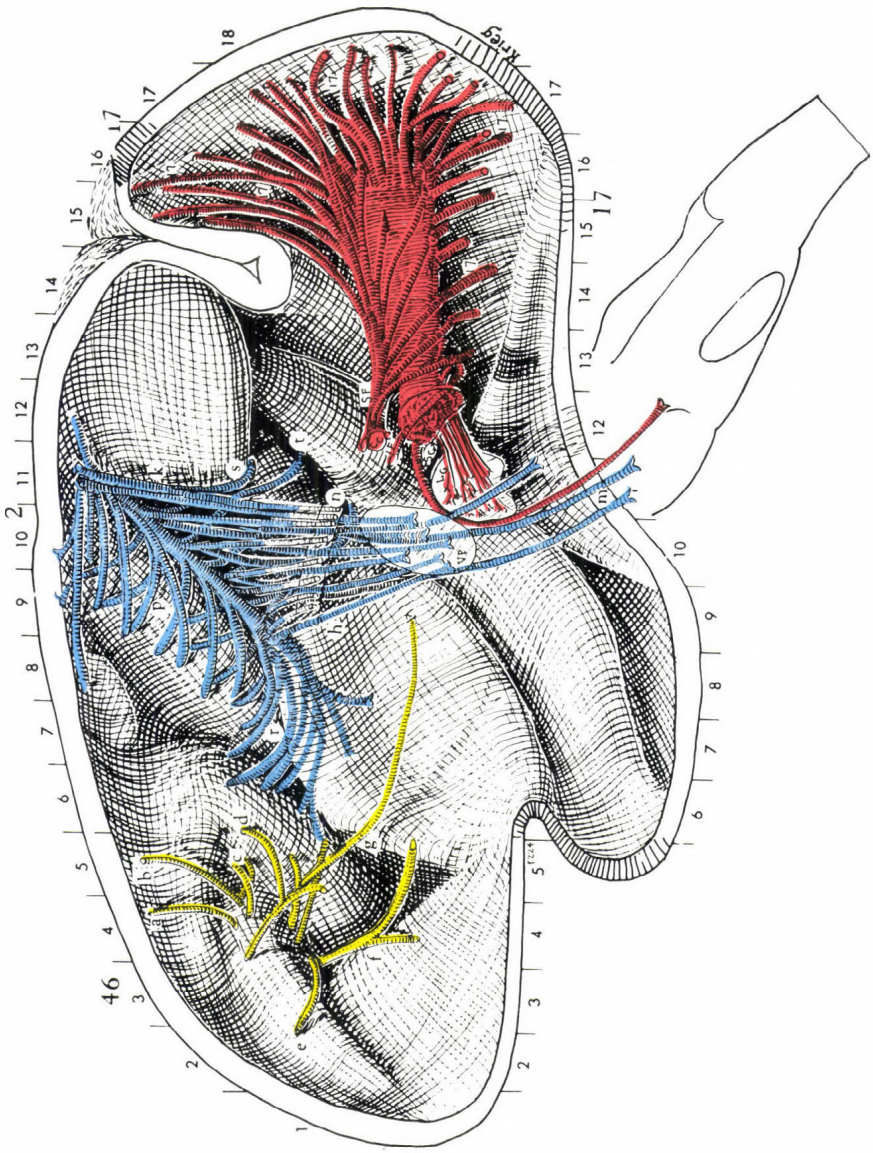

Fig. 11.—Corticifugal connections of Areas 46 (yellow), 2 (blue) and 17 (red) in the macaque

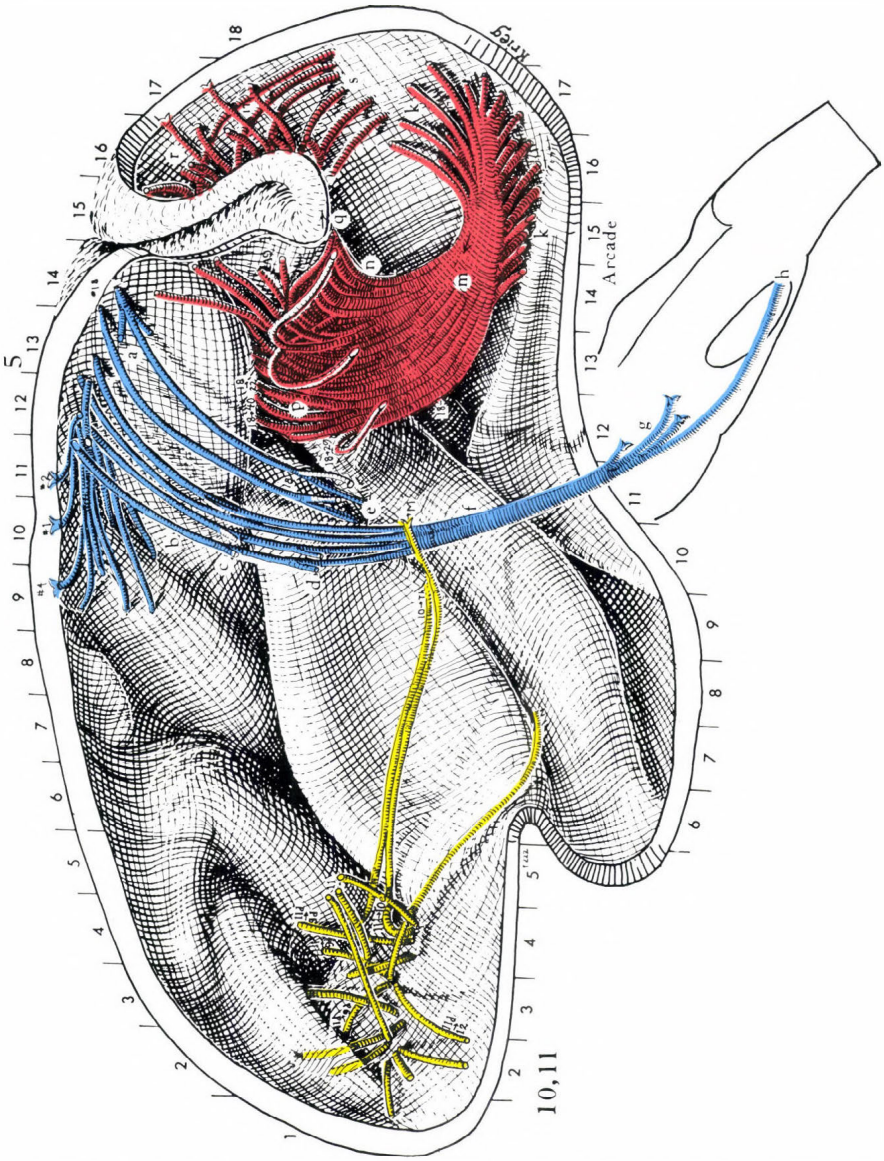

Fig. 12.—Corticifugal connections of Areas 10, 11 (yellow), and 5 (blue) and the occipital arcade (red) in the macaque

greatly in the human brain, supporting its interpretation as being intellectual It receives richly from medialis nucleus, from superior temporal gyrus, and also from Area 9 around it.

Areas 10 and 11 may be grouped together as occupying the orbital surface and the frontoorbital gyrus. Consonant with their prefrontal nature they have very few efferent connections, and none reach the peduncle (Fig. 12, yellow). They receive abundantly from medial nucleus, and send to it likewise. On the cortical side they receive abundantly from superior temporal and insular regions. Stimulation to this region in animals by Kaada elicits autonomic activity, and in man by Penfield evokes moods and states of mind with autonomic concomitants. Sufficient associations to Area 9 exist to enable us at this time to forge a concatenation of connections from temporal to 10 and 11, thence to 9, thence to 8, so to 6f and 6g, finally to 4. We have traced the discrete motor act back from formulation to planning and finally to a state of mind which is induced by an abstraction of total sensory experience as represented in the temporo-insular field.

We have noted a decreasing number of efferent connections as we have traced frontally. Contemplation, reflections, moods, do not need direct projections but they must be able to translate their decisions into activity, broadly speaking, behavior.

In beginning with the motor area we have been forced to work backwards toward the source (premotor, prefrontal) but the advantage has been that of proceeding from the known and concrete to the poorly understood and the abstract. The remainder of the neocortex is under the direct dominance of the three great senses of touch, hearing and vision. To understand the non-frontal cortex we must trace each of these senses into the cortex and see what is done with them, which means we must trace the thalamocortical radiations. The thalamus is intimately bound to the cortex, so to understand the cortex, we must understand the thalamus.

Each of the triad of discriminative senses (touch, hearing, sight) sends a projection from a discrete thalamic nucleus to a localized region of the cerebral cortex, and most of the other larger nuclei project to one or other non-sensory cortical region, so that most of the cortical surface is served by some part of the thalamus. We ordinarily think of the cortex as a shell or dome, and of the thalamus as a central mass sending out radiations at all angles toward this surface.

If the picture were this simple, our troubles would be over, but by making direct small stereotaxic lesions in the thalamus we have come to some unexpected and disquieting discoveries. One of them is that the thalamocortical radiations do not follow the corticifugal fibers in a reverse direction but, as is best exemplified by the medialis-frontalis projection, they accumulate under the internal capsule until they can break through, which they do abruptly. Once on the cortical side they tend to run directly under the cortex for long distances, accumulating under gyral crests. Another is that after penetrating the capsule they send off great systems of true collaterals at right angles into gyri the fibers are passing. This may be repeated, and it is probable, though difficult to demonstrate, that throughout their long subcortical course they send off collaterals into the cortex. This means that one thalamic cell discharges into a strip rather than a point of cortex. These phenomena are not confined to the

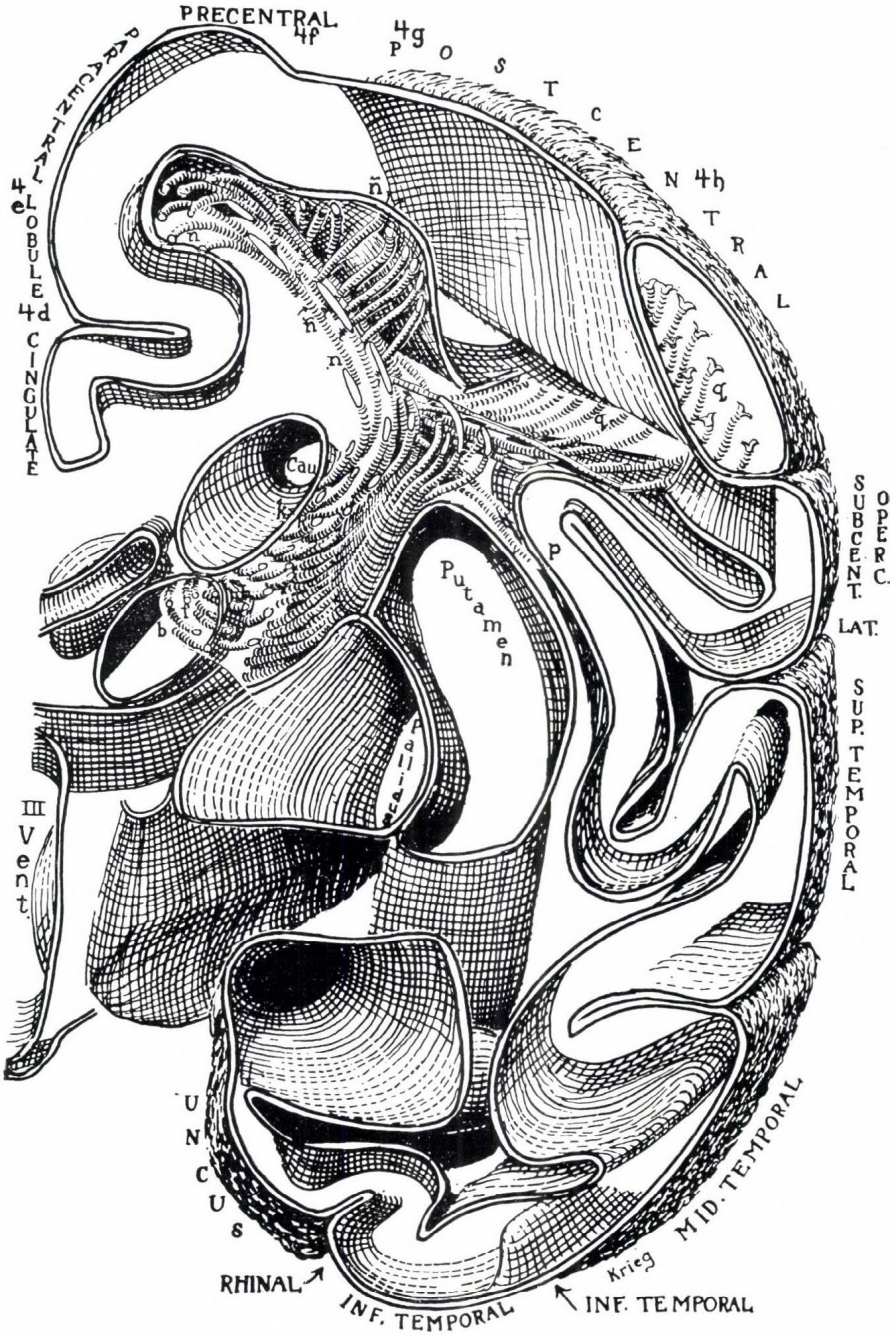

Fig. 13. — Thalamocortical connections

medialis-frontalis radiation, but heavily shared by somesthetic and visual projection system. The somesthetic radiation is primarily directed toward the precentral gyrus which the main trunks run through, and then turn frontally at the dorsal edge of the cerebrum (Fig. 13). Under the central sulcus stout parallel collaterals are given off at a sharp right angle. These enter the postcentral gyrus and thus the somesthetic cortex, but they too give off collaterals to the inferior parietal lobule, while the main stalks turn occipitally at the dorsal edge. This distribution by collaterals is also true of the visual radiation, but only Area 17 is served (Fig. 14). This arrangement seems redundant in view of the punctate localization of the visual field. However awkward for strict localization, this situation is a fortunate one for the organism, since it would apparently allow one region of the cortex to function vicariously for another which is damaged, and suggests that the use made of the information transmitted depends on the character and connections of the cortical neurons encountered.

Turning now to the parietal region, the realm of the somesthetic system, we begin with the postcentral gyrus. The Brodmann Areas 3, 1 and 2 which compose it are usually recited together as though they were a unit, but this is from ignorance of the specificity built into them. It is true that all three receive terminals from the ventralis posterior nucleus, though to greatly differing degree, Area 3 receiving the richest supply, Area 2 the least. Area 3 sends a richer cortico-thalamic feedback than any other cortical area, but it neither sends nor receives many associational fibers (Fig. 10, yellow). This is the role of Area 1, which sends to adjacent gyri in front of and behind it. The subcentral lamina from Area 1 to Area 4 is very well developed, constituting the shortest interareal cortical circuit of the cerebrum. The posterior group curve under the intraparietal sulcus to inferior parietal lobule, or run straight backward along the dorsomedial edge, to Area 5. A few stout fibers reach pontile nuclei, but activity upon postcentral stimulation depends upon the subcentral connection. The role of Area 2 is different again (Fig. 11, blue). In addition to well-developed connections to the somesthetic nuclei, a very strong callosal connection, and a fair number of fibers into pons, it makes connections with more distant cortex, not only with inferior parietal lobule and temporal lobe, but sends a strong forward connection to motor and prefrontal cortex, furnishing somesthetic information for and incitement of motor acts The postcentral gyrus handles exteroceptive sensations, while Area 5 of th. superior parietal lobule deals with proprioceptive.

Area 5 (Fig. 12, blue) receives terminals of the main stalks of somesthetic fibers, but whether these are enough for all the proprioceptive information from the body is uncertain. Possibly some integration to avoid duplication takes place in the thalamus. It also receives from the postcentral gyrus, especially from Area 2. More projection fibers from it reach the peduncle than from any other parietal area, but they end in the pontile nuclei, to relay to the cerebellum, as would be expected of a proprioceptive area.

Area 5 is quite selective in its associations. It sends a strong bundle forward under the dorsomedial edge, which terminates in the more dorsal part of Areas 2 and 1, but especially in the motor area. Certainly the conscious proprioception area should connect strongly with the principal effector area. It does not associate with Area 7 directly behind, which is

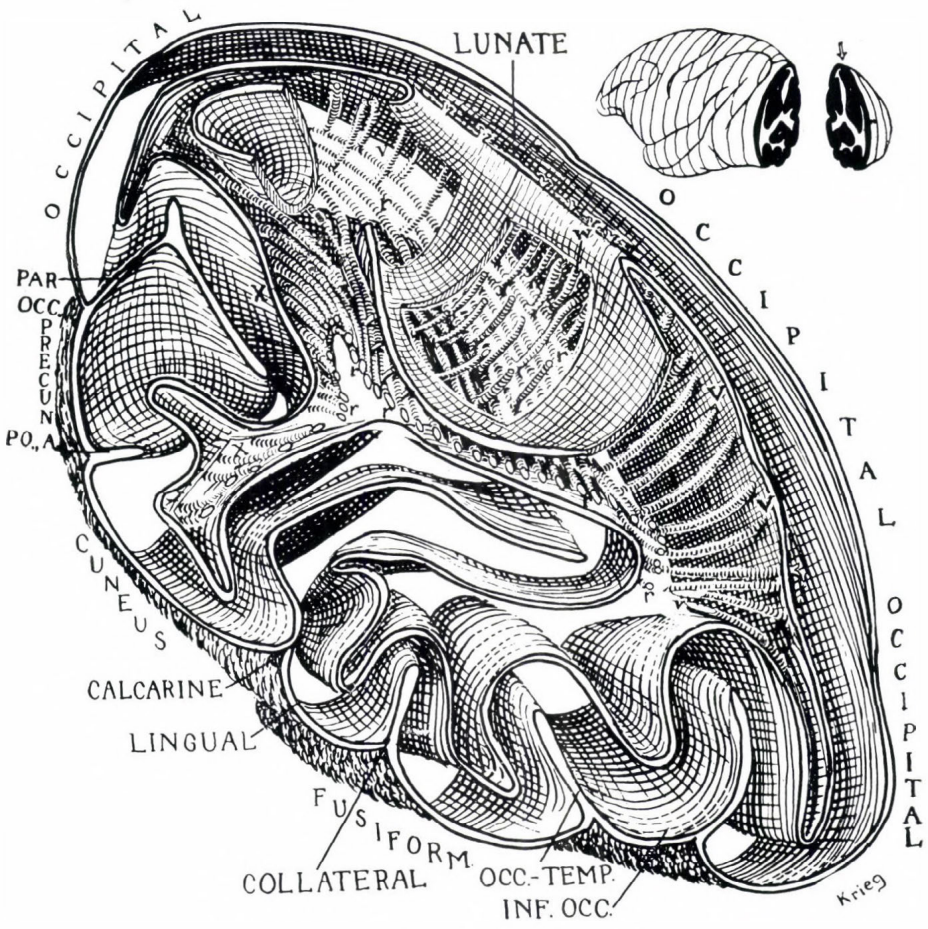

Fig. 14.–Thalamocortical connections

in a high and narrowing gyrus. Such observations as this, and there were many during the course of the study, give evidence of the specificity of cortical areas, that they do not just associate with what lies near at hand.

The inferior parietal lobule, destined to expand into the language centers of the human brain, is composed of Areas 40, in front, and 39, behind. These have rather differing connections: 39 receives from both somesthetic and visual psychic areas (Fig. 9, blue), so is in a position to synthesize an interpretation to a total experience. It projects to pontile nuclei and to the somesthetic thalamic nucleus, and associates to Area 5, and most important, perhaps, is a well marked forward-running bundle to the frontal operculum, where in man the motor speech area of Broca is situated.

Area 40 receives from 39 and from secondary somesthetic areas but sends almost nothing out. It is thus in a position to integrate and store the highest syntheses of the discriminative senses—namely, experience. We are gradually learning, in this work, not to judge the efficiency or

high calling of a cortical area by the wealth of its associations. As in the world, those who are freest to go their own way, receiving the most refined and sophisticated callers, are the most esteemed.

The occipital region is entirely taken over by the visual system, no fiber of any other nature penetrates it. We have come to think of it as a color television screen, with built-in recording camera and computing machine. This is no mere flight of fancy, we feel that by interpreting the occipital lobe in this way, using different elements, it is true, we will go further in understanding the role of the occipital lobe than by feeling we must send the visual images elsewhere for utilization. The fact is that there are very few efferent connections going elsewhere, either, except to superior colliculus.

We have seen that Area 17, the visual area (Fig. 11, red), receives the projection of the visual image from the lateral geniculate body. The visual radiation goes to no other cortical area, and all parts of Area 17 receive it equally. We have long been aware that the visual field is spread out on the visual area in an orderly manner, though with greatly distorted coordinates. However, the corticifugal connections of the visual area have not been known.

The projectional-callosal stalks are present here, too, but they are short because they almost immediately encounter the occipital continuation of the internal capsule—the sagittal stratum—and bifurcate into projection collaterals which run forward in the sagittal stratum, and into callosal stalks which form the forceps of the callosum and end, according to Meyers, in Area 18, not 17. The projection fibers penetrate the pulvinar without synapsing and are distributed to the superior colliculus. A few hardy individuals enter the cerebral peduncle and continue to the pontile nuclei. Mixed with the projection fibers, or collaterals of them, we are not sure which, are thalamic recurrents, in this case to lateral geniculate body, of course. Cortical points and sectors of the geniculate correspond, for connections in both directions.

One might have the notion that all points of the visual area would be elaborately interconnected. Paradoxically, there are very few intraareal subcortical connections for Area 17, nor is there room in the very thin white matter of the occipital lobe for such, when the known afferents and efferents are subtracted.

However, one unsuspected and rather remarkable association system was found, and we have christened it the occipital arcade (Fig. 12, red), for it takes a different course from all other association tracts and forms a great system of arches in the lateral wall and in the parietooccipital boundary zone. It consists of fibers from the lower part of the visual area, which run forward just outside the sagittal stratum. On reaching the parieto-occipital boundary they climb dorsally as a broad lamina and run occipitally again under the parieto-occipital sulcus. A large number end in Area 19 befote the recurrent limb begins, others are lost to Area 18 on the way but many continue into the occipital gyrus, under Area 17, forming their own lamina outside the attenuated remnant of the sagittal stratum, and end in the upper half of Area 17. The disseminated distribution in Area 19 shows us we are not to look for a direct transfer of spatial localization in the visual synthesizing areas.

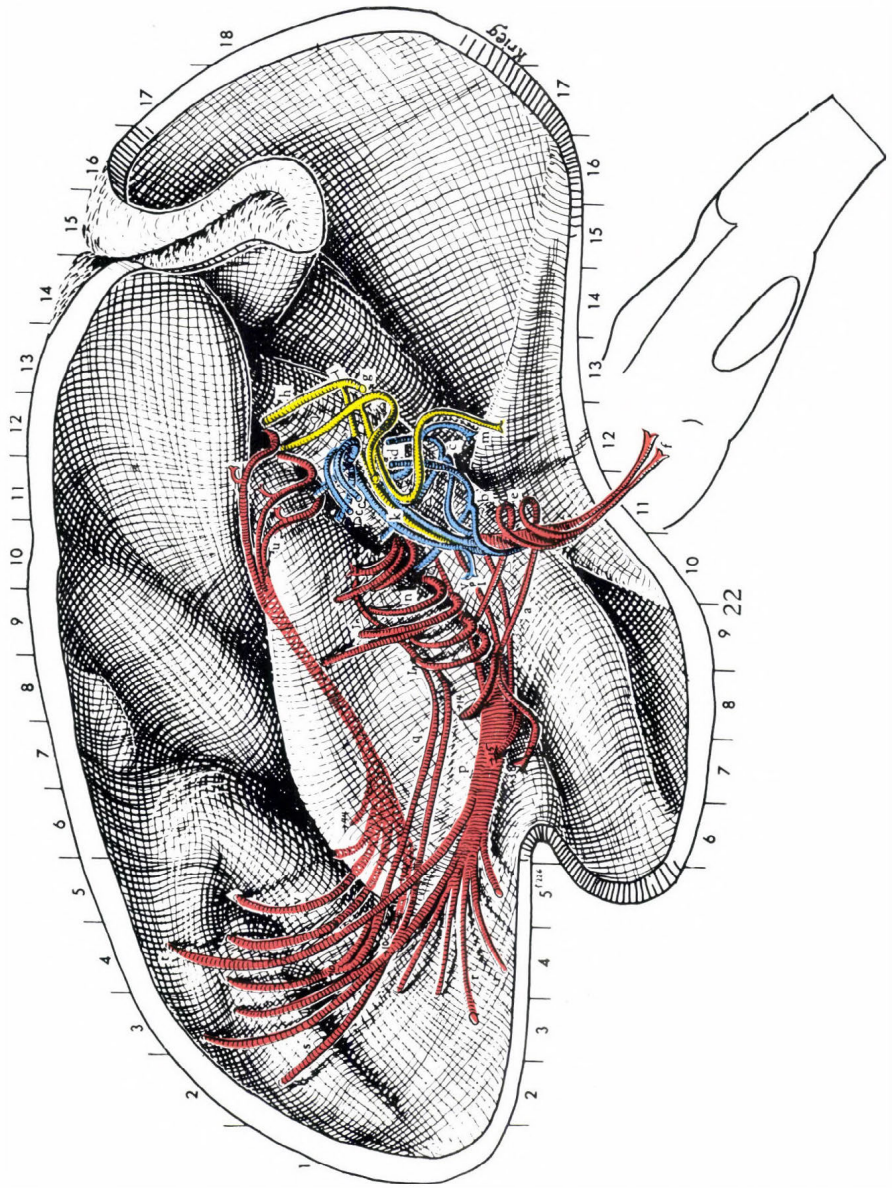

Fig. 15. — Corticifugal connections of Area 22 in the macaque

Areas 18 and 19 have much more in common than their appearance in section. Area 18 entirely surrounds the visual area, and Area 19 comletely surrounds Area 18 with Area 17. The three form the occipital lobe. Neither receives from any thalamic nucleus, both project exclusively into superior colliculus (Fig. 6, blue), both receive abundantly from visual area but neither send many connections back in return, nor do they communicate

widely with adjacent areas or at all with distant ones. The occipital lobe is its own world.

The fact that there is little interconnection between stations of the visual area and that there are few cortical associations outside occipital lobe teach us important lessons about the nature of cerebral cortex and leave us in the speculative realm. When we ask where and how the visual picture is actually seen, we must answer, in Area 17, and each minute part is synthesized subjectively to make a meaningful colored image which we can imagine only as 'seen' by something else as an image on a screen. But this image screen is its own eye, a concept difficult for us to imagine. This is where we live, visually, not elsewhere, and we should not always shift the burden to some other part of the cortex we are not thinking about at the moment. Callosal fibers between the two halves of the visual field are non-existent, yet our image is without a trace of a seam. This should be argument enough for the integrational viewpoint. So if we want to know more about the mechanism of vision we must examine the visual cortex more closely. The single cell studies of Hubel and Wiesel show that there are specialized cells in the visual cortex of all specialized sorts: shift to left, shift to right, on, off and on-off, etc. This is only the beginning of the detailed analysis of the various cells of the various areas in the cerebral cortex, which will be one of the principal fields of future research.

The temporal region retains much of its mystery, though we know much more about it than before. Stimulation in conscious human subjects by Penfield evokes the most subjective and bizarre results—visual phantasmagoria and forgotten episodes, remembered melodies, strange or uncomfortable or ill-defined emotions, peculiar states of mind such as detachment of self from the body, unreality and *déjà vu*.

What parts and what connections does such a lobe possess? When we look at its afferents, except for the auditory radiation, we see very little and must conclude that they seem not to be massive enough for all the temporal lobe performs. The existing ones are of the right nature, however, coming from the highest parietal and occipital areas, which when combined with auditory abstractions, seem well adapted to furnishing the kind of synthesis and distillation from experience which would be required. But here again we must disburden ourselves of the notion that products of cortical activity are merely a blending of the afferent ingredients. Obviously the temporal cortex, in its more subjective aspects, is functioning for itself; it broods, so to speak, and brooders are influenced very little by the outer world. Visual and auditory memories are stored, recalled and all forms the material from which overall attitude, personality, selfhood, individuality and character are made.

The study of the temporal lobe connections has made significant revelations of its nature. In the first place, there is a sharp division hodologically between superior temporal gyrus and lower temporal gyri. No fibers connect the two divisions, and this is reflected by the great depths and length of the sulcus separating them. Repeatedly, in this study, the presence and the depth of sulci have been found to be a guide to the units of organization and their discreteness. It ought to be possible to broaden our knowledge of the cortical organization of various animal species by comparing

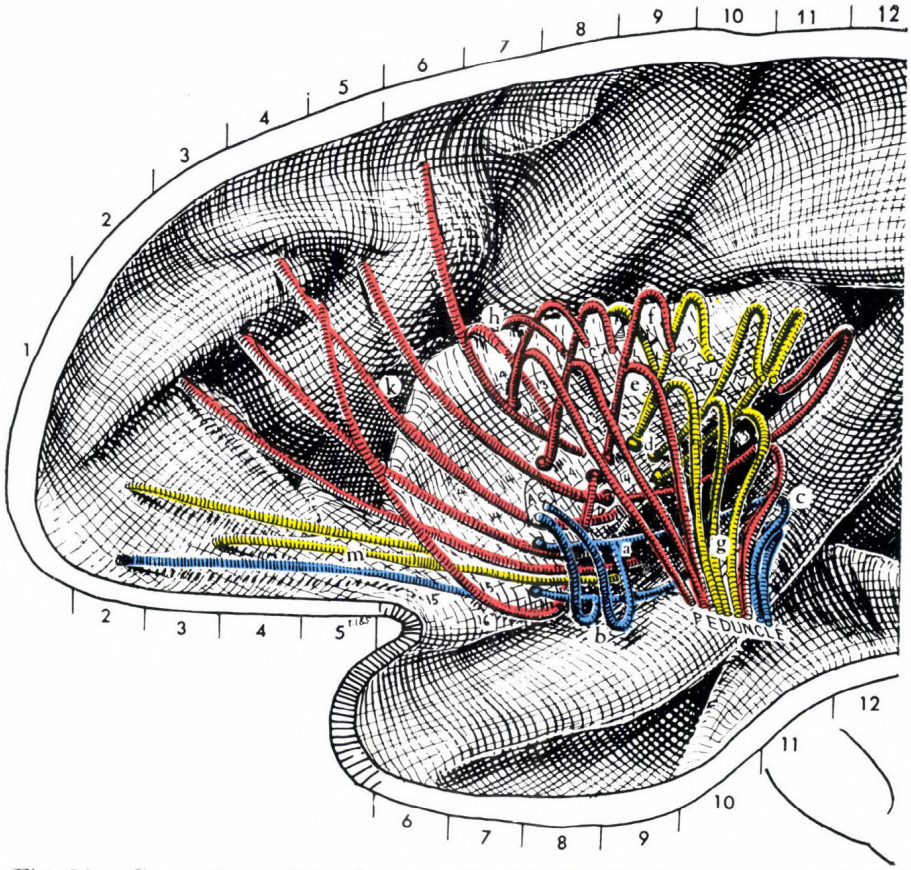

Fig. 16. — Connections of insula on parietal half-shell of monkey's cerebral hemisphere

their convolutional patterns, with some knowledge of the distribution of areas. Indeed, we may be on the threshold of a new phrenology of man, dependent on an interpretation of the convolutions. It is no job for the tyro, let us remember that some the most massive and important U fibers cross the central sulcus.

The efferent fibers of the superior temporal gyrus, Area 22, vary considerably in the different parts of its extent (Fig. 15). Posteriorly, the projection fibers are more abundant. Here, as elsewhere, there is a stalk which bifurcates at the retrolenticular corona into callosal and projectional divisions, the callosal keeping its smooth curvature, the projectional diverging to enter the lateral extreme of the peduncle, to end among pontile cells. One wonders what use the cerebellum makes of the information the temporal lobe sends it, but judging from connections, the cerebellum must be kept informed of what is occurring in nearly every part of the neocortex except motor area and primary sensory areas. The three-dimensional ordering of this system is beautiful to contemplate but difficult to

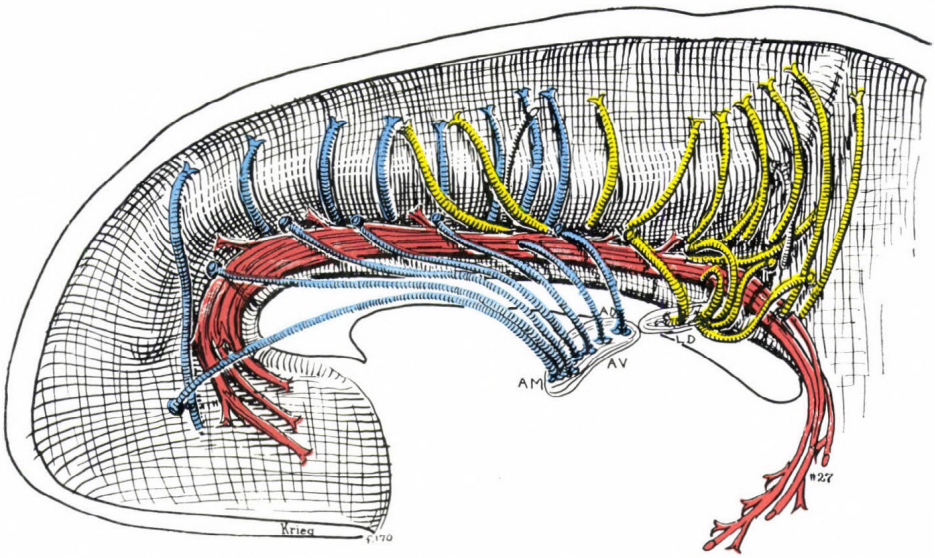

Fig. 17. — Reconstruction of connections of cingulate cortex. Lateral view of cortex of medial surface

explain. No cortico-thalamics have been seen. No associations pass backward or ventrally, but the insula is well supplied. The principal discovery here is that Area 22 sends associations richly to the entire prefrontal and orbital regions. It does this by three separate systems—below, lateral to, and above the putamen. It is in this way that the temporal lobe is able to pass along its commentaries on experience in order to influence intellectualization, contemplation and finally behavior.

Although the sulcal cortex forming the high walls of the superior temporal gyrus on either side aggregate much more area than the gyral surface, the combined cross section of the stalks arising from the walls is much less. We have found gyral and sulcal cortex in many other, but not all, parts of the cerebrum to differ radically in the extent of their connections, even though clearly belonging to the same general area. Fewer afferents reach sulcal cortex, fewer projections and fewer associations come from it. Perhaps the connections of the thalamo-cortical stalks, travelling in the crests of the gyri, have enriched the life of these favored strips, leaving the sulcal parts for ancillary work and for elaborations. The greater thickness of their supragranular layers corroborates this interpretation. The cortex at the fundus of a sulcus is still further reduced and ordinarily has still fewer connections. In some situations, notably on the basal surface of the occipital lobe, the sulci are simple foldings, without alteration of structure or proportion of connections.

The middle and inferior temporal gyri, comprising, respectively, Areas 21 and 20, though extensive, neither receive nor send many connections of any kind (Fig. 10, blue), but the architecture of their stalk is an interesting test of the universality of the bifurcation-collateral theory. After passing through the gyrus, and on reaching the counterpart of the corona radiata at the edge of the sublenticular capsule, the collateral branches turn into

the lateral extreme of the peduncle as Türck's bundle. The stalk continues, but converges into the anterior commissure. It is the anteroventral position of the anterior commissure which permits the existence of a sublenticular capsule. If the crossing fibers had to cross in the callosum, the bifurcation would have to be much further posterior.

The connections and significance of the insula have always been a mystery. Larson, in our laboratory, has made a special study of insular and cingulate lesions. Brodmann Areas 13 to 16 comprise the insula, while the inner surfaces of upper and lower opercula include Areas 50 and 52, respectively, Areas 15 and 16 are small and at the orbito-insular transitional zone. Areas 13 and 14, comprising the flat face of the insula, so well visualized in the halfshells, send out stalks that divide as do those from other parts of the cortex. From the lower part of the insula the crossing branch passes through the anterior commissure, while the projection joins the sublenticular capsule (Fig. 16). Those stalks from the main part of the insula are flattened into the external capsule but bifurcate as usual. Since all internal capsular fibers must clear the putamen, they surround it above and behind and pass into the peduncle; and since all callosal fibers must clear the caudate nucleus they run dorsally before turning medially. No thalamic connections are seen.

The insula sends numerous fibers forward through external capsule as a sheet which expands in the prefrontal region, terminating like the temporofrontal group, but they extend more medially in the orbital region into Area 12, and further back, into the forward parts of Area 6.

The connections of the cingulate region are unique (Fig.17). There are no stalks, no callosal component, and no projection fibers whatever, from any true cingulate Area 23, 24, 25, nor probably from 31 or 32. Contributions are made to and received from the longitudinal cingulum bundle, which arches over the top of the corpus callosum, and shows a strong tendency to continue into the lower limbic cortex, terminating freely in Area 27 of the parahippocampal convolution. The projections of the anterior nuclei follow this course also, many terminating in Areas 23 and 24, but apparently the main stalks tend to continue around the callosal splenium into the parahippocampal convolution.

The associations may be grouped as curving around the cingulate sulcus to end in the portions of the neocortical areas of the medial cerebral surface which adjoin them.

The corticothalamics are traced with ease around the caudate and into the nucleus anterior medialis from Area 25, the anterior ventralis from Area 24; and into the lateralis dorsalis from Area 23. This is the little nucleus, shaped like an almond, which sits on top of the lateralis posterior, and has always been an orphan among thalamic nuclei.

We have now described briefly and illustrated summarily the connections of the principal areas of the cerebral cortex. Each has its own portrait, which like human portraits differs subtly from others, and each has its own set of connections which fit together to form a continuum. These connections fall into four categories, each of which may now be very briefly summarized.

All parts of the cortex send off a stalk which proceeds in a parallel and orderly manner to the corona radiata, through the core of a gyrus if one

is present, directly if not. There the main stalk continues, generally without abrupt change of direction through corpus callosum, still keeping an orderly arrangement, usually to the same point on the opposite side, without much scatter. Area 2 shows a wider distribution, and we are told that the callosal connections of Area 17 end in Area 18.

As the stalk passes across the internal capsule it sends off at a right angle if local conditions permit, a collateral which follows the capsule for a variable distance to thalamus, to pontile nuclei, to tegmentoreticular nuclei, or to primary motor nuclei. Myelinated fibers from cortex do not project to corpus striatum, to hypothalamus, or to subthalamic structures. It is not determined whether corticothalamic connections are collaterals or independent axons or both. They generally pass to thalamic nuclei directly medial to the segment of the capsule which passes them, and generally to the thalamic nuclei which project to their origin, so that the arrangement of the thalamus reproduces *in parvo* the arrangement of the cortex, except that the thalamus is three-dimensional, the cortex two-dimensional.

The motor area alone projects to primary motor nuclei but not to pontile nuclei.

The premotor Area 6 projects to reticular substance and to pons.

Some areas project to pons but not at all or only slightly to other stem or cord structures. They are 6, 9; 1,2; 22, 21, 39, 13, 14.

Some areas project to superior colliculus but only slightly to pons: 17, 18, 19.

The projections of some areas never go further than the thalamic nuclei in the monkey, though they may in man (8, 10, 11, 3, 38, 40).

There are some areas which have only very slight projections, or none at all (12, 20, 35, 36).

Finally, we must make a class for areas which project to brain stem but have no thalamic connections (13, 14, 22, 21).

Surveying the interareal connections in the broadest possible way, we find that the cerebral cortex can be divided into realms of influence which have elaborate but hierarchical systems within themselves, but which intercommunicate but little.

Any notion that the cerebral connections consist of a primitive reticulum from which pathways are later worn by use must be abandoned. For the most part, these realms coincide, fortuitously, with the classical lobes of the cerebrum, except that we would form a sensorimotor category including the swath 6, 4, 3, 1, 2, 5 as an integrated unit. Within this unit are direct sensorimotor connections from somesthetic areas. There are somewhat larger connections from secondary somesthetic areas to motor, and to premotor. This involves a longer circuit, more possibility of choice and of modification. Outside this there are interregional connections of a higher order; these are the connections from the inferior parietal lobule to the premotor and posterior prefrontal areas. Finally is the long circuit, the temporo-insulo-frontal system, which might involve the following concatenations 3→1→2→7→40→21→9→8→6→4. For higher intellectual considerations this one is the most important, for it permits an enormous abstraction and subjective modification from crude sensation, through the successive modifications of interpretation, combination, appreciation,

abstraction, subjectivization, mood or inclination, reflection and contemplation, judgement, decision, planning, activation, organization, motion. This long chain is the most likely to break under stress or with abnormal wear, and a chain is no stronger than its weakest link. We consider this viewpoint the most significant thing we have learned in the whole project.

It should be emphasized that quantitatively the connections differ enormously, for this cannot be well shown in drawings. The corticifugal system of the motor Area, 4, is by far the richest of any, not only in the brain stem but also in the cerebrum, and there the fibers are the largest. The back part of the premotor Area 6 perhaps comes next, but its fibers divide away as one traces them down. The forward part of the premotor area has many fewer fibers than the near part. Area 8 sends out still fewer and finer fibers. In the entire prefrontal region the connections from fairly large lesions may even be difficult to follow in the capsule.

Turning to the non-frontal cortex, the corticothalamics from the postcentral gyrus are heavy and rich, and all connections from Area 2 and 5 are fairly robust, but those from the inferior parietal lobule are thin. Corticifugal fibers from the occipital lobe are fairly prominent and mediumly large of fiber. The temporal lobe connections show great differences dependent on the position in the lobe. From the dorsal and occipital end they are as abundant as from the middle parietal areas, but they grow more rarefied as we pass forward, until from the polar and ventromedial parts they can be exasperatingly difficult to follow at all.

It is obvious that the connections of cerebral cortex differ quantitatively even more than qualitatively, and in appraising an area this is very important.

It should now be clear that cortical areas differ not only in details of their connections, which could not all be the same, anyway, but in their character and hence in their function. It is clear that there are classes and hierarchies, just as among men, not only are they identified by their occupations, but placed in hierarchical classes dependent on these occupations.

The lowest level is the driven cortex—the motor area and the primary sensory areas. These are entirely ordered and at the mercy of the outer world and must function when the individual's body is awake. Next is conceptualizing cortex, Areas 1, 2, 5, 7, 18, 42; a cut higher is synthesizing cortex, Areas 39, 40, 22. At this level on the motor side comes organizing cortex, the complex called 6, plus 8 and 44. Then, continuing in order, is abstracting cortex, which from experience builds notions and concepts: Areas 19, 22a, 21, 20, and on the prefrontal side Areas 10 and 11, and finally autonomous, contemplative, independent cortex 9, 46, possibly 38 and sulcal parts of other high areas.

We can sharpen the picture if we think of cortical cells as people, people with telephones, tending to be independent and individual and self-contained unless acted on by outside influences. The laborers are, like driven cortex, at the mercy of the demands of the outer world. The creative workers—artists, writers, researchers—are the autonomous, inner motivated group. Next, below them are the professional men, rendering a high grade of service. Somewhere below are the corporation men and the businessmen, serving the outer world and dependent upon it, but less directly than the

driven. All classes are necessary for the complete functioning society. Together they form a population, as do all the areas of the cerebral cortex considered together. As a hierarchy of a large population they automatically establish new principles of activity which amount to special laws of conduct, and are the basis of organized society—in the case of the cortex they are called the laws of mental activity. Thus a study of the cerebral cortex, properly pursued, is an analysis of the sociology of large working populations of varied units.

DISCUSSION

Környey : The lecture brings up important viewpoints to both neuropathologists and clinicians. A part of the connexions revealed may be found in pathological human cases (e. g. in amyotrophic lateral sclerosis the degeneration extending towards the corpus callosum), the others will have to be looked for in cases with foci in appropriate sites.

Szentágothai : I am wondering whether you have noticed any difference of projection from the optic cortex to the lateral geniculate according to central and peripheral parts of this region. In the cat there is some evidence that the corticofugal fibers from the optic region to the lateral geniculate body—that are considered inhibitory—are arising rather from its borders than from its center.

Engström : Are the results constant from animal to animal, and may size or convolutional variation interfere with generalization?

Kiss : It was a pleasure for me to see Professor Krieg's excellent drawings of the monkey brain, illustrating the subject in a new and unique manner which shows great competence and skill. The optic radiation in the monkey should, in my opinion, give information also in human relations. As a human anatomist I should like to inquire whether Professor Krieg could, on basis of his recent investigations, give us new data on the human cerebral tracts. We could use them and Dr Krieg's skill in designing in our efforts to demonrate these things in our text books and atlases.

W. S. Krieg : (to Dr Környey) Selected examples of multiple sclerosis should be very useful in extending the findings of degeneration in the monkey, to man. While the lesions in multiple sclerosis may be larger, in myelin-stained sections, than the path of degeneration from them, in old lesions one should be able to trace continuous trails of granules that would have the value of a Marchi series.—(to Dr Engström) We order animals with only a small range of head size, so the brains we use are nearly constant in size. The macaque has a remarkably constant convolutional pattern, and the findings from similar lesions in different examples have corresponded. We have tried to be as objective as possible, and often lesions would be reconstructed without the recollection that similar ones had been already studied. If the results had not been quite consistent, one should have grown discouraged and abandoned the project before fifteen years had passed.—(to Dr Kiss) Actually we have extrapolated our findings to the human brain so far as possible and have matching half-shell reconstructions of the human brain for all of those of the monkey and a comprehensive series of slice reconstructions showing the internal architecture of the human cortical fibers. All these will appear in the new book *Connections*

of the Cerebral Cortex to be published this fall.—(to Dr. Szentágothai) I did not notice if there was any difference, but there might have been. At least it was not enough to attract my attention. I would recall though that there are great differences in the usual life of cats and monkeys. Monkeys are bright light animals with macular vision, cats are active in dark or dim light. The cats' visual field is more sensitive to objects entering the periphery. The monkey uses the macula for discriminative vision.

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EXPERIMENTAL ANATOMICAL STUDIES OF THE CORTICO-SPINAL AND CORTICO-RUBRO-SPINAL CONNECTIONS IN THE CAT

by

A. Brodal

ANATOMICAL INSTITUTE, UNIVERSITY OF OSLO
NORWAY

Recent neurophysiological research on the supraspinal control of spinal reflexes has demonstrated the need for a detailed knowledge of the exact sites and modes of termination of descending fibres to the cord. Although several of these connections have been studied by previous authors, the sites of termination of the various fibre systems have not been mapped in sufficient detail to permit correlation with neurophysiological data. It is now possible as a result of Rexed's (1952, 1954) studies, to indicate precisely the sites of termination of descending fibres with reference to the ten laminae which can be distinguished cytoarchitectonically in the spinal grey matter.

In the experimental studies in the cat, performed in our laboratory, the Nauta (1957) and Glees (1946) methods have been used to map the sites of termination of corticospinal, corticorubral and rubrospinal fibres following lesions of the cerebral cortex and the red nucleus, respectively. Horizontal and sagittal sections of the cord have been studied in addition to transverse sections, since thin fibres and small numbers of degenerating fibres are difficult to identify in transverse sections. The modified Gudden method (Brodal 1939, 1940) has been used to study retrograde cellular changes in the red nucleus following lesions of the spinal cord.

Cases with lesions of the sensorimotor cortex demonstrate that the majority of the *corticospinal fibres* terminate in the spinal grey matter on the side contralateral to the cortical lesion, more precisely in Rexed's laminae IV—VII (Nyberg-Hansen and Brodal 1963). However, fibres from the 'motor' cortex end more ventrally and laterally (mainly laterally in laminae V and VI and in the dorsal part of VII) than those from the 'sensory' cortex (mainly medially in laminae IV and V and in the dorsomedial part of lamina VI) as seen from the diagrams in Fig. 1. This distribution is found throughout all levels of the cord (except for the difference resulting from the absence of lamina VI in the thoracic cord). On the ipsilateral side there are a few terminations in the same positions as contralaterally, derived from fibres descending in the uncrossed corticospinal tracts. In longitudinal sections some degenerating corticospinal fibres can be ascertained even at lumbar levels in the ipsilateral lateral and both ventral corticospinal tracts, and degenerating fibres can be seen to enter the grey matter from all four corticospinal tracts.

Our studies of the cortico-rubro-spinal pathway were prompted by the observation that there is within the *rubrospinal projection* in the cat a distinct somatotopic localization. This was determined by studying the distribu-

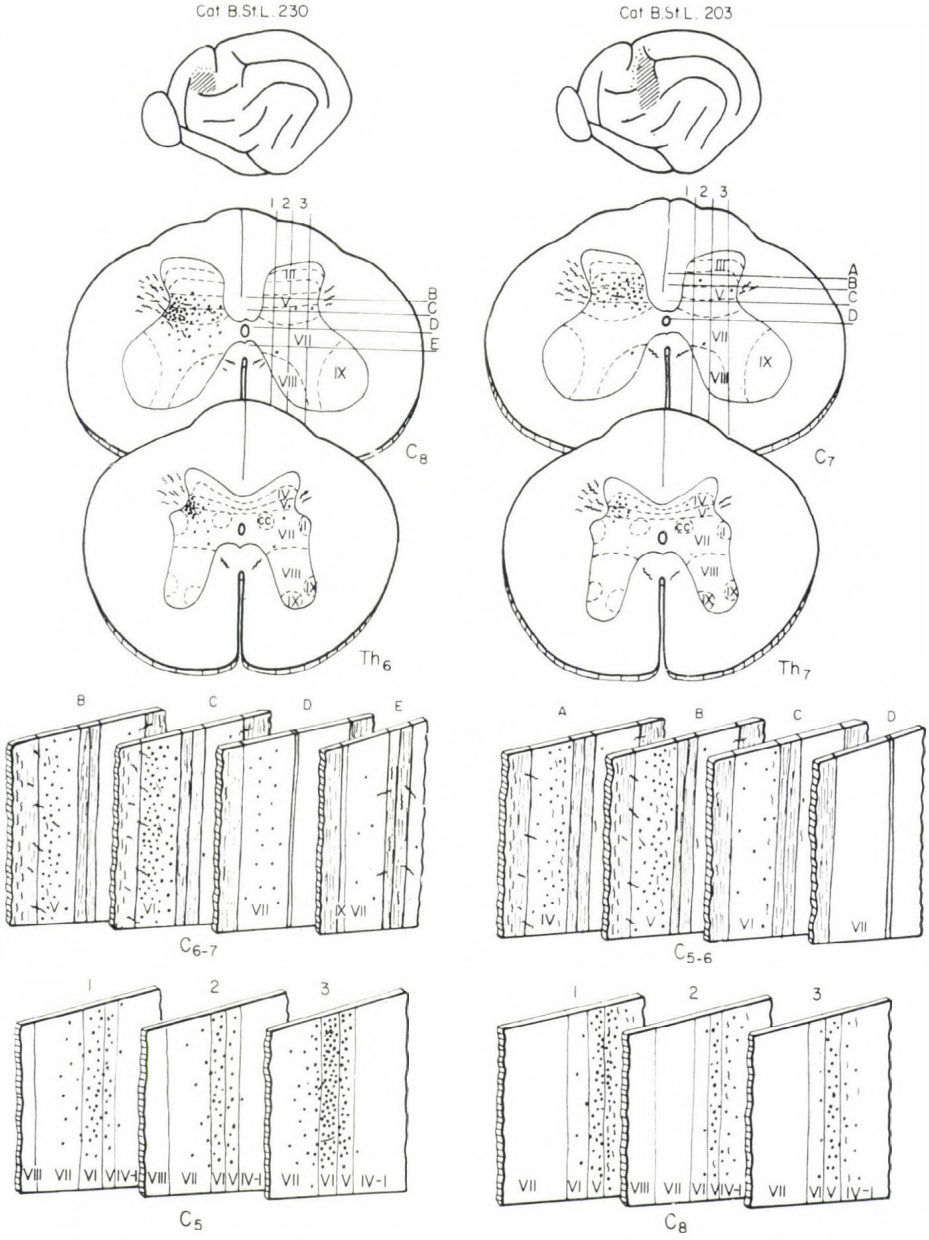


Fig. 1.—Diagrammatic representation of the difference in distribution of degenerating terminal fibres (dots) within the cervical and thoracic grey matter in two cases (B. St L. 230 and 203) with relatively isolated lesions of the forelimb 'motor' and 'sensory' cortices, respectively. Above, the extent of the lesions as seen on the surface of the left hemisphere. From the thoracic cord only transverse sections, while transverse as well as horizontal and saggittal sections are shown from the cervical enlargement. The positions of the horizontal and saggital sections are shown in the transverse sections. The white matter is not shown in the saggittal sections. From Nyberg-Hansen and Brodal (1963)

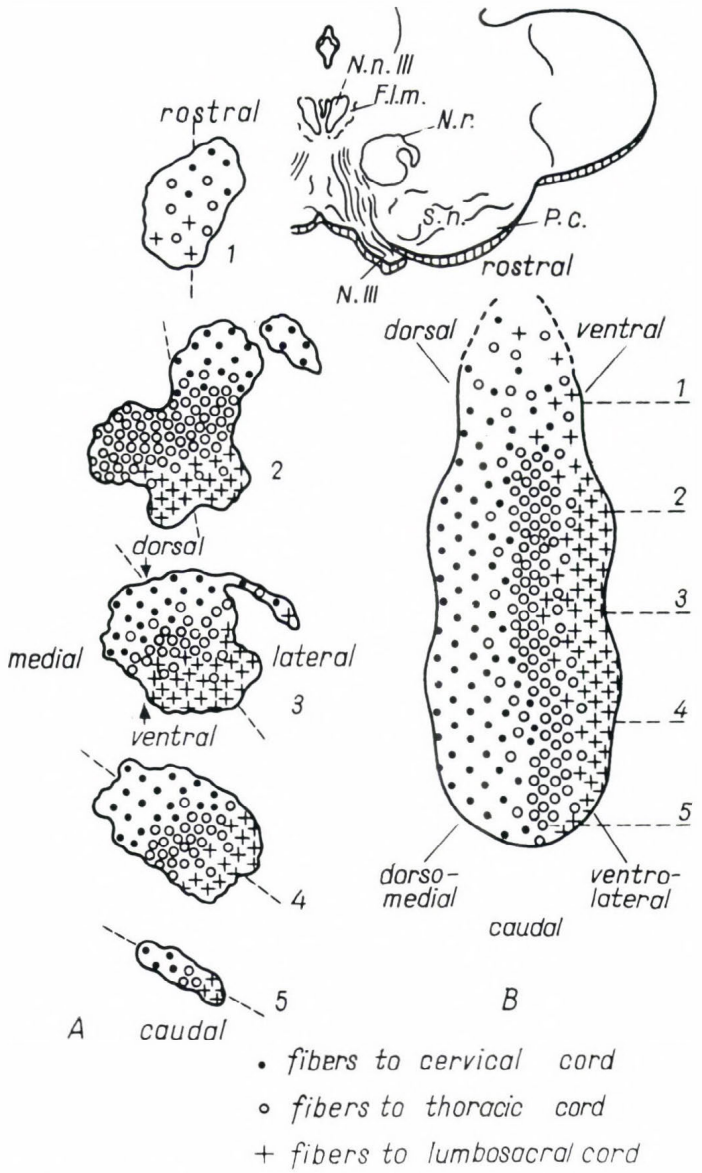


Fig. 2.—Diagram showing the somatotopical projection of the red nucleus onto the spinal cord, as determined experimentally. To the left (*A*) a series of transverse sections through the red nucleus, to the right (*B*) a longitudinal reconstruction of the red nucleus. From Pompeiano and Brodal (1957)

tion of retrograde cellular changes in the red nucleus following lesions at various levels of the cord (Pompeiano and Brodal 1957). As seen from Fig. 2, the ventrolateral part of the nucleus projects onto the lumbosacral cord,

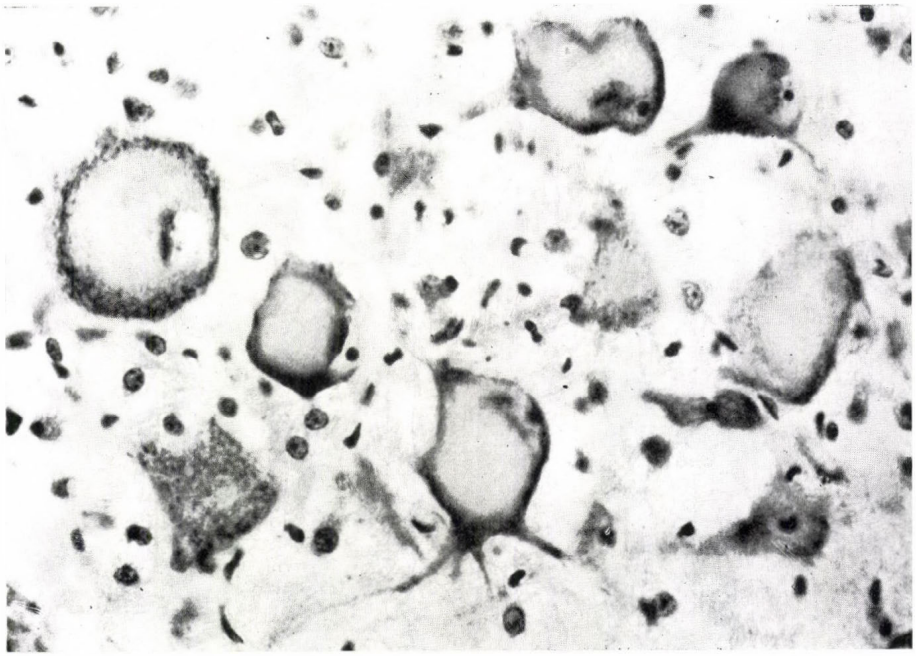


Fig. 3. — Photomicrograph of cells in the red nucleus of the cat, showing retrograde changes following transection of the ventrolateral funiculus of the cord

the dorsomedial onto the cervical cord, while the intervening part sends its fibres to the thoracic cord. A further observation of interest is that not only the large cells of the red nucleus but a great number of small ones as well project onto the cord, as evidenced by the occurrence of typical retrograde changes in both types of cells following lesions of the cord (Fig. 3).

The demonstration of the somatotopic pattern in the rubrospinal projection naturally raised the question whether the *cortico-rubral projection* is likewise somatotopically organized. This problem was studied by mapping the terminal degeneration in the red nucleus as seen in silver impregnated sections following lesions in the cerebral cortex (Rinvik and Walberg 1963). It turned out that while the 'motor' cortex projects amply onto the red nucleus, the 'sensory' cortex gives off relatively few fibres to it (supplying largely the reticular formation dorsolateral to the nucleus). Furthermore, the cortical projection to the red nucleus shows a clear-cut somatotopic pattern (Fig. 4). The fibres from the forelimb region of the cortex end dorsomedially, those from the hindlimb region ventrolaterally, i.e. in the fore- and hindlimb regions of the nucleus, respectively. Thus the entire cortico-rubro-spinal projection is somatotopically arranged, just as the direct corticospinal pathway.

Further points of resemblance between these two routes for impulses from the cortex to the cord were found when the *sites of termination of the rubrospinal fibres* were investigated. Following stereotaxically placed electrolytic lesions of the red nucleus, the ensuing terminal degeneration in

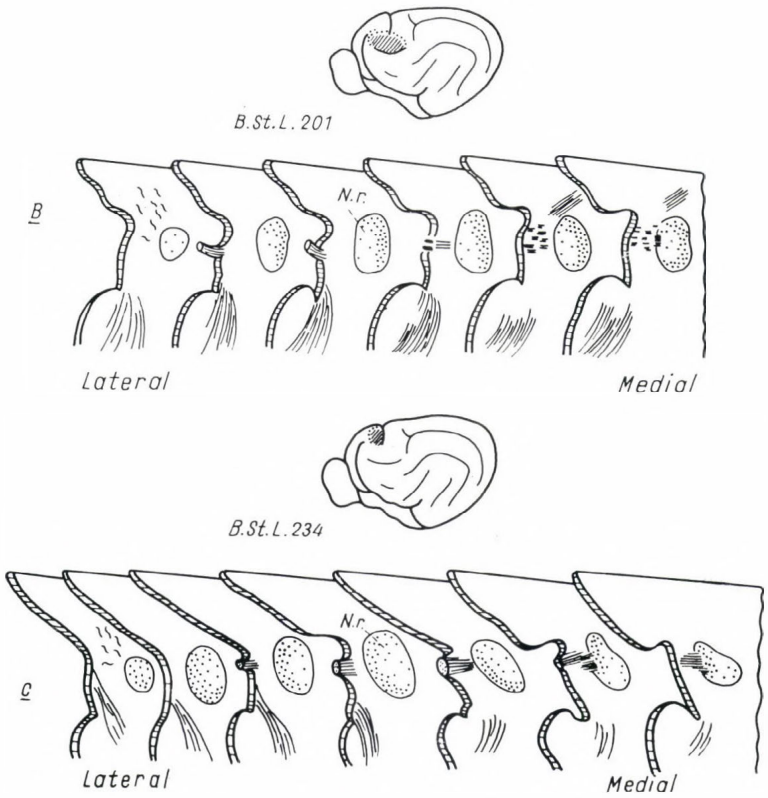


Fig. 4.—Diagrammatic representation of the distribution of terminal degeneration (dots) in the red nucleus of the cat as seen in series of sagittal sections following lesions of the cortical forelimb (*B*) and hindlimb regions (*C*). (cf. Fig. 2.) From Rinvik and Walberg (1963)

the cord was studied with silver impregnation methods (Nyberg-Hansen and Brodal 1964). The sites of termination of rubrospinal fibres were found to be the lateral half of Rexed's lamina V, lamina VI and the dorsal and central parts of lamina VII (Fig. 5). The rubrospinal fibres descend in the dorsal half of the contralateral lateral funiculus and undergo a shift in the dorsolateral direction during their descent. They reach to the lowermost levels of the cord. The somatotopical pattern in the rubrospinal projection (Fig. 2) was confirmed in cases with lesions involving restricted parts of the nucleus.

As will be seen, the sites of termination of rubrospinal fibres (Fig. 5) coincide with those of the corticospinal fibres derived from the ‚motor' cortex, a finding of particular interest, since the corticorubral fibres come chiefly from this part of the cortex. Furthermore, the corticospinal and rubrospinal fibres both establish synaptic contact with cells of all types present in their laminae of termination, and with somata as well as dendrites. In Nauta sections pericellular arborizations of degenerating fibres are seen

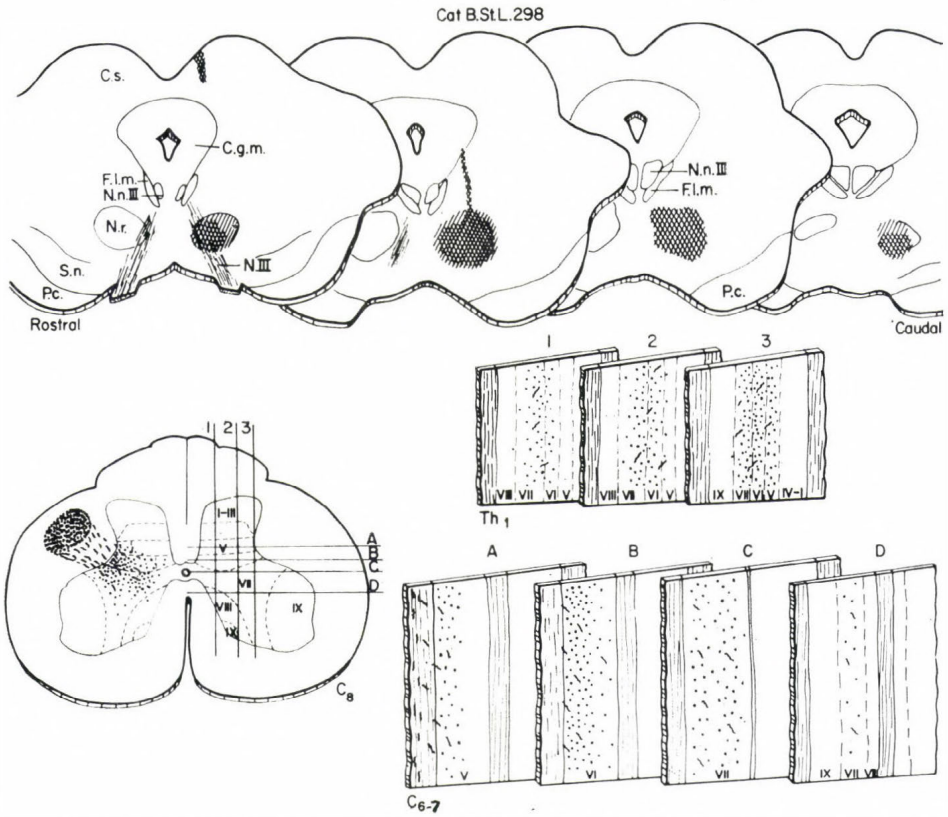
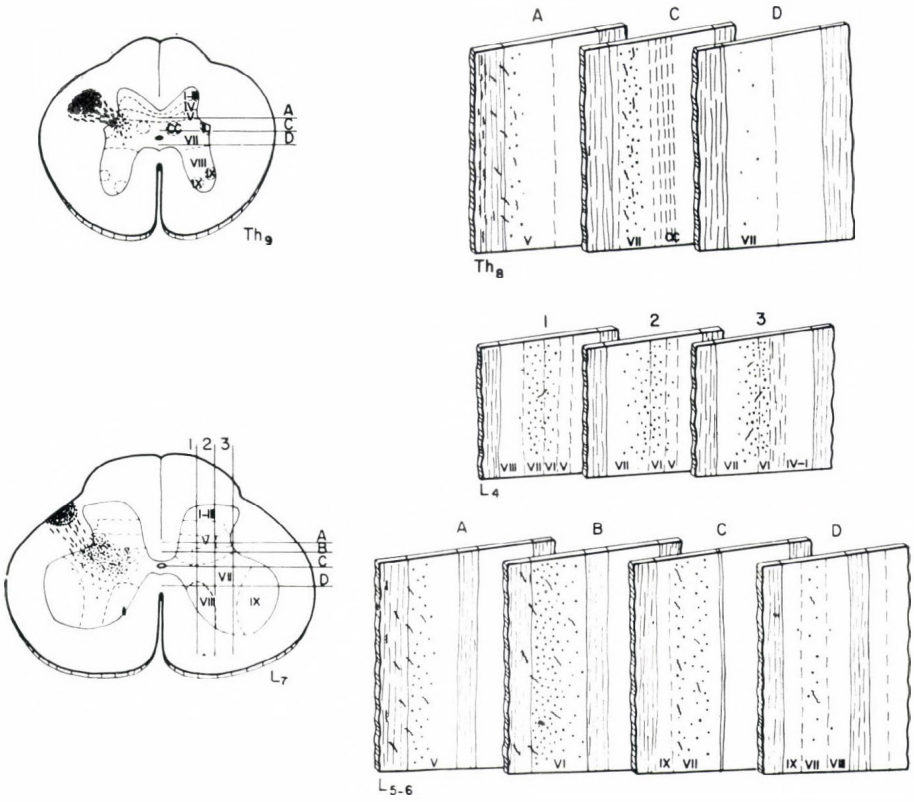


Fig. 5.—Diagrammatic representation of the distribution of degenerating coarse (wavy lines) and preterminal and terminal fibres (dots) within the spinal grey matter in cat B. St. L. 298 with a total lesion (hatchings) of the left red nucleus (above). From the thoracic cord only transverse and horizontal sections are shown, while sagittal sections as well are shown from the cervical and lumbar enlargements. The position of the horizontal and sagittal sections are indicated in the drawings of the transverse sections. The most peripheral parts of the white matter are not shown in the longitudinal sections. The roman numerals refer to Rexed's (1952, 1954) laminae of the spinal grey matter. From Nyberg-Hansen and Brodal (1964) (Fig. extends overleaf)

(Figs 6*b*, 6*d*) as well as terminations of fine degenerating fibres on dendrites (Fig. 6*a*). In Glees sections degenerating terminal boutons can be identified (Fig. 6*c*). In no case could we find degenerating fibres in contact with motoneurons of Rexed's lamina IX.

The terminations of corticospinal—and to a lesser extent—rubrospinal fibres in the cord, have in the past been studied by a number of authors, most of them using the Marchi method. This, however, is not well suited for a study of this subject, since it does not give precise information of the sites of termination of degenerating fibres. Unfortunately, most recent authors using silver impregnation methods have indicated the sites of termination only by referring to 'the base of the dorsal horn', the 'intermediate zone' and so forth, terms which are not well defined. Furthermore, most of them have not devoted particular attention to synaptic relation-



ships, i.e. whether fibres end on cells of different types and whether contacts are established with dendrites and/or somata of nerve cells. For references to the pertinent literature the reader is referred to our original publications. Suffice it to mention a few points. The absence of terminations on motoneurons in the cat has been noted previously as concerns the corticospinal fibres, among others by Szentágothai-Schimert (1941), Walberg and Brodal (1953), and Chambers and Liu (1957), and as concerns the rubrospinal fibres by Szentágothai-Schimert (1941) and Staal (1961). The differential distribution of terminations of corticospinal fibres derived from the 'motor' and 'sensory' cortices found in our study (Fig. 1) appears to be in general agreement with Kuypers' (1960) observations in the monkey. The origin of corticorubral fibres from the 'motor' cortex has been advocated among others by Levin (1936) and Mettler (1947), while no previous students have apparently recognized the existence of a somatotopical pattern in the corticorubral and rubrospinal projection. (The latter has been physiologically confirmed by Pompeiano, 1957.)

It may be objected against our describing degeneration with reference to particular laminae of the spinal grey matter that this is of limited value, since it is well known that dendrites of cells often extend for considerable distances beyond the perikaryon. However, even if our findings do not exclude that some cells outside the laminae showing degeneration may be

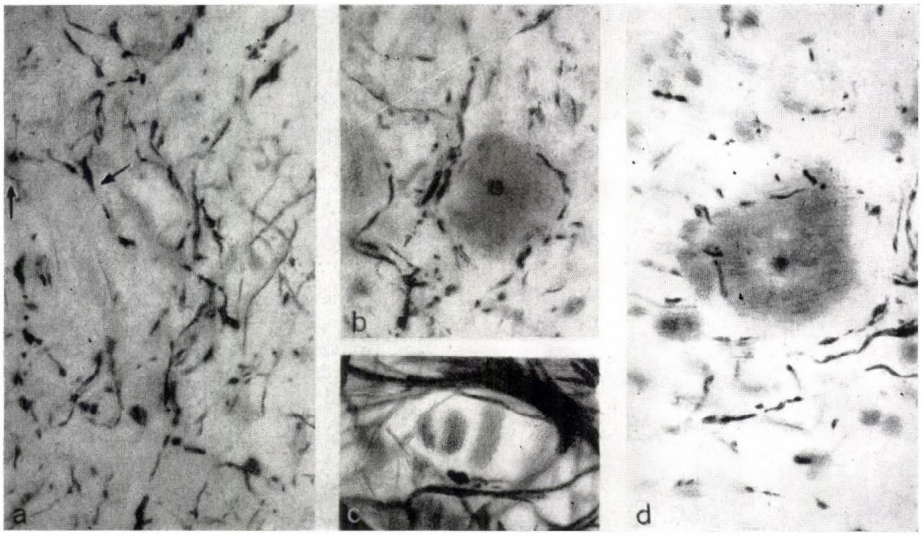


Fig. 6.—Photomicrographs of silver impregnated sections from the grey matter of the spinal cord following lesions of the cerebral cortex (a and b) or the red nucleus (c and d). *a* = degenerating fibres in contact with a dendrite (arrows) and the perikaryon of a large cell laterally in lamina VI at C_6. Nauta (1957) method, × 500. From Nyberg-Hansen and Brodal (1963). *b* = pericellular arborization of degenerating fibres around a large cell in lamina VI at C_7. Nauta (1957) method, × 500. From Nyberg-Hansen and Brodal (1963). *c* = degenerating terminal bouton in contact with a small cell medialy in lamina VI. Glees (1946) method, × 1100 (oil immersion). From Nyberg-Hansen and Brodal (1964). *d* = pericellular arborization of degenerating fibres around the soma of a large cell in lamina VII at L_7. Nauta (1957) method, × 500. From Nyberg-Hansen and Brodal (1964)

synaptically contacted by corticospinal or rubrospinal fibres, the density of degeneration in certain laminae, or even subdivisions of these, leaves no doubt that most of the contacts are established with cells in these laminae. Furthermore, reference to Rexed's laminae serves to make possible precise indications of importance for comparisons between anatomical and physiological findings (see below).

From a *functional point of view* two main problems will be briefly touched upon. The first concerns the *role of the red nucleus*. The demonstration that there is in addition to the corticospinal tract another pathway from the cortex to the cord which is somatotopically organized, namely the cortico-rubro-spinal, raises the question whether this pathway may be concerned in the execution of skilled voluntary movements. Some support for this view may be derived from the observations of Bucy (1957) and others that transection of the middle 2/3 of the cerebral peduncle in man is followed by only moderate impairment of the capacity to perform skilled movements. The objection which may be raised against the assumption that the cortico-rubro-spinal system is responsible for this, is that the rubrospinal tract is rudimentary in man. This generally accepted notion is, however, not well documented (for some data see Brodal 1962; Rinvik and Walberg 1963).

The second functional aspect to be mentioned is the *correlation of anatomical data with physiological studies* of the supraspinal influences on the cord (discussed in some detail by Nyberg-Hansen and Brodal 1963, 1964). The absence of terminations of corticospinal fibres on the motoneurons in the cat is in agreement with physiological data which show that the effects on motoneurons following stimulation of the cerebral cortex are mediated via internuncials. (In the monkey, however, anatomical and physiological data show that some corticospinal impulses may reach the motoneurons directly.) If, as it appears from the study of Eccles, Eccles, Iggo and Lundberg (1960), the γ neurons are located within the group of α motoneurons supplying the same muscle, the cortical and rubral effects on the muscle spindles likewise must involve internuncials in the cord. According to the anatomical findings, these internuncials must be located somewhere within laminae IV—VII. It is of interest that according to Eccles and collaborators the interneurons mediating inhibition of Ia impulses from the muscle spindles are found in lamina VI, where corticospinal fibres end, and that stimulation of these fibres increases this inhibition (Lundberg and Voerhoove 1962). Furthermore, the facilitation of flexor motoneurons obtainable on stimulation of the red nucleus (Pompeiano 1957) according to our findings must be mediated via interneurons in laminae V—VII. Finally, it is of interest that corticospinal fibres, particularly those ending in laminae IV—V and coming from the sensory cortex, may be involved in the cortical influence on the central transmission of sensory impulses, even if it appears from the physiological studies of Hubbard and Oscarsson (1962) that secondary sensory neurons (of the ventral spinocerebellar tract) may be found as far ventrally as in lamina VII. Presynaptic inhibition of impulses in primary dorsal root fibres occurring on stimulation of the 'sensory' but not of the 'motor' cortex was observed by Andersen, Eccles and Sears (1962). On the basis of our findings this most likely occurs by way of corticospinal fibres ending in laminae IV and V. It is interesting to note that the termination of corticospinal fibres in laminae IV and V of the cord appears to be paralleled by the termination of corticobulbar fibres in the sensory nuclei of the brain stem, such as the trigeminal nucleus (Brodal, Szabó and Torvik 1956; Kuypers 1958, 1960; Szentágothai and Rajkovits 1958), the dorsal column nuclei (Walberg 1957; Chambers and Liu 1957; Kuypers 1958, 1960) and the nucleus of the solitary tract (Brodal, Szabó and Torvik 1956).

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LOCATING AND IDENTIFYING POSTSYNAPTIC INHIBITORY SYNAPSES BY THE CORRELATION OF PHYSIOLOGICAL AND HISTOLOGICAL DATA

by

P. ANDERSEN* and J. C. ECCLES

DEPARTMENT OF PHYSIOLOGY, AUSTRALIAN NATIONAL UNIVERSITY
CANBERRA, AUSTRALIA

A. GENERAL PRINCIPLES OF DESIGN

For the proper operation of the central nervous system inhibitory synaptic action is indispensable. We must envisage postsynaptic inhibition as having just one function, which is to prevent the discharge of impulses from nerve cells. In most types of nerve cell, impulses are generated in the initial segment of the axon and propagation down the axon starts without appreciable delay. Usually, the impulse also propagates back into the soma and some way up the dendrites of the neurone, but this propagation has no functional significance when we consider that neurone as a unit component of a neuronnal network. All that matters in that context is whether or not the cell discharges an impulse along its axon to activate its synaptic endings. With some neurones such as hippocampal pyramidal cells (Cragg and Hamlyn 1955; Andersen 1960a; Spencer and Kandel 1961b; Fujita and Sakata 1962) and chromatolyzed motoneurones (Eccles, Libet and Young 1958) the generation of impulses or local responses may occur in the dendrites so that impulse propagation occurs down to the soma and so to the axon and its synaptic terminals. With such a functional arrangement postsynaptic inhibition could be very effectively exerted by synapses located on the dendrites in close proximity to the regions of impulse generation. But such an arrangement would have the disadvantage that these inhibitory synapses would be very unfavourably located for controlling impulse generation in dendrites remote from their location. For example, inhibitory synapses on the apical dendrites of hippocampal pyramidal cells would have very little control of impulse generation in the basal dendrites. This disadvantage could be overcome if there were also inhibitory synapses on the basal dendrites. And this wide dispersal of inhibitory synapses has been actually or tacitly assumed in all physiological discussions of excitatory-inhibitory interaction. Of course, it has also been recognized that postsynaptic inhibition can function at a distance by virtue of electrotonic transmission of the IPSP. But with the very extended dendrites of cortical pyramidal cells, for example, electrotonic transmission would be severely decremented, and there is no other way in which postsynaptic inhibition can spread.

* Present address: Department of Anatomy, University of Oslo, Norway

However, if we consider the general principles of neuronal operation in the central nervous system, we will appreciate that even this postulated wide dispersal of inhibitory synapses over the dendrites and soma can be criticized on the grounds of poor functional design. A neurone is a functional unit, because it is functionally effective only when it discharges an impulse along its axon. It has been frequently pointed out that a neurone resembles a binary system of a digital computer in having only two states, that of rest or that of all-or-nothing action. There can be no functional selectivity between the various types of excitation converging onto a neurone. They achieve a common functional significance when the neurone discharges an impulse down its axon and so in turn acts by its synapses on the various neurones next in sequence. It is, therefore, difficult to see a functional meaning in arrangements whereby a neurone is specifically inhibited with respect to one type of excitatory synaptic action, but is free to discharge impulses in response to another type of synaptic excitation: for example, if a cortical pyramidal cell was inhibited in respect of all excitation on its apical dendrites but not on its basal dendrites. It might be suggested that a balanced inhibitory control of all types of synaptic excitation could be secured if the inhibitory synapses were dispersed over the whole soma-dendritic structure; but even this would be a poor functional design, because submaximal inhibitory operation probably would fail effectively to oppose excitation at all sites, so that a relatively weak excitation might evoke impulse discharges, because its synapses were close to the initial segment of the axon and remote from regions of strong inhibitory action. This argument leads to the conclusion that in the most effective functional design the inhibitory synapses are located at the confluence of all excitatory actions, and particularly in close proximity to the site of discharge of the impulse down the axon.

A zonal location of inhibitory synapses on the axon hillock is an essential component of Gesell's (1940) polar hypothesis of excitatory and inhibitory synaptic action. It was supposed that at rest the dendrites are more depolarized than the soma-axon-hillock region, so that extracellularly there is current from this latter region to the dendrites. Synapses on the dendrites would increase this current and so cause the discharge of impulses down the axon. Contrariwise, depolarizing synapses on the axon hillock would decrease this current and are thereby supposed to depress the discharge of impulses, so having an inhibitory function. Intracellular recording from neurones has falsified this polar hypothesis of inhibitory action, but it had the merit of suggesting (cf. Retzlaff 1954) that the remarkable synaptic structures around the axon hillock of the Mauthner cell and of the Purkinje cell had some special function in inhibiting the discharge of impulses. With the Mauthner cell, Furukawa and Furshpan(1963) have shown that impulses in the helicoidal structure of fibres inhibit the discharge of impulses by exerting a brief anelectrotonic action on the axon hillock; and there is also a later inhibitory postsynaptic potential apparently produced by these same impulses acting by chemical transmission. Retzlaff (1954) illustrated a similar structural arrangement for the basket cell endings around the origin of the axon from the Purkinje cell of the cerebellum and Furshpan (personal communication) has suggested that this structure may also have an electrical inhibitory action.

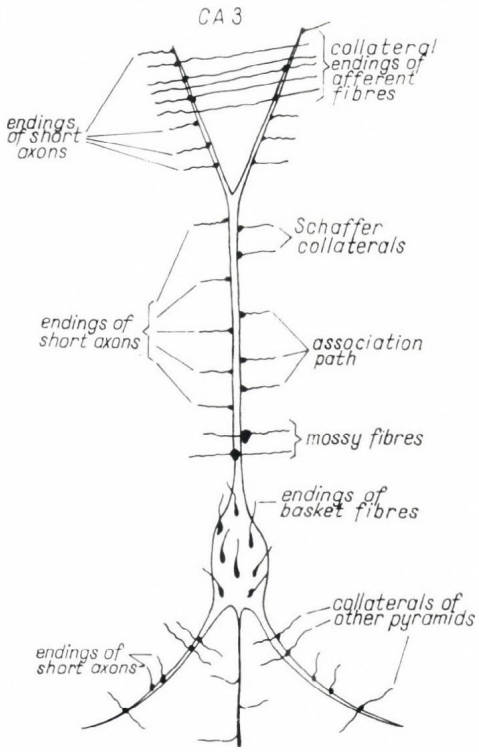

Fig. 1.—Diagram taken from Lorente de Nó (1934) of a hippocampal CA3 pyramidal cell, showing the lamination, or zoning of synapses, from various sources on different parts of the cell

B. INHIBITORY ACTION BY THE BASKET CELLS OF THE HIPPOCAMPUS

One of the most important aspects of hippocampal histology, making this area a preferred target for physiological studies, is the zoning of the synapses on the pyramidal cells. For all practical purposes these cells are arranged in one layer and parallel to each other, and with their long axes perpendicular to the ventricular surface. Since histological investigations have shown that the various afferent systems to the hippocampus form synapses in sharply restricted band-like structures, parallel to the surface (Cajal 1911, Lorente de Nó 1934, Blackstad 1956, 1958), one may conclude that a particular afferent route can influence synaptically a restricted part of the pyramids only. For example, in CA1 the basal dendrites receive commissural afferents, whereas the apical dendrites are influenced by afferents from at least the following four sources that are located respectively at increasing distances from the cell body: the septum, the contralateral hippocampus, the ipsilateral CA3 neurones, and the ipsilateral entorhinal area. The soma itself receives afferents almost exclusively from the basket

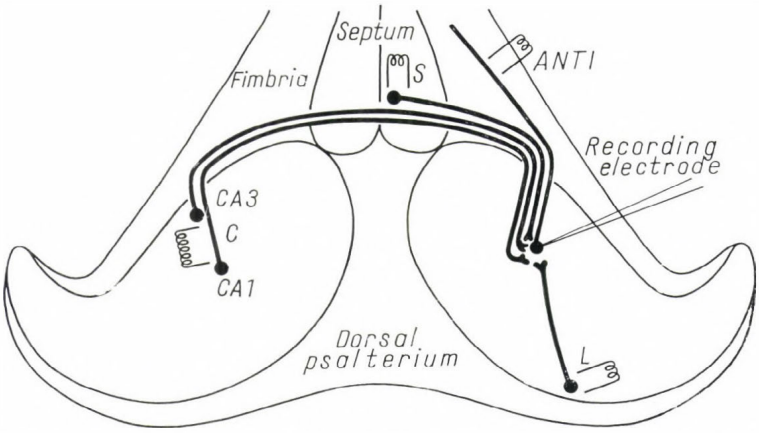

Fig. 2.—Diagram of the hippocampal formation as seen from above with the electrode arrangement used in this study. Heavy lines indicate the pathways stimulated. C = commissural, S = septal, L = local stimulation

cells. In CA3 the situation is similar (Fig. 1) except that the shafts of the apical dendrites are influenced by the longitudinal association path and not by commissural afferents. Furthermore, the effective mossy fibre system ends on the base of the apical dendrites.

Very significant physiological implications are, first, that it is possible to activate restricted portions of the hippocampal pyramids by the isolated stimulation of one of the sources for these afferents and, second, that some afferent systems can have but little effect on the generation of impulses by the pyramids since their synapses are located so peripherally, the entorhinal being the most obvious example.

The diagram in Fig. 2 shows the outline of the hippocampal formation as seen from above after the overlying neocortex and corpus callosum have been removed. The heavy lines indicate the neurones and their axons in the three distinct pathways that were activated: the commissural C, the septal S and the local L afferents. The fibres of these three afferent routes converge upon the pyramidal cells of the region into which the microelectrode was inserted. The efferent axons from the pyramidal cells course rostrally to the fimbria where they may be activated by a stimulus applied through the electrodes labelled ANTI. Besides this antidromic activation of pyramidal cells, there will also be excitation of fibres in the fimbria that have an orthodromic excitatory action.

The responses illustrated in Fig. 3 were evoked by the C, S and L excitatory inputs. In each of the triple assemblages there are from above downwards, the surface potential at high amplification with negativity upwards, the extracellular potential outside the pyramidal cell somas, and the intracellular potential. Both these potentials mentioned last are at the same low amplification and with positivity upwards. The sweep speeds were progressively slower from A to C, as shown by the time scales. All three inputs are seen to produce the very large and long IPSP of the pyramidal cell that have already been reported (Andersen, Eccles

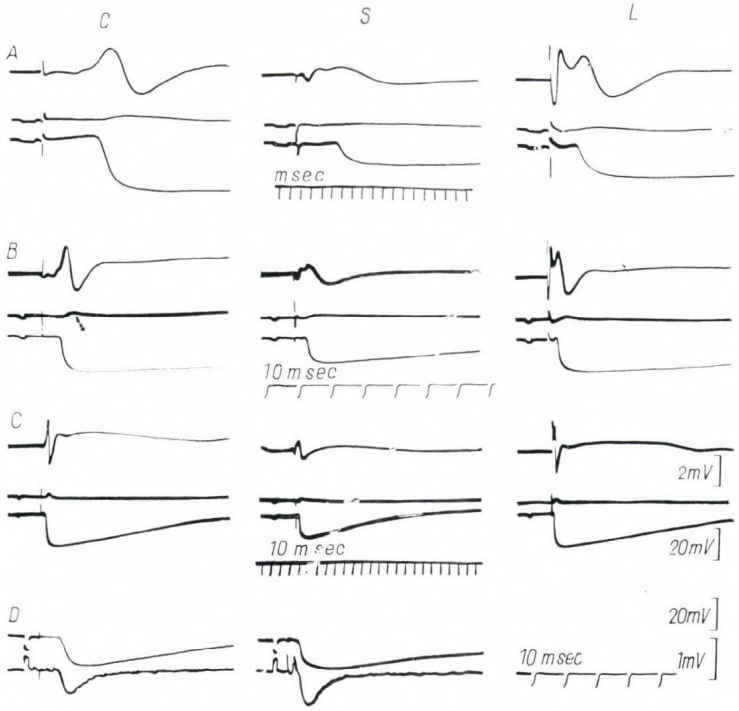

Fig. 3.—*Inhibitory postsynaptic potentials and associated responses in hippocampal pyramidal cells.* In A-C each assemblage of three traces shows from above; the surface record, the extracellular, and the intracellular records, the two last being taken with the same gain and polarity. C, S and L = response to commissural, septal and local stimulation, respectively. All three inputs produce large and long-lasting IPSPs recorded with decreasing sweep speeds from A to C. D is from another cell. The upper traces are intracellular, the lower are extracellular records, the latter taken with opposite polarity and higher gain than the former, as indicated to the right. Note the simultaneous onset of the two potentials, the opposite polarity and the shorter duration of the extracellular records compared to the intracellular. Calibration bars in C also for A and B, calibration for D in the lower right corner

and Løyning 1963), and as were originally observed by Kandel, Spencer and Brinley (1961) and by Spencer and Kandel (1961c) in response to fimbrial stimulation. Such large IPSPs were observed in virtually all pyramidal cells impaled. Careful examination of the fast records, especially the commissural of Fig. 3A, shows that the potentials outside the somas have the same latency and duration of the rising phase as the intracellular potentials, but differ in that they are of the reverse polarity and of much lower amplitude. This relationship is well shown in Fig. 3D for both C and S stimulation in another experiment in which the extracellular recording was at higher amplitude than the intracellular, and with reversed polarity.

Fig. 4A displays assemblages of extracellular potentials produced by commissural, septal and local stimulation and recorded at various depths along a track that penetrated vertically to the surface to approach the terminals of the apical dendrites of the CA3 pyramidal cells, as is indicated

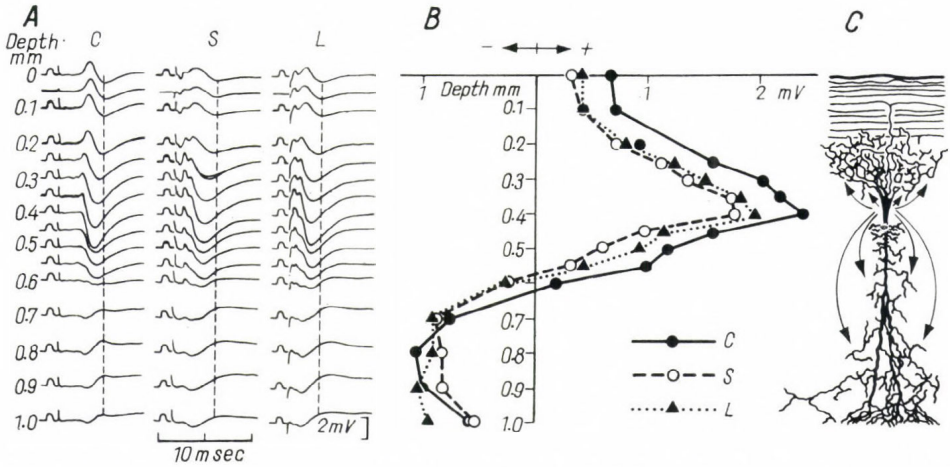

Fig. 4.—*Hippocampal field potentials associated with inhibitory action.* A = depth recordings of CA3 responses to three different inputs, C = commissural, S = septal, L = local stimulation, B = graph of the size of the potentials plotted against depth. The potentials are measured at the time indicated by the stippled lines in A to avoid contamination by the early and superficial negativity. All inputs give a peak positivity at a depth of 0.4 mm. C = diagrammatic representation of a hippocampal pyramidal cell, drawn to scale to allow comparison with the graph in B. The arrows indicate the flow of current associated with the potentials shown in A

by Fig. 4C. All three stimulations evoke an initial negativity at superficial levels, which is probably due to synaptic excitation of the basal dendrites of the pyramidal cells (Andersen, 1960b); but the dominant potential down to 0.5 mm is the large positive wave that attains a maximum at about 5 msec after the stimulus. This wave was measured at the fixed intervals shown by the broken lines in Fig. 4A, so as to minimize contamination by the initial surface negativity, and plotted in Fig. 4B by the appropriate symbols that are labelled C, S and L. respectively. There is a remarkable similarity in these three plots of potential fields, which all have a sharp maximum at a depth of 0.4 mm.

The interpretation of such potential field is simplified by recognizing that, because of their length, density and orientation, the pyramidal cells are the only neurones that could generate such large extracellular fields. Furthermore, the cell bodies of these neurones are arranged in a layer at a depth of 0.4 to 0.5 mm below the surface of the alveus, and the electrode track is perpendicular to this layer and runs along the length of the cells from their basal to their apical dendrites, as shown in Fig. 4C. It can thus be concluded that the field potentials of Fig. 4A must be produced by a powerful source of potential at or near the somas of the pyramidal cells, and that, in the extracellular medium, current flows from this source both superficially to the basal dendrites and deeply to the apical dendrites, as indicated by the arrows in Fig. 4 C.

In interpreting this laminated potential field it is essential to recognize that it is concurrent with the large intracellular hyperpolarizations of the pyramidal cell somas (Fig. 3D). There is virtual simultaneity, within

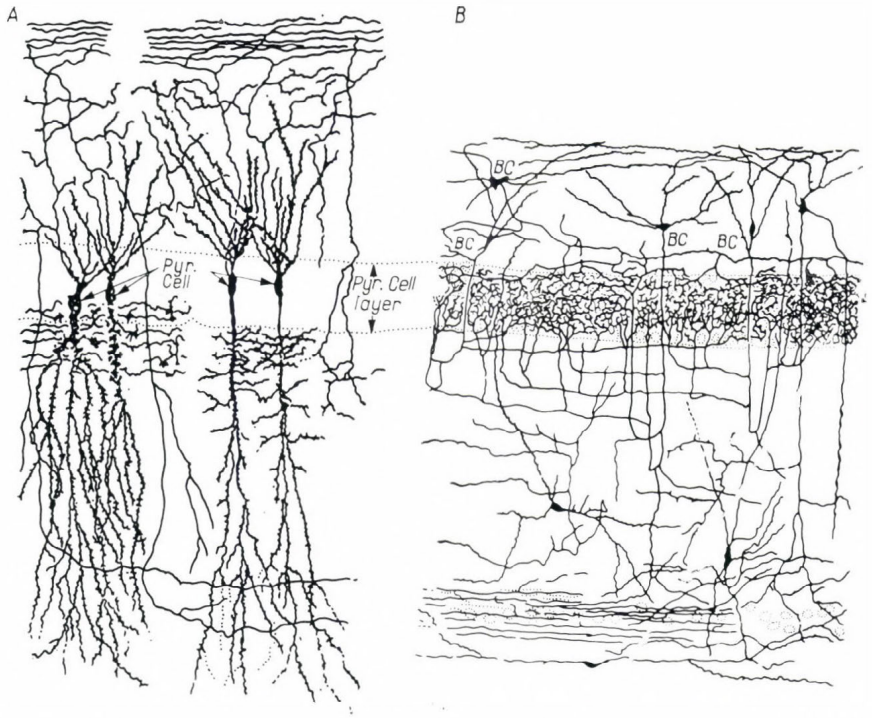

Fig. 5.—*Basket-cell endings in the hippocampus.* Drawings taken from Cajal (1911) to show two typical pyramidal cells as seen in Golgi sections (A), and the plexus in the pyramidal layer created by the profusely branching axons of the basket cells BC in B. Each basket cell axon ends on a large number of pyramidal cell somas

1 msec, between the onsets of these IPSPs and of the positive potentials recorded outside the somas in response to all three inputs. This correlation of intracellular with extracellular potentials makes it certain that they are generated by an active synaptic hyperpolarization of the somatic region of the pyramidal cells, that is of an inhibitory synaptic action concentrated on the soma. Such a location would give a source for current flowing extracellularly into two sinks, one in the region of the apical dendrites, the other in the opposite direction in the region of the basal dendrites, as is shown diagrammatically in Fig. 4C. However, it can be seen in Fig. 4A that the development of a subsequent negative potential in the apical dendrites obscures the prolonged low hyperpolarizing current that would be expected to flow from inhibitory synapses generating an IPSP with a half decay time of 50 to 80 msec in a membrane that has a time constant of about 10 msec (Spencer and Kandel 1961a).

The wide distribution of the inhibition, and the finding that all three inputs produce inhibitory postsynaptic potentials in virtually all pyramidal cells, suggest that there is some special type of cell mediating this inhibition, that is an inhibitory interneurone. This suggestion is strengthened by the observation of a latency difference of 0.8 to 3.2 msec between the EPSP

and the inhibitory potential when both are recorded from the same cell. The specifications of this inhibitory neurone can be derived from the physiological findings and would be: first, that its axon should have extensive ramifications and should be distributed to a large number of pyramidal cells; secondly, that the synaptic terminals of this cell should end on the soma of the pyramids; and thirdly, that the inhibitory neurone should be activated by all three inputs employed, either directly, or more likely, indirectly by the axon collaterals of pyramidal cells that were excited by the three inputs. The last type of operation would be another example of recurrent inhibition.

These postulated inhibitory neurones can be identified both anatomically and physiologically. The detailed histological investigations of Cajal (1911) and Lorente de Nó (1934) reveal a special type of cell, the basket cell (Figs 5 and 6) which exactly corresponds to the postulated inhibitory neurone, and there is no alternative. The axon of each basket cell ramifies profusely and distributes itself to the somas of 200 to 500 pyramidal cells, making a dense plexus restricted to the layer of the cell bodies of the pyramidal cells and enclosing them in a basket-like structure that ends in terminal synapses (Fig. 5B).

The physiological evidence of inhibitory cell activity is usually given by a brief, high frequency (500—1000/sec) ripple resembling that given by Renshaw cells in the spinal cord. This ripple appears on the initial phase of the positive wave, is largest at a depth of 0.3 to 0.4 mm and is given by all the inputs. Occasionally, it is possible to record selectively from one of the rhythmically firing cells (Fig. 6B—F), which then displays the properties expected for an inhibitory cell. It is activated by all the inputs, and its first discharge often just precedes the onset of the positive extracellular wave and continues during its rising phase. It is not antidromically invaded when pyramidal cell axons are stimulated; and when recorded intracellularly (Fig. 6D, middle record) it differs from pyramidal cells in showing a prolonged excitatory postsynaptic potential with superimposed spikes and no IPSP. Furthermore, this cell was at a depth of only 0.2 mm, where basket cells, but no pyramidal cells, may be found.

The proposed inhibitory pathway is shown semi-diagrammatically in Fig. 6A, where a basket cell receives excitatory synapses from axon collaterals of pyramidal cells and itself forms many inhibitory synaptic endings on the somas of pyramidal cells. As indicated in Fig. 6A, the C, S and L inputs employed in Figs 3, 4 and 6 would all excite pyramidal cells and so indirectly activate the basket cells.

Thus, it seems possible for the first time to give an example from the mammalian nervous system of a recurrent inhibitory pathway where both the inhibitory neurone and its synapses (Cf. BC in Fig. 5B and Fig. 6) are histologically identifiable. It will be appreciated that from strategic considerations inhibitory synapses located on the soma are optimally placed for controlling the generation of impulse discharge, because they would be sited between the excitatory synapses on the apical and basal dendrites and the axon hillock where the impulse generation probably occurs, either in response to synaptic depolarization of the basal or apical dendrites, or triggered off by a spike generated more peripherally and travelling somatopetally in the apical dendrite.

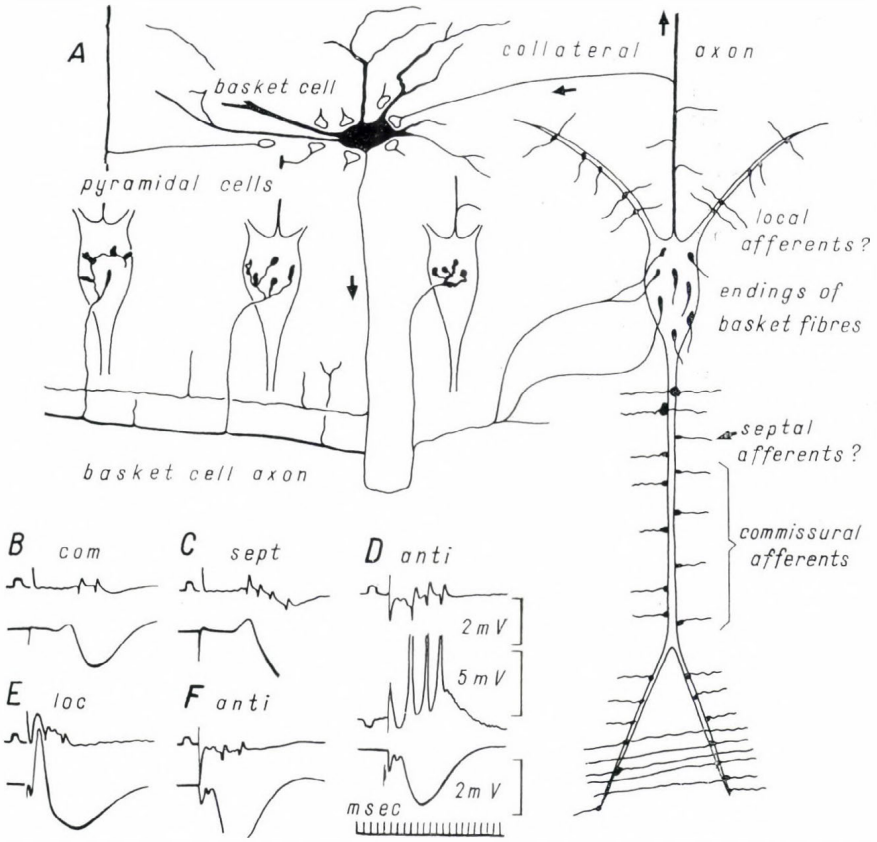

Fig. 6.—*Hippocampal basket-cell activity.* A is a diagram showing a pyramidal cell to the right with the zoning of synapses from various inputs. The basket cell (black) is excited by axon collaterals from this and other pyramids. The basket cell axon distributes itself to the somas of four pyramidal cells. B–F show recordings from a presumed basket cell in the upper trace and the surface record in the lower, in response to commissural COM, septal SEPT, local LOC and antidromic ANTI stimulation. In D the middle trace is an intracellular record showing a long EPSP with three superimposed spikes (truncated). Recording depth 0.2 mm

C. INHIBITORY ACTION BY BASKET CELLS OF THE CEREBELLUM

The basket cells of the cerebellum provide a most remarkable concentration of synapses on the soma and axon hillock of Purkinje cells (Cajal 1888, 1911; Jakob 1928; Scheibel and Scheibel 1954; Fox and Barnard 1957). The Purkinje cells (P-cells) therefore present a very favourable site for a further investigation (Andersen, Eccles and Voorhoeve, 1963) into the general proposition that the synapses responsible for postsynaptic inhibition are localized on the somas of neurones. Granit and Phillips (1956) have referred to the occasional presence of spontaneous hyperpolarizing responses of Purkinje cells.

Fig. 7 is a simplified diagrammatic representation of the most important cellular elements in the cerebellar cortex and of the electrode arrangements that we have used. The afferent mossy fibres (mf) synapse with the granule cells (G) which send their axons vertically into the molecular layer, where by a T-shaped junction each fibre divides into two branches which are called parallel fibres (pf) because they course strictly parallel to the surface of the folium for at least 1.5 mm in each direction. During this traject they penetrate a large number of P-cell dendritic trees, all of which are oriented in a plane transverse to the long axis of the folium. The P-cell axons (Pa) represent the efferent link from the cerebellar cortex. Each of them has a number of recurrent collaterals said to end upon the soma and initial dendrites of other P-cells (Cajal 1888, 1911; Jakob 1928). In the lower part of the molecular layer there are a number of special cells, the so-called basket cells (B). These have extensive axon ramifications (Ba) that run largely transverse to the long axis of the folium and just above the P-cell layer. Numerous branches form basket-like plexuses around the somas of the P-cells, as indicated in Fig. 8, especially at the axonal poles, hence the name. The vast majority of synapses on the lower part of the somas of P-cells do, therefore, belong to the basket cell axons.

The P-cells have been excited (Fig. 7) either by local stimulation exciting parallel fibres (Dow 1949) or by antidromic activation through a concentric needle electrode (Granit and Phillips 1956). Recordings were made by microelectrodes filled with 4M NaCl or 3M KCl or 2M K-citrate. The cerebellar cortex is extremely sensitive to mechanical or circulatory damage. By covering the surface with 4 per cent solution of agar in Ringer-Locke after the electrodes were positioned, the respiratory and circulatory movements of the cortex were controlled when the agar jelly had set. When agar-covered, the cerebellar cortex remained in good condition for many hours. The microelectrode can be withdrawn and reinserted through the agar gel.

When the depth of microelectrode penetration was 0.3 to 0.4 mm, it frequently impaled nerve cells. This was indicated by the recording of a resting potential, of repetitive spike potentials in the positive direction and of synaptic potentials. Usually these responses suggested that there was extensive cell damage, and there was rapid deterioration. However, resting potentials above -40 mV and large synaptic potentials have been recorded from several cells for a sufficiently long time to allow a discriminative investigation. Since the intracellular recording was always at the depth of the somas of the Purkinje cells, and since the intracellular responses conformed to a standard pattern, including antidromic invasion, it may be concluded that in fact our intracellular recording was restricted to Purkinje cells.

As shown in Fig. 9A, a weak local stimulation of a folium evoked, after a latency of about 4 msec, a prolonged hyperpolarization that had a rising phase of more than 10 msec and a total duration much in excess of 100 msec. In Fig. 9B there are two superimposed traces, the one set up by the stronger stimulation showing an additional depolarization with a latency about 1 msec briefer than for the hyperpolarization. These potentials correspond in every respect to excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs). The IPSP had the standard properties: reversal to a delaporizing response when a sufficiently strong hyperpolarizing current was

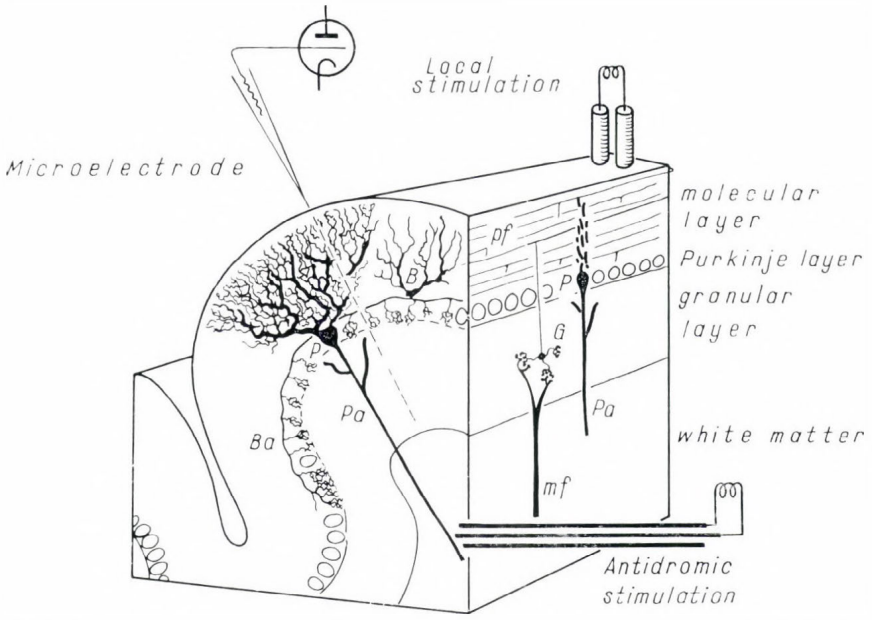

Fig. 7.— *Simplified diagram of the cerebellar cortex.* The local stimulation will excite the parallel fibres which in turn will synaptically excite the Purkinje cells (P). Antidromic activation is produced by a concentric electrode placed in the white matter under the recording site. The microelectrode track is stippled. B = basket cell, Ba = basket-cell axon, G = granule cell, mf = mossy fibre, Pa = Purkinje cell axon, pf = parallel fibres

Fig. 8.—*Cerebellar basket cell.* Drawings taken from Cajal (1911) of a basket cell from a rat, Golgi section. The axon distributes itself to the somas of 8 Purkinje cells. A = Purkinje cell, B = basket cell, a = pericellular ramifications, b = thin terminal part of basket cell axon, c = basket cell axon

passed out of the microelectrode; large increase during the passage of a depolarizing current; reversal to a depolarizing potential when Cl^- ions were injected out of a recording electrode filled with 3M KCl. Local stimulation produced these large and prolonged IPSPs in every P-cell from which we have recorded. In contrast, antidromic activation of P-cells by the stimulation as in Fig. 7 did not poduce IPSPs, though with strong stimulation IPSPs were produced, presumably in response to the stimulation of the mossy fibres with serial synaptic activation of granule cells and basket cells.

The inhibitory action of the prolonged IPSP is illustrated in Fig. 9C, where the spontaneous rhythmic discharges of P-cells are inhibited for as long as 800 msec by the same surface stimulation of the folium that evokes the IPSP. This powerful and prolonged inhibitory action on spontaneous discharges of a P-cell has invariably been produced by a surface stimulust hat excited the parallel fibres projecting towards that P-cell.

Just as with the pyramidal cells of the hippocampus, the organization of the P-cells of the cerebellum gives ideal conditions for investigations into the location of synaptic action on them. The somas are arranged in a single layer parallel to the surface of the folium (Fig. 7) and the cells are essentially bipolar with the dendritic trees projecting from one pole up to the surface and with the axon from the other pole into the depths of the folium (Fig. 9G). In Fig. 9D, E are shown typical series of potentials recorded at the indicated depths along a microelectrode track and evoked by a weak (30 V) and a strong (50 V) local stimulation. The weak stimulation was remarkable in that it showed the development and the decline of an almost pure positive potential with progressive penetration. With the stronger stimulation there was at depths from 0.1 to 0.5 mm also an earlier negative response that overlapped with the onset of the positive potential. A trace of this negativity can be seen also with superficial records at the weak stimulation.

In order to obtain measurements of the field potential produced by the positive component relatively uncontaminated by this earlier negative component, measurements have been made at 6.7 to 5.7 msec after the stimulation at the stippled lines in D and E, respectively, and are plotted in Fig. 9F. The positive wave is seen to reach a maximum at a depth of 0.35 to 0.4 mm with both strengths of stimulation; and more superficially it rapidly diminishes and reverses to a surface negativity, which can be recognized as a distinct component following the initial surface negativity in Fig. 9D, E at depths of 0.1 and 0.15 mm. Beyond 0.4 mm the positive wave also declines, but much less rapidly, and no reversal is seen. The curves of Fig. 9F show that current is flowing outwards from the region of the somas of the P-cells very intensely up towards the dendrites all the way to the surface, and very weakly in the reverse direction towards the axon, as shown by the arrows in Fig. 9G. This is precisely the extrinsic current that would be generated by an inhibitory synaptic action concentrated on the P-cell somas. It cannot be due to depolarizing synaptic action on the P-cell dendrites, because in that case intracellular recording from the somas must give a depolarizing potential, owing to electrotonic spread of the EPSP, and not the hyperpolarization that is invariably observed. In every respect this field analysis of the cerebellum cor-

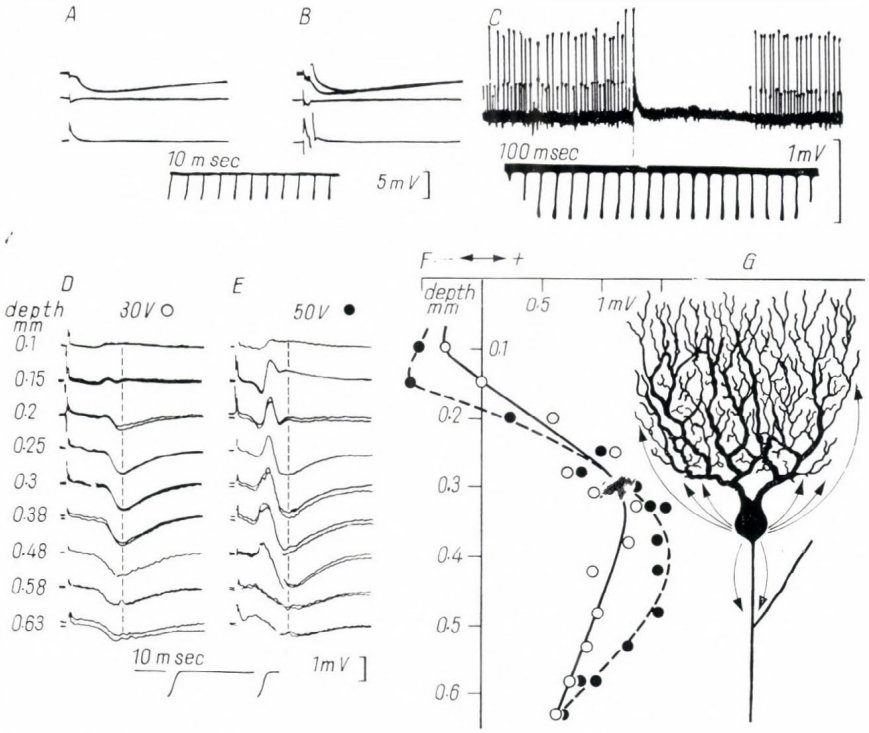

Fig. 9.—*Inhibition and associated potentials in the cerebellar cortex.* A = upper trace shows a large and long-lasting IPSP recorded from a Purkinje cell. Middle trace is the extracellular and the lower is the surface record. B = similar to A but with a superimposed record due to stronger stimulation giving an additional EPSP with latency shorter than that of the IPSP, C = cessation of spontaneous discharge of two Purkinje cells produced by a single local stimulus. Inhibition lasts for 800 msec. D and E show records taken at the indicated depths in reponse to 30 V and 50 V local stimulation. The sizes of the positive potentials in D and E, measured at the stippled lines, are plotted against depth in F. G is a diagram of a Purkinje cell drawn to scale to facilitate comparison with the graph F. The arrows indicate the currents associated with the positive potentials re corded in D and E

responds very closely with the field analysis of the hippocampus (Fig. 4).

From these various experimental investigations it may be concluded that powerful postsynaptic inhibition is exerted on the somas of the Purkinje cells. Since this is observed in all successfully impaled cells, there must be a concentration of inhibitory synapses on their somas. Evidently, the basket cells are the cells responsible for mediating this inhibitory action. This identification is further supported by the finding that stimulation of the folium may produce an inhibition virtually uncontaminated by a preliminary excitation of P-cells if it is off the direct line of parallel fibres to the recording electrode, which was the case with the weaker stimulation in Fig. 9D. Under these conditions the inhibition must be produced by mediation of interneurones having axons transverse to the folium, which is the case with basket cells (Figs. 7, 8). The parallel fibres traverse the dendritic trees

of the basket cells as well as the P-cells, and probably synaptically excite both. This would explain the difference in latency of only about 1 msec between the EPSP and the IPSP (Fig. 9B), for the latter would have an additional synaptic delay.

This concentration of the inhibitory synapses on the somas of Purkinje cells, exactly parallels the situation with the hippocampal pyramids. However, there is a remarkable difference in the pathways activating the inhibitory basket cells in these two locations. In the cerebellum the basket cells are not excited by axon collaterals of Purkinje cells. In contrast to the situation in the hippocampus (Fig. 6D) antidromic stimulation of P-cell axons failed to give postsynaptic inhibitory potentials in spite of readily demonstrable antidromic invasion into the cell bodies of large numbers of P-cells. An additional parallel between the basket cells of the cerebellum and of the hippocampus is that in both locations the inhibitory synapses in the somas have the characteristics of synapses that Gray (1959) designated Type 2 (Palay, McGee-Russell, Gordon and Grillo 1962; Blackstad and Flood 1963).

D. IDENTIFICATION OF OTHER INHIBITORY SYNAPSES

Since the somatic location of inhibitory synapses now has been established experimentally in two locations, it may be postulated that there may be some degree of generality about this arrangement. In the further investigation of this postulate it is desirable to choose locations where there are histological structures corresponding to the terminal arborizations of basket cells.

The dentate area of the hippocampus is presently being investigated for this reason. In this structure the predominant cell type, the granule cell, has a small cell body and an extensive bush-like dendritic structure, not unlike that of the Purkinje cell. The dendrites are synaptically influenced by perforant path fibres from the entorhinal area (Cajal 1911; Lorente de Nó 1934; Blackstad 1958), from the contralateral hippocampal formation (Blackstad 1956) and from the septum (Andersen, Bruland and Kaada 1961). The synapses on the soma are largely derived from the basket cells of the dentate area, which are situated below the granule cell layer (Cajal, 1911; Lorente de Nó, 1934). Their name reflects the structures which their axon terminations make around the granule cell somas. Just as in the CA1 and CA3 of the hippocampus and in the cerebellar cortex, one basket cell sends axon branches terminating on a large number of granule cells. The activation of the basket cells are through collaterals of the granule cell axons, the mossy fibres. One advantage in this system is that the size and density of the basket cells ought to make it possible to record from them.

Other examples of heavy synaptic covering on the soma of central neurones are the pericellular networks of the neocortical pyramidal cells (Cajal 1911). The size of the IPSP recorded intracellularly from such cells (Albe-Fessard and Buser 1953, 1955; Phillips 1956, 1959, 1961; Albe-Fessard 1960; Lux and Klee 1962), suggests that these potentials are generated at or close to the cell bodies where the electrode impales.

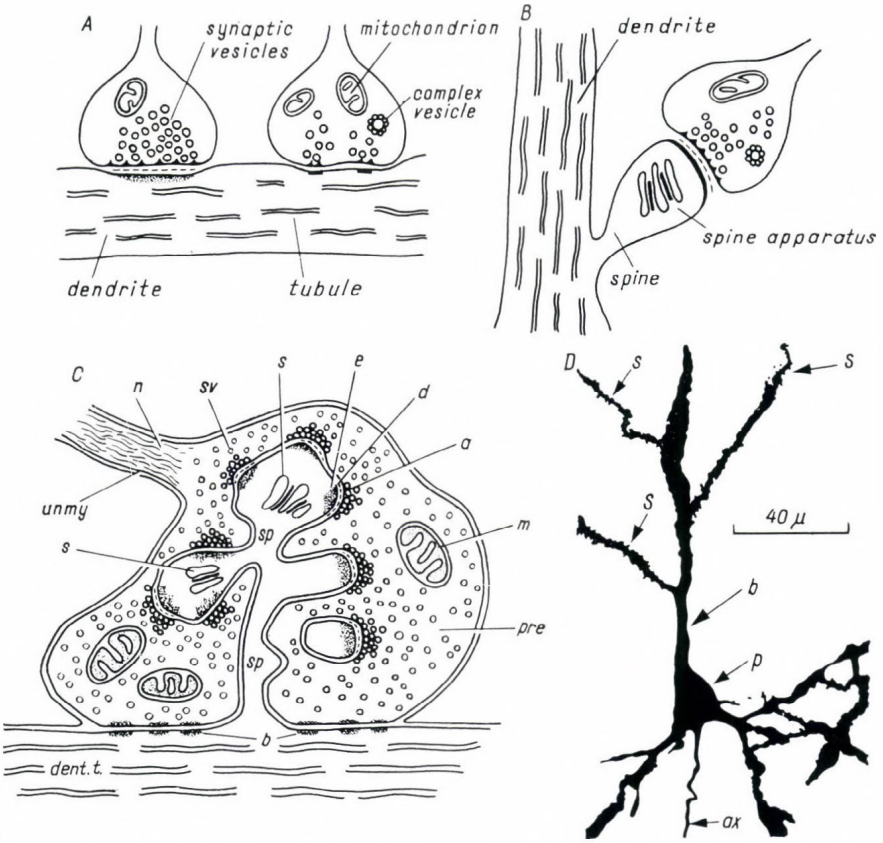

Fig. 10.—*Diagram over cortical synapse types.* (Whittaker and Gray 1962). A = schematic representation of Type 1 (left) and Type 2 synapses. B = excitatory synaptic knob in contact with a dendritic spine of a pyramidal cell. C = specialized axo-dendritic contact from the mossy fibre layer of CA3 in the hippocampus. A dendritic spine invaginates a large presynaptic ending, having numerous areas typical of Type 1 synapses. D = part of a cortical pyramidal cell as seen in Golgi sections. Soma (p) and initial dendritic (b) membranes are smooth, whereas the rest of the dendrites have numerous spines (s). These enter into contact with excitatory synaptic knobs

E. THE MORPHOLOGY OF INHIBITORY SYNAPSES

All the synapses on the somas of hippocampal pyramidal cells in both CA1 and CA3 areas, i.e. the basket cell synapses, have the structural features characteristic of Type 2 synapses (right synapse of Fig. 10A) as defined by Gray (1959) and Blackstad and Flood (1963). A relatively small fraction of the total area of contact is specialized to form 'active zones' (Couteaux 1961) with an increased density of the membranes on both sides of the synaptic cleft and some accumulation of vesicles (Fig. 11A; Blackstad and Flood 1963). By contrast, the synapses on the dendritic spines of hippocampal pyramidal cells are Type 1, being characterized by large areas of

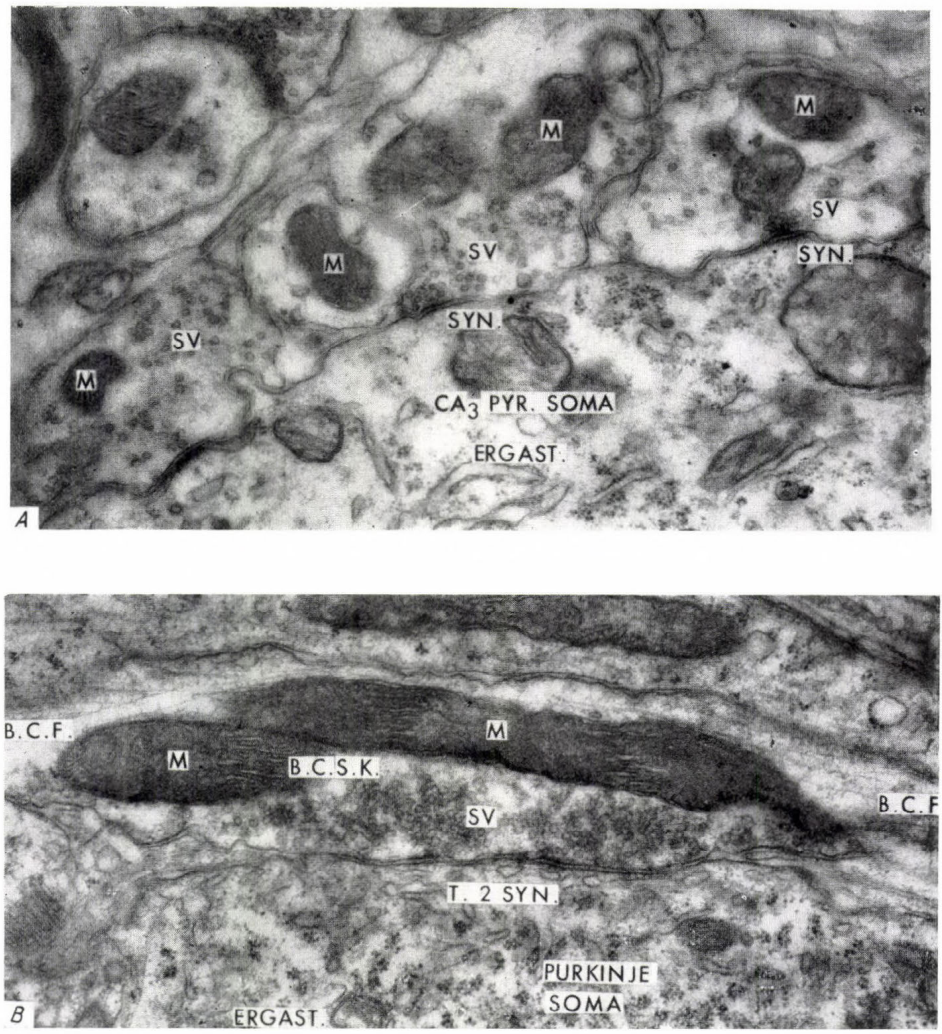

Fig. 11. — *Electron microscopy of somatic synapses.* A = electron micrograph of three synapses forming active zones on the soma membrane of a CA3 pyramidal cell of the rat hippocampus. The synapses are of Type 2, and belong to basket cell axons. ERGAST = ergastoplasma, M = mitochondrium, SV = synaptic vesicles, SYN = synaptic cleft with active zone (Blackstad, personal communication). B = electron micrograph of *bouton en passage* in contact with the soma membrane of a cerebellar Purkinje cell in rat. The synaptic knob belongs to a basket cell axon. B. C. F. = basket cell fibre, B. C. S. K. = basket cell synaptic knob, M = mitochondrium, SV = synaptic vesicles, T. 2 SYN = Type 2 synapse (Palay, personal communication)

active zones that have dense staining of the membrane, especially on the postsynaptic side of an expanded synaptic cleft, and large accumulations of vesicles (Fig. 10 A, C and Fig. 11; Blackstad and Kjaerheim 1961; Hamlyn 1962).

This same difference between axo-somatic and axo-dendritic synapses also occurs with the Purkinje cells of the cerebellum (Palay, McGee-Russell, Gordon and Grillo, 1962; Palay, personal communication). The synaptic endings of basket cells on the somas of Purkinje cells can be seen in Fig. 11B to resemble the axo-somatic endings of basket cells on the hippocampal pyramids, and to be typical Type 2 synapses. The synapses of the parallel fibres on the dendritic spines and of the climbing fibres on the dendrites have the distinguishing features of Type 1 (Palay, McGee-Russell, Gordon and Grillo 1962; Szentágothai 1963).

This histological differentiation of synapses corresponds at least in part to their functional differentiation. The inhibitory synapses on the somas of these two species of nerve cells are uniformly of Type 2; and undoubtedly the great majority of the Type 1 synapses on the dendrites of these cells is excitatory. There is a similar distribution of Types 1 and 2 on the pyramidal cells of the neocortex (Gray 1959; de Lorenzo 1961), the synapses on the dendritic spines being always of Type 1 (Fig. 10B), and the axo-somatic synapses being always Type 2. There is a transitional zone where Type 2 synapses occur on the surface of large dendrites in areas between the dendritic spines with their Type 1 synapses (Gray 1959). It seems likely that the axo-somatic synapses on neocortical pyramidal cells are formed by the pericellular network of fibres that has been suggested above as being homologous with the basket cell arborizations around the somas of hippocampal pyramidal cells and Purkinje cells. The granule cells of the fascia dentata of the hippocampus provide another example of a dense perisomatic plexus of nerve fibres, and the axo-somatic synapses are there also uniformly of Type 2 (Blackstad and Dahl 1962).

The functional distinction between Types 1 and 2 is further indicated by the fact that, on every occasion where a single presynaptic ending enters into synaptic relationship with two different postsynaptic structures, the synapses are either both Type 1 or both Type 2 (Gray 1961a). At least it can be concluded that this speculation gives hope that it may be possible to distinguish between excitatory and inhibitory synapses on morphological grounds. By means of ingenious degeneration experiments Szentágothai (1958, 1961) obtained evidence that, with motoneurones, oculomotor neurones and dorsal spinocerebellar tract cells, inhibitory synaptic action is exerted by an extremely fine meshwork of fibres ramifying over the cell body. Whittaker and Gray (1962) suggest that the associated inhibitory synaptic knobs would not be stained by the silver techniques, because they have no ring of neurofibrils, and hence would be undetectable by light microscopy.

F. THE GROWTH OF AXONS TO SPECIFIC SYNAPTIC LOCATIONS

The role of functional specificity in determining the site of synaptic contacts on neurones in the central nervous system is suggested by the very restricted location of inhibitory synapses on the somas of the hippocampal pyramidal cells (Fig. 4; Andersen, Eccles and Løyning 1963; Blackstad and Flood 1963) and of Purkinje cells in the cerebellum (Fig. 9; Andersen, Eccles and Voorhoeve 1963). As argued above, it is good physiological

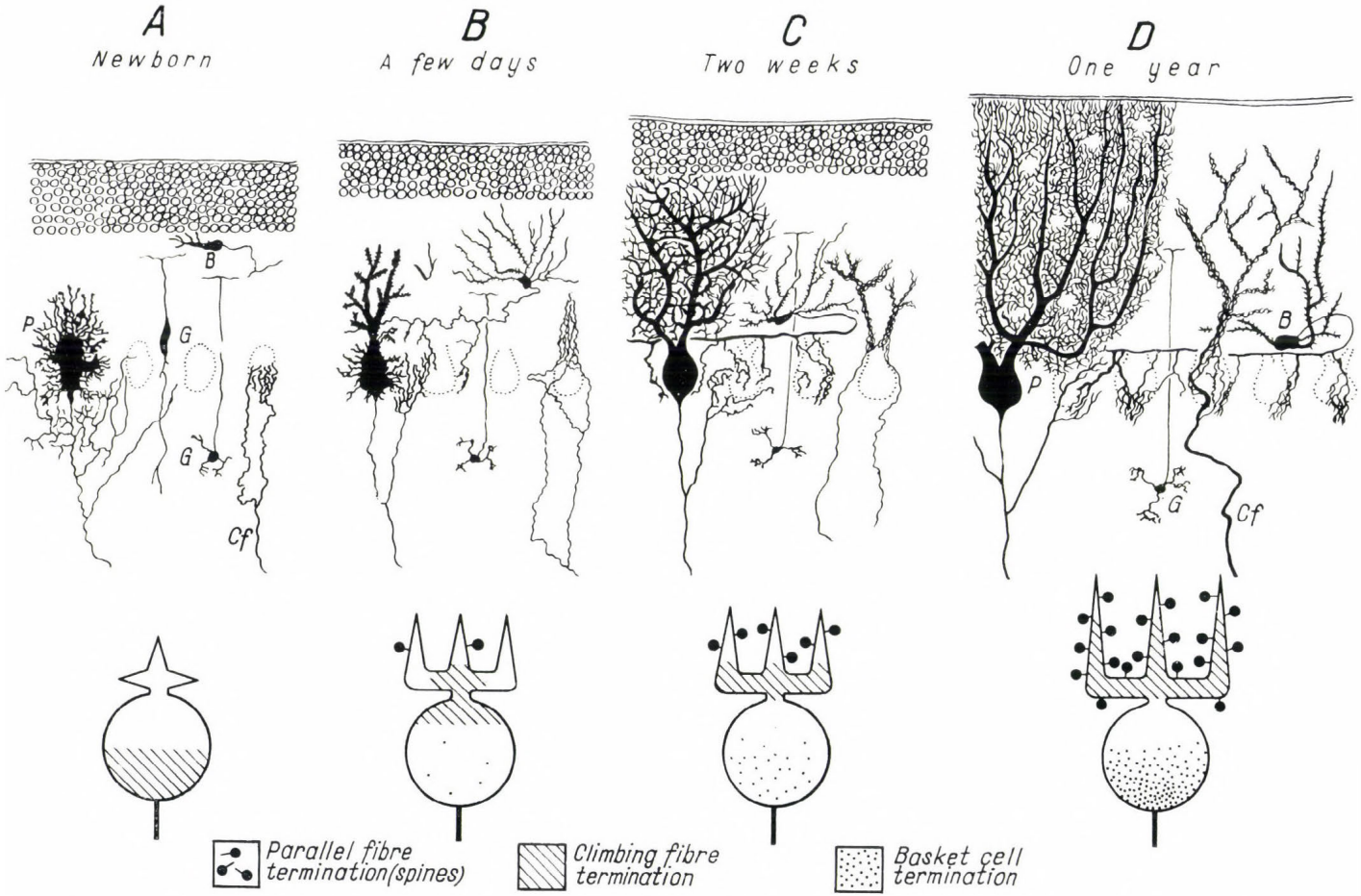

Fig. 12. — *Diagram of histogenesis of cerebellar cortex.* P = Purkinje cells, G = granule cells, B = basket cell, and Cf = climbing fibre. The lower part illustrates schematically the evolution of the synaptic territories of three afferent systemson the Purkinje cells

design to have inhibitory synapses located on the soma, because they would there exercise the most effective control of the discharge of impulses down the axon. But in the present context it can be asked, what developmental control ensures that the inhibitory synapses are concentrated on the soma and the excitatory more remotely on the dendrites, particularly on the dendritic spines?

Evidently the simplest hypothesis is that these specific locations result from contact guidance and chemical sensing by the growing nerve terminals, as postulated by Weiss (1947) and Sperry (1951a, 1951b, 1958), and that the discrimination between different parts of the neuronal surface is effected, because in the proximity of the nucleus the metabolism of the nerve cell (pyramidal or Purkinje) is different from that in the remote dendritic regions. In the mammalian central nervous system the excitatory and inhibitory neurones are chemically unique and have unique synaptic connections; hence it is envisaged that the inhibitory type of fibre is attracted by the special chemical character of the surface membrane in proximity to the nucleus, whereas the excitatory type of fibre would be repelled, and on the contrary is attracted by the dendritic surface remote from the nucleus and by the even more remote dendritic spines.

The relatively simple and standardized structure of the cerebellar cortex provides a particularly good situation for testing this postulate of specific growth control in relation to the known developmental processes (Cajal 1911; Jakob 1928). Four stages of development of the cerebellar cortex in small mammals are shown diagrammatically in Fig. 12.

At birth (Fig. 12) the Purkinje cells have profusely branched but short dendrites devoid of spines, including perisomatic dendrites. The climbing fibres appear to enter into synaptic contact with the basal areas of Purkinje cell somas. Already some granule cells have migrated down towards the ingrowing mossy fibres leaving their T-branched axons to form the parallel fibres in the molecular layer, though as yet there are probably no synapses on the Purkinje cell dendrites. Other granule cells are still in the molecular layer, though cell processes have grown downwards along the path that the somas are soon to follow. Basket cells are still virtually undifferentiated.

A few days later (Fig.12B) the Purkinje cell dendrites have greatly developed and grown a few spines, so that excitatory synapses may already be formed by the parallel fibres. However, the most interesting development is that the climbing fibre contacts have now migrated to the dendritic pole of the soma and the bases of the dendrites, while basket cells of the molecular layer have already established contacts in the vacant area at their final site at the axonal pole of the somas.

At 2 weeks after birth there is further development in the same direction. There are more dendritic spines on the expanded dendritic arborization, and the climbing fibres have lost all contact with the soma in their movement up the dendrites. Meanwhile the basket cells have established much denser contact areas on the soma. The final development (Fig.12D) results merely from further evolution of these various changes.

The temporal relationship of the climbing fibre and basket cell contacts on the soma is particularly significant. The climbing fibres show a progressive movement of their site of contact from the axonal pole of the soma until

it is exclusively on the tertiary dendritic branches (Szentágothai 1963). It appears as if these areas remote from the perikaryal region were progressively more attractive. On the contrary the basket cell axons apparently are not attracted by the Purkinje cell dendrites, which they pass on their way to the overwhelmingly attractive sites on the axonal pole of the soma. It seems that the postulate of a discriminative sensing by excitatory and inhibitory axonal growth cones respectively gives a coherent explanation of the sequences of development depicted in Fig. 12.

Experimental embryology provides pertinent information on factors involved in directional axonal growth. In the normal development of the chick the cells destined to become the pontine nuclei wander ventrally from the rhombic lip to attain a position in the ventral part of the contralateral half of the medulla (Harkmark 1954). They then send out their axons which normally find their way to the contralateral half of the cerebellum. Since the anlages to the cerebellar cortex, and to the pontine nuclei are located at different places in the developing chick embryo, it is possible to destroy selectively the area giving rise to one half of the cerebellum without severing the pontine anlage. Following such an operation, the migration of the cells to form the pontine nuclei occurs as before, and they also send out their axons towards the contralateral cerebellum in the normal manner (Harkmark 1956). However, these axons are unable to make synaptic contacts, because the appropriate part of the cerebellum has not developed. Upon this synaptic failure, but not till then, both the axon and its parent cell disintegrate. Two conclusions may be drawn: directional axonal growth occurs even when the target of the axonal avant-garde is not there to have an attractive action; and second, the establishment of functional synaptic contact is a prerequisite for the survival of the neurone.

In the development of the cerebellar cortex two growth zones occur, the deeply situated zone arising from the neural epithelium covering the ventricular surface and a superficial zone, the external granular layer (circles in Fig. 12). By making a lesion of the tissue along the free edge of the cerebellar plate in 5 day old chick embryos the formation of the external granular layer is prevented (Forstrønen 1962). No granule cells or parallel fibres develop, but still some large neurones appear, identified as Purkinje cells. Thus, the granule cells normally take their origin from the external granular layer and descend with their cell bodies (Fig. 12A), leaving the axons behind to form the bundles of parallel fibres. On the other hand, the Purkinje cells develop from the deeper layer, moving towards the surface and sending their dendrites to make contact with the already existing parallel fibres (Fig. 12A-D). Thus, specific synaptic locations are not due only to the searching growth of the axonal cones, but also to the growth of the dendrites towards the axons, the dendritic spines actually invaginating the axons in order to make synapses (Szentágothai 1962).

Evidently new insights have been gained by the correlation of our physiological investigations with the structural features displayed both by light microscopy and electron microscopy. It may now be asked whether the concepts we have developed with respect to the zonal location of inhibitory and excitatory synapses have more general validity.

It is remarkable that the spines which are the postsynaptic components of so many synapses do not occur on the soma and adjacent dendritic stumps, as may be seen in Figs. 5 and 10 D. The synapses formed on the dendritic spines appear to be uniformly Type 1 (Gray 1959; Blackstad and Kjaerheim 1961; Hamlyn 1962; Westrum and Blackstad 1962). In some of these locations, e.g. on the hippocampal pyramids and the Purkinje cells, these Type 1 synapses are excitatory. It may be asked how far this identification applies generally throughout the central nervous system.

In view of the functional desirability attaching to a somatic location of inhibitory synapses it is of particular interest to make a general survey of somatically located synapses in order to discover if they have a general resemblance to the basket cell synapses on hippocampal pyramids and Purkinje cells. Amongst possible examples we can instance the plexus of fine fibres around neocortical pyramidal cells, the cells of the lateral vestibular (Deiters) nucleus, the ventral cochlear nucleus and the mitral cells of the olfactory bulb.

DISCUSSION

Walberg : The demonstration that the situation in the cerebellum appears to be similar to that in the hippocampus is very interesting. I would like to ask Professor Eccles whether he thinks all axo-somatic synapses may be inhibitory. To my knowledge all axo-somatic synapses hitherto described are either of Gray's 2nd type or unspecialized ones.

Krieg : I believe that Cajal would be delighted to see how Professor Eccles has used the trails he worked out as isolable physiological units to answer complex physiological questions. In the same it will be very interesting to see the application of these methods to the neocortex, using the pathways which have now been worked out and portrayed with exactness of location.

Szentágothai : Most axo-somatic synapses in the cerebral and cerebellar cortex are in fact of Gray's 2nd type. I am not yet sure, but it often looks as if the same axon terminal might have a Type 1 synapse with a dendrite and a Type 2 contact on its opposite side with a soma. Would this not be difficult to reconcile with the generalization of the inhibitory nature of Type 2 synapses?

Eccles : In reply to the first question (Walberg) I would suggest that with many types of neurone the synapses on the somas are inhibitory, but on the other hand there are examples of axosomatic synapses that must be excitatory. A notable example is the synapses responsible for monosynaptic excitation of motoneurones, and which by degeneration procedures Dr. Szentágothai has shown to be the large synapses on the bases of the dendrites and on the soma. With the Clarke's column neurones, the synapses of the primary afferent fibres are similarly shown to be on the dendrites and even encroach onto the soma. In reply to dr Szentágothai's question, I think it means that we have to be more cautious in giving functional significance to the special features that distinguish synapses and such categories as Type 1, Type 2 and undifferentiated ones.

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STUDIES OF BOUTON DEGENERATION IN THE INFERIOR OLIVE

by

F. WALBERG

ANATOMICAL INSTITUTE, UNIVERSITY OF OSLO
NORWAY

Previous studies have shown that the inferior olive receives fibres from various regions of the central nervous system. Experiments in which the Glees silver method has been used have revealed that spinal afferents ascending in the ventral funiculus reach the caudal part of the nuclear complex. The fibres terminate mainly in the caudal half of the medial and dorsal accessory olive (Brodal, Walberg and Blackstad 1950). The fibres descending from higher levels of the brain have likewise particular regions of termination, partly terminal areas of their own, partly areas in common with fibres from other regions (Walberg 1956, 1960). The pattern of termination of the afferent fibres is shown in Fig. 1.

An important contribution of afferent fibres is that from the periaqueductal grey substance of the mesencephalon. The fibres from this part terminate in the principal olive, its ventral as well as dorsal lamella, and in the rostral part of the caudal half of the medial accessory olive (Fig. 1). Another important contribution comes from the dorsal part of the reticular formation in the region of the red nucleus. These fibres reach the rostral half of the medial accessory olive, particularly the ventral region (Fig. 1).

Following lesions which include the periaqueductal grey matter and the dorsal part of the mesencephalic reticular formation, Glees sections show a heavy degeneration of terminal fibres and boutons in the corresponding regions of the inferior olive. The degenerating fibres show an increased argyrophilia, and appear beaded and fragmented. Likewise, the number of visible boutons is increased, owing to an increased argyrophilia of these structures. Some of the degenerating boutons appear as heavily stained dark rings, others are compact and irregular in shape.

The basis for argyrophilia of boutons in the central nervous system is not known, and an important question is whether the argyrophilia can be related to certain structures in the boutons. Important papers dealing with this problem are those of Gray and Guillery (1961), Gray and Hamlyn (1962) and Colonnier and Gray (1962). Based on normal studies of the spinal cord and experimental investigations of the tectum opticum and visual cortex, the authors conclude that argyrophilia of boutons is only related to the presence of filaments in these. Gray and Guillery (1961) conclude that neither the mitochondria, the synaptic vesicles, the cytoplasm nor the surface membrane of the boutons are responsible for their staining properties.

In a recent electron microscopical study of the normal inferior olive of the adult cat, it was observed that the boutons contain mitochondria,

synaptic vesicles, vesicles of a special type and other organelles (Walberg 1963a). In none of the boutons were filaments present. However, Glees sections reveal that normal boutons show argyrophilia and that a various number of boutons are visible in the different subdivisions of the olive (Blackstad, Brodal and Walberg 1951).

To get more precise knowledge concerning argyrophilia of degenerating boutons, lesions have been placed in those regions of the mesencephalon which have been shown to send fibres to the rostral half of the medial accessory olive and to the ventral lamella (see above). The experiments were also made to obtain information on the fine structure of degenerating terminal fibres and boutons in the olive.

7, 14 and 36 day stages have been observed. The lesions of the mesencephalon have been made either with a Horsley-Clarke stereotaxic apparatus or by hand. The animals have been perfused intravitally with Ringer solution and the formalin fixative introduced by Holt and Hicks (1961), following a procedure described previously (Walberg 1963a). The brain stem has been dissected out, and the medulla cut in thin horizontal slices with a razor blade. Pieces of the inferior olive have been selected under the dissecting microscope and subsequently transferred to chilled osmium tetroxide for further fixation. The pieces have then been embedded in Araldite.

The rostral half of the medial accessory olive and the ventral lamella have been selected for study. The ultrathin sections were made with an LKB Ultrotome and studied with a Siemens Elmiskop I. Primary magnification was either 10 000 × or 15 000 ×.

After 7 days the boutons have degenerating mitochondria with fragmented cristae, and a matrix which is electron dense. Some boutons appear shrunken. The mitochondria often fill the entire bouton (Fig. 2).

The synaptic vesicles are either aggregated and clumped together in the degenerating bouton, or are no longer visible as separase structures. No filaments are present in the degenerating boutons.

At the 14 day stage the findings are almost similar to those described above. The surface membrane of the degenerating bouton cannot always be recognized.

On the 36th day most of the degenerating boutons have the same appearance.

The changes in the small myelinated nerve fibres are also marked. The mitochondria are enlarged, the cristae are fragmentedand the matrix is dark. The axoplasm has changed to a granular, electron dense mass in which the degenerating mitochondria are lying. Other structures are also present in degenerating axons. Some of these are dense, finely granular bodies, about 1/2 to 1 μ in diameter. They are round or oval, and can partly be surrounded by a membrane. In addition, bodies built up of groups of concentrically arranged lamellae, similar to myelin, are found.

The degenerative changes described here, are present in small myelinated fibres to about 1μ in diameter. The available material does not allow definite conclusions concerning the fine structure of the myelin sheath of these fibres, but the findings indicate that the changes in the myelin sheath take their beginning at relatively late stages.

At the 14 and 36 day stages many dendrites adjacent to degenerating boutons differ from those found in normal animals. Thus, while in normal

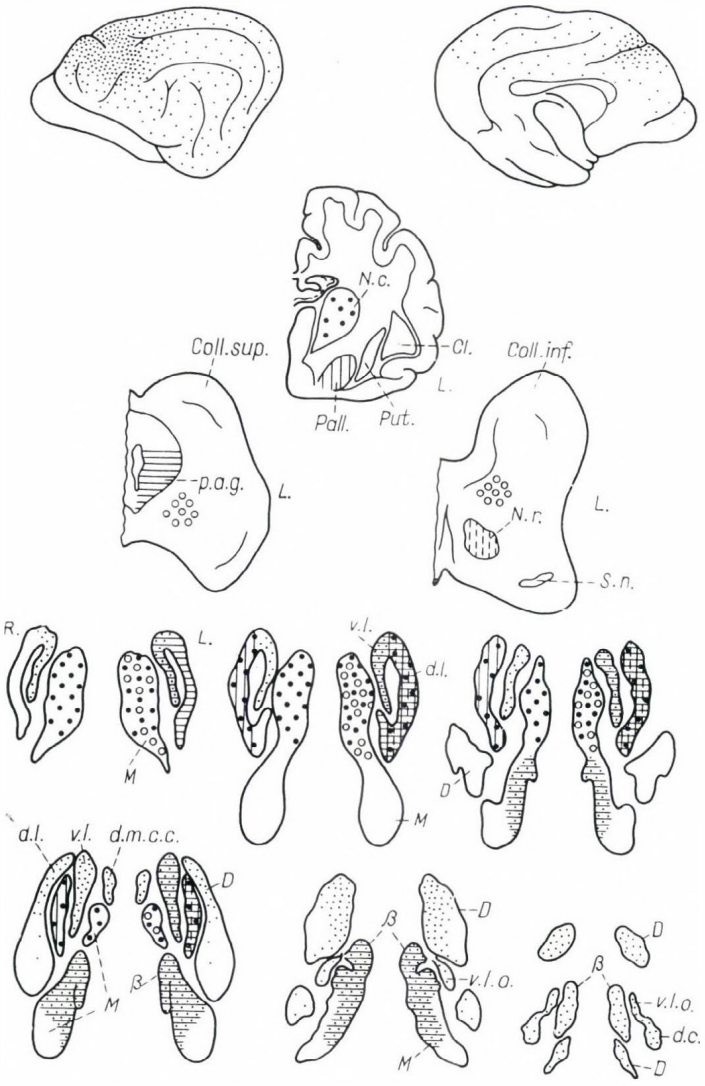

Fig. 1.—Diagram of the projection of various regions of the central nervous system to the inferior olive. Above, structures giving rise to afferent fibres to the olive, indicated with different symbols. Below, receiving areas of the olive indicated with same symbols as the structures projecting onto them. The areas of the olive not indicated with symbols receive fibres from the spinal cord. Note the terminal areas of the fibres from the periaqueductal grey region and the reticular formation of the mesencephalon. (Walberg, 1960). ß = nucleus ß, Cl. = claustrum, Coll. inf. = colliculus inferior, Coll. sup. = colliculus superior, D = dorsal accessory olive, d.c. = dorsal cap, d.l. = dorsal lamella of principal olive, d. m. c. c. = dorsomedial cell column, L. = left, M = medial accessory olive, N. c. = nucleus caudatus, N.r. = nucleus ruber, p. a. g. = periaqueductal gray substance of the mesencephalon, Pall. = globus pallidus, Put. = putamen, R. = right, S. n. = substantia nigra, v. l. = ventral lamella of principal olive, v.l.o. = ventrolateral outgrowth

cats only some dendrites in the olive show the organelles which have been called multivesicular inclusion bodies (compound vacuoles), many dendrites close to degenerating boutons contain inclusion bodies. Some dendrites may also show more than one body. The functional importance of such inclusion bodies is not known, but there is some indication that they are concentrated in areas where certain enzymes such as acid phosphatase are present (for references see Novikoff 1961).

Furthermore, it is to be noted that the surface membranes of some of the dendrites show invaginations of various types. They may be shallow or form large pits. These invaginations are present where the surface membrane of a dendrite lies adjacent to degenerating boutons.

Dark material, presumably originating from degenerating boutons, is present in the pits, and a radial arrangement of dark material is indicated on the dendritic side.

The pits described here are very similar to those shown by Roth and Porter (1962) occurring on the surface of developing mosquito oocytes, and interpreted by these authors as regions of protein uptake.

The following conclusions can be drawn from these findings:

The observations are not in agreement with those made by Gray and Guillery (1961), Gray and Hamlyn (1962) and Colonnier and Gray (1963) concerning argyrophilia of boutons. They show that argyrophilia of boutons in the inferior olive does not depend upon the presence of filaments, but is probably related to chemical and physical properties of one or more of the structures present in the boutons. Therefore, the Glees and Nauta methods can not be referred to as neurofibrillar silver stains. (For a more complete discussion of the problem of argyrophilia, the reader is referred to Walberg (1963b).

The findings made in the present study in addition give information on the structural changes taking place in regions where degenerating boutons are being removed. Obviously, at the 14 day stage the degenerating boutons have not changed notably from those found at the 7th day. The same is the case at the 36 day stage. At this time the pits are found on the surface membrane of dendrites.

One can probably not exclude that the degenerating boutons are in part removed by pinocytosis of adjacent dendrites, and that the engulfed material is further dissolved by enzymes present in the multivesicular inclusion bodies (see Walberg 1964c). However, pinocytosed vacuoles have not been observed. Most of a degenerating bouton is probably removed by lytic processes, in which enzymes produced in the bouton are active. To what extent enzymes produced in inclusion bodies of dendrites take part in this process, is not known.

Only few signs of glial activity have been found at the stages studied. The reason for this probably is that in the inferior olive the majority of the glial element of the neuropil are processes of fibrous astrocytes. These processes are entirely filled with filaments. In addition thin sheets, either extending from these processes of from the cell body of fibrous astrocytes, are encountered.

The processes and sheets are found close to degenerating boutons, but none of these structures appear to have phagocytotic activity. The findings in the olive are therefore not similar to those made in the cerebral cortex by

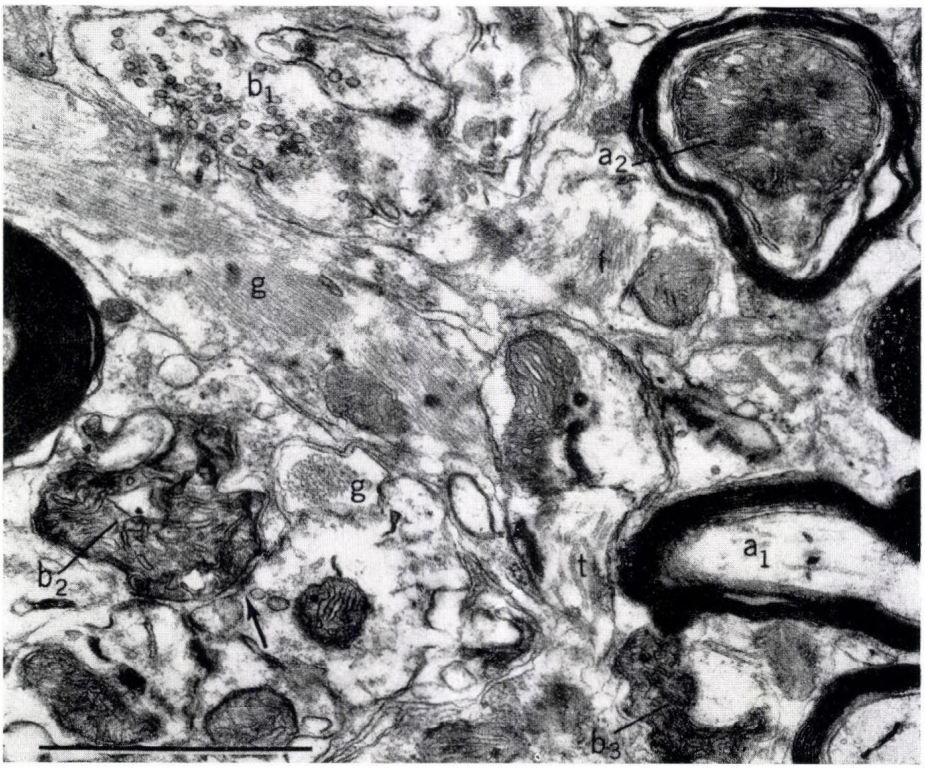

Fig. 2.—Electron micrograph from ventral lamella of the inferior olive in a cat 7 days after a lesion of the mesencephalon. b_1 = a normal bouton, in upper part of picture, b_2 and b_3 = degenerating boutons. Arrow points to synaptic contact between bouton b_2 and a dendrite. a_1 = a normal myelinated axon, a_2 = a myelinated axon in a state of degeneration. For details, see text. g = glial process, f = filaments, t = tubuli. Scale line 1μ.

Colonnier and Gray (1962). These authors found that degenerating boutons were engulfed in glial processes and also present in the glial cell body where they possibly had moved for enzymatic destruction. Probably, therefore, the morphological processes related to the disappearance of degenerating boutons are different in different regions of the central nervous system, depending, among other things, upon whether protoplasmic or fibrous astrocytes are present.

DISCUSSION

Szentágothai : I was highly impressed by the strange invaginations seen in degenerated material. In degeneration there is, of course, always some tortuousness of the contact lines between different profiles. Some of them appear to be regular invaginations, as you showed them. In the lateral

geniculate body we have also normally small axon terminals invaginated into the large optic axon terminals. If by cutting the optic nerves we let the large terminals degenerate 2 months later, the small ones are found to be invaginated into medium sized host axon endings in which we usually never find other invaginated axonal profiles. It is possible that the small ones have the tendency to invade large ones, and if we destroy the primary host endings, they look for the second best possibility where to impress themselves.

Picard : (1) Regarding the increase of numbers and increased argyrophilia of boutons, has any other technique been used, such as technique based on the use of haematoxylin?(2) Have you an opinion as to the significance of 'dense core vesicles' encountered together with empty vesicles in the boutons of olive in the normal state?

Walberg : In answer to your first question I can say that I have only used the Glees and Nauta methods in the study of terminal degeneration.— According to what has been found in our laboratory, the so-called dense core vesicles are present in boutons in many regions of the central nervous system. However, we have not made a particular study of these vesicles, so we cannot say whether they are present in larger numbers in boutons in certain nuclei.

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THE SYNAPSES OF SHORT LOCAL NEURONS IN THE CEREBRAL CORTEX

by

J. SZENTÁGOTHAI*

DEPARTMENT OF ANATOMY, UNIVERSITY MEDICAL SCHOOL
PÉCS, HUNGARY

Short neurons and their synaptic relations are routinely investigated by the classical Golgi impregnation and its modifications. Whatever we know of these today had, therefore, been known already about the turn of the century. Apart from the generally known facts concerning short neuron systems in the cerebellum and the Golgi second type neurons in the cerebral cortex, not much attention has been paid to them for some decades. Interest for these types of connexions and neurons has first been revived by the fundamental ideas of Lorente de Nó (1938) on the arrangement of neurons in recurrent and multiple chains, both in reflex arcs—considered originally as of simple linear design—and in higher integrative centers. These ideas have been verified and further developed far beyond expectation by the recent spectacular development of the unit-level neurophysiology, so we may consider the thorough investigation of short neurons and short connexions as one of the most important tasks of neurohistology.

The Golgi methods, however good, have certain disadvantages rendering it impossible to rely exclusively on them in the study of short connexions. If, as is the case, for example, in the cerebellar cortex, the arrangement of neurons has some well-defined and regular orientation in space and there are relatively many neurons of each kind, it is fairly easy to recognize their connexions correctly. If, however, there is no such uniformity of spatial orientation, or the number of short neurons or connexions is smaller, or the shape and structure of the entire grey matter is complex geometrically—as e.g. in the spinal cord—it may be very difficult if not impossible to get a true picture of the short connexions from Golgi preparations alone. In addition, the Golgi picture is often equivocal as regards the question what is connected to what. In order to be sure whether or not synaptic contact is effected between two elements, seen in close attachment under the light microscope, information by the electron microscope is required. It is therefore of crucial importance to identify under the electron microscope the synapses belonging either to short neurons or to short connexions, for example to initial collaterals of axons.

A new approach to these questions is by the technique of 'persisting elements' in chronically isolated parts of central nervous tissue. Under favourable anatomical conditions it is possible to isolate slabs of grey matter leaving intact its blood supply. It is therefore a natural condition

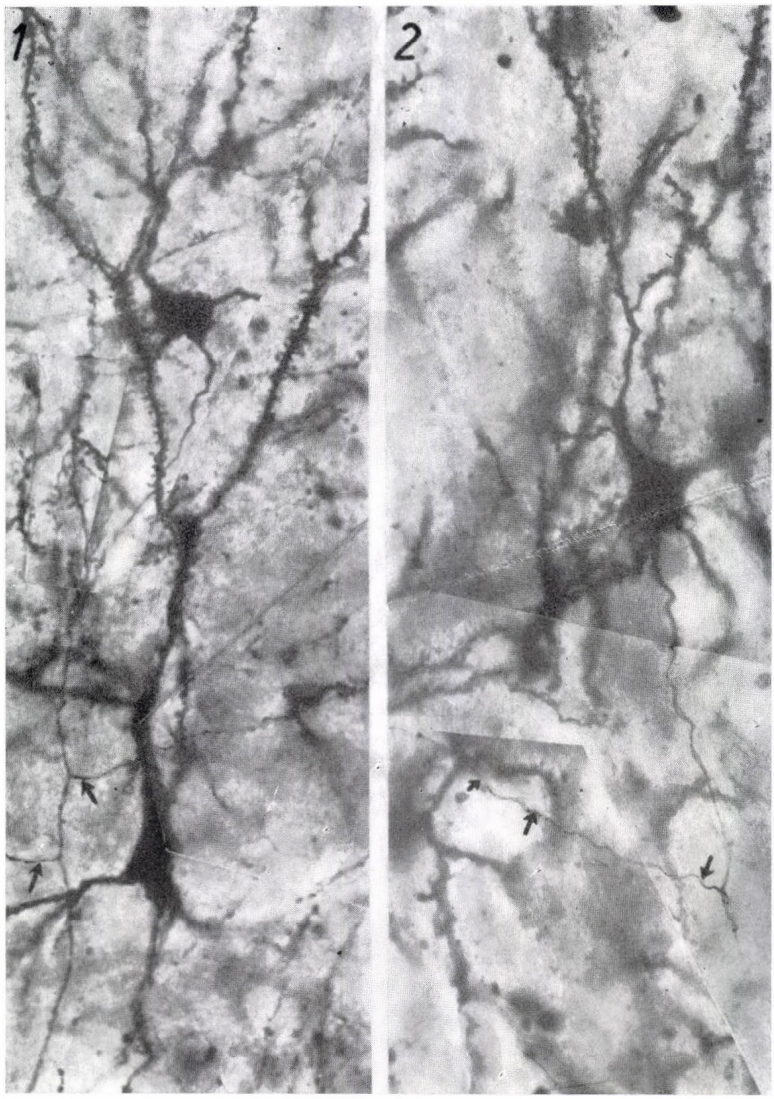
* Present address: Department of Anatomy, University Medical School, Budapest, Hungary

of this technique that the blood supply of the isolated grey matter be secured, at least partially, from some region of the pial surface nearby. This condition is fulfilled in many regions of the CNS: cerebral and cerebellar cortex, hypothalamus, superficial regions of the brain and even in the spinal cord, so the technique can be used quite extensively. Neurons situated in such isolated slabs can survive together with their dendritic and axonal ramifications for very long periods without major changes of the arborization patterns. After two months of survival time, when all extraneous elements have undergone degeneration and have already been resorbed, any synaptic terminal encountered intact may safely be considered as arising either from the axon of a short neuron or from an initial collateral of an axon from a nerve cell that has survived in the isolated slab. This procedure fulfils both requirements following logically from the considerations in the preceding paragraph: (1) short neurons and short connexions can be investigated in the isolated slabs by the aid of several staining procedures for light microscopy and (2) the synapses involved in these connexions can be easily identified under the electron microscope. This technique has already since several years been exploited in our laboratory for the investigation of the short neuronal connexions on the light microscopy level in the spinal cord (1958, 1964) in the cerebral cortex (1962a) and in the cerebellar cortex (1965). This paper deals with the electron microscopic identification of short connexions in the cerebral cortex.

The technique of isolation of slabs of the cerebral cortex has been described elsewhere (1962a). The cortex is undercut by introduction of the hooked apical part of a handled needle along the curvature of the hook, so that its tip should emerge again on the pial surface. Then using the two points, through which the needle passes the pia, as hinges, the needle is turned around its axis first 90° in one direction, then backwards 180° in the other, until the curvature appears under the pial surface but does not cut through it. The curved part must be flattened and sharpened on both edges like the surgical needles, in order to cut neatly through the brain tissue. This way lens-shaped slabs of the cortex can be isolated from the surrounding parts. The blood supply of such slabs is fairly well secured from the practically uninjured pial surface. By using needles with different radii of their curved parts and by introducing them into different depth, slabs of various diameter and various thickness can be cut. From slabs containing only the two superficial cortical layers to those having all the six layers preserved, various kinds have been investigated after two months postoperative duration with light microscopical and some with electron microscope techniques.—Isolated slabs were prepared from the primary optic region and also from the gyrus lateralis.

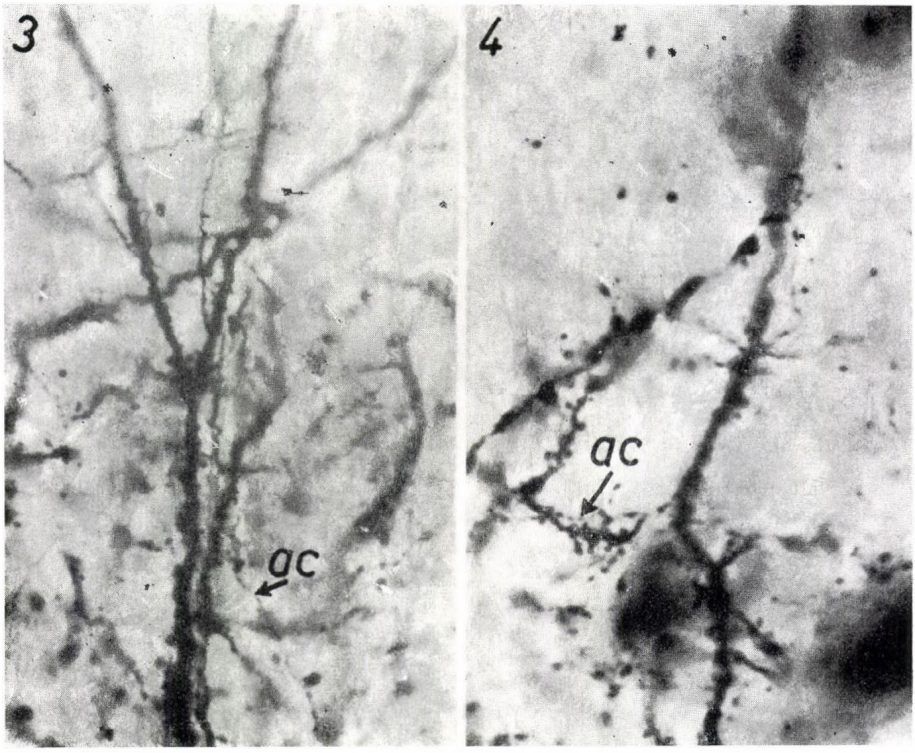
LIGHT MICROSCOPY OF ISOLATED CORTICAL SLABS

As seen in Figs 1 and 2, the nerve cells fairly well preserve their characteristic shape and dendritic arborization patterns. At first sight even the dendritic spines appear to be unchanged. Axons and their initial collaterals, as well as the arborizations of the latter, seem to show no significant changes either. No evidence of abnormal sprouting could be observed, with



Figs 1 and 2. — Neurons in chronically isolated slabs of the cerebral cortex. Cat, rapid Golgi procedure, × 580 — The upper neuron of Fig. 1 is a 2nd layer star pyramid cell with two of its axon collaterals (arrows) visible. Lower neuron is a 3rd layer pyramid cell with seemingly well preserved spines. — In Fig. 2 the axon of star pyramid cell can be traced downwards and its collaterals to the left (indicated by arrows) can be followed to basal dendrites of other neurons

the exception of a zone bordering the lesions where together with a strong glial reaction, there are the well-known signs of abortive regeneration of fibers. Being, however, interested here exclusively in the elements belonging to the original structure of the cortex, we shall leave them out of consideration. Concerning the value of this technique, it is of crucial importance—in



Figs 3 and 4. — Synaptic entanglement of star pyramid or pyramid cell initial axon collaterals *(ac)* with apical dendrites of pyramidal neurons. — Chronically isolated slab of cerebral cortex, cat, rapid Golgi procedure, × 900

order to avoid serious errors—to exclude 'sprouting' of nerve elements. As it appears from these figures, there is nothing to be seen, especially of axons or axon branches, in the well-preserved central regions of the slabs, that could not be seen in Golgi preparation of the normal cortex in the same region. — In the neurofibrillar picture the intercellular neuropil is reduced; the more so the fewer layers the slab consists of. Strangely, the density of the neuropil, in general, does not depend on the diameter (in tangential direction) of the slab. This already suggests that the intrinsic connexions of neurons running parallel with the surface might contribute only in a small extent and above all for comparatively small distances to the intercellular neuropil. Even in the outermost layer of the *stratum zonale*, which is a layer of tangential axonal plexus *par excellence*, most of the axons are rather short. According to our degeneration studies (1962a), the secondary degeneration of axons, in tangential direction, cannot be traced farther than four mm after a superficial cut into the first layer; but the far majority of degenerated fibers disappear already at a distance of about two and a half mm. From the scarcity of the nerve cells in the first layer and the shortness of the axons, it immediately became obvious that the majority of the axons in this layer must originate from deeper layers. Indeed, there

are practically no axons preserved in the first layer of the chronically isolated slabs—not even in those of large diameter—if only the outer four layers are preserved. They begin to increase in number in slabs of five layers and are practically preserved in full number in isolated slabs of six layers (Szentágothai 1962a). This shows that the tangential plexus of the zonal layer mainly arise from ascending Martinotti (1895) cell-axon ramifications but never from recurrent pyramidal cell-axon collaterals. We have not yet been able to determine the distances bridged in tangential direction by axons or collaterals in the deeper layers, but they are probably smaller than found in the zonal layer.

The initial axon collaterals of pyramidal cells can easily be seen and traced in isolated slabs (Figs 1 and 2). Wherever they can be traced into the immediate vicinity of another well-stained neuron, they appear to establish synapses with the basal dendrites or the apical dendritic shaft of pyramidal cells and its lower side branches (Figs 3 and 4). They practically never contribute significantly to the zonal layer. There was no clear evidence of pyramidal initial axon collaterals that would establish contacts with cell bodies or participate in baskets around cell bodies. On the other hand, one can see very clearly in good neurofibrillar preparations of isolated slabs that the pericellular baskets surrounding pyramidal cells are preserved.

ELECTRON MICROSCOPY

The differences between the structure of chronically isolated cortical slabs and the normal cortex of the same region can easily be recognized. Most characteristic for the normal cerebral cortex are the numerous axo-dendritic synapses of the intercellular neuropil, being mostly of Gray's Type 1, from which especially the axon spine synapses dominate the picture (Fig 5). Axo-somatic synapses, almost exclusively of Gray's Type 2 are also frequent (Fig. 5), although there are considerable parts of the cell body surface left free from immediate contact with presynaptic elements. In the chronically isolated slabs there is always some gliosis, very often with characteristically hypertrophic fibrillar apparatus of the glial cells. There is also some 'withering' of the preserved axonal and dendritic elements, as the margins of their profiles have lost somewhat of their smooth curves and appear to be much more irregular. Especially characteristic of the 'withering' of nerve elements is the shrinkage of the axon inside the myelin sheaths with the appearance of the innermost coils of the mesaxons (Fig. 6). The network of intercellular spaces, although more irrregular than in the normal cortex, is not significantly changed. What is most significant, however, is the disappearance of most of the characteristic spine synapses, accompanied by a considerable reduction of other axo-dendritic contacts. It would be impossible to find visual fields of the same size in the normal cortex of an intercellular neuropil without several axon-spine synapses as shown in Figs 6, 7 and 10 in the isolated cortex. Small profiles which can be recognized with high probability as atrophic spines can be seen quite frequently (Fig. 6), but they have lost their differentiated structure completely. Axon-spine synapses of the usual appearance, especially those invaginated into axonal profiles, rarely occur in the isolated cortex (Fig. 10, inset).

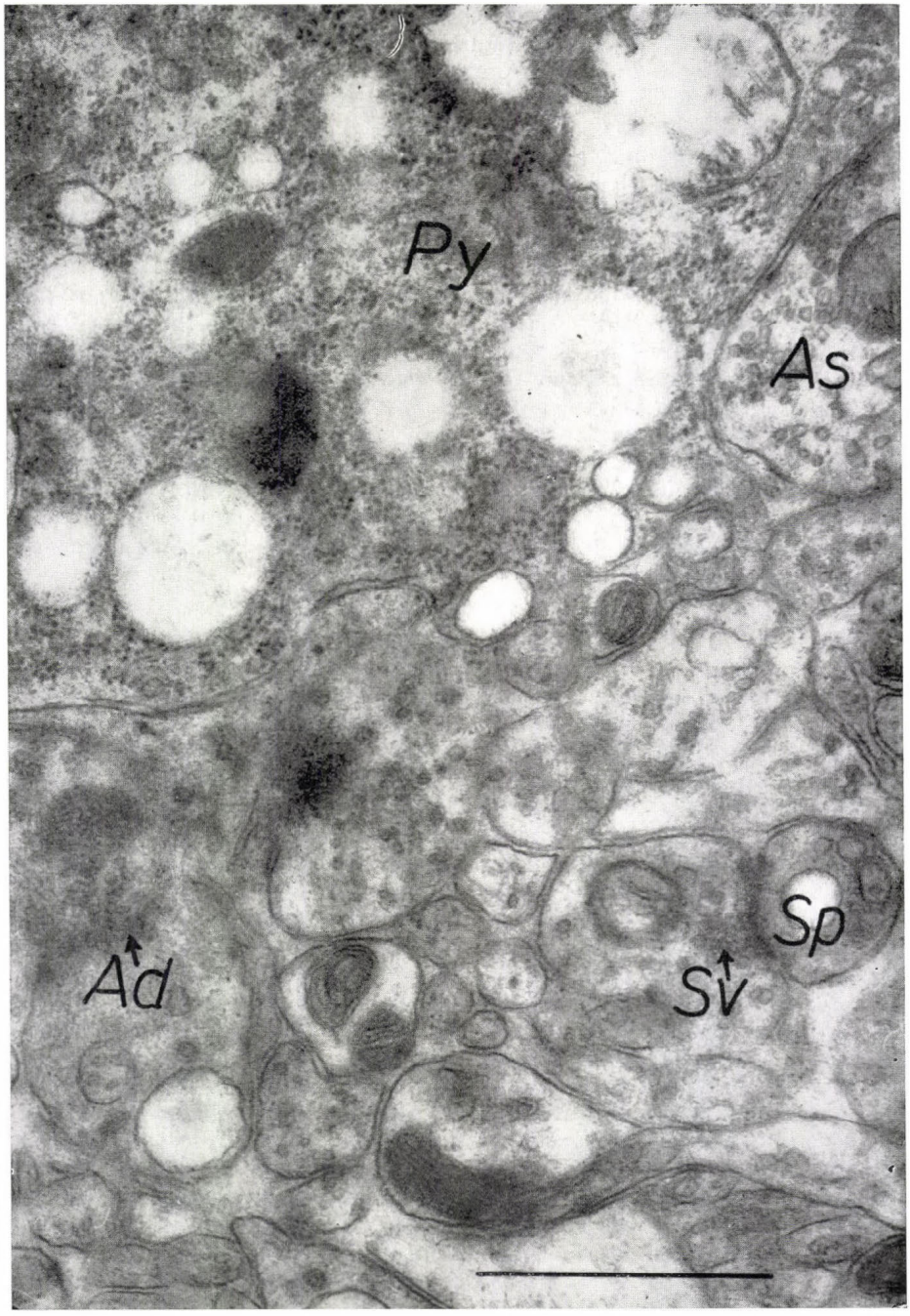


Fig. 5.—Part of the surface of pyramid neuron *(Py)* in contact with axo-somatic *(As)* ending. This contact is of Gray's Type 2. Axo-dendritic *(Ad)* synapse to the left is cut obliquely, to the right small axon profile with synaptic vesicles *(Sv)* has Type 1 synaptic contact with dendritic spine *(Sp)*. Cat, optic cortex. Scale = 1 micron

Fig. 6.—Chronically isolated optic cortex of the cat, showing part of the neuropil and hypertrophic glial profiles *(Gl)*. Shrinkage of axons *(Ax)* inside the myelin sheaths is characteristic. Almost no true axo-dendritic synapses, with specialized regions of contact, in spite of close packing of dendritic *(D)* and axonal profiles with synaptic vesicles *(Sv)*. Small profile (indicated by arrow) is probably atrophic dendrite that, although in close relation to an accumulation of synaptic vesicles of an axonal profile, has no synaptic contact with it. Other similar small profiles might be interpreted with respect to Figs 1—4 as atrophic and dedifferentiated spines

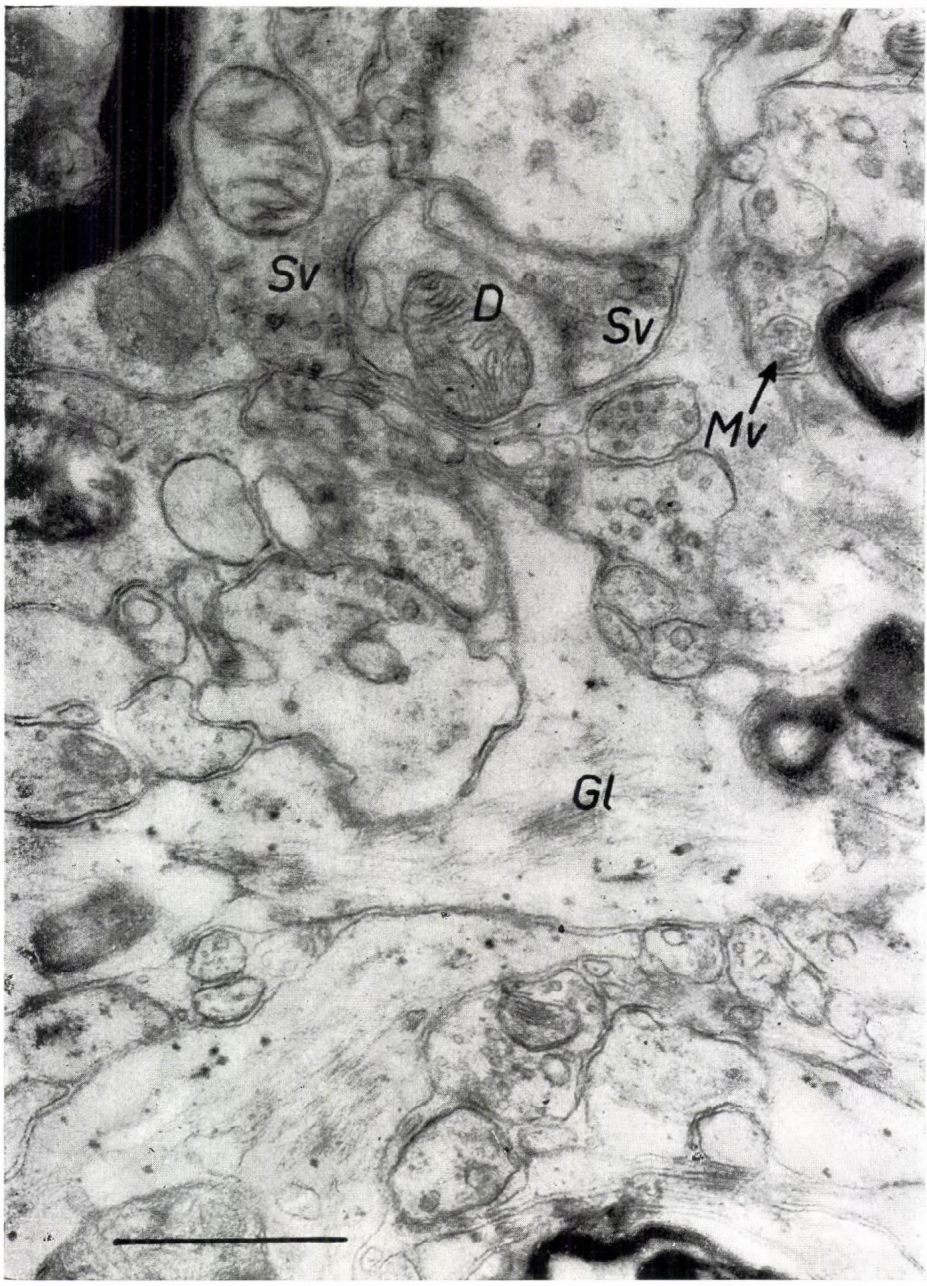


Fig. 7.—As Fig. 6, with large glial profiles *(Gl)* containing hypertrophic filaments and small dense bodies; probably glycogen. Well-preserved synaptic contacts between dendrite *(D)* and axonal profiles, the synaptic vesicles *(Sv)* of which are somewhat more irregular in size as normally. *Mv* = multivesicular body

Fig. 8.—Part of surface of pyramid neuron *(Py)* with nucleus *(Nu)* and Golgi systems *(Go)* visible. Intact axonal profiles with synaptic vesicles *(Sv)* are seen in contact with cell surface. Large axonal profile has somewhat atrophic, but still clearly recognizable specialized synaptic attachments (indicated by arrows) to neighbouring dendrite *(D)* and cell body. On the top right preserved axo-dendritic synapse *(Ad)*. Chronically isolated optic cortex of the cat

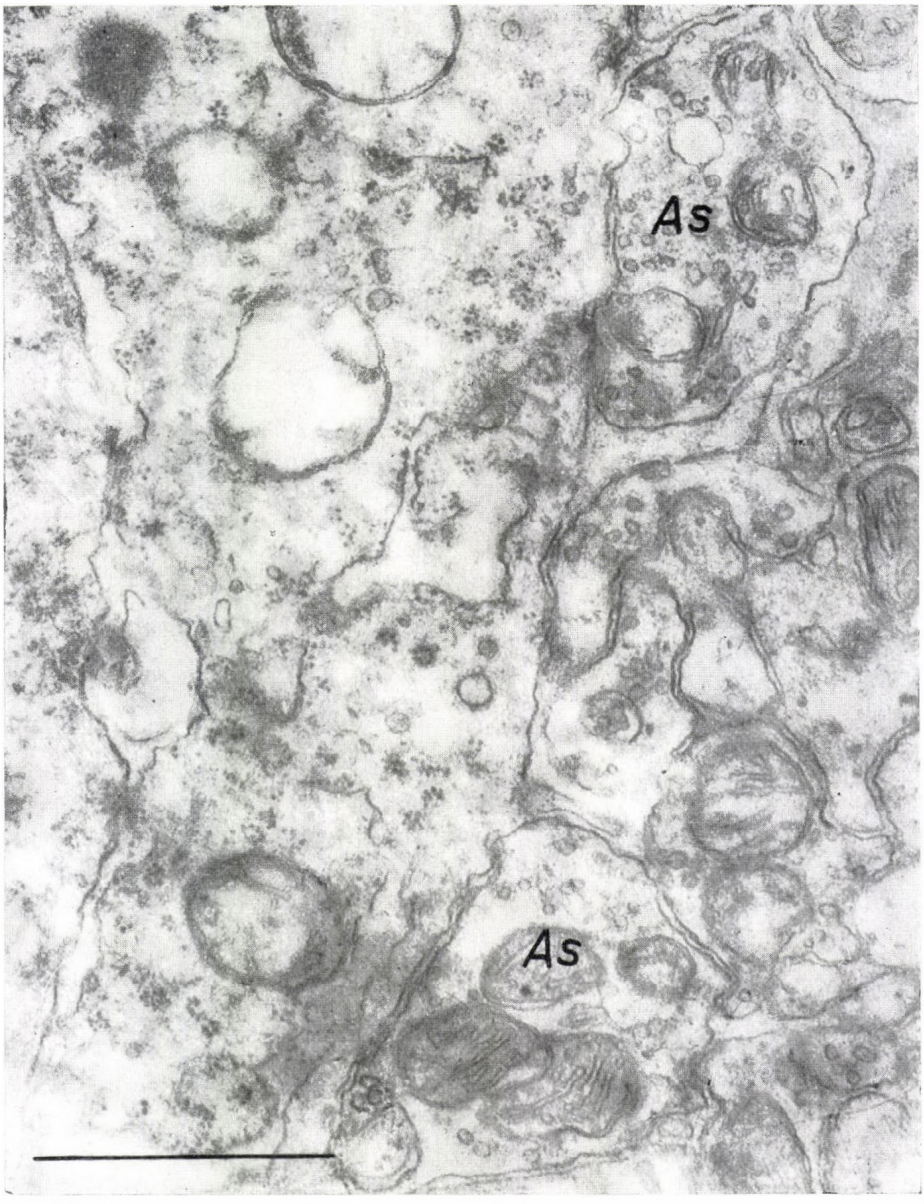


Fig. 9.—Relatively well-preserved axo-somatic synapses of Type 2 *(As)* on the surface of pyramid neuron. Cat, chronically isolated optic cortex

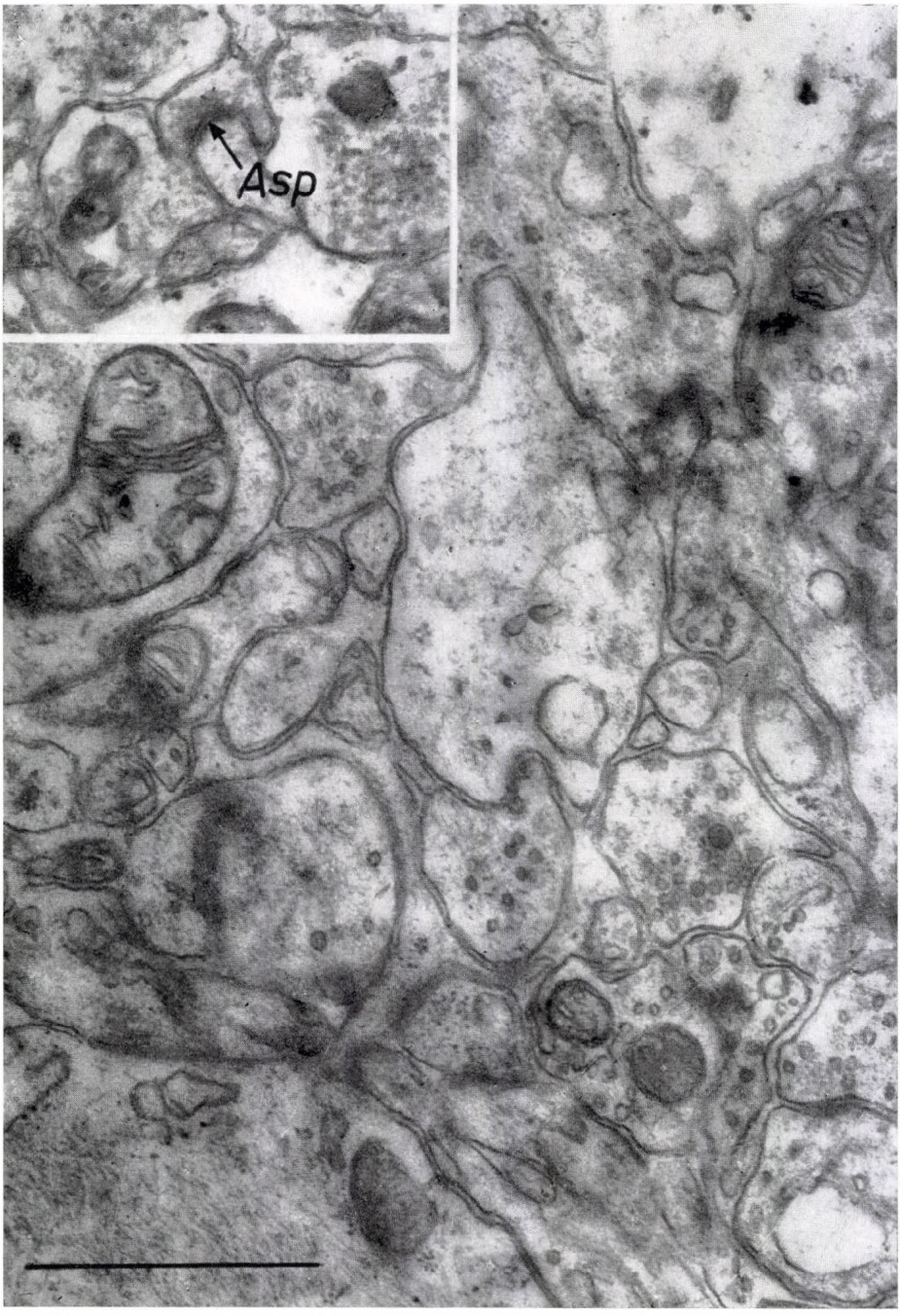


Fig. 10.—Neuropil of chronically isolated cortex with almost no differentiated synaptic attachment regions. Rare, relatively intact axon-spine synapse *(Asp)* is shown in upper inset microphotogram

In contrast, the axo-somatic synapses are well preserved in all isolated slabs and show no appreciable reduction in number (Figs 8 and 9). They even appear to occur in higher density than normal, owing most probably to the slight shrinkage of the nerve cells. The elementary structure and organelles of the persisting nerve elements do not show major changes in the chronically isolated cortex.

CONCLUSIONS

Several interesting points are worthy of discussion in connexion with this material. It is on the whole amazing how little the elementary structure of the nervous tissue of the cortex is changed, if deprived from all extraneous neuronal connexions. There is, of course, some general atrophy of the persisting elements, as shown especially by the shrinkage of axons inside their myelin sheaths. As all extrinsic axons and consequently also myelin sheaths have already undergone complete degeneration and have been resorbed with the rare exception of occasional larger myelin bodies—containing, of course, no axons—the remaining myelin sheaths with shrunken axons must be those of the pyramidal cells that have survived in the slab. The general 'withering' of the persisting dendritic tree and axonal ramifications appears from the irregular borders of the profiles, and the lack of their usual smooth convexities is indicative of a loss in the turgescence of the individual cell processes.

One of the most significant changes is the sharp decrease in number of the axo-dendritic and particularly of the axon-spine synapses. Those present represent obviously only a small fraction of the original number and probably serve as postsynaptic sites to short intrinsic connexions of the cortex. These connexions are clearly visible in Golgi preparations of chronically isolated cortical slabs. They belong mainly to initial axon collaterals of pyramidal neurons or star pyramids (Fig. 1) and can be traced to the dendrites of neighbouring pyramidal and star pyramid cells (Figs 2, 3 and 4). Although this is difficult to decide from Golgi preparations, such kinds of connexions do not participate in the baskets that surround the bodies of pyramidal cells. Most synapses that persist are direct axo-dendritic contacts (Figs 7 and 8) of Gray's Type 1 in which the presynaptic side does not change significantly, whereas the dendrites have lost many of their structural details and have become much lighter, although their large mitochondria are usually well preserved. The synaptic vesicles are much less uniform in size than normally. The thickenings of the subsynaptic membrane are also much less clear than in normal material.

The large reduction in number of the characteristic axon-spine synapses might raise the question whether the spines of the dendrites have not been lost entirely in the isolated cortex. As seen from the Golgi preparations (Fig. 1), this is not the case, although their quantitative relations would be difficult to judge from simple inspection alone. Nevertheless, from some of the details (e.g. Figs 3 and 4) one gets the impression that the spines may have undergone considerable shrinkage and, even if in immediate contact with a presynaptic axonal surface, may have lost their postsynaptic differentiations (Fig. 6). If the isolated slab is very small—not only in depth, but also in tangential extension—and contains only a small number (in

the order between ten and hundred) of nerve cells, we see a considerable reduction of the number of spines in the Golgi preparation (Szentágothai 1965), without any major change otherwise in the gross dendritic arborization pattern. Thus the reduction in number of the axon-spine synapses may be due not only to their majority being established between extraneous axons and the dendritic spines, but probably to the fact that the most distal parts of the dendritic tree—the spines—might be those parts of the neuron that suffered most, having lost all-over functional connexions. As we shall point out in a forthcoming paper, one important transneuronal change of the neuron in general—visible only by electron microscope—is the quick and almost 'degeneration-like' reduction of certain most apical or peripheric parts of the dendritic tree.

Exactly the reverse holds true for axosomatic synapses. Their number is increased rather than decreased in isolated cortical slabs. This is, of course, simply due to the moderate shrinkage of the cell body, which ought to lead necessarily to an increase in the density of axosomatic synapses on the cell surface, supposing that the absolute number of that type of synaptic terminals has not decreased. Figs 8 and 9 indicate clearly that synaptic terminals—of Gray's Type 2—although having undergone some shrinkage, are fairly well preserved and their elementary structure is practically unchanged. This observation is particularly significant if one considers the fact, clearly visible in neurofibrillar or Nissl preparations (Szentágothai 1962a) that there is no major loss in the nerve cells in isolated cortical slabs. One cannot, therefore, interpret this observation by assuming a passive rearrangement of the elements, i.e. that after a considerable part of the nerve elements are lost, the remaining ones would be rearranged, and the axosomatic endings would be those that have come into a random contact with the cell surface. First of all, with the exception of the axons of foreign source, there is no real loss of nerve elements, so there is not much place for such 'rearrangement'. Secondly, the large reduction in number of axodendritic contacts under circumstances when terminal axons and dendritic branches are present everywhere in the neuropil (as seen in Figs 6, 7 and 10), most clearly shows that passive spatial rearrangement does not lead to the formation of new synapses. If the presence of a postsynaptic locus that has lost its presynaptic contact would simply 'invite' an axonal branch that happens to be nearby to establish a new contact, one should expect much more preserved synapses, as appears to be the case in the periphery, especially striated muscle. The intercellular neuropil of isolated slabs is full of axonal and dendritic profiles wedged closely together, in most cases without the slightest indication of real synaptic contacts. In the normal cortex a considerable part of the dendritic surface is covered by synaptic terminals and is thus a surface 'receptive' for synaptic contacts. The great loss in axo-dendritic synapses, in spite of the large number of axon branches available, indicates that either the postsynaptic or the presynaptic elements, or both, must be highly specified as regards the type of contact they accept or establish themselves.—This reasoning leads up to the assumption that whatever synapse is encountered intact in an isolated cortical slab, it must have been there originally, and no secondary rearrangement of the persisting elements can lead—on major scale—to the formation of new synapses.

Therefore the preservation of almost all axo-somatic synapses, as substantiated also by the observation of many pericellular baskets under the light microscope, indicates that the axo-somatic synapses must be of local origin. This had been assumed already by Cajal (1911) who described the fibre baskets surrounding pyramidal cells as deriving from nerve cells with short axons, mostly of stellate neurons.—Lacking the simple and obvious geometric order in the cerebral cortex, which so clearly governs the arrangement of neurons and their connexions in the cerebellar cortex, one might be baffled by the erroneous concept that the connexions in the cerebral cortex—besides perhaps some broader spatial design—might be arranged in the details in a haphazard manner. This observation demonstrates very clearly how wrong such a view would be and that, in spite of the lack in simple all-over geometric design, the connexions of the cerebral cortex are nevertheless very specific and strictly determined.

Axo-somatic synapses in two instances have been identified recently as of specific inhibitory function. The axosomatic synapses of the basket cells have been shown (Andersen, Eccles and Løyning 1963) to exercise an inhibitory influence on the pyramidal cells in the hippocampus, and the basket cells of the cerebellum on the Purkinje neurons (Andersen, Eccles and Voorhoeve 1963). Both synapses are of Gray's Type 2 (Hamlyn 1963 hippocampus; Szentágothai 1963 cerebellum), so the idea of the possible excitatory function of Type 1 and of an inhibitory role of Type 2 contacts, as proposed by Sir John on this conference, might perhaps be the correct solution of the problem. The short stellate neurons of the neocortex, which establish pericellular baskets with Type 2 contacts around the bodies of pyramidal cells have an architectonic arrangement highly analogous to the basket neurons both in the hippocampus and in the cerebellar cortex. Although such inference seems rather premature at present, it cannot be put beyond all expectation that these stellate cells and their pericellular baskets will finally turn out to be the (or one of the) mechanism(s) of inhibition in the neocortex.

DISCUSSION

Eccles : Were there any differences in the pictures of residual elements that were obtained after various depths of undercutting—e. g. if only at a depth of 2 layers as against a depth of 6 layers?—Was it possible to identify the type of neurone on which the residual synapses occurred? For example, I would expect the axon collateral of pyramidal cells would largely be excitatory for star cells and that the star cell axons would in turn have inhibitory synapses in the pyramidal cell bodies, forming the pericellular network that Cajal has described. I think that the basis of this expectation lies largely on analogy with the hippocampus.

Krieg : Did you see many degenerating cells in the isolated cortex? Our experience has been that the cells in undercut cortex do not degenerate. *(Szentágothai :* No, they were remarkably well preserved). We explain this difference by the presence of collaterals in the cortex. When collaterals make connections, the cell can function, and so can stay alive.

Ábrahám : What is the difference between the local synapses and those which underwent degeneration because of the isolation of the cerebral

region in question. Is there any difference between the two kinds of synapses and if so, what is it?

Szentágothai : With the electron microscope we have not been able so far to investigate many slabs of different depths of undercutting. From light microscope pictures one can expect that the number of intact synapses decreases with decreasing vertical diameter of the slab; it should be rather independent from the dimensions of the slab in tangential direction up to a certain minimum of a few hundred micron diameter. If the slab contains only very few cells (in the order of the tens) the synapses practically disappear, however well the preparation would be preversed otherwise.—The pericellular network is well preserved in slabs of two and a half layers' thickness.—It is difficult to identify under the electron microscope to which type of neurons a certain cell body belongs. I still would think it probable that most cell bodies with intact axo-somatic contacts were of pyramidal cells.—I would agree with Dr Krieg in his explanation given to the good preservation of nerve cells.—To Dr Ábrahám I can only answer that apart from the fact that the majority of preserved synapses are of Gray's Type 2, it would be difficult to point out clear differences between synapses that are preserved by undercutting and those that degenerate. For this one would need better and especially some quantitative information on the several kinds of synaptic contacts in the normal cortex.

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SECTION FIVE

NEUROSECRETION AND MISCELLANEOUS

Symp. Biol. Hung., **5**, 269—276 (1965)

НЕКОТОРЫЕ ДАННЫЕ ОБ ЭМБРИОГЕНЕЗЕ ЯДЕР ТРОЙНИЧНОГО НЕРВА ЧЕЛОВЕКА

Л. Е. ГОНЧАРЕНКО

ОТДЕЛ ЭВОЛЮЦИОННОЙ МОРФОЛОГИИ (ЗАВ. – АКАДЕМИК АН УССР В. Г. КАСЬЯНЕНКО) ИНСТИТУТА ЗООЛОГИИ АН УССР КИЕВ, СССР

Возникновение и развитие зачатков ядер тройничного нерва человека изучено недостаточно. Полностью отсутствуют данные о значении процессов митотического и амитотического деления клеточных элементов и их миграции в развитии различных ядер тригеминального комплекса. Большинство исследователей (*М. Маршаль*, 1950; *Кейбле и Молл* 1911; *Кунтц*, 1947; *Арей*, 1948, *Гамбургер*, 1952 и др.) считает, что основное значение в увеличении количества клеточных элементов нервной трубки принадлежит многочисленным митозам в эпендимном слое её, а в образовании зачатков ядер — процессу миграции клеток в соответствующие участки плащевого слоя. Имеются единичные указания о наличии фигур амитоза в стенке различных отделов нервной трубки (*Чайльд*, 1907; *С. Б. Иванова*, 1954; *В. М. Минаева*, 1961 и др.). Наши наблюдения (*Л. Е. Гончаренко*, 1955) показали, что соотношение процессов митотического и амитотического деления клеток и их миграции различно при возникновении и развитии двигательного, мезенцефалического, главного чувствительного и спинального ядер тройничного нерва.

Были исследованы 63 серии поперечных срезов соответствующей части ствола мозга эмбрионов и плодов человека от 5 недель до 9 месяцев эмбриогенеза. Препараты окрашены гематоксилин-эозином, тионином; кроме того была проведена гистохимическая реакция Фельгена для выявления в клетках дезоксирибонуклеиновой кислоты.

На 5—6-ой неделе эмбриогенеза в тройничном сегменте нервной трубки четко различимы 3 слоя: матрикс, очень узкий плащевой слой и краевая вуаль. Во внутренних участках матрикса наблюдаются многочисленные фигуры митоза (89,64 $\pm$ 0,87 на каждом поперечном срезе нервной трубки).* В плащевом слое, в отличие от матрикса, видны единичные фигуры амитоза (12,04 $\pm$ 0,44 на поперечном срезе нервной трубки). В краниальном отделе тройничного сегмента наблюдается утолщение вентральной стенки нервной трубки, соответствующее расположению возникающих

* Подсчет и обработка цифрового материала произведены следующим образом $\bar{x} = \frac{\Sigma' x}{n}$ ($\bar{x}$ — среднее арифметическое, Σ' — суммирование, x — значение вариант, n — число вариант в вариационном ряду, $n = 50$); вычисляли квадратическое отклонение (σ) по формуле: $\sigma = \pm \sqrt{\frac{\Sigma'(x - \bar{x})^2}{n}}$ и стандартную ошибку (m) по формуле: $m = \pm \frac{\sigma}{\sqrt{n}}$.

симметричных зачатков мезенцефалического ядра. Светлые клеточные ядра нейросимпласта в этом участке расположены с определенной направленностью, являющейся морфологическим свидетельством процесса миграции их из матрикса. К концу 2 месяца эмбриогенеза происходит интенсивное расширение плащевого слоя. При этом фигуры митоза в эпендимном слое почти исчезают. В плащевом слое появляется большое количество фигур амитоза (8,02 $\pm$ 0,23 в каждом поле зрения).

В течение 7 недели эмбриогенеза возникают симметричные зачатки главного чувствительного, спинального и двигательного ядер тройничного нерва, внешне отличающиеся друг от друга, что обусловлено различными процессами, лежащими в основе их формирования. Светлые зачатки двигательного ядра образованы развивающимися нервными клетками, содержащими светлые, округлые ядра (8—10 μ), которые окружены, в отличие от других участков плащевого слоя, угловатыми протоплазматическими ободками. Фигуры деления клеток в зачатке ядра почти отсутствуют. Однако между эпендимным слоем и зачатками двигательного ядра видны тяжи мигрирующих клеточных ядер, которые по мере миграции значительно дехроматизируются и окружаются узкими ободками цитоплазмы. Многие из них амитотически делятся (8,13 $\pm$ 0,89 амитозов в каждом поле зрения). В течение 3—5 месяцев эмбриогенеза происходит цитологическая и цитоархитектоническая дифференциация зачатков двигательного ядра тройничного нерва *(Л. Е. Гончаренко*, 1963), в результате которой к 9-му месяцу определяется их дефинитивная структура. В этот период фигуры деления клеток в зачатках отсутствуют.

Развитие мезенцефалического ядра в начале (5—6 неделя эмбриогенеза) имеет сходные черты с развитием двигательного ядра тройничного нерва. В этот период интенсивно происходит процесс миграции клеточных ядер из эпендимного слоя в плащевой, в частности, в зачатки мезенцефалического ядра. К 7—9 неделе процесс миграции ослабевает, но 'вспыхивает' процесс амитотического деления в зачатках. Количество амитозов нарастает до 9 месяца эмбриогенеза, а затем резко уменьшается.

В возникших на 7 неделе зачатках главного чувствительного и спинального ядра среди густо расположенных темных клеточных ядер нейросинцития видны многочисленные фигуры амитотического деления, количество которых увеличивается до 6 месяца эмбриогенеза. При этом в различных отделах чувствительных ядер тройничного нерва процессы амитотического деления проходят с различной интенсивностью*: на 3 месяце эмбриогенеза — наиболее интенсивно в зачатках главного чувствительного ядра (48,76 $\pm$ 4,26 амитозов на поперечном срезе ядра); к 4 месяцу количество фигур амитоза в этом отделе уменьшается (23,61 $\pm$ 2,18), но значительно увеличивается в каудальном отделе спинального ядра (51,73 $\pm$ 3,21); в течение 5—6 месяцев эмбриогенеза происходит вспышка амитотического деления и в главном чувствительном ядре (86,61 $\pm$ 3,41) и в краниальном отделе спинального ядра (80,92 $\pm$ 3,6). Увеличивается количество амитозов и в других отделах спинального ядра (56,27 $\pm$ 4,61).

* Исходя из особенностей цитоархитектоники, мы условно делим спинальное ядро n. V в кранио-каудальном направлении на три отдела: краниальный, расположенный между главным чувствительным ядром n. V и краниальным концом нижних олив; средний — на уровне нижних олив; каудальный — от уровня каудального конца нижних олив до первых сегментов шейного отдела спинного мозга.

К 7 месяцам эмбриогенеза количество фигур амитотического деления в чувствительных ядрах n. V резко уменьшается (12,68 ± 0,78), что, по-видимому, связано с процессом цитологического 'созревания' зачатков (появляются нервные клетки, уменьшается количество малодифференцированных элементов, способных к делению).

В течение 3—6 месяцев эмбриогенеза в зачатках чувствительных ядер фигуры митоза почти отсутствуют. Миграция клеточных элементов из эпендимного слоя в зачатки не выражена.

На основании наших наблюдений мы считаем возможным рассматривать митоз как более ранний вид деления клеток в развитии тройничного сегмента нервной трубки человека, а амитоз — вид клеточного деления, которое возникает позднее, но приобретает решающее значение в дальнейшем развитии. Увеличение количества фигур амитоза (начиная с 6 недели эмбриогенеза) в плащевом слое, наряду с полным исчезновением митозов в эпендимном (8 недель эмбриогенеза), ограничивает во время камбиальное значение эпендимного слоя.

Нами установлено, что в формировании, в развитии зачатков двигательного ядра тройничного нерва человека основная роль принадлежит процессу миграции клеточных элементов из эпендимного и плащевого слоя в зачатки. Деления клеток в зачатках почти не происходит.

В начале формирования зачатков мезенцефалического ядра также происходит интенсивная миграция клеточных элементов из эпендимного слоя в зачатки, но в дальнейшем развитии первостепенное значение приобретают процессы амитотического деления в зачатках.

В возникновении и развитии зачатков главных чувствительных и спинальных ядер ведущее значение имеют процессы амитотического деления в самих зачатках. Миграция клеточных элементов в зачатки выражена чрезвычайно слабо.

Приведенные данные позволяют предположить, что процесс миграции клеточных элементов имеет основное значение в возникновении и развитии рано появляющихся и быстро развивающихся зачатков ядер (двигательное и мезенцефалическое ядро тройничного нерва); в тех же ядрах, у которых темпы развития медленнее (главное чувствительное и спинальное ядра тройничного нерва), первостепенное значение имеет процесс амитотического деления в самих зачатках *(Л. Е. Гончаренко*, 1962, 1962, 1963).

Изучая развитие тригеминального сегмента нервной трубки и, в частности, становление ядер тройничного нерва человека, мы наблюдали на различных этапах развития выход гранул дезоксирибонуклеиновой кислоты (ДНК) из клеточных ядер в цитоплазму и за ее пределы. В литературе описаны подобные явления в клетках различных тканей эмбрионов *(С. А. Усов*, 1924; *Эрнст*, 1926; *Глюксман*, 1930, 1950; *Спер и Глюксман*, 1938; *Калиус*, 1931; *А. П. Дыбан*, 1959; *Гамбургер, Леви-Монталчини*, 1949; *Гамбургер*, 1952). Однако тригеминальный ядерный комплекс в этом отношении не был изучен. Наличие большого числа серий препаратов позволило нам наблюдать некоторые этапы выхода гранул ДНК из развивающихся клеток в зачатках двигательного, мезенцефалического, главного чувствительного и спинального ядер n. V.

В ранее опубликованных исследованиях *(Л. Е. Гончаренко*, 1955, 1962, 1962, 1963) мы отмечали присутствие в развивающихся зачатках ядер n. V. три типа дифференцирующихся клеток, ядра которых отличаются коли-

чественным содержанием ДНК: 1. клетки с крупным (6—8 μ) овальным светлым ядром, содержащим небольшое количество различных по размеру гранул ДНК; 2. клетки с тёмным ядром (4—5 μ), наполненным густо и равномерно распыленными мельчайшими гранулами ДНК; 3. клетки с ядром (5—6 μ), по содержанию ДНК и тинкториальными свойствами, являющимся промежуточным между первыми и вторыми. Выход гранул ДНК наблюдается в развивающихся клетках всех трёх типов, однако этот процесс более интенсивен в клетках, содержащих тёмные гомогенные ядра (2-го типа). В некоторых таких ядрах равномерно распыленные по всей кариоплазме гранулы ДНК, сливаясь, образуют более крупные глыбки, часть которых расположена вблизи оболочки ядра (рис. 1а, б). Следующим морфологическим этапом, по-видимому, является прохождение различного размера гранул ДНК через ядерную оболочку. При этом можно четко видеть расположенные в кариолемме гранулы ДНК, которые нарушают её ровные очертания (рис. 1в, г, д). Вокруг некоторых ядер в цитоплазме и за пределами её расположены мигрировавшие из ядер гранулы ДНК (рис. 1а, г, д, е, ж, з, и, к, л). Выход гранул ДНК сопровождается заметным обеднением ядер ДНК — ядра становятся более светлыми, иногда в них сохраняется не более 2—5 глыбок ДНК. Признаки гибели исходных ядер мы обнаруживали очень редко. Наряду с описанным выше процессом мы наблюдали превращение клеточных ядер, напоминающее 'физиологическую дегенерацию', описанную *Глюксман* и *Спер*, 1938). В ядрах при этом более ярко выражена преципитация хроматина (хромопикноз). Часть гранул ДНК расположена у ядерной оболочки, очертания которой не всегда чётки. В некоторых участках ядерная оболочка не видна, а гранулы ДНК располагаются на фоне цитоплазмы или нейросинцития. Такого рода изменения чаще наблюдаются среди клеток 1-го и 3-го типа. Кроме того, довольно часто можно наблюдать слияние глыбок ДНК в 2—4 крупные (1—2 μ), тесно прилегающие друг к другу гранулы, которые заполняют почти всё ядро, сохраняя при этом его первоначальную форму. Контуры ядерной оболочки становятся менее чёткими, а затем исчезают. Гранулы оказываются расположенными на фоне цитоплазмы (рис. 1м, н, о, п). Затем они несколько отдаляются друг от друга. Дальнейшую судьбу гранул ДНК, вышедших из клеток, нам не удалось проследить с достаточной отчетливостью.

Необходимо подчеркнуть чёткую зависимость интенсивности процесса выхода ДНК от степени цитологической дифференцированности того или иного ядра n. V. Так, на 5-ой неделе эмбриогенеза в матриксе и плащевом слое тройничного сегмента нервной трубки на каждом поперечном срезе видно от 3 до 5 (4,21 $\pm$ 1,71) клеточных ядер, из которых выходят гранулы ДНК. В 6—7 недель количество таких ядер в этих участках уменьшается — на 4—5 поперечных срезах видно 1—2 (0,73 $\pm$ 0,08) ядра с выходящими глыбками ДНК. Однако в возникших зачатках ядер n. V процесс выхода гранул ДНК происходит довольно интенсивно: в зачатке мезенцефалического ядра на каждом поперечном срезе видно 2—3 (2,64 $\pm$ $\pm$ 0,26) таких ядра; в главном чувствительном и спинальном ядрах — 4—6 (5,38 $\pm$ 0,26); в зачатке двигательного ядра этот процесс менее интенсивен — на 4—5 поперечных срезах видно не более одного ядра с выходящими гранулами ДНК (0,81 $\pm$ 0,04). На 8—9 неделе эмбриогенеза интенсивность выхода гранул ДНК в зачатках мезенцефалического и двига-

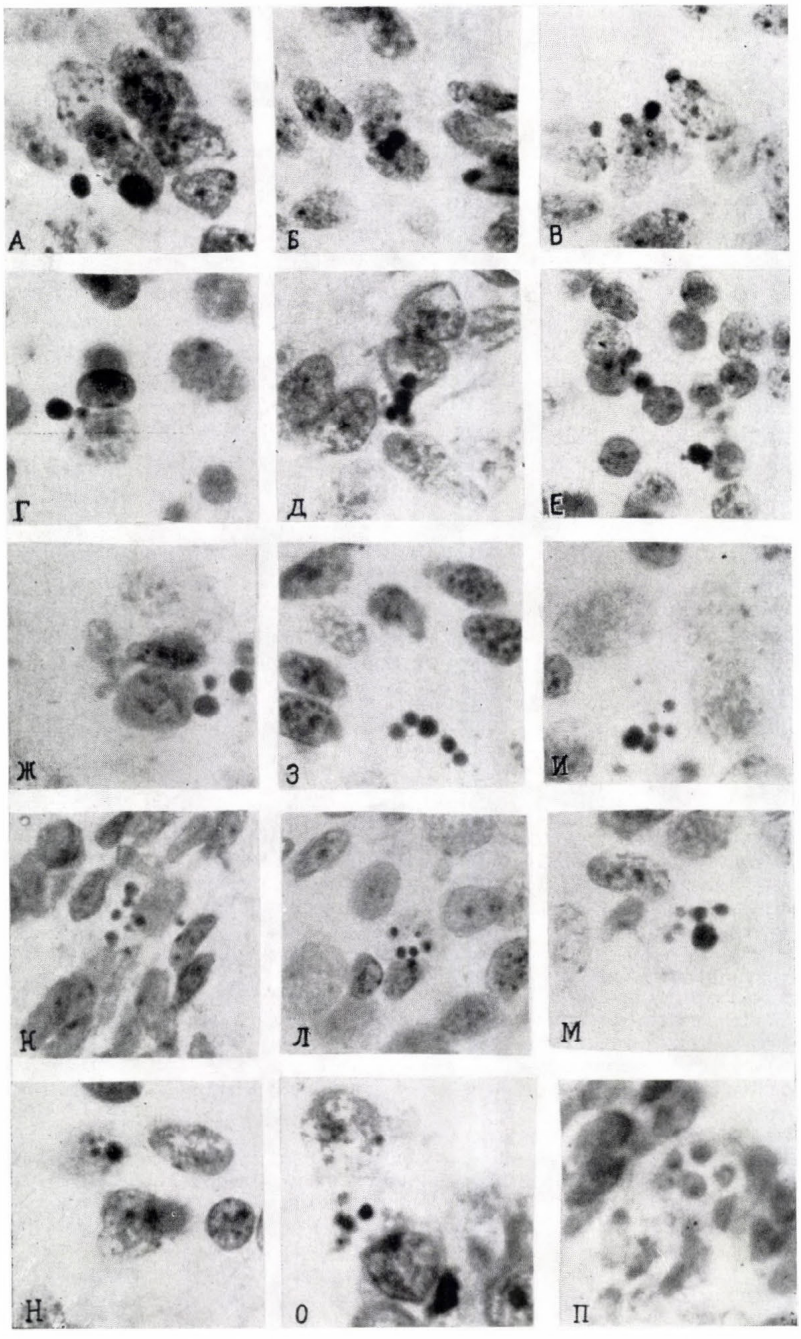

Рис. I, — Выход гранул ДНК из развивающихся клеточных элементов зачатков ядер n. V. человека. Реакция Фельгена. Ок. 10, об. 90

тельного ядра резко уменьшается, и в дальнейшем подобные процессы полностью исчезают. Но в главном чувствительном и спинальном ядрах выход гранул ДНК из дифференцирующихся клеток всё более активизируется, достигая максимальной интенсивности на 10—15 неделях; на каждом поперечном срезе зачатка видно от 6 до 12 (8,41 ± 0,63) клеточных ядер, из которых выходят глыбки ДНК. В течение 16—18-ой недели выход гранул ДНК уменьшается и к 20-ой неделе совсем прекращается.

Из приведенных данных следует, что активность выхода гранул ДНК снижается по мере цитологического 'созревания' зачатков ядер n. V, а с появлением нервных клеток исчезает совсем. В зачатках мезенцефалического и двигательного ядер нервные клетки появляются значительно раньше (8—9-ая неделя эмбриогенеза), чем в главном чувствительном и спинальном ядрах n. V (20—22 недели эмбриогенеза), в соответствии с этим находятся и сроки интенсивности описываемого процесса.

На данном этапе исследования мы считаем невозможным сделать конкретные выводы о значении наблюдаемых явлений в формировании зачатков ядер n. V. Постоянство процесса выхода гранул ДНК, чёткая приуроченность к определённому времени развития зачатка каждого ядра и явная зависимость от степени цитологической 'зрелости' их, — всё это позволяет предположить, что данный процесс является физиологической дегенерацией, связанной с гистогенетическими процессами в тригеминальном ядерном комплексе. Однако следует особо отметить количественное несоответствие между гибнущими клетками и клетками с выходящими гранулами ДНК. Последние явно преобладают. Поэтому не все случаи выхода гранул ДНК следует относить к явлению физиологической дегенерации.

РЕЗЮМЕ

Изучение процессов возникновения и развития зачатков ядер тройничного нерва человека показало, что митотическое деление клеток в эпендимном слое активно происходит в течение первых 6 недель эмбриогенеза, а затем фигуры митоза исчезают. В это время в плащевом слое появляется большое количество амитозов, наблюдаемое в чувствительных ядрах до 6—7 месяцев эмбриогенеза. В возникновении и развитии зачатков двигательного ядра тройничного нерва решающая роль принадлежит процессу миграции клеточных элементов из эпендимного слоя в зачатки. Деления клеток в зачатках почти не происходит. Формирование зачатка мезенцефалического ядра сопровождается миграцией клеточных элементов из эпендимного слоя, которая сменяется вспышкой амитотического деления в зачатках. В зачатках главных чувствительных и спинальных ядер тройничного нерва в течение 2—6 месяцев эмбриогенеза активно происходит амитотическое деление клеток. Миграции клеточных элементов не наблюдается.

Данные автора позволяют предположить, что процесс миграции имеет первостепенное значение в возникновении и дальнейшем развитии равно появляющихся и быстро развивающихся зачатков ядер (двигательное и мезенцефалическое ядро тригеминального комплекса). В ядрах со сравнительно замедленными темпами развития (главное чувствительное и спинальное ядра n. V) основное значение имеет процесс амитотического деления в зачатках.

Во время эмбриогенеза зачатков ядер тройничного нерва человека происходит выход гранул ДНК из развивающихся клеточных элементов (реакция Фельгена). Установлено, что интенсивность этого процесса связана со степенью цитологической дифференциации зачатков ядер n. V, с появлением в зачатках развивающихся нервных клеток выход гранул ДНК прекращается. Наиболее активен этот процесс в мезенцефалическом и двигательном ядрах n. V на 6—8-ой неделях эмбриогенеза, в главном чувствительном и спинальном ядре n. V — на 10—15-ой неделе.

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CONTRIBUTIONS TO THE DEVELOPMENT OF THE TRIGEMINAL NUCLEI OF MAN

Our studies on the development of the trigeminal nuclei in man have shown that a high mitotic activity in the ependymal layer can be observed during the first 6 months of embryogenesis. At this time a great number of amitotic figures appear in the pallium layer, which can especially be found in the sensory nuclei up to 6th or 7th month of embryonal development. The formation of the motor nucleus is connected with wandering of cellular elements from the ependymal layer. Cell cleavage in the anlage of this nucleus occurs very rarely. The formation of the mesencephalic nucleus is related with wandering of cells from the ependymal layer into the anlage. This process is followed by an explosion-like amitotic cleavage of the cells. In the primary sensory and the spinal nuclei of the trigeminal nerve a very strong amitotic activity can be observed during the period between the 2nd and 6th month of embryogenesis. No migration of cellular elements was observed during this time.

These data seem to indicate that the above-mentioned migration process has a primary importance in the development of the motor and mesencephalic nuclei of the trigeminal nerve, whereas in the primary sensory and spinal nuclei—which are developing relatively slowly—amitotic cleavages are found to have an important role in development.

During embryogenesis of trigeminal nuclei extrusion of DNA granules from cellular elements could be observed (Feulgen reaction). The intensity of this process seemed to parallel with the rate of cytological differentiation of the trigeminal nuclei. This process is most pronounced in the 6th to 8th weeks in the motor and mesencephalic nuclei and in the 10th to 15th week in the primary sensory and spinal nuclei.

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KRITISCHE BEMERKUNGEN ZUR ‚AUSSCHLEUSUNG' DES NUKLEOLUS IN GANGLIENZELLKERNEN

von

W. MÜLLER

MAX-PLANCK-INSTITUT FÜR HIRNFORSCHUNG (ABTEILUNG FÜR TUMORFORSCHUNG UND EXPERIMENTELLE PATHOLOGIE) UND NEUROCHIRURGISCHE UNIVERSITÄTSKLINIK
KÖLN, DEUTSCHE BUNDESREPUBLIK

1932 wurde von Berg über den Durchtritt von Kernstoffen in das Zytoplasma bei Lebern verschiedener Warm- und Kaltblütler berichtet. Er beobachtete, daß sich kompakte Nukleolen in Blasen umwandelten, die zur Kernmembran wandern, mit ihr verschmelzen und schließlich ihren Inhalt an das Zytoplasma abgeben. Diesem Vorgang wurde die Bezeichnung ‚Schleusenmechanismus' gegeben. Von zahlreichen anderen Autoren wurde über das gleiche Phänomen in anderen Organen berichtet (Lit. s. Altmann, 1949, 1955). Die bekannten Vorstellungen Casperssons (1941) und von Vogt und Vogt (1947) über die Rolle des Nukleolus im Eiweißstoffwechsel der Zelle ließen dieses Phänomen in neuem Licht erscheinen. 1949 diskutiert Altmann die Abgabe von Kernstoffen in das Protoplasma unter dem Gesichtspunkt eines Ereignisses von allgemeinbiologischer Wichtigkeit. In neuerer Zeit widmete Höpker (1953) dem Nukleolus der Nervenzelle eine morphologische Studie, in der er die Ausbildung von Vakuolen in diesem Zellorganell beschreibt. Neben dem Vorkommen und der Ausbildung derartiger blasiger Kerneinschlüsse, auf deren Genese hier nicht eingangen werden soll (Lit. s. Pecchiai (1957) und Pecchiai u. D'Ancona (1958)), finden sich in der Literatur Angaben über den Durchtritt ganzer Nukleoli aus dem Zellkern ins Zytoplasma (z. B. Dittus, 1941; Harms, 1921; Schiller, 1944; Altmann, 1952; Hild, 1951; Metuzals, 1951). Wir berichten im folgenden über entsprechende Befunde in der Infiltrationszone von Hirntumoren. Neben den vordringenden Tumorzellen finden sich noch häufig Ganglienzellen, die histologisch mehr oder weniger pathologisch verändert erscheinen. Ganglienzellveränderungen lassen sich in dem darüberliegenden Cortex ebenfalls noch beobachten (s. a. Stern und Odom, 1947). Häufig finden wir Ganglienzellen, deren Nissl-Substanz verschwunden ist (Tigrolyse). Parallel zu dem Schwund der Nissl-Schollen treffen wir meistens einen sehr stark vergrößerten Kern an (Abb. 1). Die Durchmusterung derartiger Areale zeigt nicht selten folgende Bilder: der Nukleolus ist ebenfalls vergrößert und liegt der Kernmembran dicht an; die Kernmembran erscheint nach außen vorgebuckelt, und in dieser Ausbuchtung findet sich der Nukleolus; der Nukleolus liegt zum Teil noch innerhalb des Kernraumes, zum Teil jenseits der Kernmembran; der Nukleolus liegt vollkommen außerhalb des Kernes; der Nukleolus liegt um das Vielfache seines Durchmessers von der Kernmembran entfernt im Zytoplasma der Ganglienzelle oder sogar außerhalb der Ganglienzelle (Abb. 2).

Überträgt man diese Befunde auf die Literaturangaben, so würde dies bedeuten, daß dieses Phänomen durch die Aktivierung des Zellstoffwechsels

Abb. 1.—Ganglienzelle mit Kernödem und Tigrolyse im Infiltrationsbereich eines Astrozytoms. Diese wie Abb. 2 und 3 Gallocyanin-Chromalaun-Erythrosin

zustande gekommen wäre. Gleichzeitig damit müßte man jedoch erwarten, daß die chemische Zusammensetzung des ‚ausgeschleusten' Nukleolus eine Änderung erfahren würde. Aus diesem Grunde versuchten wir mit der Gallocyanin-Färbung zur Darstellung von Ribonukleinsäure in Erfahrung zu bringen, ob sich bereits histochemisch irgendwelche Abweichungen an diesen Nukleolen finden. Die Färbung ergab keine Abweichung von den normalen Nucleolen in den Zellkernen. In gleicher Weise zeigte auch die Fluorochromierung mit Acridin-Orange gegenüber den normalen Nukleoli nichts Besonderes. Wir wählten die Acridin-Orange-Fluorochromierung, da sie nach den Untersuchungen von Zeiger und Harders (1951), Zeiger, Harders und Müller (1951) sowie von Schümmelfeder, Krogh und Ebschner (1958) als ein ausgezeichnetes Testverfahren für die Darstellung von Ribonukleinsäure zu gelten hat.

Bei der intensiven Suche nach Ganglienzellen mit ‚ausgeschleustem Nucleolus' fiel jedoch auf, daß innerhalb des Schnittes der Austritt der Nucleolen stets auf derselben Seite des Kernes zu finden war. Dieser Befund spricht dafür, daß es sich eher um ein Artefakt handelt, das bei der technischen

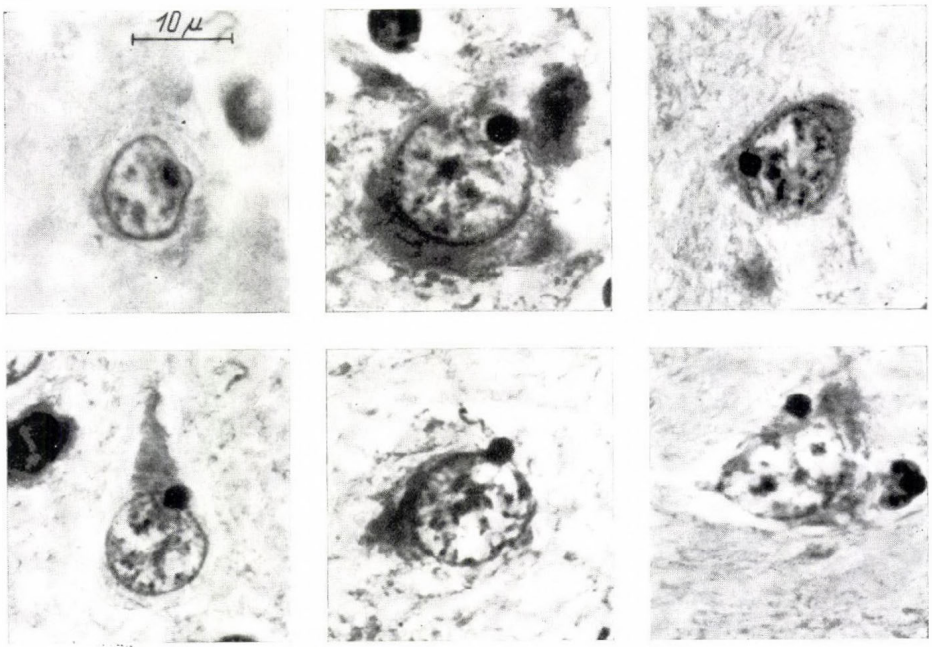

Abb. 2.—Verschiedene Stadien der ‚Nukleolusausschleusung'

Bearbeitung des Materials, sehr wahrscheinlich beim Schneiden des Paraffinblockes, entstanden ist. In dieser Vermutung wurden wir durch vereinzelte Befunde bestärkt, wie in Abbildung 3 dargestellt ist. Wir sehen sehr deutlich, daß sich hinter dem Nukleolus ein Riß befindet, der offensichtlich durch das Herausschieben des Kernkörperchens während des Schneidevorganges zustande gekommen ist. Die Untersuchung normaler Rindengebiete ließ so gut wie niemals das Phänomen eines außerhalb des Kernes liegenden Nukleolus erkennen.

Fassen wir die Befunde zusammen, so ergibt sich folgendes: 1. Die ‚Ausschleusung' eines Nucleolus im Randgebiet eines Hirntumors scheint sehr wahrscheinlich ein Artefakt zu sein, das auf die mikrotechnische Verarbeitung des Materials zurückzuführen ist. Diese Nukleoli weichen, was die histochemische Darstellbarkeit von Ribonukleinsäure anbetrifft, nicht von normalen Kernkörperchen ab. 2. In normalen Ganglienzellen ist dieses Phänomen so gut wie nie zu beobachten. Wir glauben, nicht ausschließen zu dürfen, daß die Kernvergrößerung eine Rolle für das Zustandekommen des Artefaktes spielen muß. Das Kernödem ist auf die besondere Stoffwechsellage der Ganglienzellen zurückzuführen. Durch die Einlagerung von Flüssigkeit in das Karyoplasma entspricht dessen Konsistenz nicht mehr der eines Normalkernes. Dieser Konsistenzunterschied wird, was zu vermuten ist, auch noch am fixierten und Paraffin-eingebetteten Präparat vorhanden sein. Es scheint uns aus diesem Grunde möglich zu sein, daß die vergrößerten Kerne viel leichter für eine Artefaktentstehung zugänglich sind. Liest man in den Arbeiten von Vogt und Vogt (1947), von La Velle (1956) und Beheim-Schwarzbach (1957) nach, die sich sehr eingehend mit der Struktur der Ganglienzellen beschäftigen, so findet man keinerlei Angaben über den Austritt von Nukleolen. Der Durchtritt von Kernsubstanzen in das Zytoplasma ist eine gesicherte Tatsache. Zahlreiche elektronenmikroskopische Untersuchungen an den verschiedensten biologischen Objekten förderten derartige Ergebnisse zutage. Bei der Plastizität biologischer Membranen ist es sicherlich denkbar, daß auch die Porenweite der Kernmembran variabel ist. Inwieweit jedoch eine Erweiterung der Kernmembran bis zur Möglichkeit des Durchtritts eines unversehrten Nukleolus vorkommen kann, muß vorerst dahingestellt blei. ben, obwohl bei verschiedenen Objekten dieses Phänomen beobachtet wurde. Die hier vorgetragenen Befunde sprechen jedoch mit größerer Wahrscheinlichkeit dafür, daß es sich um eine Artefaktbildung handelt.

Abb. 3.—Die artifizielle Entstehung des Nukleolenaustritts wird an dem Einriß in Kern und Zytoplasma sehr deutlich. Man erkennt noch die ehemalige Lage des Nukleolus

DISKUSSION

Röhlich Es war recht interessant, zu hören, daß der Austritt des Kernkörperchens als Kunstprodukt nicht nur eine Folge der Mikrotechnik ist, sondern auch mit dem vorherigen pathologischen Zustand der Zelle zusammenhängt. Ebenso greift auch in der Ultramikrotomie die Metakrilat-Explosion in erster Reihe jene Zellen an, die sich bereits vorher in einem pathologischen Zustand befanden (z. B. Degeneration).

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UTILITÉ ET INCONVENIENTS DE LA FIXATION PAR PERFUSION EN CYTOCHIMIE NERVEUSE

par

D. PICARD

LABORATOIRE D'HISTOLOGIE I, FACULTÉ DE MÉDECINE
MARSEILLE, FRANCE

Un des objectifs de toute investigation histochimique est évidemment de contrôler tous les événements qui se produisent dans l'objet étudié depuis l'instant de la mort (ou même avant, si la mort est précédée par des manipulations préparatoires) jusqu'à celui où la préparation est prête à être examinée; cet objectif n'est pratiquement jamais atteint, et c'est pour tenter de s'en approcher, qu'il convient de réfléchir à ces événements dont l'ensemble constitue le pré-traitement, si l'on entend ce terme dans son sens le plus large. Dans le cas où l'investigation porte sur du matériel fixé, ce qui est le cas le plus fréquent en ce qui concerne le tissu nerveux des centres, la nature chimique du liquide fixateur est évidemment un des facteurs les plus importants de ce pré-traitement; mais indépendamment de sa nature, le procédé d'utilisation de ce fixateur joue en matière de neurohistochimie un rôle certainement important et qui est loin d'être élucidé. On doit à cet égard considérer principalement les trois points suivant: (1) comment la fixation conserve-t-elle les structures, donc la localisation exacte des constituants mis en évidence? (2) la fixation évite-t-elle l'apparition de propriétés histochi miquesnon vitales (artefacts)? (3) la fixation entraîne-t-elle la disparition de certains constituants? Sous ces trois rapports: on peut se poser la question de savoir si la fixation par perfusion est meilleure ou moins bonne que la fixation par immersion. Il n'est pas question de faire ici une discussion exhaustive de ce problème; mais nous prendrons seulement quelques exemples, dont certains ont fait l'objet de travaux dans notre laboratoire, pour montrer plutôt la difficulté du problème et la nécessité de ne pas le sous-estimer.

I

Le principe de la fixation par perfusion vasculaire est de porter le fixateur rapidement et de façon homogène au contact de tous les points du tissu étudié, à la fois dans l'intention d'éviter toute modification autolytique de celui-ci, et pour éviter des inégalités de conservation ou de comportement des constituants, se traduisant par des gradients qui répondent à la diffusion plus ou moins rapide du fixateur. Le procédé comporte toujours une perfusion préalable par une solution physiologique ou autre, destinée à chasser le sang pour assurer la perméabilité des plus petits vaisseaux en évitant la coagulation du plasma par le fixateur. Ce procédé a été très en vogue, en ce qui concerne le système nerveux, depuis l'apparition de la méthode de

Bodian (1935, 1936); non seulement avec l'alcool à 80° de cette technique, mais avec tous les mélanges fixateurs employés par de nombreux auteurs depuis Scharrer (1938), on obtient ainsi avec une grande régularité une conservation qui paraît excellente des caractères morphologiques des neurones, et de leur environnement; les résultats sont particulièrement bons avec les fixateurs mercuriels ou à base d'autres sels de métaux lourds, par exemple le mélange formol-chlorure de cadmium-urée préconisé dans notre laboratoire par Seite et coll.; on évite les dilacérations dues à des rétractions inégales et on voit disparaître les artefacts comme les espaces péri-vasculaires et péri-neuronaux; les structures cellulaires (mitochondries, appareil de Golgi, corps de Nissl, neurofibrilles, nucléole) sont partout d'une grande finesse. L'impression de 'sécurité' donnée par de telles préparations est accrue lorsque des réactions histochimiques localisent de façon précise sur ces structures nettement préservées des constituants à réactions caractéristiques, et il semble que dans ces conditions on puisse à juste titre faire confiance aux localisations histochimiques du fait même de l'intégrité des structures.

Cette opinion est considérablement renforcée depuis les progrès de l'étude ultrastructurale; en effet des progrès vraiment décisifs ont été faits depuis que Palay (1960, 1962) a montré la nécessité de la perfusion vasculaire des solutions osmiées pour obtenir la fixation rigoureuse des centres nerveux; c'est elle qui a permis d'aborder et déjà de mener à bien l'étude ultrastructurale de régions complexes, comme le cortex cérébelleux par exemple. Nous voyons ainsi maintenant tous les éléments nerveux (neurones et leur prolongements, névroglie) étroitement adjacents; selon l'expression de Palay cela 'oblige l'observateur à se souvenir qu'il s'agit d'un neuroépithélium'. En effet, on acquiert ainsi la conviction qu'il ne peut plus être question d'une hypothétique 'substance fondamentale' du tissu nerveux; l'existence de celle-ci a été très débattue, et soutenue par certains, précisément, sur la base de réactions histochimiques dont la localisation intercellulaire n'est plus soutenable, maintenant qu'on connaît la structure fine du tissu nerveux; les réactions en question, si elles ne sont pas intra-neuronales, ne peuvent se situer que dans les éléments névrogliques. Les investigations histochimiques sur le tissu nerveux, si elles utilisent des méthodes impropres à conserver idéalement les structures, doivent donc en tous cas intégrer leurs résultats dans la connaissance précise des structures que nous a donnée la fixation par perfusion.

Du reste, l'histochimie ultrastructurale elle-même n'en est qu'à ses débuts, et déjà elle a abordé le tissu nerveux; c'est ainsi que l'association de l'autoradiographie et de la fixation par perfusion a fourni récemment ce très brillant résultat qu'est la localisation de la noradrénaline dans les microvésicules à granules denses de fibres adrénergiques (Wolfe, Axelrod, Potter et Richardson, 1962).

Il semble donc que la perfusion, en permettant l'accès rapide du fixateur en tous les points du tissu nerveux, assure au mieux le maintien en place des constituants recherchés par les méthodes histochimiques.

Toutefois, il faut mentionner ici deux objections; la première est hypothétique et consiste à supposer que peut-être les éléments nerveux et névrologiques ne sont rigoureusement adjacents, que parce que la perfusion préalable de solution saline a déterminé leur gonflement et effacé des espaces

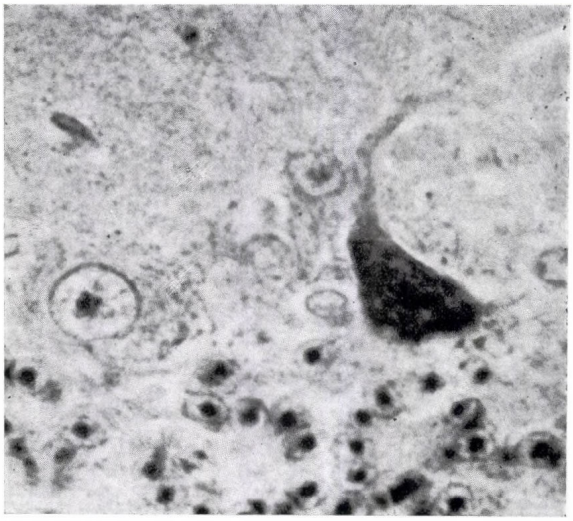

Fig. 1.—Cobaye; fixat. Carnoy (immers). Vert de méthyle-pyronine (Unna). Cellule de Purkinje hyperchromophile, pyroninophile (Cotte, 1957, *Arch. Biol.*)

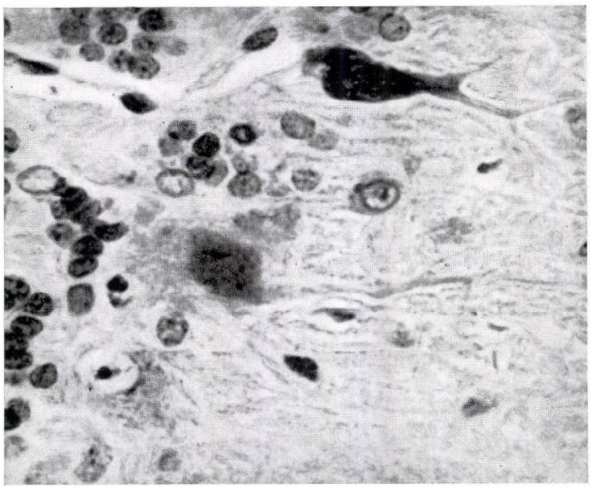

Fig. 2.—Cobaye; fixat. Helly (immers.). Crésyl-violet prolongé. Cellules de Purkinje normale, intermédiaire et hyperchromophile (Cotte, 1957, *Arch. Biol.*)

intercellulaires existants à l'état vital; ce gonflement est en effet possible; mais l'inexistance d'une substance fondamentale interstitielle n'en est pas moins établie, car on sait aujourd'hui que l'oedème cérébral pathologique ne consiste pas en une infiltration d'espaces interstitiels, mais bien en un gonflement de la glie (Gruner, 1962). L'autre objection est plus sérieuse:

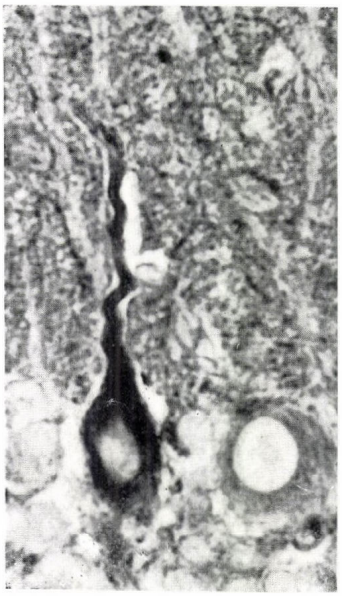

Fig. 3.— Cobaye; fixat. Helly (immers.). Cellule de Purkinje rétractée hyperchromophile soudanophile (Cotte, 1957, *Arch. Biol.*)

on doit présumer que les constituants recherchés par l'histochimie ne seront effectivement maintenus en place, dans des structures morphologiquement intactes, que s'ils sont fortement liés aux éléments même de ces structures et notamment aux protéines. Nous verrons plus loin que cette 'erreur par défaut' est loin d'être négligeable.

II

La fixation peut-elle éviter, ou au contraire laisser apparaître des caractères non vitaux, comme des réactions histochimiques à considérer comme des artefacts? Question à laquelle il est difficile de répondre; pour évaluer les avantages possibles de la perfusion, nous prendrons comme exemple la question des cellules 'hyperchromatiques' (Nissl) ou 'hyperchromophiles', que nous avons étudiées il y a quelques années avec Cotte (1957). Ces cellules, à la fois hyperbasophiles et hypercolorables par divers colorants non spécifiques, sont en même temps rétractées et leurs prolongements sinueux sont eux-mêmes devenus colorables sur une partie de leur trajet; elles n'ont cessé d'intriguer les neurohistologistes et surtout les neuropathologistes; Cotte a tenté de les caractériser par des réactions histochimiques, et a montré notamment que leur hyperbasophilie était essentiellement due à des lipides insaturés et peut-être à des glucides, ce qui a été confirmé par Diculescu et coll. (1957; galactolipides) et par Scharf et coll. (1957, 1958; lipides, glycogène et polysaccharides) pour les cellules polygonales sombres des ganglions cérébro-spinaux et végétatifs, par Preto—Parvis et Bosisio (1960) et par Loebel et Dresel (1961) pour les cellules de Purkinje. C'est par contre la signification de ces aspects cellulaires qui est actuellement encore controversée: de rares auteurs les ont considérés comme de nature dégénérative même lorsqu'ils se voient chez l'animal normal, ce qui impliquerait un renouvellement de cellules ainsi hautement différenciées que les cellules de Purkinje (Baffoni, 1956); cette interprétation a été rejetée par Cotte, puis par Preto—Parvis, par Loebel. D'autres pensent que ces aspects caractérisent des catégories cellulaires chimiquement et fonctionnellement distinctes dans un même territoire (Scharf). La plupart des auteurs les considèrent depuis Kölliker (1896) et Nissl (1899) comme des artefacts dûs à l'autolyse (Fisher et Ranson, 1933; Bywater, Glees et Hauffe, 1962) ou à diverses conditions défectueuses liées à la technique (Spielmeyer, 1922; Drooglever—Fortuyn, 1927; Fisher et Ranson, 1933; Samuel, 1958); parmi celles-ci le rôle des microtraumatismes avant la fixation a été particulièrement incriminé par Cowdry (1916), puis surtout par Scharrer (1938), par Cotte (1957), par Cammermeyer (1961); on trouvera dans ce dernier

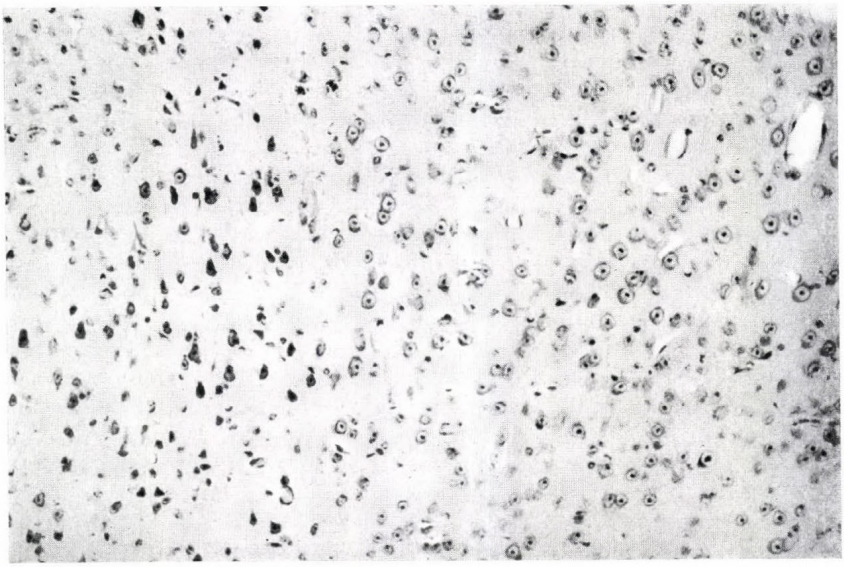

Fig. 4.—Cobaye; fixat. Helly (perfus.). Crésyl-violet. Cortex cérébral incomplètement perfusé; les cellules hyperchromophiles se trouvent uniquement dans un territoire où la perfusion n'a pas atteint les vaisseaux, qui sont collabés (Cotte, 1957, *Arch. Biol.)*

travail la revue la plus complète de cette question; il a été montré que la fixation par perfusion avec autopsie différée permettait d'éviter l'apparition de cellules hyperchromatiques chez l'animal normal (Cox, 1937; Scharrer, 1938; Picard et Cotte, 1957; Cammermayer, 1960, 1961), et dans des conditions expérimentales où leur présence avait été considérée comme significative (électro-choc: Alpers et Hugues, 1942; Siekert et coll., 1950 — Asphyxie et choc anaphylactique: Cotte, 1957; Poursines, Picard, Grebus, Cotte et Liandra, 1957). D'autres auteurs n'admettent pas ces résultats ou en critiquent l'interprétation, en général du reste sans avoir eux-mêmes pratiqué la perfusion; aussi l'opinion est-elle encore soutenue par certains que les aspects hyperchromatiques rétractés avec soudanophilie et réaction PAS + correspondent à des états fonctionnels particuliers, en général à un état actif ou hyperactif (Preto—Parvis; Loebel) pouvant être le résultat d'une stimulation. Si cette interprétation est valable pour les variations de la basophilie dûes à des variations de l'ARN, comme cela a été amplement démontré (en particulier depuis Hyden, 1943; Hyden et Hartelius, 1948), nous ne sommes pas convaincus qu'elle soit aussi valable pour les cellules hyperchromatiques. Or la question est d'importance, puisque des modifications morphologiques et surtout histochimiques peuvent être considérées par les uns comme significatives en neuropathologie, alors que d'autres leur dénient toute signification si elles n'apparaissent pas après une fixation par perfusion; nous avons attiré l'attention avec Poursines, Grebus et Cotte (1957) sur cet aspect de la question; Cammermeyer (1961) y a très fortement insisté, Bywater, Glees et Hauffe (1962) également, et plusieurs neuropathologistes ont adopté cette façon de voir qui oblige à réviser bien

des données pathologiques classiques, et à modifier les conditions techniques du prélèvement ou de l'autopsie à visée neuropathologique.

A titre d'hypothèse, on peut envisager que l'aspect hyperchromatique résulte d'une dénaturation partielle des protéines protoplasmiques (Cotte), suivie de perte d'eau (d'où la rétraction) et de libération de groupes réactifs par rupture de cénapses; c'est ainsi que l'apparition des réactions des lipides serait une sorte de lipophanérose post-mortem; ce phénomène pourrait être consécutif à des microtraumatismes avant fixation, ce qui est indubitable; mais il pourrait aussi se produire in vivo comme altération pathologique authentique, et dans ce cas il pourrait être réversible (observations de Höpker, 1957, sur la substance réticulée bulbaire après chocs hypoglycémiques chez le Rat); le caractère réversible correspondrait bien au fait que la modification hyperchromatique n'est pas en soi dégénérative. On ne doit pas s'étonner de voir survenir des phénomènes de dénaturation protéique réversibles dans l'élément nerveux, puisque l'on sait de tels phénomènes sont démontrés dans la fibre nerveuse en relation avec le processus fonctionnel de la conduction de l'influx (cf. Ungar, 1957); le protoplasme nerveux est particulièrement instable dans certaines de ses structures physico-chimiques, et c'est pourquoi tant de questions techniques se posent pour son étude, qui ne se posent pas pour d'autres tissus.

Il est du reste fort possible qu'une même perturbation n'ait pas sur toutes les cellules d'un territoire donné le même effet, non seulement parce qu'elles sont placées dans des conditions mécaniques différentes (position dans la lamelle cérébelleuse par exemple), mais aussi parce qu'elles sont dans un état fonctionnel différent; ainsi les cellules hyperchromatiques apparues après fixation par immersion pourraient-elles éventuellement désigner des cellules dont l'état à ce moment les disposait à réagir de cette manière (Cotte, 1957). C'est à une façon de voir assez semblable que conduisent les premières investigations ultrastructurales sur ces cellules (Gansler, 1962, 1963): apparues seulement après fixation sans perfusion et absentes après perfusion, les cellules hyperchromatiques apparaissent surtout avec une matrice cytoplasmique très dense et osmiophile entre les formations ergastoplasmiques, mais sans altération importante des structures elles-mêmes et (fait important) avec tous les termes de passage entre cet état extrême et l'état sombre dont nul ne conteste qu'il soit lié à une augmentation de l'ARN cytoplasmique, par exemple après stimulation modérée; les cellules hyperchromatiques seraient donc des 'semi-artefacts', dûs à ce que des cellules dans un état fonctionnel déterminé seraient plus aptes que les autres à réagir par rétraction à diverses perturbations, p. ex. les manipulations qui précèdent la fixation.

Il n'en reste pas moins vrai que pour des raisons purement mécaniques la répartition de ces aspects dans le tissu nerveux non perfusé n'est pas rigoureusement significative, ni du point de vue fonctionnel, ni du point de vue pathologique, et peut donc conduire à des interprétations erronées basées sur des constatations histochimiques étroitement dépendantes du procédé de fixation. Mentionnons que Friede (1959) pense aussi devoir attribuer à des changements post-mortem les variations capricieuses et non significatives de l'activité phosphorylasique dans la substance grise, liées à l'apparition de cellules hyperchromatiques.

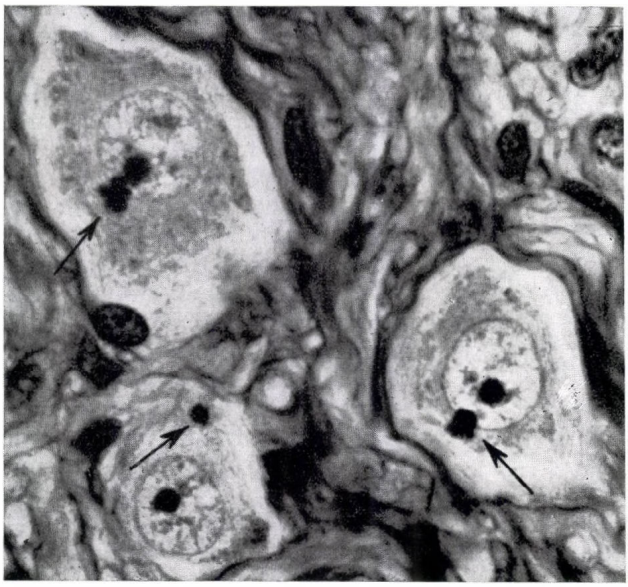

Fig. 5.—Chat; fixat. Helly (immers.). Azan-Ganglion cervical supérieur: inclusions lipoglycoprotéiques (↗) colorées ici par l'azocarmin, dans le cytoplasme et à-cheval sur la membrane du noyau où elles ont pris naissance au voisinage du nucléole (par M. Seite)

III

Si la fixation vise à maintenir en place et aussi peu altérés que possible les constituants cellulaires, il est bien certain que ce résultat n'est jamais obtenu que très partiellement. En ce qui concerne plus particulièrement les éléments nerveux, Hyden (1960) rappelle que pendant les manipulations préparatives 50 à 80% de la substance de la cellule nerveuse peut être dissoute et perdue, la déperdition étant plus importante pour le noyau que pour le cytoplasme; cette déperdition est incontrôlable, et Hyden n'exagère pas lorsqu'il qualifie cette situation de 'calamité'. Il est bien certain qu'une proportion notable des substances protoplasmiques perdues le sont pendant le temps de fixation, et c'est en partie à limiter cette cause d'erreur que vise le procédé de *freeze-drying*; toutefois celui-ci n'est pas sans inconvénient, et s'il maintient peut-être mieux que d'autres les propriétés chimiques intactes, il bouleverse les structures fines plus qu'on ne l'a cru; mais nous ne discutons pas ici les méthodes histochimiques sur tissus non fixés. En tous cas, il ne semble pas qu'on ait fait une évaluation quantitative de ces déperditions en comparant la fixation par immersion et par perfusion; mais beaucoup d'auteurs redoutent explicitement qu'à cet égard la perfusion n'aggrave encore les choses; en effet, on est en droit de penser que faire passer dans les vaisseaux une solution saline revient à pratiquer une véritable 'élution' du tissu frais avant même le contact avec le fixateur. Certains auteurs ont pensé ainsi que s'il n'y a pas de cellules hyperchroma-

Fig. 6.—Comme Fig. 5. — Inclusions lipoglycoprotéiques dans un axone amyélinique (par M. Seite)

tiques dans le tissu nerveux perfusé, c'est parce que la perfusion a soustrait à ces cellules leurs constituants les plus caractéristiques. Il y aurait probablement beaucoup à apprendre d'une étude chimique des perfusats et des liquides fixateurs où les tissus sont immergés.

La pratique de la perfusion a appris à ceux qui l'utilisent qu'elle risque de provoquer un oedème, surtout si elle est trop prolongée ou effectuée avec une trop forte pression; les conditions exigées par chaque matériel expérimental doivent être déterminées, et on peut prendre comme ordre de grandeur moyen les chiffres suivants proposés par Cammermeyer: perfusion in vivo égale à 14% du poids corporel (tant pour la solution physiologique que pour le fixateur), effectuée en 2 à 4 minutes, c'est-à-dire terminée 6 à 8 minutes après injection intra-veineuse de pentobarbital + héparine. Cette nécessité de standardisation montre bien que la perfusion n'est pas une méthode parfaite, mais relative; de sorte que si elle minimise les artefacts, c'est de manière statistique plutôt qu'absolue.

Nous prendrons encore un exemple pour illustrer la réalité de cette cause d'erreur et en faire comprendre l'incidence sur une recherche histochimique. Depuis plusieurs années, sont étudiés dans notre laboratoire des phénomènes d'élaboration, à partir du noyau des neurones, de complexes chimiques lipoprotéiques généralement riches aussi en glucides et comportant des ribonucléoprotéines et des protéines sulfhydrilées; on a de bonnes raisons de penser que ces substances sont transportées le long des prolongements des neurones et que leur signification fonctionnelle, non encore élucidée, est sans doute importante. Seite, qui a plus particulièrement étudié ces phénomènes depuis 1955, a montré que les substances en ques-

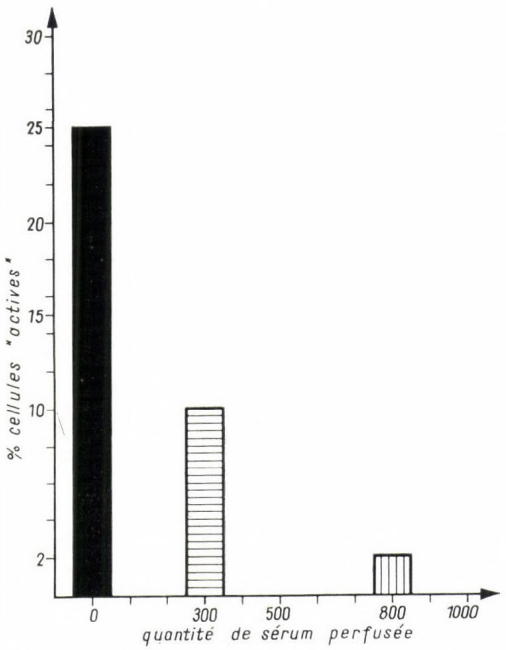

Fig. 7.—Dans le ganglion cervical supérieur du chat, la fixation par perfusion diminue le nombre de cellules possédant des inclusions lipoglycoprotéiques; cette diminution dépend de la quantité de sérum physiologique perfusé avant le fixateur (par M. Seite)

tion, qui ont après fixation un aspect figuré et qu'on a du reste pu retrouver comme des masses osmiophiles dans des fibres nerveuses examinées au microscope électronique, sont électivement conservées par les mélanges fixateurs contenant du mercure. La comparaison de divers territoires nerveux a fait sélectionner le ganglion cervical supérieur du chat adulte comme un territoire à répartition homogène se prêtant à l'étude statistique de ces phénomènes; dans ce territoire, les complexes étudiés se trouvent sous forme d'inclusions nucléaires ou cytoplasmiques dans 25% des neurones, après fixation par immersion. Si par contre on pratique une fixation par perfusion, le fixateur étant précédé par une solution physiologique en quantité équivalente à 15% du poids corporel, le nombre des neurones renfermant ces inclusions tombe à 10%; si le sérum perfusé avant le fixateur est 40% du poids corporel, il n'y en a plus que 1–2% (Seite, 1963). Il y a donc là un constituant chimique, pourtant complexe et peu diffusible, dont la conservation par la perfusion est aléatoire; dans ce cas particulier, à une fixation morphologique structurale excellente, correspond une imprécision histochimique rendant alors le matériel obtenu inutilisable pour l'étude de variations expérimentales.

CONCLUSIONS

Des diverses considérations qui précèdent, nous ne pensons pas qu'il faille garder une impression décevante. Malgré toutes les techniques possibles sur tissu nerveux frais, beaucoup d'investigations exigeront toujours une fixation; le procédé de la perfusion a incontestablement une grande supériorité pour la conservation des structures, de la morphologie, des rapports respectifs entre les constituants cellulaires, entre les neurones et la glie; il soustrait le tissu nerveux éminemment fragile à des causes de perturbations ou d'altérations incontrôlables si la moindre manipulation directe est faite avant la fixation; il évite aussi l'autolyse des zones profondes des centres nerveux et protège contre une dispersion au hasard des images normales et anormales. Le procédé de la perfusion comporte par contre des risques certains soit de gonflement tissulaire, soit surtout de déperdition de substances qui dès lors échapperont à la détection histochimique, peut-être plus encore qu'après fixation par immersion. Compte tenu de cet inconvénient, qu'il faut évaluer et tester dans chaque cas particulier, le procédé de la perfusion a le grand avantage de se prêter à une standardisation très précise, et cet avantage est majeur en neuropathologie expérimentale.

Ce qu'il faut surtout retenir, c'est qu'à l'égard de ce problème, comme pour tout autre problème biologique, il faut avant tout se garder de l'esprit dogmatique et de l'esprit de système, appliquer la vieille et solide discipline des méthodes convergentes, et utiliser les deux procédés de fixation conjointement et non pas exclusivement. Nous n'en sommes pas encore, en cette matière, à des conditions d'observation d'une rigueur absolue; nous en sommes encore à comparer ce que Nissl appelait des images équivalentes *(Aequivalentbilder)*, définissant ainsi déjà ce qu'aujourd'hui nous appelons la standardisation.

Nous pouvons donc rappeler pour finir cette phrase pleine de sagesse et d'humour de Wolman (1955) qui s'applique de façon particulièrement

pertinente au tissu nerveux: it may be safely concluded that fixation always creates structural artifacts, that a fixation is good when these artifacts occur mainly beyond the resolving power of the optical system, and that the scientist is good if he realizes that he works with *Aequivalentbilder*.

DISCUSSION

Vigh : La réaction du matériel neuro-sécrétoire a-t-elle été examinée par différentes méthodes de fixation?

Müller : Die Fixierung durch Perfusion ist in der Neuropathologie in der Regel nicht möglich. Es wird aufmerksam gemacht auf die Temperatur der Fixierungsflüssigkeit. Auf die Vorteile der Fixierung bei möglichst tiefer Temperatur des Fixierungsmittels wird hingewiesen.

Picard : La fixation par perfusion, chez les mammifères étudiés dans notre laboratoire, ne paraît pas entrainer la disparition du neuro-sécrétat dans le système hypothalamo-hypophysaire.

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ELEKTRONENMIKROSKOPISCHE UNTERSUCHUNGEN ÜBER DAS ZEREBROVISZERALE KONNEKTIVUM DER MUSCHEL ANODONTA CYGNEA, UNTER BERÜCKSICHTIGUNG DER NEUROSEKRETENTLEERUNG

von

I. Zs-Nagy

INSTITUT FÜR ANATOMIE, HISTOLOGIE UND EMBRYOLOGIE
DEBRECEN, UNGARN

K. Benkő

ZENTRALES FORSCHUNGSLABORATORIUM DER MEDIZINISCHEN UNIVERSITÄT
DEBRECEN, UNGARN

Die Daten über das Nervensystem der Lamellibranchiaten sind ziemlich spärlich. Über die neurosekretorische Tätigkeit der Nervenzellen der Muschel Unio tumidus teilte Fährmann im Jahre 1961 elektronenmikroskopische Befunde mit, und jüngst wurde von Baranyi und Salánki (1962) die Neurosekretion bei Anodonten nachgewiesen. Eine Arbeit unserer Forschungsgruppe über das elektrophysiologische Verhalten und über die Faserzusammensetzung des zerebroviszeralen Konnektivums der Muschel Anodonta cygnea ist gegenwärtig im Druck.

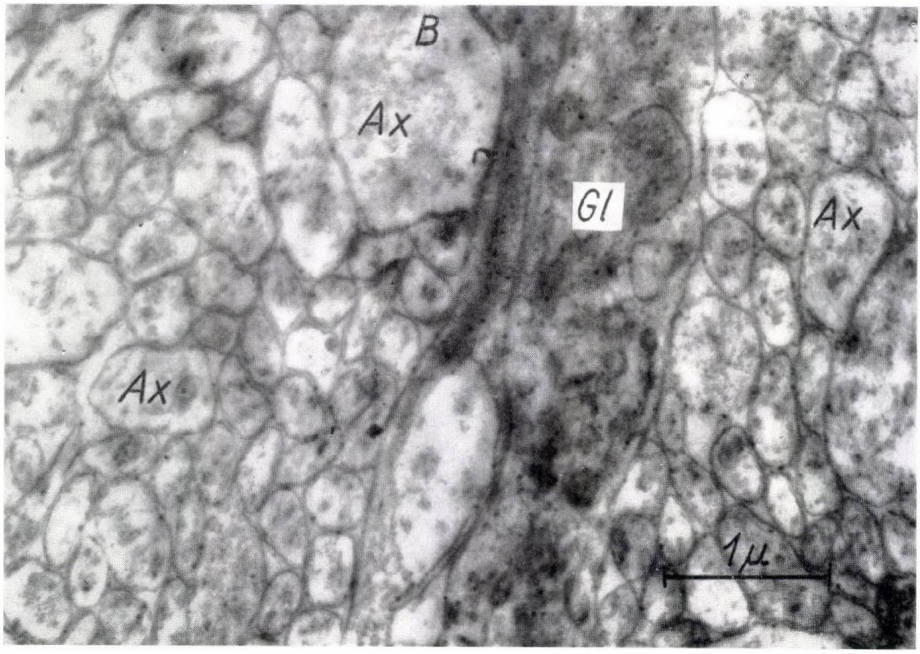

Abb. 1.—Querschnitt des zerebrovizeralen Konnektivums. *Ax* = Axone, *Gl* = Gliafortsatz, *B* = Bläschen im Axon. Araldit-Präperat

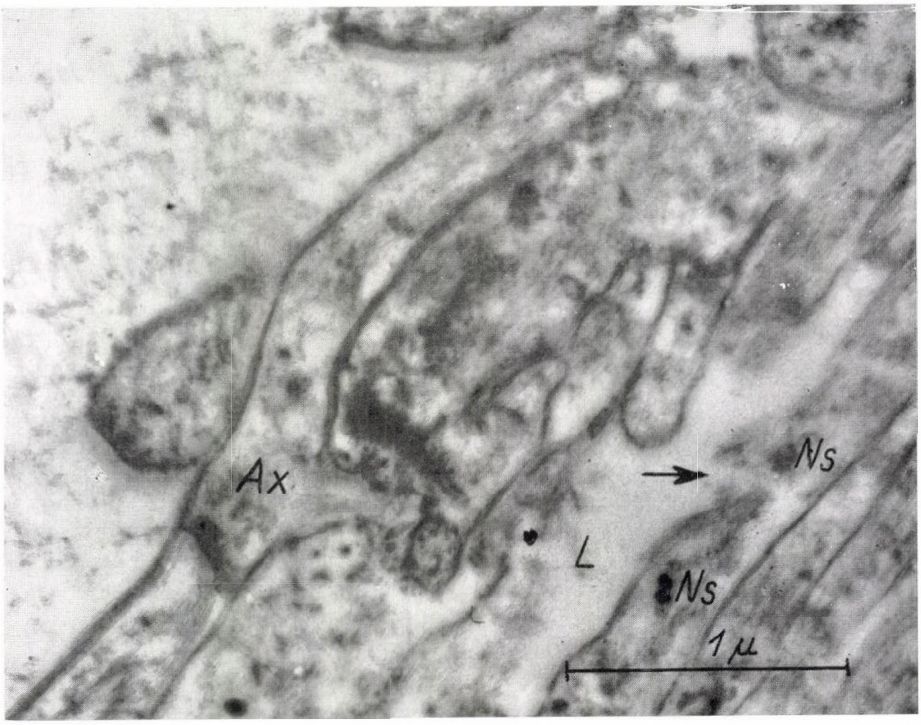

Abb. 2.—Längsschnittbild. Axonverzweigung *(Ax)* und Axolemmöffnung (Pfeil) zum Gewebsspals *(L)*. *Ns* = Neurosekret. Metakrylat-Präparat

In diesem Vortrag wollen wir über die Ultrastruktur des zerebroviszeralen Konnektivums der Anodonta cygnea berichten.

Unser Material wurde, wie üblich, in gepufferter Osmiumsäure fixiert und in Metakrylat bzw. in Araldit eingebettet. Zum Schneiden verwendeten wir ein LKB-Ultrotom, zur Herstellung der Aufnahmen ein Zeiss-D-2-Elektronenmikroskop.

Ich möchte unsere Befunde in einigen Bildern vorlegen.

Auf dem ersten Bild sieht man, daß die Axone weder mit Markscheide noch mit Schwannscher Scheide versehen sind. Die einzelnen Axone sind voneinander durch etwa 200—500 Å breites interaxonales Spatium getrennt. Größere Axongruppen sind von Gliazellfortsätzen umgeben. Das Axoplasma erscheint als eine zerstreute, feinkörnige Substanz, die von einfachen Membranen begrenzte Bläschen von einigen hundert Å Durchmesser und gebogene Röhrchen von der gleichen Wandung und vom gleichen Durchmesser enthält.

Die Axone sind von verschiedener Dicke. Im Durchmesser eines Kreises von identischem Flächeninhalt ausgedrückt schwankt die Axondicke zwischen 0,15 und 1,35 Mikron.

Zwischen den Axonen sind stellenweise Gewebsspalten von verschiedenem Kaliber ohne eigene Wandung zu sehen.

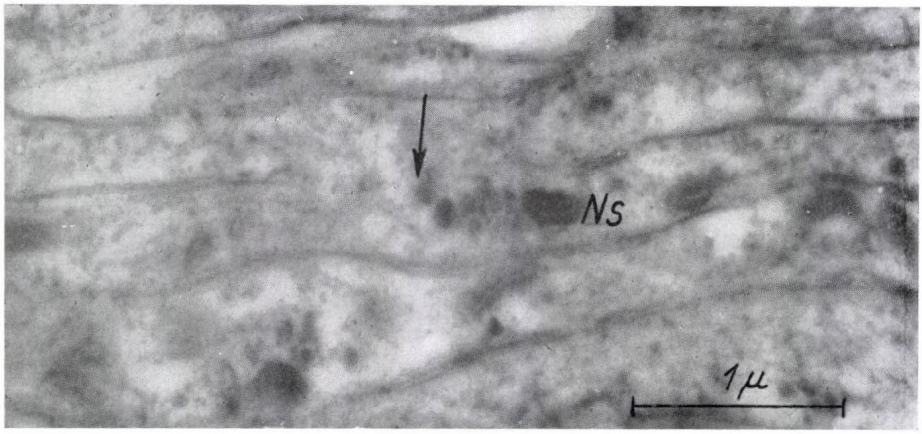

Abb. 3.—Der Pfeil weist auf die Auflockerung des Axolemms zwischen zwei parallelen Axonen. *Ns* = Neurosekret. Metakrylat = Präparat.

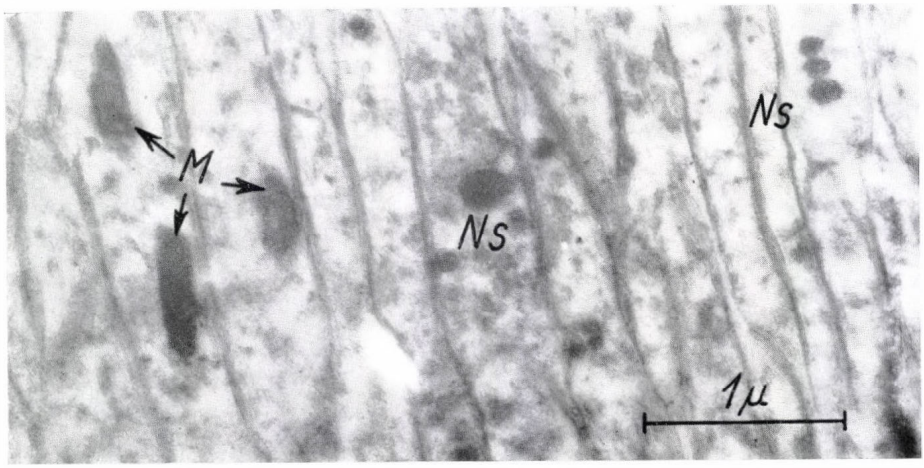

Abb. 4.—Längsschnittbild Mitochondrien *(M)* und Neurosekretgranula *(Ns)* in den Axonen. Metakrylat-Präparat

Das zweite Bild ist ein Metakrylat-Präparat. In den Längschnittbildern läßt sich die Verzweigung einzelner Axone deutlich erkennen. Diese Verzweigung ist gabelförmig, an anderen Stellen, wie im dritten Bild, sieht man die Auflockerung des Axolemmas zwischen zwei parallel laufenden Axonen und eine Vermischung des Axoplasmas.

Auch das nächste Bild zeigt ein Metakrylat-Präparat. In den Axonen sind 0.05 bis 0.3 Mikron große runde oder ovale Granula von starker Densität zu sehen, die oft einen schmalen, hellen Saum aufweisen. Diese Granula stimmen mit dem von Fährmann (1961) im zerebroviszeralen Konnektivum der Muschel Unio tumidus beschriebenen und für Neurosekret gehaltenen Körnchen überein.

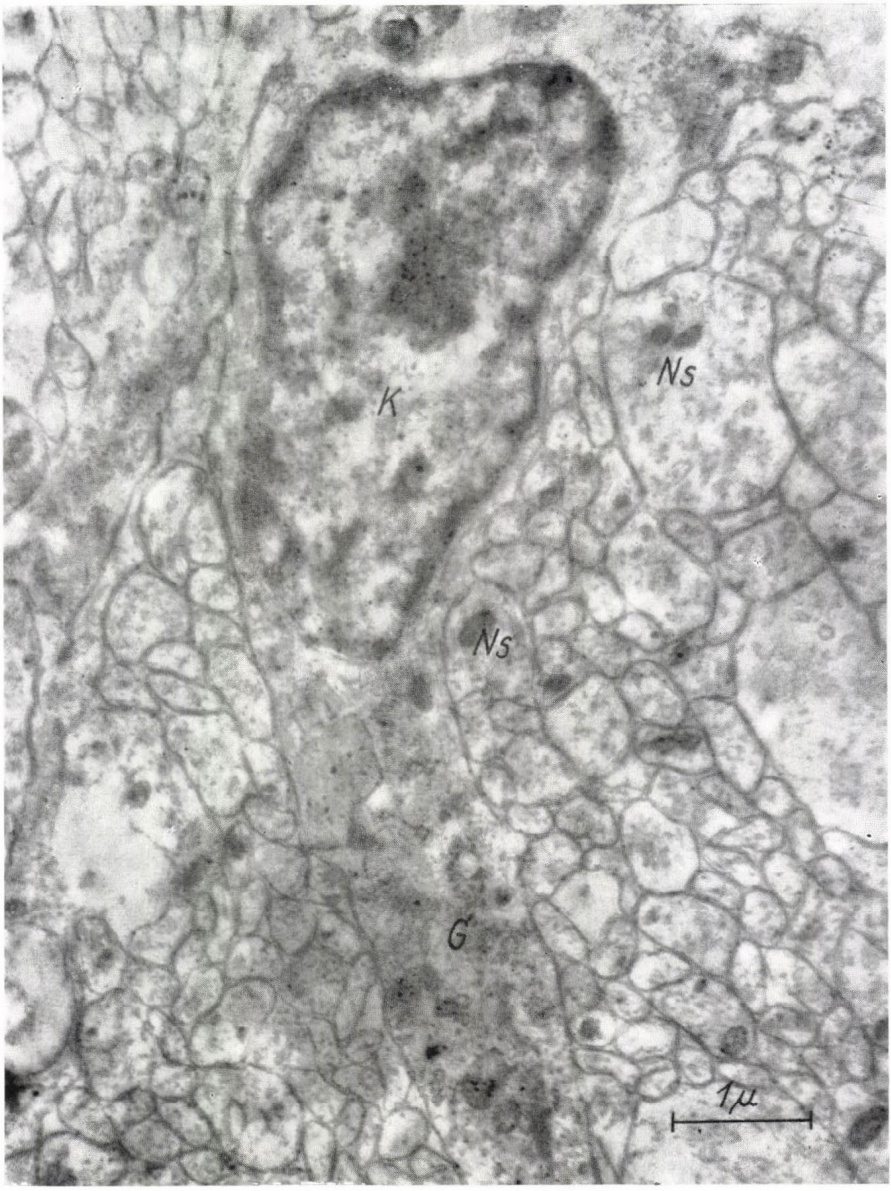

Abb. 5.—Teil einer Gliazelle. *K* = Kern, *G* = Golgi-Komplex, *Ns* = Neurosekret. Araldit-Präparat

Mitochondrien sind in den Axonen verhältnismäßig selten zu finden, ihr Längsdurchmesser ist mit der Längsachse des Axons parallel (Abb. 4).

Das fünfte Bild zeigt ein Araldit-Präparat. Man sieht im Bild eine Gliazelle. Solche Zellen sind in den Schnitten des zerebroviszeralen Konnektivums verhältnismäßig selten. Die Gliazellen haben viele Fortsätze.

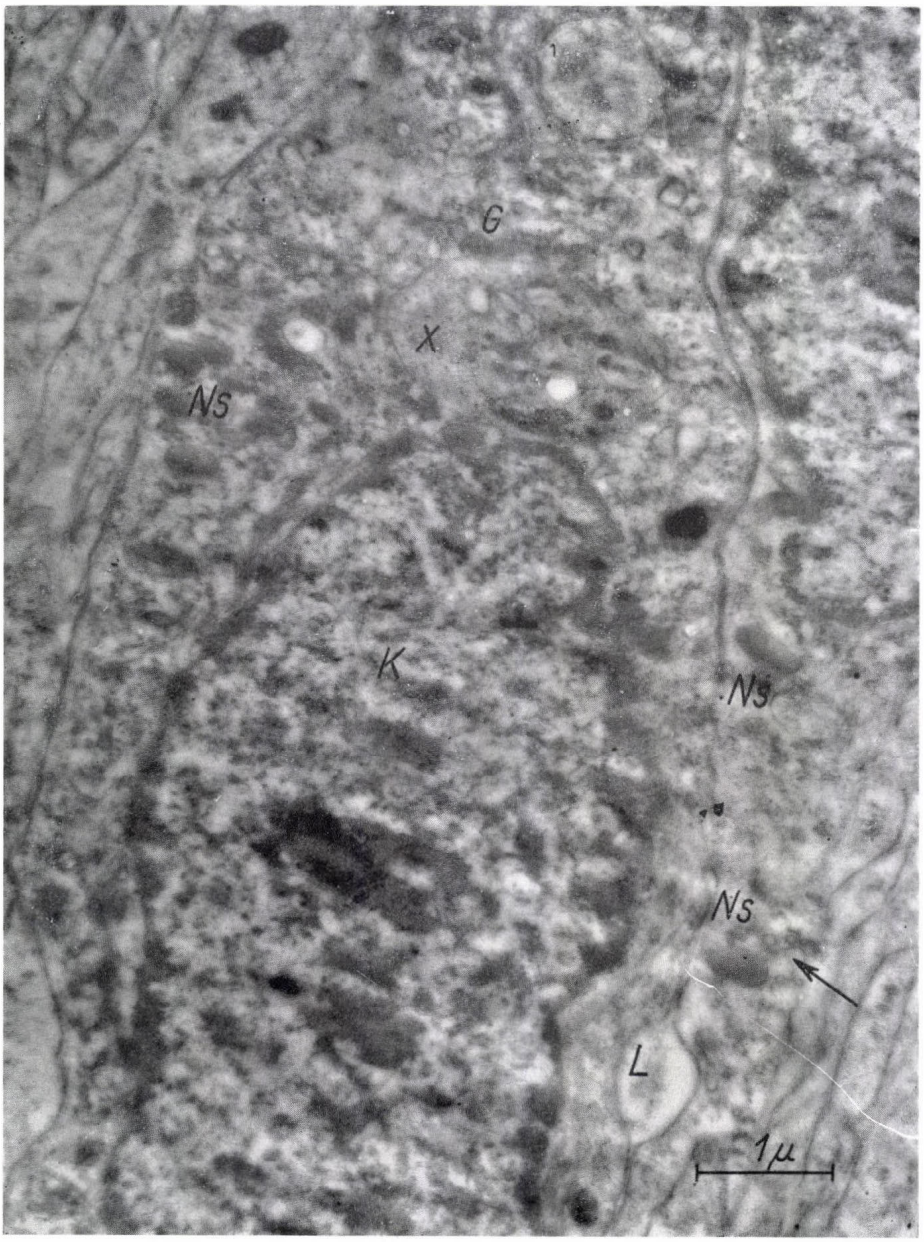

Abb. 6.—Teile zweier Gliazellen. K = Kern, Ns = Neurosekret, L = Gewebsspalte zwischen zwei Gliazellen. G = Golgi-Komplex, X = nicht identifizierte Zellbestandteil. Der Pfeil weist auf die Öffnung der Gliazelle gegen den Gewebsspalt. Metakrylat-Präparat

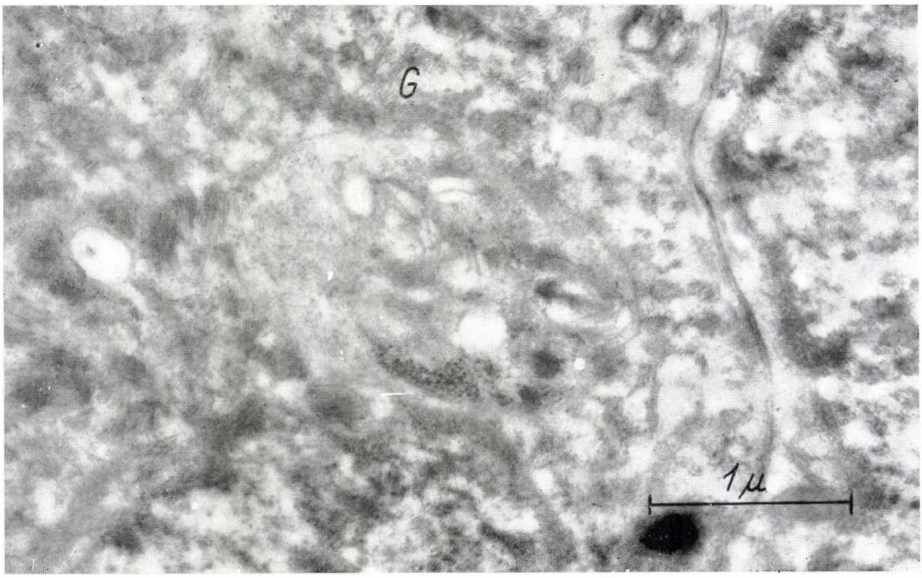

Abb. 7.—Stärkere Vergrößerung des mit *X* bezeichneten Abschnittes aus Abb. 6. Es sind Granula, Bläschen sowie lamelläre Gebilde und die Grenzmembran zu sehen. *G* = Golgi-Komplex. Metakrylat-Präparat

Im Randgebiet des Zellkernes ist eine feingranulierte Verdichtung wahrzunehmen, und eine größere Verdichtung ähnlicher Art kommt auch im Inneren des Kernes vor. In den Gliazellen ist kein typisches Ergastoplasma zu sehen, während Golgi-Substanz in blasiger und lamellärer Form reichlich vorhanden ist. In den Gliazellen und ihren Fortsätzen sieht man Neurosekretgranula von 0,1 bis 0,5 Mikron Größe (Abb. 6). In den Gliafortsätzen sind stellenweise Gliafasern zu erkennen.

In einigen Gliazellen sieht man mitunter von einer Membran umgebene leicht ovale Gebilde von etwa 1,5 Mikron Durchmesser, in welchen Granula von verschiedener Größe und Densität, kleine Vakuolen und unregelmäßige lamelläre Strukturen liegen (Abb. 7).

Man findet einzelne Axone, deren Axolemma im Abschnitt neben den beschriebenen Gewebsspalten seine Kontinuität verliert, in der Nähe der Öffnung ist innerhalb oder außerhalb des Axons ein Neurosekretgranulum zu sehen (Abb. 8).

Wir finden am Axolemma auch Öffnungen, in deren Nähe kein Neurosekretgranulum vorhanden ist, die Konkavität des Axoplasmas hat den Abdruck des an dieser Stelle wahrscheinlich ausgetretenen Neurosekretgranulums bewahrt (Abb. 9).

Gleichartige Öffnungen wurden auch an der Membran der Gliazellen beobachtet, allerdings mit dem Unterschied, daß Sekretgranula in der Nähe der Öffnung immer nur in der Zelle, und nie in den Gewebsspalten wahrzunehmen waren (Abb. 6 und 10).

Unsere Befunde lassen sich im folgenden zusammenfassen:

Das zerebroviszerale Konnektivum der Muschel Anodonta cygnea besteht ausschließlich aus marklosen Fasern, die auch keine Schwannsche Scheide haben und deren Durchmesser zwischen 0,15 und 1,35 Mikron schwankt. Größere Gruppen benachbarter Axone sind ebenso von Gliazellfortsätzen umgeben wie die marklosen Optikusfasern des Frosches, über die Maturana im Jahre 1960 berichtet hat. Die von Batham im Jahre 1961 im zerebroviszeralen Konnektivum der Aplysia californica beschriebenen 3—4 Mikron dicken marklosen Fasern konnten wir bei Anodonta cygnea nicht nachweisen.

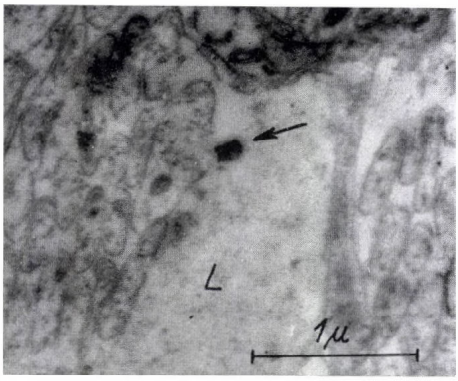

Abb. 8.—Querschnittbild. Der Pfeil weist auf ein vom Axon die Gewebsspalte *(L)* eintretendes Neurosekretgranulum. Metakrylat-Präparat

Die Verzweigung der Axone kann neben ihrer Marklosigkeit eine Erklärung für die von Kahn und Kusnezov schon im Jahre 1938 beschriebene Reizleitung mit Dekrement geben. Gabelförmige Verzweigungen sind in den Bildern ganz eindeutig zu sehen. Die zweite Verzweigungsart, die Auflockerung des Axolemmas zwischen zwei parallel verlaufenden Fasern wurde auch von Maturana (1960) im Nervus opticus des Frosches gesehen. Er deutete aber diese Gebilde nicht als Nervenverzweigungen, sondern als die Folgen schiefer Schnittführung. Unseres Erachtens können diese Gebilde durch schiefe Schnittführung nicht entstehen.

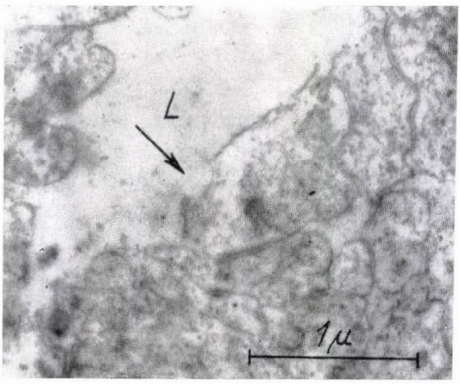

Abb. 9.—Der Pfeil zeigt den 'Abdruck' eines supponierterweise zuvor ausgetretenen Neurosekretgranulums. *L* = Gewebsspalt. Metakrylat-Präparat

Die in den Axonen gesehene Neurosekretgranula dürften sicherlich in den Nervenzellen entstanden und von den Zellen in das Axoplasma gelangt sein. Die Wanderung des Neurosekrets längs der Axone wird heute in der Literatur allgemein akzeptiert (Scharrer, 1951; Bargmann und Knoop, 1957).

Das Vorhandensein von Neurosekretgranula in der Nähe der Axolemmaöffnungen betrachten wir als ein Zeichen dafür, daß sie aus dem Axon in die Gewebsspalten eintreten. Für diese Ansicht spricht der Befund von Axolemmaöffnungen, in deren Nähe der Lymphstrom die in die Gewebsspalten eingetretenen Sekretgranula wahrscheinlich bereits weggespült hat. Die Rolle und das weitere Schicksal der in die Gewebsspalten gelangten

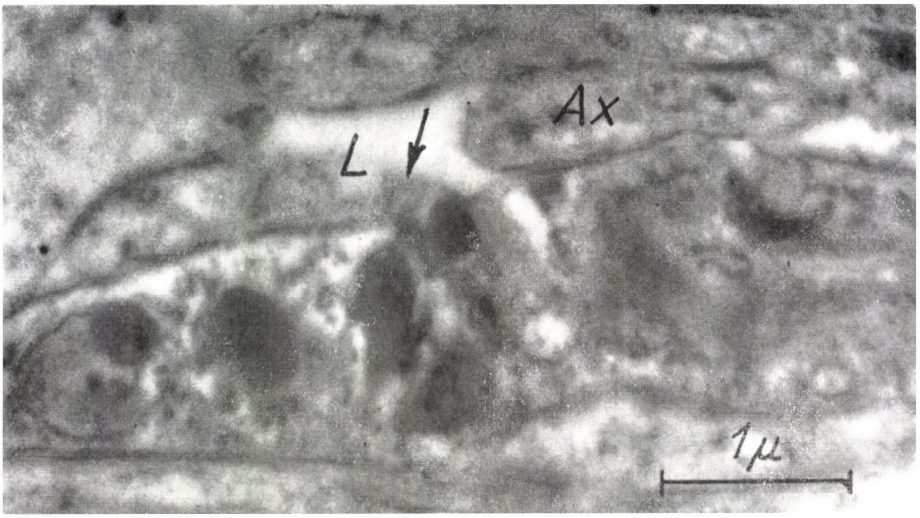

Abb. 10. Teil einer Gliazelle. Der Pfeil weist auf ein die Zellmembran vorwölbendes Neurosekretgranulum. L = Gewebsspalt, Ax = Axon, Metakrylat-Präparat

Sekretgranula sind noch ungeklärt. Das Übertreten von Neurosekretgranula in die Körperflüssigkeiten wurde an Asterias glacialis von Unger (1962) im letzten Jahre beschrieben, obwohl er kein Elektronenmikroskop benutzt hat.

Es sei bemerkt, daß wir die Axolemma-Öffnungen in den Metakrylat-Präparaten als Artefakte bewertet hatten, aber später fanden wir diese auch in den Araldit-Präparaten. Demnach konnten die Axolemmaöffnungen nicht durch die Explosion des Metakrylats entstehen.

Der Klärung bedarf auch der Konnex von Gliazellen und Neurosekret. Ob die Gliazellen das Neurosekret nur speichern oder auch an dessen Produktion beteiligt sind, ist vorläufig eine offene Frage. Wenn die Gliazellen nur als Speicherorgane fungieren, dürften die Granula durch die nachgewiesenen Öffnungen der Gliazellmembran in die Zelle gelangen. Da die Entstehung des Neurosekrets neuerdings von Bern, Nishioka und Hagadorn (1961) bzw. von Röhlich, Aros und Vigh (1962) mit der Golgi-Substanz in Zusammenhang gebracht wurde, und die Gliazellen diese reichlich enthalten, ist die Teilnahme der Gliazellen an der Produktion des Neurosekrets durchaus plausibel.

Die in einzelnen Gliazellen gesehenen, von einer Membran umgebenen ovalen Gebilde, die Granula, Bläschen und Lamellen enthalten, konnten wir mit keinem Organellum der Zelle identifizieren, und so wissen wir auch über die Rolle desselben nichts Näheres.

Zusammenfassend können wir feststellen, daß das zerebroviszerale Konnektivum der Anodonta cygnea marklose Fasern enthält, die auch keine Schwannsche Scheide haben. Größere Axongruppen sind vom Gliaplasma umgeben. Die Axone weisen Verzweigungen auf. In den Axonen und Gliazellen sowie in den Gewebsspalten wurden Neurosekretgranula von 0,05 bis

0,5 Mikron Größe nachgewiesen. Die Neurosekretgranula scheinen aus den Axonen in die Gewebsspalten einzutreten. Der Konnex zwischen Gliazellen und Neurosekretgranula ist ungeklärt.

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THE STRUCTURE OF THE NEUROSECRETORY SYSTEM OF THE EARTHWORM

by

B. Aros, B. Vigh and I. Teichmann

DEPARTMENT OF HISTOLOGY AND EMBRYOLOGY, MEDICAL UNIVERSITY
BUDAPEST, HUNGARY

Neurosecretory phenomena in Lumbricidae were first described in 1937 by Scharrer and Scharrer in the cerebral ganglion of *Lumbricus terrestris*. Ten years later their examinations were confirmed by Schmid (1947), Harms (1948), Hubl (1953, 1956), Herlant-Meewis (1956a, 1956b, 1957, 1961), Aros (1963), Aros and Bodnár (1960), Aros and Vigh (1961a, 1961b, 1962a, 1962b), Brandenburg (1956), Otremba (1961), Michon and Alaphilippe (1959), as well as Marapao (1959), who investigated the neurosecretory system of different species. Scharrer and Brown (1961), Röhlich, Aros and Vigh (1962) dealt with the electron-microscopy of the neurosecretory cells, their histochemistry was studied by Herlant-Meewis (1956a), Aros (1963). Particulars on the general morphology of the nervous system of the earthworm are to be found in the papers of Lenhossék (1892), Langdon (cit. in Laverack 1963), Szüts (1915), Hess (1925), Hanström (1928), Prosser (1934), Aros and Vigh (1961b).

This paper has been designed to summarize our investigations into the morphological and histochemical conditions of the neurosecretory system of the earthworm *(Eisenia foetida, Lumbricus rubellus, Lumbricus herculeus)*.

THE SECRETORY SYSTEM OF THE CENTRAL NERVOUS SYSTEM

The nervous system of the earthworm may be divided into a central and a peripheral part, the structures of which do not differ essentially from one another in the species investigated.

The central nervous system consists of the cerebral ganglion, the hypopharyngeal ganglion and the ganglia of the ventral nerve chain, as well as of the connectives, which link together the ganglia.

CEREBRAL GANGLION

The cerebral ganglion (Figs 1, 2 and 2) consists of two symmetrical halves. The cells (i.e. cortical substance) are situated dorso-caudally, as well as laterally, close beneath the capsule of connective tissue, while the neuropil, a network of fibres originating from the cells has a ventral position. Between the fibres the anterior, medial and posterior commissures are situated. Ventrally there are only a few cells in the cerebral ganglion. The cells may be divided into a dorsal, a lateral and a ventral group, each having an ante-

Fig. 1.—Frontal section of the pharyngeal segment of the earthworm. a = cerebral ganglion, b = hypopharyngeal ganglion, c = pharyngeal connective, d_1 and d_2 = 'vegetative' ganglia, e = pharynx, Chromhematoxyline-phloxine stain

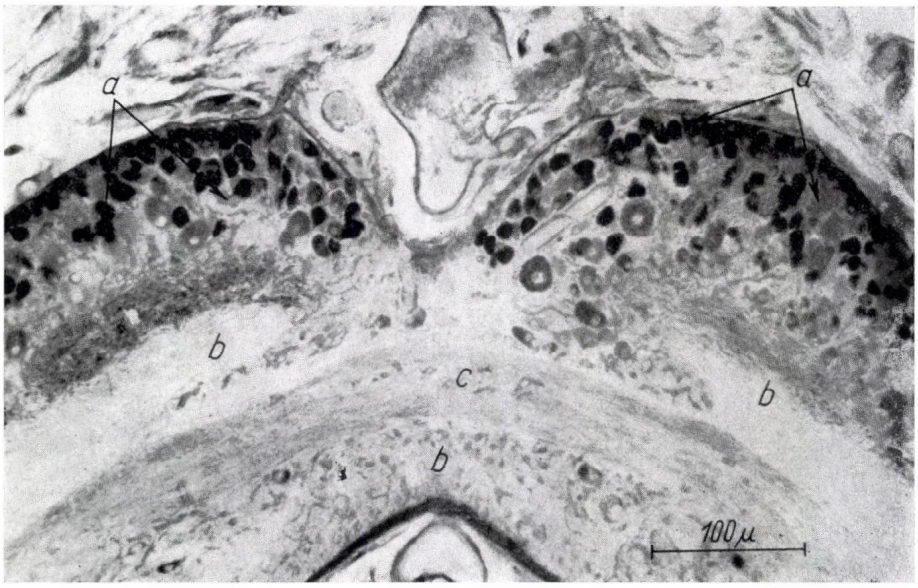

Fig. 2.—Frontal section of a cerebral ganglion. *a* = cellular layer, *b* = neuropil, *c* = commissure, Paraldehyde-fuchsin stain

rior and a posterior subdivision. Besides the nerve cells numerous glial cells and pericyte-like connective tissue cells are to be found.

From the various types of nerve cells, those termed as 'A' cells or 'small' cells are of secretory character (Figs 3 and 4). These 'A' cells are generally pear-shaped, unipolar, but bi- or multipolar shapes may be found as well. The 'A' cells are situated near the capsule of the cerebral ganglion and especially in the *Lumbricus herculeus* make two distinct layers (Figs 2 and 6). The superficial layer consists of mostly multipolar cells situated immediately below the capsule. The plasma of the cells is granulated and stains moderately with chromhematoxyline and paraldehyde-fuchsin. The deeper layer consists of more loosely arranged cells which stain rather dark; these cells are mostly unipolar, their processes filled up with Gomori-positive granules may be well traced as far as to the boundary of the cortex and the fibrous layer (Fig. 3). Here the ends of the fibres thicken. The string-like shaped processes resembling Herring-bodies form a broad, Gomori-positive granulated area, the so-called 'storage zone' (Figs 3 and 7) (Aros and Vigh 1961b). It is along the axon that the neurosecretory granules appearing in the 'A' cells get to this area, where they accumulate and probably empty into the capillaries to be found here. On the basis of these observations two ways may be surmised for the emptying of the neurosecretory material: from the cells the granules either directly get into the capillaries adjacent to the perikaryon, or they migrate along the axon to the 'storage zone', where they accumulate and finally also get into the capillaries. The role of the Gomori-positive granulated 'storage zone' in the cerebral ganglion of the earthworm may be similar to that of neurohypophysis in vertebrates, or that of the sinus-gland in *Crustacea*.

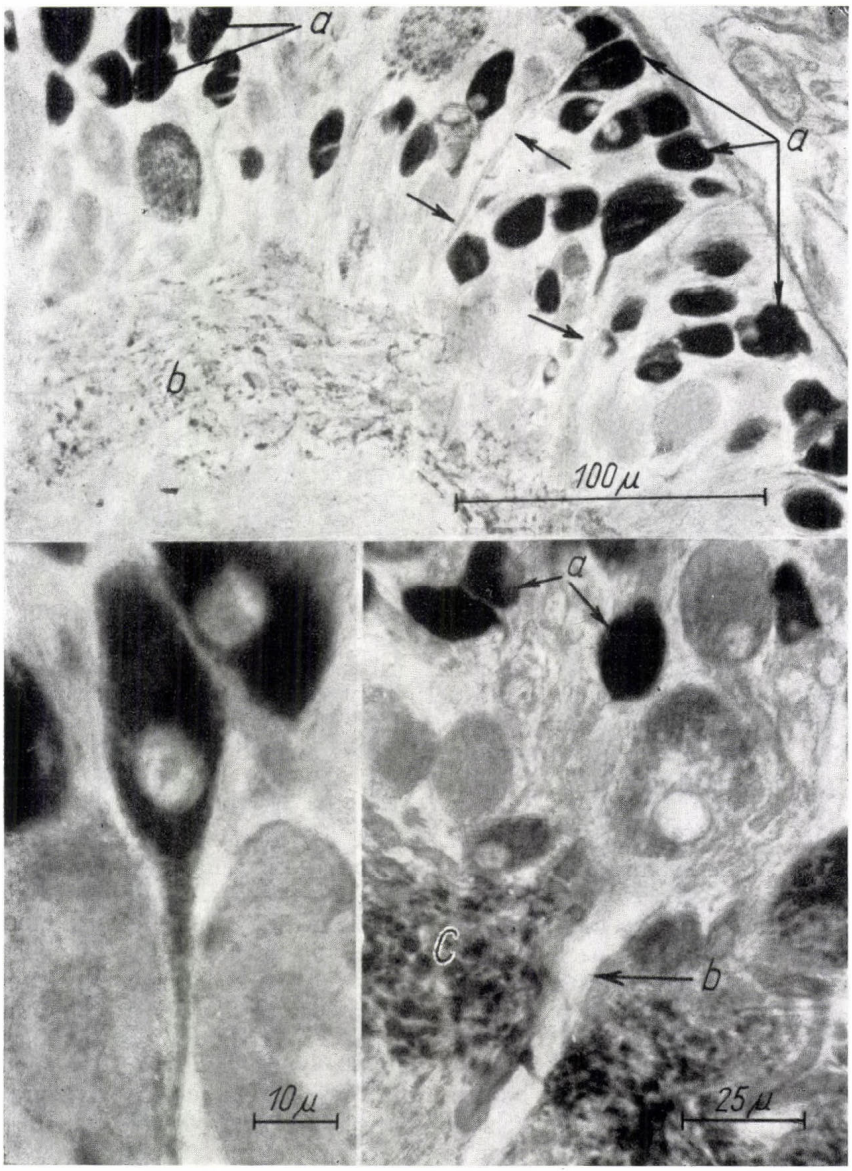

Fig. 3. – A part of the cerebral ganglion. a = 'A' cells, b = storage place. The arrows show processes of the small cells coursing to the storage place. Paraldehyde-fuchsin stain

Fig. 4. – 'A' cell. The secretory granules filling the process are also easily seen. Paraldehyde-fuchsin stain

Fig. 5. – 'B' = cell. a = 'A' cells, b = process of the 'B' cell passing across the storage place, c = storage place. Paraldehyde-fuchsin stain

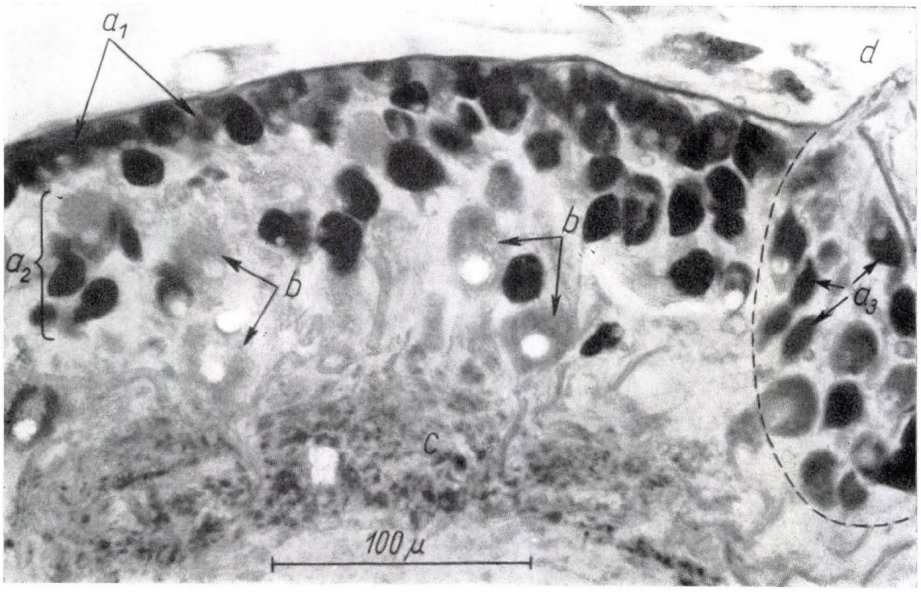

Fig. 6.—A part of the cerebral ganglion with paraldehyde-fuchsin stain. a_1 = superficial row of the 'A' cells, a_2 = deep row of the 'A' cells, a_3 = group of the bipolar 'A' cells (within the jagged line), b = 'B' cells, c = storage place, d = nerve trunk emerging from the celebral ganglion

The layer of the 'B' or 'large' cells (Figs 5 and 6) may be found among the cells of the deep layer of the 'A' cells, as well as beneath them. These 'B' cells often contain Gomori-positive granules, their secretory character, however, is not so explicit as that of the 'A' cells. These large cells are unipolar, their strikingly thick processes proceed across the storage zone and enter into the cerebral commissures.

The 'C' or lateral cells of also non-secretory character are mostly situated on the lateral area of the ganglion. When leaving the cortical substance the fibres of these elongated unipolar cells break at a sharp angle and enter the connectives of the ipsilateral side (Figs 8 and 9).

On the dorso-medial part of the cerebral ganglion a smaller group of cells consisting of 'A' cells deliminates caudally. These cells are of secretory character and often bipolar. Some of their processes filled with Gomori-positive granules proceed towards the neuropil, while others in the opposite direction towards the surface, where, forming a thinner nerve stem, they leave the cerebral ganglion. The nerve stem courses to the vessel situated behind the ganglion. There may be found a good number of Gomori-positive granules in the cells of the nerve stem. The granules also fill up the processes of the nerve cells and can be followed in the nerve stem too. (Aros and Vigh, unpublished). The role of the above-mentioned neurosecretory cell group and nerve stem and their relation to the vessel are not yet elucidated.

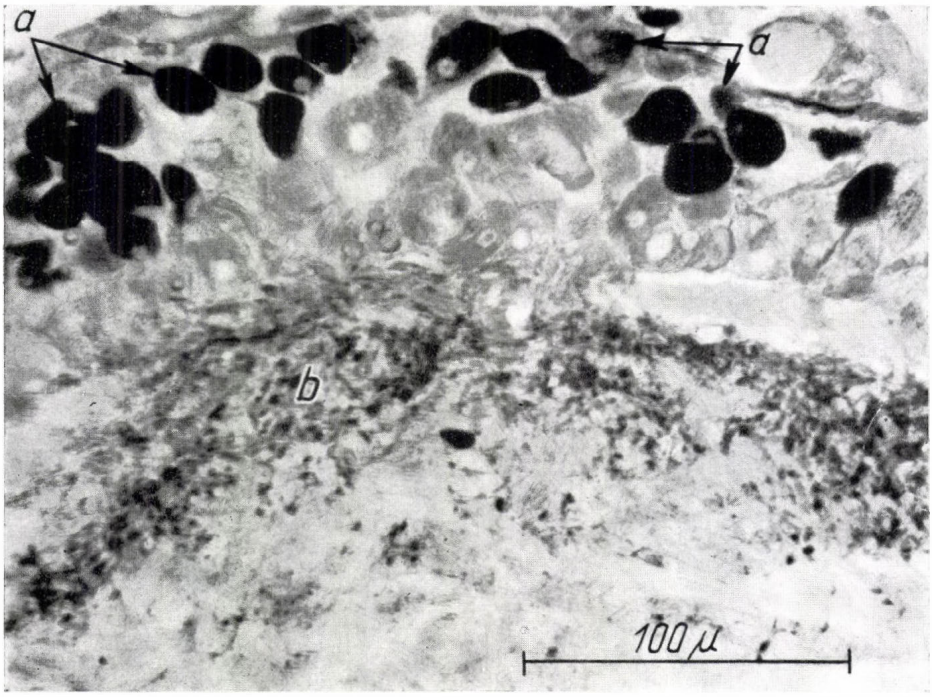

Fig. 7.—The thin, string like Herring-bodies of the storage place. *a* = 'A' cells, *b* = storage place. Paraldehyde-fuchsin stain

Fig. 8.—Lateral cells. Their processes, breaking in a sharp angle, enter into the pharyngeal connective of the ipsilateral side. Paraldehyde-fuchsin stain

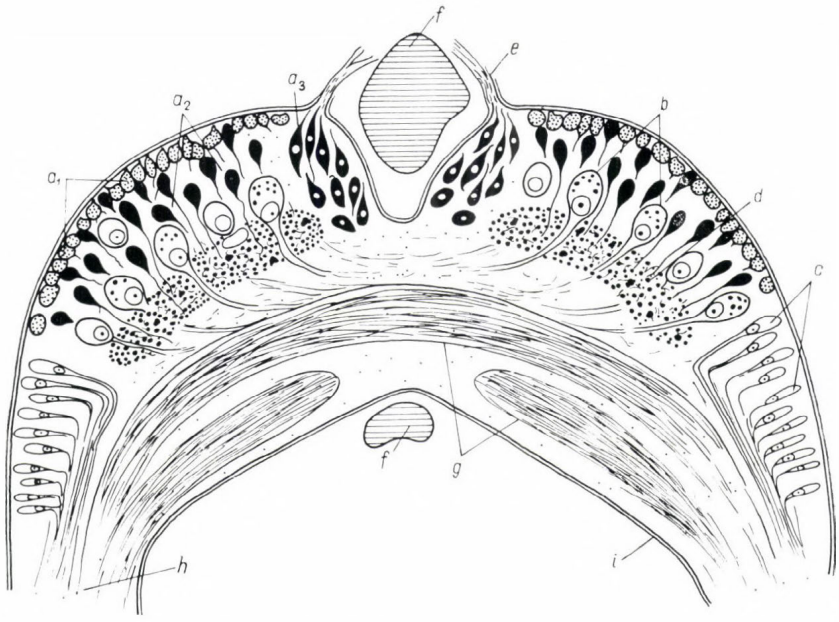

Fig. 9.—Schema of the cerebral ganglion. a_1 = superficial layer of the small 'A' cells, a_2 = deep layer of the small 'A' cells, a_3 = the cells of the nerve-trunk proceeding from the cerebral ganglion to the vessel behind it, b = 'B' cells, c = lateral cells, d = storage zone, e = the nerve-trunk proceeding from the cerebral ganglion to the vessel behind it, f = the vessel running on the dorsal surface of the cerebral ganglion, g = anterior and median commissure, h = site of emerging of the pharyngeal connective, i = the connective tissue sheath of the cerebral ganglion

HYPOPHARYNGEAL GANGLION

The hypopharyngeal ganglion results from the fusion of the first and second of the ventral nerve chain ganglia. In both of these parts we find anterior and posterior groups of cells which further divide into medial and lateral cells. The two sides of each ganglion are connected by two transverse commissures.

In the rostral part of the ganglion we find large cells, maximally filled with Gomori-positive substance, which are arranged in anterior, medial and lateral cell groups (Fig. 10). From the denomination 'Unterschlundganglion' Hubl (1956) has termed these cells 'U'-cells. They deeply penetrate the pharyngeal connective extending between the hypopharyngeal and cerebral ganglion. Morphologically these 'U' cells may be compared with the 'A' cells of the cerebral ganglion, but they are larger and unipolar. In the processes of the 'U' cells coursing perpendicularly towards the middle of the ganglion the Gomori-positive granules may be traced to the midst of the ganglion. Here the terminations of the processes show thickenings like Herring bodies and similarly to the storage place of the cerebral ganglion they form a Gomori-positive area (Fig. 11). There are two Gomori-

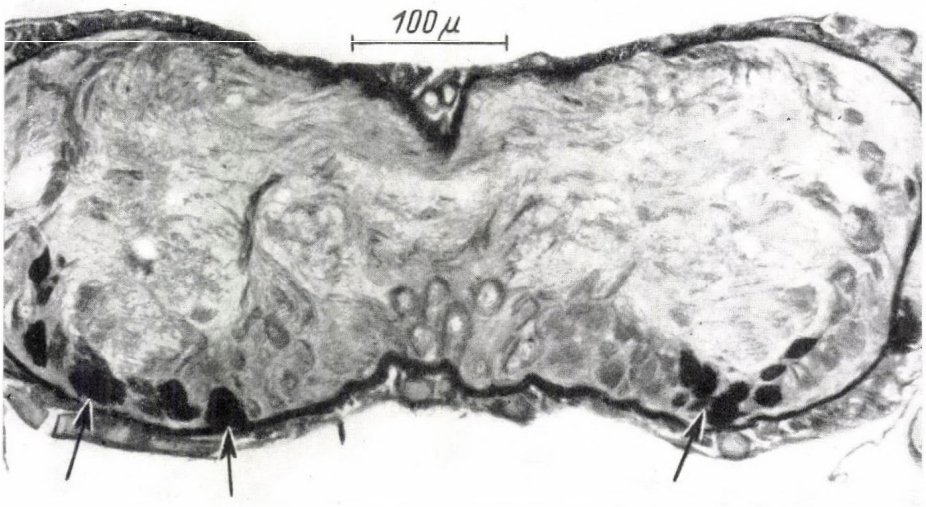

Fig. 10.—Transverse section of the hypopharyngeal ganglion. Arrows show 'U' cells. Paraldehyde-fuchsin stain

positive areas, one in the left half of the hypopharyngeal ganglion and the other in the right one. The storage zones have a diameter of about 50 μ.

Apart from the 'U' cells, Gomori-positive cells may be found also in other areas (Aros and Vigh 1961b), so in the cell groups of the rostral and of the caudal ganglion half.

VENTRAL GANGLION CHAIN

In the ganglia of the ventral nerve chain two commissures and six cell groups can be distinguished. In each ganglion there are rostrally and caudally two lateral and one ventral groups. Each of the mentioned six cell groups has many cells containing Gomori-positive material (Aros and Vigh 1961a, 1961b; Herlant-Meewis and N. van Damme 1962).

In every ganglion the secretory activity of the anterior cell groups is generally more intensive than in those of the posterior ones. In the ventral cell groups there are multipolar cells, especially large and showing a rather strong Gomori-positivity. These cells occur regularly at the same place in each ganglion.

Comparing the different ganglia of the ventral nerve chain, we find that in caudal direction the secretory activity gradually decreases.

Accumulation zones of granules as described in the cerebral ganglion and hypopharyngeal ganglion may be also found in the ventral ganglia.

PERIPHERAL NERVOUS SYSTEM

From the ganglia of the ventral nerve chain, two nerve trunks originate from each segment on both sides. The posterior trunks are paired. After passing through the ventral muscle layers, these nerves proceed towards

Fig. 11.—The zone of Gomori-positive granules in the hypo-pharyngeal ganglion. *a* = 'U' cells, *b* = granulated zone. At the arrows fibres of the 'U' cells are extending towards the granulated zone. Paraldehyde-fuchsin stain

the ventral bristle pair. Between the longitudinal and circular layers of the subepidermal musculature they bend toward the dorsal side and course along the border of the two muscular layers in the form of the so-called annular nerves. At the emerging of the annular nerves, as well as at their cranial end, groups of ganglion cells may be observed. The nerve cells of the ganglia—though staining with chrom-hematoxyline and paraldehyde-fuchsin—do not show significant neurosecretory features. The same may be established for the so-called bristle ganglia, the localization of which corresponds to the bristles. No secretory activity could be observed in them.

VEGETATIVE NERVOUS SYSTEM

Numerous fibres emerge from the ventral ganglion chain of the earthworm. These fibres supply the glands, the visceral tract, as well as the proper muscles of the sheath of the nervous system. A separate system may be found around the pharynx, which in many respects resembles the vegetative nervous system of animals of higher orders (Hess 1925; Aros and Vigh 1961b).

The mentioned system, consisting of numerous ganglia and nerve fibres, innervates the whole foregut and especially the pharynx. The structure of this system is the following. The circum-esophageal connective gives off

4 to 5 short thick nerve trunks in its upper and middle third part. After a short course the stems pass into a large ganglion situated between the pharynx and the pharyngeal connective. In the structure of this ganglion an outer cellular and an inner fibrous layer may be distinguished, the whole ganglion being surrounded by a fibrous sheath. The nerve fibres originating from this ganglion penetrate into the numerous minute ganglia situated along the intestinal wall. These ganglia consist of a few cells and fibres. The fibres emerging from here run towards the basis of the intestinal epithelial cells, where nerve cells are also discernible at many places. In the wall of the digestive tube, especially in its first portion, in front of the pharynx and in the dorsal longitudinal fold of the foregut resembling the typhlosolis, elongated, long cells, similar to the cutaneous sensory nerve cells are seen, which seem to continue as nerve fibres.

In each of the enumerated ganglia, cells containing Gomori- positive granules are to be observed. The granules can be traced far in the processes of the nerve cells. In the two large ganglia situated at both sides of the pharynx, numerous Gomori-positive granules may also be observed along the fibres (Aros and Vigh 1961b). Thus it seems to be most likely that the 'vegetative' ganglia also contain cells with secretory character and function, though we have no knowledge about the physiological significance of the phenomenon.

THE FINE STRUCTURE OF THE NEUROSECRETORY CELLS

In the secretory cells of the cerebral ganglion—similarly to higher species—the neurosecretory elementary granula appear as round formations consisting of electrodense substance, surrounded with membrane. Their diameter is about 250 to 300 mμ. The formation of elementary granules is related to the Golgi apparatus (Scharrer and Brown 1961, Röhlich, Aros and Vigh 1962). The electrodense substance characteristic for the neurosecretory material appears at first in the flat cysterns of the Golgi-apparatus and then in the Golgi-vesicles.

Among the nerve cells forms of different types are found (Röhlich, Aros and Vigh 1962). Some cells (the storage cells) are filled with neurosecretory granules. Such cells are poor in ergastoplasm and Golgi-apparatus. In others empty vesicles or ergastolplasm are to be found in abundance (resting cells). Some cells show a 'vacuolated' form, due to the numerous widened ergastoplasmic cysterns. Presumably, the cell-forms mentioned represent different functional phases (see also the chapter on the secretory cycle).

Apart from the mentioned elementary granules large, dark, round corpuscles with fine-granulated, homogeneous or lamellar content occur in the nerve cells of the cerebral ganglion. Their diameter is of 0.5—2.5 μ. Many of such formations may be observed in the glial cells.

The capillaries of the cerebral ganglion consist of a thin basic membrane and a myoendothelial cell layer. In the adjacent endothelial cells, between the cells and the membrana basalis, electrodense formations resembling the neurosecretory elementary granules are frequently observed. Such formations are also found in the intercellular dilatations (Röhlich, Aros and Vigh 1962). They most probably represent the secretory granules released from the cell and passing into the vascular pathway.

As known, Gomori's chrome-hematoxyline and paraldehyde-fuchsin staining cannot be considered as a histochemical reaction. Probably both dyes—when used after oxidation—are mostly bound to the sulphur-containing acid groups resulting from oxidation, whereas without oxidation they are bound to the acid groups also originally present in the tissue, mostly to the acid mucopolysaccharides (lit. in Teichmann 1964). According to Rodeck (1959), the Gomori-positivity would reveal the sulphon (So_3H) groups, Konecny and Pliczka (1958), however, believe the dye to be—apart from the SO_3H-groups—possibly also bound to the SO_2H, COH, OSO_3H, and eventually to other acid groups (COOH). Thus the 'Gomori-positivity' in itself gives only an orientation about the chemical nature of a substance.

Herlant-Meewis (1956a) has given particulars on the histochemical nature of the neurosecretory system of the earthworm. Investigating earthworms on different development stages, she found the neurosecretory cells to show PAS-positivity before the appearance of the Gomori-positive substance. PAS-positivity disappears simultaneously with the accumulation of Gomori-positive material. The PAS-positive substance may be supposed to be a precursor of the neurosecretory material.

The problem was investigated in detail by Aros (1963) who also examined earthworms *(Eisenia foetida)* of different age, including mature ones.

The following positive reactions can be found in mature earthworms: tetrazonium-reaction, eriochrome nigrum T staining, demonstration of the protein-bound SH and SS groups, and tryptophane reaction. All these reveal the presence of protein. PAS reaction gives a more intensive reaction without digestion, though PAS-positivity does not totally disappear after digestion either. These observations suggest that there must be a substance in the cytoplasma of the neurosecretory cells that contains both glycogen and some other polysaccharide. Trypaflavine staining is positive, which according to Takeuchi, would indicate the presence of mucopolysaccharides containing sulphur.

However, the localization of the subtance of polysaccharide nature does not correspond morphologically to the area where the Gomori-positive substance is found. The same may be said of the sudanophilia of the cells. One has to conclude from these observations that the neurosecretory material itself contains neither carbohydrate nor lipid substances. The protein reactions, however, reveal a different situation, as their positivity corresponds to the localization of the Gomori-positivity. Only the protein-reactions showed positivity at the so-called storage place, where a considerable quantity of 'pure' neurosecretory material should be found. Thus, as a final conclusion, one has to surmise that the neurosecretory substance found in the cells of the earthworm is of protein-nature (Aros 1963).

The intensity of the reactions vary in the cerebral ganglia of earthworms according to age. PAS-reaction seems to decrease with the growth of the animal and the development of the neurosecretory system, while the intensity of the protein-reactions increases with the growing intensity of Gomori-positivity (Herlant-Meewis 1956a, Aros 1963). This observation also seems to confirm that the neurosecretory material in the earthworm is a sort of protein.

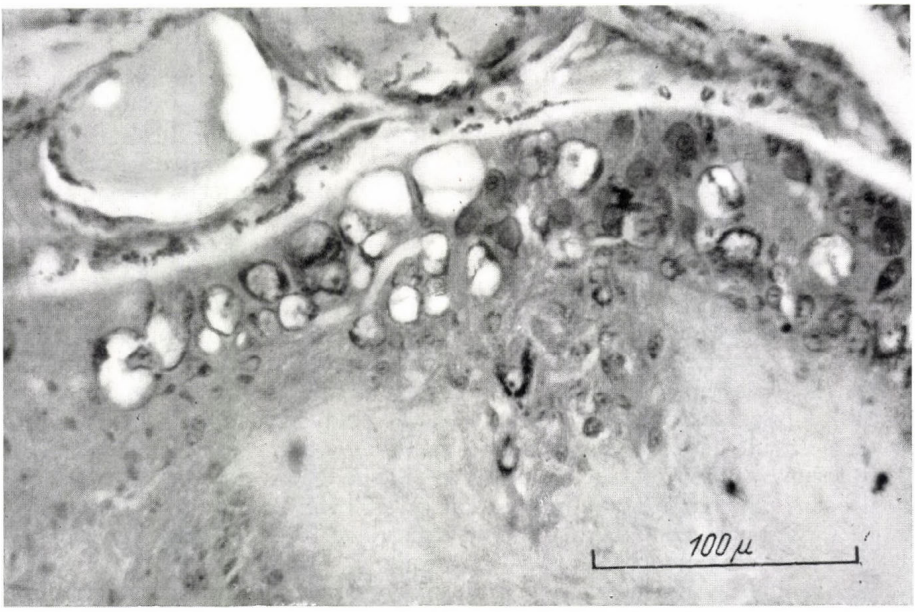

Fig. 12.—Hypervacuolated degenerating cells in the cerebral ganglion. Chromhematoxyline-phloxine stain

THE SECRETORY CYCLE OF THE NEUROSECRETORY CELLS

Among the neurosecretory cells, especially the 'A' cells—as mentioned already by Schmid (1947)—different cell-forms may be observed, independently. The cells are mainly filled—though to a different degree—with Gomori-positive granules, but cell forms without granules may also be found, as well as such ones completely filled with secretory material. The mentioned variations most likely represent the various phases of the production of the neurosecretion, i.e. of the secretory cycle.

The electron-microscopical picture also supports this assumption, since it fairly well reveals the connexion between the formation of the elementary granules and the different cellorganells in the different cell forms. The 'resting' cells, which do not contain neurosecretory granules, have a well developed ergastoplasm. In the cells of 'secretory' character the appearance of an electrodense substance in the Golgi apparatus and its accumulation in the Golgi-vesicles may be observed, close to which more or less secretory granules are situated in the plasm. The 'storage' cells are practically filled with elementary granules, and the ergastoplasm, as well as Golgi-apparatus, is only present in a negligible amount (Röhlich, Aros and Vigh 1962). According to Scharrer and Brown (1961) the precursor of the neurosecretory material develops in the ergastoplasm and appears then in the Golgi-apparatus. The elementary neurosecretory granule is formed by the secretory substance accumulated in the Golgi-vesicle.

The vacuolated cell-forms discernible with both the light-and electron-microscope most likely represent the stage after emptying of the secretory

material. Apart from the vacuolation, however, cells showing degeneration and cell shadows—disintegrated cells—may also be found. Degenerated cells appear in an especially high number, when—with different effects—an intensive secretion is brought about in the cerebral ganglion (Aros and Vigh 1962a). Hypersecretion in such cases is always accompanied by strong vacuolation and disintegration of cells (Fig. 12). This reveals that in the case of hypersecretion a phenomenon similar to the holocrine gland secretion occurs: concomitant with the process of neurosecretion the neurosecretory cells disintegrate. The disintegration of cells is followed by a rapid increase of the undifferentiated nerve cells always present in the ganglia (Aros and Vigh 1962a). As all transition forms of cells ranging from undifferentiated ones to neurosecretory nerve cells do occur, the phenomenon may be interpreted in a way that the undifferentiated cells are able to make good for the loss of integrated neurons. The probability that the neurons are thus easily replaced is also confirmed by the good regenerative capacity of the earthworm. In the extirpated cerebral ganglion, namely, the neurosecretory system completely regenerates within 8 weeks or so (Herlant-Meewis 1961, Aros and Vigh 1962b). On the basis of the above observations we may surmise the neurosecretory cycle to be a process similar to that of complete holocrine secretion.

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