MIKLÓS GÁBOR

THE ANTI-INFLAMMATORY ACTION OF FLAVONOIDS

FOREWORD
BY ALBERT SZENT-GYÖRGYI

AKADÉMIAI KIADÓ, BUDAPEST

MIKLÓS GÁBOR

THE ANTI-INFLAMMATORY ACTION OF FLAVONOIDS

With a foreword by
Albert Szent-Györgyi

Natural substances — among them flavonoids — have always represented an interesting field of drug research. For this reason a short and up-to-date monograph dealing with the anti-inflammatory activity of flavonoids is very timely and useful.

The first part of the book gives concise information to the reader on the present state and new trends of flavonoid research, as well as on the anti-inflammatory activity of this group of compounds. The second part is a report on the lectures of the author and his collaborators presented at the Round Table Conference of the 3rd Hungarian Bioflavonoid Symposium (Debrecen, 1970), under the title "The Anti-inflammatory Action of Flavonoids".

The book is of interest to pharmacologists, pathophysiologists, and pharmaceutical chemists.

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THE ANTI-INFLAMMATORY ACTION OF FLAVONOIDS

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FOREWORD BY

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FOREWORD

Serendip was an Indian prince who sent out his three sons to find a treasure. They all came home with something different and more valuable. Finding something one does not seek is called 'Serendipity' in science. 'Vitamin P' was an example of Serendipity. When I was on my way to isolate ascorbic acid, my friend and colleague, Professor I. Rusznyák, had a patient with subcutaneous capillary bleedings. We thought that these might have something to do with a lack of Vitamin C, so Professor Rusznyák proposed administering to the patient some of my impure Vitamin C preparations. I obliged and the patient was cured. Later when I had pure Vitamin C we again gave it to a patient with hemorrhages expecting a still more striking effect. There was none. Evidently, something was in my impure preparations which was responsible for the therapeutic action and was not Vitamin C. I guessed that this 'something' might be a flavone. Thus we tested flavones, and they worked. It looked as if these would have a vitamin nature. I was not sure. Vitamins are usually designated with letters of the alphabet. However, if I used the next available letter and it turned out that flavones are not vitamins, then this would have caused confusion. So I picked a letter on the far unoccupied side of the A B C. Should flavones not be vitamins, correction could be made without confusion.

Subsequently several cases of so-called 'Henoch Purpura' were cured and I had no doubt that we had discovered a very important new drug.

American science did not take in a friendly spirit to Vitamin P and the name 'Vitamin' was dropped. More than that: discussions have been going on to strike the flavones altogether from the list of drugs, since no therapeutic action has been found.

I think that the contradiction is due to the fact that in the USA citrus fruits belong to people's regular daily diet. They are rich in flavones, so a lack in flavones is very rare and if there is no deficiency, a vitamin has no action. In contrast to this, in countries where citrus fruits are expensive, the lack of flavones may cause trouble and their medication may show favorable effects.

While these discussions were going on, important experimental material was collected in Hungary which, to my mind, leaves no doubt about the vitamin nature and the biological activity of flavones. The present volume is an important contribution in this line and I welcome its appearance.

Albert Szent-Györgyi, M. D., Ph. D.

INTRODUCTION

No one has ever given a comprehensive picture of the anti-inflammatory action of the numerous different flavone derivatives. The larger review works, if they mention this question at all, treat the relevant results in at most a few lines.

In recognition of the interest of the theme, the 3rd Hungarian Bioflavonoid Symposium (Debrecen, 21–23 May, 1970) dealt with the anti-inflammatory action of flavonoids at a round-table conference. The purpose of this monograph is primarily to review the anti-inflammatory action of flavonoids (Part One, II) and to introduce the lectures delivered at that conference (Part Two). By way of introduction, with no efforts at completeness, the present state and newer trends of flavonoid research are dealt with (Part One, I).

As a result of the experimental nature of this monograph, it is not wished, nor is it attempted, to take a stand in the debate which has arisen on the therapeutic action of flavonoids. However, it is undoubtedly worth noting that while the known highly effective anti-inflammatory agents do possess undesired side-effects, no side-effects of flavonoids used in therapy were described until 1969.

According to the most recent studies, the parenteral application of commercially available rutin or its sodium salt causes inflammatory phenomena and morphological changes in the uriniferous tubules and liver–gall ducts of mice, rats and guinea pigs. In contrast, the applica-

tion even in extremely large doses of the sodium salt of the sulfuric acid ester of rutin and O-(β-hydroxyethyl) rutin as pharmaceuticals does not produce a similar change in experimental animals.

The road to the discovery of further effective flavone derivatives which can be used in therapy means the preparation of new synthetic compounds and the research into still unstudied naturally occurring flavonoids. It is hoped that this monograph will provide help for future work.

I should like to take the opportunity in this introduction to express my grateful thanks to Professor Szent-Györgyi for sparing some of his valuable time to write a foreword to my work. I also express my thanks to my colleagues for their invaluable cooperation in the work treated in Part Two of the monograph as well as to Mrs. E. K. Kállay for her careful editorial work.

PART ONE

I. PRESENT POSITION AND RECENT TRENDS OF FLAVONOID RESEARCH

Attention was drawn to the curative effects of the flavonoids, which give a rich variety of colours to the vegetable kingdom, by the studies of Rusznyák and Szent-Györgyi (1936). Whereas their known biological effects were few at that time, Böhm (1959) could already enumerate 40 different pharmacodynamic effects of this group of compounds.

In this introductory Chapter I wish to give an account — based on the available literature data — of the present position and some of the more important recent results of flavonoid research. The order is as follows:

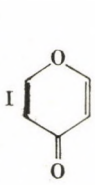
1. Effect on enzyme activity
2. Metabolism
3. Tachyphylaxis
4. Liberation of histamine
5. Increase of two-phase capillary resistance
6. Protective effect on ultrastructural cerebral damage caused by ischaemia
7. Effect on the heart of synthetic flavone derivatives
8. Antihepatotoxic effect
9. Therapeutic application and side-effects of flavonoids.

For the benefit of the reader, before the recent results are dealt with, the different flavone types appearing in this work are introduced (Table 1).

The chemical structures of some flavonoids which occur in this monograph are given in Table 2.

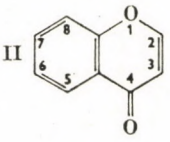
TABLE 1

Naturally occuring flavonoids and some related compounds (basic skeletons)



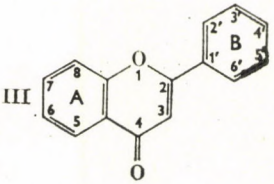
I

γ-Pyrone



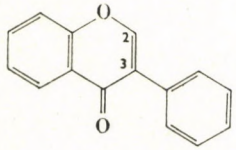
II

Chromone
(benzo-γ-pyrone)



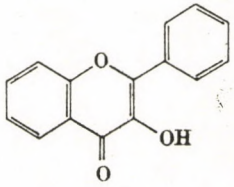
III

Flavone
(2-phenylbenzo-γ-pyrone)



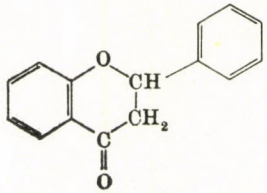
IV

Isoflavone
(3-phenylbenzo-γ-pyrone)



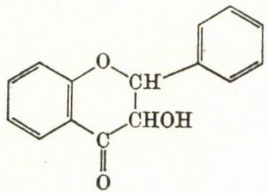
V

Flavanol
(3-hydroxyflavone)



VI

Flavanone
(2,3-dihydroflavone)

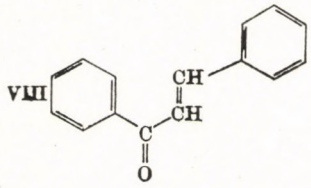


VII

Flavanonol
(3-hydroxyflavanone)

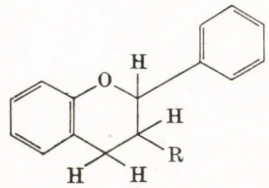
TABLE 1 (*continued*)

VIII



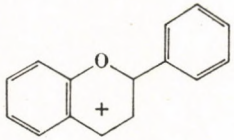
Chalcone
(benzalacetophenone)

IX



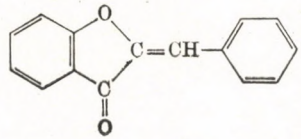
R=H Flavan
R=OH Catechin

X



Anthocyanidin
(flavylium salt)

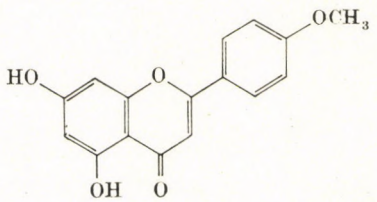
XI



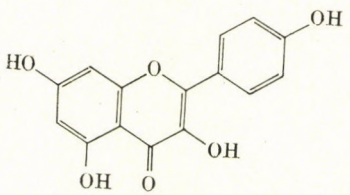
Aurone (2-benzalcoumaranone-3)

TABLE 2

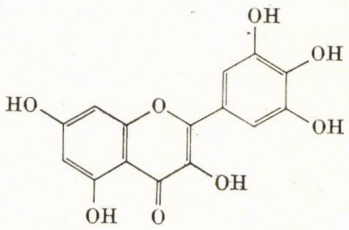
The chemical structures of some flavonoids which appear in the monograph



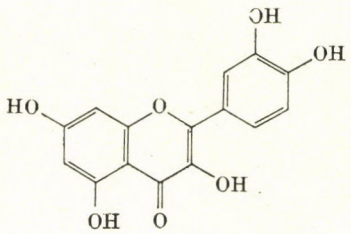
Acacetin



Kaempferol

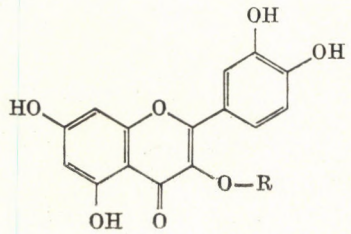


Myricetin



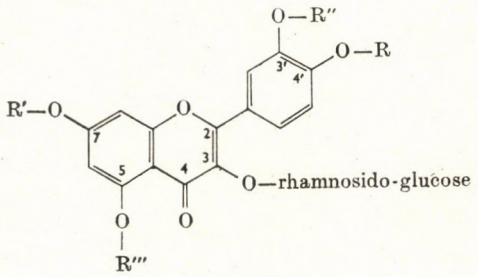
Quercetin

TABLE 2 (*continued*)

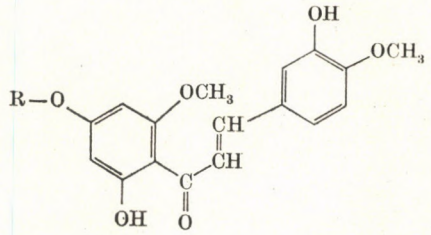


Rutin

R = rhamnosido-glucose



Hydroxyethylrutin

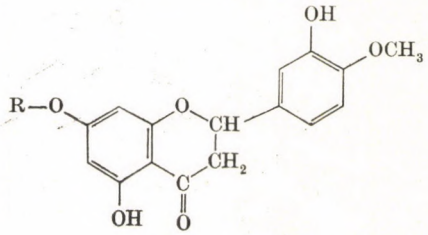


Hesperidin methylchalcone

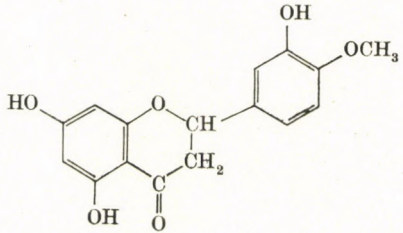
		R	R′	R″	R‴
5,7,3′4′	— tetrahydroxyethylrutoside	HE	HE	HE	HE
5,7′4′	— trihydroxyethylrutoside	HE	HE	H	HE
7,3′4′	— trihydroxyethylrutoside	HE	HE	HE	H
7,4′	— dihydroxyethylrutoside	HE	HE	H	H
4′	— monohydroxyethylrutoside	HE	H	H	H

HE = $—CH_2CH_2OH$

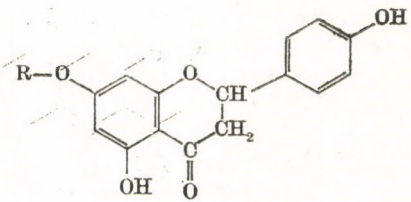
TABLE 2 (*continued*)



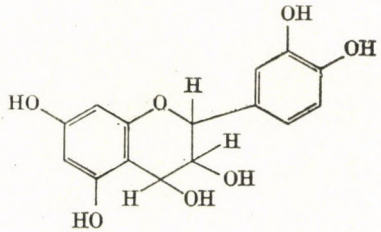
Hesperidin
R=rhamnosido-glucose



Hesperetin®



Naringin



Leucocyanidin

O-(β-hydroxyethyl)rutin (Venoruton®, Zyma, Nyon) contains a mixture of hydroxyethylrutins in fixed proportions, prepared by the hydroxyethylation of rutin. The five major components of the mixture have been identified by UV spectrophotometry (Courbat *et al.* (1966)); they are: 5,7,3′,4′-tetrahydroxyethylrutin, 5,7,4′-trihydroxyethylrutin, 7,3′,4′-trihydroxyethylrutin, 7,4′-dihydroxyethylrutin and 4′-monohydroxyethylrutin.

1. EFFECT ON ENZYME ACTIVITY

The effects of flavonoids in inhibiting hyaluron idase histidine decarboxylase, cholinacetylase and xanthin oxidase have long been known (Böhm (1968)). From recent studies the relations between the chemical structures of the flavonoids and their enzyme-inhibiting effects are also known. Thus Stenlid (1965) has shown that flavonoids substituted in the 4′-position stimulate the action of indolacetic acid oxidase, and this stimulating effect is conspicuously increased by a hydroxyl group in the 7-position. However, flavonoids containing 3- and 4-hydroxyl groups inhibit the activity of the enzyme.

It is worth mentioning that according to recent experiments by Stenlid (1970) various flavonoids inhibit the production of adenosine triphosphate in plant mitochondria. Flavones are more inhibitory than the corresponding flavanones. Substances without any substituent in ring B are very active and the presence of substituents at the 3′- and 4′-positions is of minor importance for the effect upon ATP formation. Glycosides are less active than the corresponding aglycones.

Mori and Noguchi (1970) have studied the effect of flavonoids on the activity of bovine pancreas ribo-

nuclease Type 1. They found that the activity of RNase Type 1 was considerably inhibited by 7,3′,4′-trihydroxyflavones and flavonols. The inhibiting action of flavonoids is attributable partly to the 4-keto group and the 7-hydroxyl group, and partly to the 4′-hydroxyl group. 6- and 8-methoxyl groups decrease the inhibiting effect. Flavanones and flavanols have no action on the activity of RNase Type 1.

From among the most recent investigations, those of Carpenedo *et al.* (1969), Diamond and Gelboin (1969), Gelboin *et al.* (1970), and Kokarovtseva and Fedurov (1970) should also be mentioned. Carpenedo *et al.* showed that quercetin inhibited Mg^{2+}-dependent, Na^{+}- and K^{+}-stimulated ATPase.

According to Diamond and Gelboin (1969) α-naphthoflavone inhibits the aryl hydrocarbon hydroxylase activity in homogenates of induced hamster embryo cells and in liver microsomes from rats previously treated with polycyclic aromatic hydrocarbons.

Aryl hydrocarbon hydroxylase is also inhibited when α-naphthoflavone is added to homogenates of mouse skin epidermis (Gelboin, Wiebel and Diamond (1970)).

Kokarovtseva and Fedurov (1970) found that tea catechins, rutin and sodium gallate, when added to the liver homogenates of rats, brought about a fall of the tyrosine-aminotransferase activity. The effects produced by aesculetin and hesperidin were less marked.

2. METABOLISM

On several occasions in the past it has been asserted that our knowledge relating to the metabolism of flavonoids is very scanty.

In their critical work on the flavonoids Shils and Goodhart (1956) suggest that the newer biochemical methods should be used to shed light on the absorption

and metabolism of these substances. A good deal later Goodman and Gilman (1970) mention available evidence indicating that little orally administered flavonoid is absorbed but the literature on this subject shows conside tabledisagreement. These facts have made reasonable a more detailed survey of the metabolism of flavonoids. (Data can be found on this topic in the works of Böhm (1968) DeEds (1968) and Lavollay and Neumann (1969).)

The first attempts of Clark and MacKay (1950) relating to the metabolism of certain flavonoids (rutin, its soluble complexes with methylglucamine and methenamine, 'methylated hesperidin chalcone', the sodium salts of aesculetin-4-carboxylic acid and of quercetin-6′-sulfonic acid, 'calcium flavonate glucoside', citrin, gossypin and xanthorhamnetin), made by means of various chemical methods, were unsuccessful.

It was found in human experiments that urinary excretion following large oral doses (50 mg/kg of body weight) of flavonoid compounds was negligible, that these materials were not recoverable from the stools, and that rutin was destroyed when incubated in an aqueous stool suspension. In animals the percentage of the fed doses which were absorbed from the intestine and excreted in the urine were negligible, some of the fed compounds being fairly completely recoverable from the gastrointestinal tract. The results suggested that a trace (less than 1%) of the fed dose of sodium quercetin-6′-sulfonate may be absorbed and excreted by rats.

The application of paper chromatographic and spectrophotometric methods to study the possible modes of transformation of flavonoids in the organism gave the following information.

Murray *et al.* (1954) reported that when rutin or quercetin was given orally to rabbits, substantial

amounts were absorbed, metabolized, and largely excreted in the urine within twenty-four hours. Evidence for the excretion of at least four compounds was obtained. One of these compounds was isolated and identified as 3,4-dihydroxyphenylacetic acid, which accounted for at least 25% of the quercetin given.

Not long afterwards evidence was presented which permitted the identification of two additional metabolites in the urine after the animals had been given rutin or quercetin orally, namely *m*-hydroxyphenylacetic acid and 3-methoxy-4-hydroxyphenylacetic acid. 3,4-Dihydroxyphenylacetic acid, also a metabolite of quercetin, was shown to be the precursor of the two metabolites first mentioned. The identification of these urinary metabolites of a flavonoid such as quercetin was offered as evidence of intestinal absorption having taken place (Booth *et al.* (1956)).

According to the results of Lang and Weyland (1955) the liver and kidney contain very active enzyme systems which break down rutin and quercetin under aerobic conditions. Only rutin injected in large doses (2.0 g/kg) is transformed in the organisms of rats and rabbits. A very small amount of rutin can be isolated unchanged from the urine, together with 3,4-dihydroxyphenylacetic acid.

With a paper chromatographic method, Masquelier *et al.* (1965) were able to demonstrate the presence of phloroglucinol glucuronide formed by rupture of the molecule, together with 3,4-dihydroxyphenylacetic acid and 3-methoxy-4-hydroxyphenylacetic acid, in the urine of rats treated with leucocyanidine (5,7,3′,4′-flavan-3,4-diol) (80 mg/kg p.o. or 50 mg/kg i.p.) and with quercetin (100 mg/kg p.o.).

Kallianos *et al.* (1959) reported the presence of radioactive protocatechuic acid in the gastrointestinal contents of rats fed quercetin labelled with ^{14}C.

This group (Petrakis *et al.* (1959)) also reported that following oral administration of quercetin — randomly labelled with ^{14}C — radioactive homovanillic and *m*-hydroxyphenylacetic acids were found in the urine of normal rats. On the other hand, following intraperitoneal injection of the radioactive quercetin, radioactive vanillic acid was found. Within 12 hours after feeding, radioactivity was found in the expired CO_2, in the lungs, in the gastrointestinal walls, and in the gastrointestinal contents.

It was suggested that quercetin might possibly be degraded in the rat by at least two metabolic pathways.

Further experiments of Booth *et al.* (1958a) throw light on the metabolism of flavanones and flavones. When hesperidin, hesperetin, diosmin, diosmetin, eriodictyol and homoeriodictyol were administered to rats by stomach tube, without exception *m*-hydroxyphenylpropionic acid was found in the urine, together with smaller amounts of *m*-coumaric acid and the aglycones. The aglycones occurred both in the free state and conjugated with glucuronic acid. This was proof not only that absorption from the intestinal tract had occurred, but also that cleavage of the middle ring of the flavonoid had taken place, accompanied by dehydroxylation, demethoxylation, or demethylation followed by dehydroxylation, to yield *m*-hydroxyphenylpropionic acid.

When rabbits kept on a purified diet (starch, Cerelose, casein, Cellu flour, salts (mixture U.S.P. XIV, 1950), oil and vitamins) were fed hesperidin, in their urine, in addition to small amounts of hesperetin and its glucuronide, at least seven other metabolites were clearly in evidence, including 3,4-dihydroxyphenylpropionic acid, 3-methoxy-4-hydroxyphenylpropionic acid, *m*-coumaric acid (*m*-hydroxycinnamic acid), *m*-hydroxyphenylpropionic acid, *m*-hydroxyhippuric acid,

m-hydroxybenzoic acid and 3-methoxy-4-hydroxybenzoic acid.

It is further worthy of note in the experiments of Booth *et al.* (1958a) that the composition of the diet exerted marked effects upon the absorption of hesperidin by rabbits.

In studies on man, after the ingestion of 1 g hesperidin or hesperetin, the major metabolite was identified as 3-hydroxy-4-methoxyphenylhydracrylic acid which was detected in the 7 to 21 hour urine sample. Thus, a species difference was observed between the rat and human when hesperidin was ingested.

The studies of Kapétanidis and Mirimanoff (1964) showed that O-(β-hydroxyethyl)rutin was completely metabolized by the human organism. The products of metabolism did not seem to be phenolic substances or very reducing phenols.

In another of their works, Booth *et al.* (1958) reported that after rats were fed naringin or naringenin, in addition to *p*-hydroxyphenylpropionic acid, small amounts of *p*-coumaric acid, *p*-hydroxybenzoic acid and the ethereal sulfate of *p*-hydroxybenzoic acid could be demonstrated in the urine.

Several papers deal with the metabolism of catechins. Griffiths (1962) detected *m*-hydroxyphenylpropionic acid in relatively large amounts in the urine of catechin-fed rats. From (+)-catechin administered orally in rat experiments, Griffiths (1964) found *m*-hydroxyhippuric acid in addition to *m*-hydroxyphenylpropionic acid in the urine.

In rabbits, however, the metabolites are vanillic acid, *m*-hydroxybenzoic acid and protocatechuic acid together with certain phenyl-γ-valerolactones (Oshima *et al.* (1958), Oshima and Watanabe (1958), Watanabe (1959a, b).

In more recent studies on the guinea pig, the major

phenolic acid metabolite of (+)-catechin was shown to be *m*-hydroxybenzoic acid. *m*-Hydroxyphenylpropionic acid was present only in trace amounts. Several phenyl-γ-valerolactones were also formed. The major lactone was δ-(3-hydroxyphenyl)-γ-valerolactone (Das and Griffiths (1968)).

In further experiments with (+)-catechin with the two rings equally labelled with ^{14}C, Das and Griffiths (1969) showed that the phenolic acid metabolites of catechin arose from ring B, since radioactivity was demonstrated in these metabolites. For a study of the metabolic fate of ring A, (+)-catechin was used which had been selectively labelled with ^{14}C in ring A. The phenyl-γ-valerolactones, which were postulated to arise in part from ring A (Das and Griffiths (1968)), were shown to possess radioactivity.

Das and Griffiths (1968) also made the very interesting finding that degradation of (+)-catechin in the guinea pig is effected at least in part by the intestinal microflora and is suppressed by aureomycin plus phthaloylsulfathiazole.

Das (1969) showed too that (+)-catechin can be degraded by a suspension of rat intestinal contents. After 48-hour incubations, analyses showed the presence of metabolites. Of these, the compounds that were positively identified were *p*-hydroxyphenylpropionic acid, *m*-hydroxyphenylpropionic acid, δ-(3-hydroxyphenyl)-γ-valerolactone, δ-(3,4-dihydroxyphenyl)-γ-valerolactone and unchanged catechin.

At this point it should also be mentioned that today numerous microorganisms (*Aspergillus flavus*, *Aspergillus niger*, *Pullularia fermentans*, etc.) are known which, bred in the presence of rutin or quercetin, induce the metabolism of these flavonoids (Hattori and Noguchi (1959), Westlake *et al.* (1959), Westlake and Spencer (1966)). According to Krishnamurty *et al.* (1970) *Buty-*

rivibrio sp. C-3 degrades rutin anaerobically to yield phloroglucinol, carbon dioxide, 3,4-dihydroxybenzaldehyde and 3,4-dihydroxyphenylacetic acid.

From the work of Child *et al.* (1963) with rutin labelled at the C-3 atom it is known that the degradation results in protocatechuic acid and phloroglucinolcarboxylic acid.

The metabolism of rutin by mycelial cultures of the lignivorous Hymenomycete, *Coniophora puteana* (Schum. ex. Fr. Karst.) has been studied recently by Armand-Fraysse and Lebreton (1969).

The metabolic pathway: rutin → isoquercitrin → quercetin → ribosyl-4′-quercetin → diribosyl-4′,7-quercetin was deduced from structural and kinetic data.

The studies of Smiths and Griffiths (1970) have provided evidence on the metabolism of myricetin. Administration of myricetin (200 mg) to rats gave rise to a major metabolite in the urine that was identified as 3,5-dihydroxyphenylacetic acid by chromatographic and spectral methods. No glucuronide conjugates of myricetin were detected.

In vitro studies have shown that microorganisms of the intestines degrade incubated myricetin and myricitrin. The metabolites are 3,5-dihydroxyphenylacetic acid and 3,4,5-trihydroxyphenylacetic acid.

3. TACHYPHYLAXIS

It has been shown by Cession-Fossion *et al.* (1968) that O-(β-hydroxyethyl)rutin (Z 4,000, Zyma-Galen) results in a lowering of blood pressure when administered i.v. to rats in 5 mg/100 g doses. The blood pressure returns to the original level 5–10 minutes later. Renewed administration of the rutin derivative in a similar dose produces no effect, the blood pressure is no longer

lowered, i.e. the phenomenon of tachyphylaxis is observed.

If the rats are previously treated with promethazine (1.5 mg/100 g s.c. or i.p.), the intensity and length of time of the lowering of the blood pressure induced by the first 5 mg/100 g dose of the rutin derivative are significantly diminished and the appearance of tachyphylaxis is accelerated. A preliminary treatment of the animals with atropine, hexamethonium, or guanethidine does not inhibit the fall in blood pressure brought about by the rutin derivative. When administered to rats in a dose of 10 mg/kg i.p., O-(β-hydroxyethyl) rutin does not lower the blood pressure (Lecomte *et al.* (1969)).

4. LIBERATION OF HISTAMINE

A very large dose of O-(β-hydroxyethyl)rutin (100 mg/100 g i.v.) leads to oedema on the paw, cheek and scrotum of the rat. According to Cession–Fossion *et al.* (1968) both the manifestation of the oedema and the fall in blood pressure can be explained by the liberation of histamine as a result of the action of the rutin derivative. Their theory is supported by perfusion experiments on the isolated hind quarters of the rat. When the preparation is injected into the aorta, a vasodepressive substance appears in the perfusate. Histamine is the substance in question since in cats these effects can be terminated by treatment with promethazine (1 mg/kg). The amount of histamine liberated by 10 mg O-(β-hydroxyethyl)rutin can be estimated at 6 μg (expressed as the dihydrochloride).

More recent experiments of Lecomte and Cession-Fossion (1970) have confirmed that O-hydroxyethyl derivatives of rutin are dextran-like amine-releasers.

The studies of Mélon and Lecomte (1970) show that the above rutin derivative does not cause either a local or a general histamine reaction in man. Even very large doses (1 g i.v.) do not induce the liberation of histamine.

5. INCREASE OF TWO-PHASE CAPILLARY RESISTANCE

Today numerous pharmacons are available for the control of capillary resistance (Gábor (1960, 1971)). The action of flavonoids in increasing capillary resistance has been known since the studies of Armentano *et al.* (1936) and is still a subject of research. The studies of Parrot and Canu (1964) and Masquelier (1969) represent new trends in this research.

As a result of their experiments on guinea pigs, Parrot and Canu (1964) were the first to report that the effect of catechins exerted on capillary resistance is two-phase. After an initial increase of capillary resistance which corresponded to the effect of rutin and its derivatives, a renewed slower increase was observed which attained its peak about 72 hours after injection.

Masquelier (1969) reported that flavan-3,4-diols (e.g. leucocyanidin, Flavan®, Labor. Gueyne, Bordeaux), even in doses approaching the mg range, cause the increase of capillary resistance in man, and this property sharply distinguishes them from other flavonoids, which display this effect only in much larger doses. Apart from this, the vascular response is completely specific: after a single dose of catechin or leucocyanidin, an early and transitory increase of capillary resistance can be observed which is followed by an even larger and more lasting increase. This secondary effect attains its maximum in general in 72 hours and lasts for several days.

(Neither the applied dose nor the number of cases was reported.) Thus, the two-phase response which Parrot and Canu (1964) observed in guinea pigs is also found in man. In contrast, other flavonoids cause merely the early increase of capillary resistance and the curve obtained is only one-phase.

From earlier studies (Masquelier *et al.* (1965)), Masquelier (1969) concluded that the early and transitory increase of capillary resistance can be attributed to the competitive inhibition of catechol-O-methyltransferase. The normal biological role of this enzyme is that it induces the initial inactivation of adrenaline. As a result the action of adrenaline and catecholamines are in general extended.

For its interest, Masquelier's (1969) assumption, which requires verification, should be mentioned that flavan-3,4-diols behave like provitamins, and the delay of the secondary phase is necessary for the organism in order that these provitamins transform to vitamins.

6. PROTECTIVE EFFECT ON ULTRASTRUCTURAL CEREBRAL DAMAGE CAUSED BY ISCHAEMIA

The studies of Pratesi *et al.* (1969) deserve special attention. Local ischaemia was produced in the cortex of the rabbit by ligature of one or both carotid arteries. The cortical ultrastructural changes resulting from the ischaemia were studied with an electron microscope 12 or 36 hours after the ligature was applied. The results were compared with the cortices of control rabbits, in which no ischaemic region could be demonstrated.

Another group of the experimental animals received for 45 days a daily treatment of 100 mg/kg 5,7-diacetoxy-flavone. The studies showed that the flavone therapy inhibited the development of ultrastructural damage

brought about by the ischaemia in the cerebral capillaries and the neuroglia. Flavonoid treatment alone, without ischaemia, does not lead to significant ultrastructural changes.

As regards an explanation of the phenomenon, Pratesi *et al.* (1969) confine themselves to hypotheses. They consider among others the finding of Capelli *et al.* (1968) according to which the flavonoids under *in vitro* conditions are essential for the preservation and development of the normal structure of the blood vessels.

It is mentioned at this point that we have observed severe pathological changes in rats maintained on a flavonoid-free diet: a significant increase of cerebral oedema and subpleural haemorrhage (Benkő *et al.* (1970)). The treatment with flavonoids (hesperidin methylchalcone, O-(β-hydroxyethyl)rutin) of the experimental animals previously maintained on the deficient diet led to a statistically significant decrease in the manifestation of the changes.

7. EFFECT ON THE HEART OF SYNTHETIC FLAVONE DERIVATIVES

The effect on the heart of the naturally occurring flavonoids has been known since the studies of Akamatsu (1929) and Jeney and Czimmer (1936). It was found that rutin, quercitrin and quercetin increased the cardiac contractility and the output and decreased the frequency rate of isolated and in situ frog heart. Many similar studies have followed these experiments; the reader is referred to the excellent review of Böhm (1968).

Crataegus extract (Crategutt®, Schwabe, Karlsruhe), which contains natural flavonoids (quercetin, hyperoside, vitexin, epicatechin, cyanidin), has found appli-

cation in therapy; it dilates the coronary blood vessels and improves the circulation in the myocardium (Böhm (1956), Schwabe and Neu (1960), Hahn *et al.* (1960), Trunzler and Schuler (1962)). Jaursch *et al.* (1969) have recently reported on its use in drug combination.

In addition to the naturally occurring flavonoids, the main purpose of the present work is to draw attention to the coronary dilating and other cardiac effects of some synthetic flavone derivatives.

According to Ferrari and Finardi (1955), the skeleton of flavone itself (2-phenylchromone) possesses coronary dilating action. Jourdan and Faucon (1958) reported a similar effect of 3-methylchromone in experiments on dogs.

The studies of Setnikar and Zanolini (1956) showed that flavone-7-ethyl oxyacetate (Recordyl®, Recordati, Milano) dilates the coronaries in isolated rabbit heart perfused by the Langendorff method. Several authors have reported its favourable clinical effect; others, however, did not observe an improvement on its use. Morin *et al.* (1964) confirmed its coronary dilating and antispasmodic effect in animal experiments. Compared with khellin, this flavone derivative is effective towards the coronaries in 20 times smaller doses and the duration of its action is much longer.

Setnikar *et al.* (1961) described the coronary dilating action of another flavone derivative, morpholinoethyl-3-methylflavone-8-carboxylate.

Szirmai (1961) suggests the 1,3-dimethylxanthine-7-acetic acid salt of 7-(β-dimethylaminoethoxy)flavone (Perflavon®, Voigt, Berlin), as a coronary dilating drug, for the treatment of patients suffering from angina pectoris, myocardial infarction and coronary sclerosis and as a prophylactic for cardiac infarction. In experi-

ments on dogs the increase of the coronary circulation could be demonstrated on the action of this flavone derivative.

From further studies on experimental animals or on isolated organs, Szirmai (1965) reported the antiphlogistic, choleretic, spasmolytic and antihistamine actions of Perflavon. In small doses Perflavon has a positive inotropic effect, it decreases the vascular resistance and it increases the oxygen utilization of the myocardium.

According to the studies of Grisk and Scheler (1965) flavone-7-oxyacetic acid-(γ-cyclohexylaminopropyl) amide has a particularly strong effect on the impulse-generating and conducting system of the heart. Both in isolated heart preparation and in the whole animal, a more pronounced bradycardiac effect could be demonstrated than for quinidine, procainamide and ajmaline. The cardiac fibrillation produced by electrical stimulation and also by strophantine and aconitine, and the ventricular arrhythmia due to coronary ligation, are favourably affected by these flavone derivatives.

In recent researches Weinges *et al.* (1971) report the coronary-dilating activity of dehydrocatechin.

8. ANTIHEPATOTOXIC EFFECT

Study of the silymarin antihepatotoxic effect means a new trend in flavonoid research. The chemical structure of silymarin, the active ingredient of the medicinal plant milk thistle (*Silybum marianum* L. Gaertn.) is known from the work of Wagner *et al.* (1965, 1968). A drug prepared from silymarin is available under the name Legalon® (Madaus, Cologne).

Its antihepatotoxic effect was revealed by Hahn *et al.* (1968) in the following experiments: it antagonized a liver defect which was caused by CCl_4 and could be

demonstrated by prolongation of the sleeping period after administration of 5-(1-cyclohexenyl)-1,5-dimethyl-barbituric acid (hexobarbital); it had a protective effect on a liver defect caused by CCl_4 which could be proved by inhibition of hepatic metabolizing of *p*-hydroxy-phenylpyruvic acid; it had a protective or curative effect on the death rate and survival time of mice after intoxication by α-amanitine; it influenced beneficially the extent and time course of weight losses in rats after application of α-amanitine, as well as the blood level of sorbite dehydrogenase increased by α-amanitine; it antagonized cirrhosis-like liver defects caused by chronic feeding of thioacetamide to rats.

9. THERAPEUTIC APPLICATION AND SIDE-EFFECTS OF FLAVONOIDS

Many publications have appeared in recent years on the therapeutic effectiveness or ineffectiveness of flavonoids.* As has been emphasized by several authors, the chief use of the flavonoids has been in the treatment of disease states characterized by capillary bleeding associated with increased capillary fragility. These include degenerative vascular disease, allergic states, diabetes mellitus, and various other disorders. The purpose of therapy has been to reduce the incidence of capillary haemorrhage and thereby prevent the sequels therefrom (Goodman and Gilman (1970)).

In connection with the therapeutic application of rutin, Griffith *et al.* (1955) have already drawn attention to the fact that in the cases which showed negative results the treatment lasted for extremely short periods,

* *Federal Register* **23**, 1 (1968); *The Medical Letter*, **9**, 2 (1968); *Chemical Week*, **20**, 1 (1968); *British Medical Journal*, **1**, 235 (1969); Taubmann (1969), Hofmeister (1970), etc.

1–3 months. The most serious mistake in the application of flavonoids in the view of Kátó and Gözsy (1969) has been that flavonoid therapy has been suggested indiscriminately for the treatment of capillary fragility without taking into account the aetiology of the vascular damage. This has naturally led to numerous contradictory data.

As we too have stressed, the basic principle of therapy with flavonoids is that they must be applied after an adequate diagnosis, in an effective dose and for the necessary period of time (Gábor (1970)).

Side-effects. Until 1969 it was generally accepted that flavonoid therapy does not lead to serious side-effects. However, the work of Pfeifer *et al.* (1969a, b) showed that parenterally applied rutin, i.e. its sodium salt ('Rutabion'), precipitated and formed concrements and even suppurative inflammations in the liver-bile ducts and renal channels of newborn, above all, of prematurely born babies. When comparatively high doses of soluble rutin ('Rutabion') were injected into newborn mice, rats and golden hamsters for several days, or extremely high single doses were administered, the same changes as mentioned above were observed (Pfeifer *et al.* (1969c)). (As a result of these studies the preparation has been withdrawn from circulation in the German Democratic Republic.)

According to the more recent studies of Pfeifer *et al.* (1970) on adult mice, parenterally applied rutin (single doses of 2,000–3,000 mg/kg, total dose 2,000–18,000 mg/kg), which is considered to be insoluble in water, leads to concrement formation in individual cases. Treatment with the sodium salt of rutin (Rutinion®, Rhein-Pharma, Heidelberg) (single doses of 200–5,000 mg/kg, total dose 1,400–8,000 mg/kg) led to similar results. Application of the sodium salt of the sulfuric acid ester of rutin (Birutan®, E. Merck AG., Darmstadt)

did not produce any precipitations or concrements in the liver-bile ducts and kidney — not even if applied in extremely high over-dosages (single doses of 5,000–20,000 mg/kg, total dose 5,000–30,000 mg/kg). Pfeifer *et al.* (1970) found that O-(β-hydroxyethyl)rutin (Venoruton®, Zyma–Blaes AG., München) was the only compound which did not show any toxic effects even at extremely high over-dosages (single doses of 20,000–40,000 mg/kg, total dose 60,000–160,000 mg/kg). No histological changes could be observed in the liver and kidney of the mice.

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II. THE ANTI-INFLAMMATORY ACTION OF FLAVONOIDS

(Review)

In recent years great interest has been attached to flavonoids. More than a dozen reviews have appeared on this theme. However, if the largest reviews (Böhm (1968), Shils and Goodhart (1956) and Griffith, Krewson and Naghski (1955)) are examined, it is found that the anti-inflammatory action of flavonoids is dealt with in a few lines, or is not mentioned at all, although this question has already been treated in numerous publications. Widespread studies over about twenty years now require that a comprehensive picture be obtained of this field.

The aim of this chapter is to review the anti-inflammatory action of the bioflavonoids according to the following order:

1. Influence on mouse and rat paw oedema
2. Generalized oedema in rats
3. Effect on the development of Selye granuloma pouch
4. Inflammation caused by cotton pellet
5. Erythema produced by UV radiation
6. Inflammation produced by mustard oil
7. Influence on the permeability-increasing action of the inflammatory exudate
8. Influence on the inflammation produced by red paprika (*Capsicum annuum* L. Solanaceae)
9. Effect of citrus flavonoid complex on experimentally induced mucous membrane inflammation

10. Influence on experimentally induced thrombophlebitis
11. Allergic and hyperergic inflammation of the skin and joints

1. INFLUENCE ON MOUSE AND RAT PAW OEDEMA

Gross (1950a, b) reported that if rutin was administered s.c. (100 mg/kg in propylene glycol) 2 hours before the local application of egg white, then oedema formation in rats was inhibited. The solvent, propylene glycol, itself (100 mg/kg s.c.) possesses a smaller but pronounced inhibiting action.

Küchle and Wegener (1952) attempted to influence rat paw oedema induced by a sub-plantar injection of 0.1 ml egg white or of egg white diluted in a ratio 1 : 10 with physiological salt solution, by treatment with rutin (Birutan®, Merck), citrin (Citrin®, Hoechst) and epicatechin (Citrin 'E'®, Hoechst). The rats received the flavonoids in 100 mg/kg doses s.c. 1 hour before the local application. The experiments showed that rutin caused a moderate retardation of the oedema formation, and that it was inhibited by citrin by 20% on average. The effect of epicatechin on the oedema caused by undiluted egg white was not pronounced, but oedema caused by the diluted egg white was inhibited by 19% on average.

Vogel and Marek (1961) were able to decrease significantly rat paw oedema produced by sub-plantar administered 0.2 ml 24% PVP (polyvinylpyrrolidone), by the i.v. injection of rutin (100 mg/kg).

Texl (1963) found that rat oedema caused by formalin or hyaluronidase was significantly inhibited by the combined administration of vitamin C (100 mg/kg) and tea

leaf catechin complex (tea tannin 100 mg/kg s.c.). This effect was not observed in adrenalectomized rats. It is interesting to note that the application of tea tannin or vitamin C alone did not significantly affect the experimentally produced inflammation.

Likewise, in the mouse experiments of Vogin and Rossi (1963) paw oedema produced by serotonin was not affected by orally applied doses of ascorbic acid of 150, 300, 600 and 1,200 mg/kg. Pre-treatment with hesperidin methylchalcone similarly proved to be without effect. Paw oedema caused by 5-HT (5-hydroxytryptamine) was not decreased to a significant extent by the combined application of ascorbic acid (150 mg/kg) and hesperidin methylchalcone (150 and 600 mg/kg).

Martin *et al.* (1953) found that paw oedema induced in rats by egg white can be moderated to an appreciable extent with phosphorylated hesperidin (200 mg/kg or 2×200 mg/kg) administered orally or s.c. Also worthy of mention is the synergistic effect of phosphorylated hesperidin administered together with trypsin.

According to the studies of Formanek and Höller (1960), orally administered O-(β-hydroxyethyl)rutin (2 g/kg) inhibits rat paw oedema induced by the application of dextran or hyaluronidase, whereas formalin oedema and serum oedema are not affected. It was found by Gross (1950a), however, that if rutin is administered (100 mg/kg s.c.) 2 hours before the formalin injection, the acute formalin inflammation is inhibited.

Van Cauwenberge and Franchimont (1967) were able to inhibit oedema induced by formalin, dextran, serotonin and bradykinin with a O-(β-hydroxyethyl)rutin treatment, but on the other hand they could not affect the extent of oedema caused by histamine. This rutin derivative was administered (30 mg/kg i.p.) to rats 48, 24, 6 and 1 hour before the oedema-inducing injection. The intensity of the oedema in adrenalectomized rats

TABLE 3

Inhibition of different rat and mouse

Author	Flavonoid	Dose, mg/kg
Gross (1950)	Rutin	s.c. 100
Küchle and Wegener (1952)	Rutin	s.c. 100
	Epicatechin	s.c. 100
Martin *et al.* (1953)	Phosphorylated hesperidin	p.o. 200 or s.c. 2×20
Formanek and Höller (1960)	HR*	p.o. 2000
Vogel and Marek (1961)	Trimethylol-rutin	i.v. 100
Texl (1963)	Vitamin C + tea leaf catechin complex	s.c. 100
Vogin and Rossi (1963)	Vitamin C + HMC**	p.o. 150–1,200 + 150 and 600
van Cauwenberge and Franchimont (1967–1968)	HR*	i.p. 30
Bonta (1969)	Rutin	i.p. 500
Khadzhai *et al.* (1969)	Acacetin	p.o. 25–100
Riesterer and Jaques (1970)	Rutin	p.o. 300
Leuschner (1970)	HR*	gel form

* HR = O-(β-hydroxyethyl)rutin
** HMC = hesperidin methylchalcone

did not decrease after the application of O-(β-hydroxyethyl)rutin.

Khadzhai *et al.* (1969) found that mouse paw oedema induced by formalin decreased by 34–72% as a result

paw oedemas by flavonoids

Oedema inducer											
Serotonin	Histamine	Bradykinin	C.p.d. 48/80	Hyaluronidase	Egg white	Formaldehyde	Cobra venom (early phase)	Cobra venom (delayed phase) PVP	Dextran	Kaolin	Trauma
					+						
					+						
					+						
					+						
				+		−					
									+		
				+		+					
−											
+	−	+				+					
+	++		+		+		+	+		+	
						+					
											+
											+

of preliminary treatment with acacetin (25, 50, 100 mg/kg p.o.). The experimental animals received the acacetin in a 1% starch mucilage suspension 2 hours before the oedema was induced.

In the experiments of Bonta (1969), injection of a large dose of rutin (500 mg/kg i.p.) led to a pronounced inhibition (ca. 50%, or more) of rat paw oedema induced by histamine, and to moderate inhibition (25–30%) of oedema induced by serotonin, c.p.d. 48/80, egg white, snake venom, kaolin or PVP.

According to Riesterer and Jaques (1970), the extent of traumatic oedema in rats can be significantly reduced by the application of rutin (300 mg/kg p.o.). Leuschner (1970) has also reported on the similar effect of O-(β-hydroxyethyl)rutin used in gel form.

For the convenience of the reader, the results of the various studies listed above are given in table form (Table 3).

2. GENERALIZED OEDEMA IN RATS

It is well known that the i.p. injection of dextran into experimental animals leads to the development of oedema localized to the regions of the limbs, the cheeks and the scrotum. The influence of flavonoids on the generalized dextran oedema of rats was reported by Hammersen and Möhring (1968, 1970).

According to histological studies made in the subcutaneous connective tissue at the back of the feet of dextran-treated experimental animals, the endothelia of the blood vessels show the same changes, of which in particular the button-like and wart-like protrusions are the most striking. In addition, the larger intracytoplasmatic vacuoles and vesicles multiply, and this can lead to the formation of transendothelial ducts.

If O-(β-hydroxyethyl)rutin is injected (10 mg/100 g i.v.) 1 hour before the inducement of dextran oedema, or is administered for 7 days (30 mg/100 g p. o. daily), the normally occurring endothelial changes are appre-

ciably decreased. Most conspicuous is the regression of the endothelial protrusions tending towards the lumen. Apart from this, the cytoplasmatic cell changes largely disappear and the larger intraendothelial vacuoles decrease significantly. This can be attributed to the absence of transendothelial dehiscence. In contrast, the inter-cellular openings do not disappear, but their number and extent decrease.

3. EFFECT ON THE DEVELOPMENT OF SELYE GRANULOMA POUCH

Using the Selye granuloma pouch method (1953), Salgado and Green (1955) investigated the anti-inflammatory actions of calcium flavone glycoside, lemon bioflavonoid complex, hesperidin complex, hesperidin, hesperidin methylchalcone, hesperetin, naringin and naringenin. The granuloma pouch was induced in rats by the injection of croton oil; directly afterwards, and daily for 10–12 days, the animals received a bioflavonoid treatment (once daily in the case of s.c. administration, twice daily in the case of oral application). The parenteral injection produced a significant reduction in the volume of the inflammatory exudate, whereas the oral doses (200, 800 or 1,600 mg/kg) proved ineffective.

The greatest anti-inflammatory effect was observed after treatment with naringin (100 mg/kg), naringenin (43 mg/kg) and purified hesperidin (100 mg/kg), and the smallest effect after treatment with the hesperidin complex (100 mg/kg). In the case of the use of hesperidin methylchalcone (100 mg/kg), merely a very small effect or none at all was observed.

Experiments on adrenalectomized rats showed that the anti-inflammatory effect is independent of the hypophysis–adrenal gland system.

The anti-inflammatory action of O-(β-hydroxyethyl) rutin was likewise examined by Radouco-Thomas *et al.* (1964) by the Selye granuloma pouch technique. They compared the effects on rats of daily oral treatments for 10 days with the rutin derivative (1, 2 and 4 g/kg), phenylbutazone (0.1 g/kg) and acetylsalicylic acid (0.2 g/kg). Treatment was begun 3 days before the granuloma pouch was induced and the weight of the 8-day granulomatous wall and the volume of the exudate were measured. The study showed that the weight of the wall was decreased on average by 25.8% by phenylbutazone, by 24.2% by the highest dose of the rutin derivative, and by 18% by acetylsalicylic acid. The decrease of the volume of exudate after phenylbutazone and acetylsalicylic acid treatments was 52 and 35.5%, respectively. O-(β-Hydroxyethyl)rutin (4 g/kg) reduced the formation of the exudate by 36%. The effect of smaller doses of the rutin derivative was less pronounced.

It is also worthwhile mentioning that in experiments on rats Radouco-Thomas *et al.* did not observe mortalities after the oral administration of the rutin derivative in doses of 40 g/kg. (After the intraperitoneal injection of this compound the observed DL_{50} was 27 g/kg.)

van Cauwenberge and Franchimont (1968) treated rats with small doses of O-(β-hydroxyethyl)rutin (3 mg/100 g i.p.) for 9 days following injection of croton oil to induce granuloma pouch. In contrast with the previous experiments, these small doses did not affect the extent of inflammation: neither the weight of the granuloma pouch nor the volume of the exudate decreased. In more recent experiments the application of larger doses (10, 25, 50, 100 and 200 mg/kg) also proved ineffective (van Cauwenberge *et al.* (1969)).

4. INFLAMMATION CAUSED BY COTTON PELLET

Only a few data are known on the influence of flavonoids on cotton pellet-induced inflammation.

Texl (1963) implanted sterile cotton wool under the skin of rats by the method of Meier *et al.* (1950). At the same time as the implantation, tea tannin was administered in 100 mg/kg doses s.c. or by tube p.o., either alone or together with vitamin C in 100 mg/kg doses. In some cases vitamin C, too, was administered alone in 100 mg/kg. The controls were injected with physiological salt solution. The pharmaceutical treatment was repeated on the 2nd, 4th and 6th days. In certain experimental groups the granuloma was removed on the 7th day and weighed after drying.

These studies showed that if tea tannin was administered s.c. together with vitamin C, the weight of the granuloma decreased by 30.8% compared with the control. In the case of oral administration the value of the decrease was not significant. Treatment with vitamin C alone also proved ineffective.

According to the studies of van Cauwenberge and Franchimont (1968), treatment with 3, 10 and 200 mg/100 g doses of O-(β-hydroxyethyl)rutin caused no significant change, either in the total weight of the inflamed tissue in the vicinity of the implanted cotton wool, or in the weight of the isolated 'foreign body' wall. In contrast, a 50 mg/100 g dose produced a significant weight decrease of both the abscess formed near the cotton pellet and the granulomatous wall. 25 and 100 mg/100 g doses decreased only the weight of the granulomatous wall.

Also in rat experiments with foreign body implantation, Makarov and Khadzhai (1969) demonstrated the anti-inflammatory effects of quercetin, kaempferol, kaempferol-7-rhamnoside and kaempferol-3,7-dirhamno-

side. The experimental animals received the flavonoids for 7 days in a daily dose of 50 mg/kg in a 1% starch suspension p.o.

5. ERYTHEMA PRODUCED BY UV RADIATION

Only one paper had previously been written on the influence of flavonoids on UV erythema: the erythema inhibiting action of hesperidin was described by Winder *et al.* (1958).

We recently reported a procedure for the evaluation of UV-induced erythema and experiments with a flavone derivative using this method (Antal and Gábor (1970), Gábor and Antal (1970)).

The experiments carried out to examine the influence of O-(β-hydroxyethyl)rutin on UV erythema are detailed later (see Part Two, Chapter IV, p. 84).

6. INFLAMMATION PRODUCED BY MUSTARD OIL

Gross and Meier (1948) reported that the development of chemosis induced by drops of 10% mustard oil in the sac of the conjunctiva of rabbits was affected to only a small extent by rutin (25–100 mg/kg i.v. or s.c.) in propylene glycol solution. (The time of administration of the rutin was not given.) A good protecting action was attained, however, if the rabbits were treated with rutin (50–100 mg/kg s.c.) in propylene glycol 2 hours before the mustard oil drops were applied to induce the inflammation (Gross (1950b)).

In our own experiments (Gábor and Szórády (1952)), we succeeded in decreasing the inflammation induced by mustard oil on the depilated skin on the backs of rabbits with haematoxylin from among the indeno-

chromene derivatives included as bioflavonoids. As a result of the haematoxylin treatment (200 mg/kg i.p.) the skin reaction decreased in every experimental animal, without exception. Rabbits which reacted to the mustard oil plaster with very strong skin inflammation and with scale and scab formation on the control side, displayed at most a strong reddening of the skin after the haematoxylin treatment. (The extent of the skin reaction was decided 24 hours after the removal of the plaster.)

7. INFLUENCE ON THE PERMEABILITY-INCREASING ACTION OF THE INFLAMMATORY EXUDATE

Sokoloff *et al.* (1951) reported that the permeability increase induced in rabbits by the leucotaxin isolated from the inflammatory exudate (3 mg per animal s.c.) can be inhibited by a bioflavonoid ('Citrus Vitamin P') treatment (10 mg/kg s.c.). The CVP was administered 20 minutes before the leucotaxin injection. The study was made using the technique of Menkin (1940).

Menkin (1959) later studied the effect of CVP on the increase of capillary permeability induced in rabbits by the exudate, using another method. (The exudate was produced by the intrapleural injection of turpentine oil.) 5–10 mg CVP was mixed with 0.25 ml of alkaline or acidic exudate, the mixture was allowed to stand for 10–15 minutes, and then injected intracutaneously into the abdominal skin of the rabbits.

For comparison purposes 10 mg exudate mixed with cortisone acetate was injected i.c. into another region of the abdominal skin. After this a 1% trypan blue solution was injected into the ear vein of the animals. A trypan blue spot appeared on the site of injection of

the exudate alone, while merely a brown discolouration was observed on the site of the injection of exudate mixed with CVP. The CVP also inhibited the migration of the leucocytes. In a similar way, cortisone acetate impedes and decreases the outflow of the dye and the leucocytes.

On the basis of these results, Menkin (1959) notes that CVP deserves further research as an anti-inflammatory agent.

8. INFLUENCE ON THE INFLAMMATION PRODUCED BY RED PAPRIKA

(*Capsicum annuum* L. Solanaceae)

According to the experiments of Jancsó (1965), if a small piece of strongly pungent paprika is attached to the inside surface of the arm, an acute inflammatory reaction is produced. In this inflammatory process we have studied (Gábor 1970) the change of capillary resistance (CR) and the effectiveness of flavonoids on this in albino guinea pigs. The CR was determined with the Lavollay–Neumann (1949) apparatus. The diameter of the suction-bell was 8.5 mm, and suction was applied for 30 sec.

Paprika disks (1.5 cm diameter) were applied to the depilated backs of 30 guinea pigs of both sexes, weighing 270–360 g. As a result, the CR decreased significantly at the site of the paprika disk in every animal, on average by 5.4 cmHg. As is well known, the compound responsible for the hot taste of paprika is capsaicin. In another experimental series on 15 different guinea pigs we succeeded in showing that the result of an exactly similar treatment with Capsoderma® ointment (Biogal, Debrecen) containing capsaicin was a decrease of CR in every animal, on average by 4.8 cmHg.

In another series of experiments, 30 guinea pigs were treated with hesperidin methylchalcone (250 mg/kg i.p.); paprika disks were then applied to half of these animals and the Capsoderma ointment to the other half. Determination of the CR 45 minutes after the injections showed that it had not decreased. In these latter two series the CR before the application of the paprika disks and the Capsoderma ointment was 24.46 and 24.80 cmHg, respectively, and after the hesperidin methylchalcone treatment 24.33 and 24.66 cmHg, respectively.

Thus the decrease of capillary resistance can be completely prevented by a previous treatment with hesperidin methylchalcone.

9. EFFECT OF CITRUS FLAVONOID COMPLEX ON EXPERIMENTALLY INDUCED MUCOUS MEMBRANE INFLAMMATION

The mucous membrane method for the study of anti-inflammatory substances was developed by Kátó and Gözsy (1969).

One ml of diluted formaldehyde solution (1 ml formaldehyde + 14 ml distilled water) was injected by tube into the recta (washed to a depth of 5 cm with physiological salt solution) of rats. Four hours later a colloidal carbon suspension was injected into the tail vein (1 ml/100 g). (Preparation of the suspension: 30 ml 'Günther' Indian ink was suspended in 70 ml of a 3% gelatin solution prepared with distilled water.) One hour after the injection of the carbon suspension the animals were killed by decapitation and the recta taken out after laparotomy. These were cut open longitudinally, washed in running water and spread out on white paper. The determination of the intensity of the carbon deposition in the rectum was performed with

the use of an empirically prepared scale. It was found that the rectal application of dilute formalin leads to vasodilatation and the increase of vascular permeability in the mucous membrane.

Using this method, Kátó and Gözsy (1969) showed that the application of citrus flavonoid complex (CFC) inhibits the blood vessel damage caused by formaldehyde in the mucous membrane. Rats received 0.5 ml of a CFC solution i.v. on two occasions: half an hour or 1 hour before or after the formaldehyde injection.

10. INFLUENCE ON EXPERIMENTALLY INDUCED THROMBOPHLEBITIS

A method for developing thrombophlebitis experimentally has been evolved by Földi and Zoltán (1965). The hind legs of dogs were depilated and the circumference measured at the height of the patella. The animals were subjected to hexobarbital narcosis, and the homolateral vena iliaca externa was ligated. 0.2 ml turpentine oil was injected into the lymphatic lumen alongside the vena saphena in the lower leg. In this way a strong oedematous inflammation was induced and the extent of the inflammation was recorded by daily measurement of the circumference of the limb at the patella. A *Melilotus* preparation, Esberiven® (Schaper–Brümmer, Salzgitter–Ringelheim; composition: sodium salt of the sulfuric acid ester of rutin (50 mg) and coumarin (1 mg) to 2 ml), was administered twice daily to examine its effect on the experimentally produced thrombophlebitis.

An average of 17 days was necessary for the circumference of the limbs of the untreated animals to decrease to the original value, while in the treated animals only a period of 7.5 days was required. The increase in the

limb circumference of the untreated dogs was 158% relative to the starting value, and in the treated animals only 120%. The difference was statistically significant.

Recent studies by Földi *et al.* (1970) show that the daily application (i.m.) per se of rutin (25 mg/kg) and coumarin (4 mg/kg) — the essential principles of Esberiven — exerts a statistically significant effect in experimental thrombophlebitis. A potentiation of the effect is observed in the case of simultaneous administration of the two substances. According to recent research the formation of oedemas following osteotomy of the femur and osteosynthesis in dogs is largely supressed by Esberiven. The swelling is significantly less and subsides more quickly (Hopf *et al.* (1971)).

11. ALLERGIC AND HYPERERGIC INFLAMMATION OF THE SKIN AND JOINTS

Fassbender and Pippert (1954) produced allergic and hyperergic inflammation and Arthus phenomenon in the thighs and hind legs of rabbits sensitized with equine serum by reinjection of the serum s.c. For 3 days before the re-injection and for 36 hours after it the animals received a 12 hourly intravenous treatment of rutin (Birutan®, Merck) (in all, 500 mg rutin per animal). The animals were killed and the appropriate skin area and knee-joints processed histologically. The inflammatory change was classified into one of four grades of severity. In the lighter cases of Arthus phenomenon in the control animals merely moderate oedema and slight granulocytosis were observed, in the more serious cases pronounced phlegmon and necrosis. Study of the joints showed that in the mildest cases slight oedema of the articular villi and a small extent of proliferation in the

joint membrane could be observed. In the most serious cases, large-scale granulocyte infiltration, fibrinoid necrosis and granuloma could be seen in the articular capsule, and fibrin and pus cells in the articular cavity, while the surroundings of the joint were infiltrated by granulocytes and lymphocytes.

The i.v. rutin treatment of the rabbits clearly and strongly inhibited the inflammation both in the skin and in the joint. The phenomenon is explained by the capillary sealing and exudation-inhibiting actions of rutin, and by its protective effect on the capillary wall and against anaphylactic shock.

12. DISCUSSION

If the results of the studies of the anti-inflammatory action of flavonoids are considered, the following findings emerge:

(1) According to the overwhelming majority of authors (Gross (1950a, b), Küchle and Wegener (1952), Martin *et al.* (1953), Formanek and Höller (1960), Vogel and Marek (1961), Texl (1963), van Cauwenberge and Franchimont (1967, 1968, 1969), Bonta (1969), Khadzhai *et al.* (1969), Riesterer and Jaques (1970)), prior treatment with flavonoids decreases the extent of oedema induced in mouse and rat paw by different methods. The majority of the studies were made with rutin or a rutin derivative.

(2) The blood vessel endothelial changes usually appearing in generalized dextran oedema are significantly decreased by treatment with a rutin derivative (Hammersen and Möhring (1968, 1970)).

(3) According to Salgado and Green (1955), the volume of the Selye granuloma pouch inflammatory exudate can be significantly decreased by a parenteral flavonoid treatment. As a result of the oral administra-

tion of large doses of HR, Radouco-Thomas *et al.* (1964) observed a decrease of both the weight of the granuloma pouch wall and the volume of the exudate. In contrast with the previous experiments, in the studies by van Cauwenberge *et al.* (1968, 1969) O-(β-hydroxyethyl)rutin treatment did not decrease either the weight of the granuloma pouch or the exudate volume.

(4) According to Texl (1963), van Cauwenberge and Franchimont (1968) and Makarov and Khadzhai (1969), inflammation induced by foreign body implantation can be reduced to a considerable extent by a prior treatment with flavonoids.

(5) The intensity of the erythema produced by UV irradiation can be markedly reduced by the use of hesperidin or O-(β-hydroxyethyl)rutin (Winder *et al.* (1958), Gábor and Antal (1970)).

(6) Inflammation induced by the application of mustard oil to the eye or the skin is moderated by flavonoid treatment (rutin, or indenochromene derivatives) (Gross and Meier (1948), Gross (1950a, b), Gábor and Szórády (1952)).

(7) The capillary permeability increase induced by inflammatory exudate can be inhibited by a 'citrus vitamin P' treatment (Sokoloff *et al.* (1951), Menkin (1959)).

(8) The decrease of skin capillary resistance during inflammation caused by the application of red paprika can be avoided by the prior treatment of the animals with hesperidin methylchalcone (Gábor (1970)).

(9) Citrus flavonoid complex treatment inhibits the capillary permeability increase and the vasodilatation brought about in the rectal mucosa of the rat by a formaldehyde solution (Kátó and Gözsy (1969)).

(10) The recovery time from thrombophlebitis experimentally induced in the hind legs of dogs is substantially shortened as a result of treatment with

Esberiven preparation containing rutin and coumarin, and the degree of tumescence is significantly less compared with the controls (Földi and Zoltán (1965), Földi *et al.* (1970)).

(11) Fassbender and Pippert (1954) found that allergic-hyperergic inflammation in rabbits can be strongly inhibited by rutin treatment.

It can be seen from the above that the anti-inflammatory action of the flavonoids has been demonstrated in numerous model studies. The experimental results make likely the anti-inflammatory effect of certain flavone derivatives.

The mechanism of action of this group of compounds must be discussed. The recent studies of van Cauwenberge and Franchimont (1968) and Kátó and Gözsy (1969) provide very valuable data for this purpose. According to van Cauwenberge and Franchimont (1968), 1 or 2 hours after the injection of O-(β-hydroxyethyl)-rutin (3 mg/100 g i.p.) the ascorbic acid and cholesterol contents of the adrenal glands decrease significantly, while the fluorescing steroid level of the blood plasma significantly increases. The increase of the plasma steroid level follows 2–4 hours after the i.m. administration of the chemical, and 4 hours after its oral administration. Hence, the mode of action of the rutin derivative is probably based on the stimulation of the adrenal gland cortex.

Intradermal injection of histamine, one of the chemical mediators formed during the inflammation, causes vasodilatation and the increase of capillary permeability without any damage to the blood vessels. This reaction of histamine can be blocked with an antihistamine; the action of citrus flavonoid complex (CFC) is much less pronounced (Kátó and Gözsy (1969)).

Especially worthy of attention are the studies of Kátó and Gözsy (1969) relating to the role and effect

of noradrenaline. Among others, also Kátó and Gözsy emphasized that the catecholamines are the natural anti-inflammatory hormones in the early stage of inflammation. As is well known, if histamine is injected i.d. into the abdominal skin of rats immediately after the i.d. injection of noradrenaline in the same spot, it no longer causes vasodilatation and the increase of capillary permeability. If, however, the i.d. administration of histamine follows 4 hours after the injection of noradrenaline, then very considerable vasodilatation, the rupture of the capillaries, and the total destruction and degranulation of the mastocytes ensue at the site of the administration of the histamine and noradrenaline. This early blood vessel reaction is inhibited in rats treated with citrus flavonoids: vasodilatation, rupture of the venal capillaries, and destruction of the mastocytes were not observed.

In further experiments they demonstrated the potentiality of sub-effective noradrenaline doses opposing the effects of histamine. 19, 39, 75 and 150 nanogram sub-effective doses of noradrenaline do not affect the vasodilatation induced by histamine. In rats pretreated with CFC, however, the vascular effects of histamine are inhibited by the same i.d. injected doses of noradrenaline. From this latter experimental result, Kátó and Gözsy (1969) put forward the possibility that the physical or enzymatic (catechol-O-methyltransferase or monoamineoxidase) inactivation of noradrenaline is inhibited by CFC. We should like to add two notes to the above results: the effect of flavonoids on the capillary resistance was explained by Lavollay and Neumann (1941) and Lavollay (1941, 1945) by the action of flavonoids in inhibiting the autoxidation of adrenaline. Masquelier *et al.* (1965) reported on the competitive inhibiting action of leucocyanidin on catechol-O-methyltransferase.

From all this, Kátó and Gözsy (1969) concluded that the essential pharmacological action of the water-soluble flavonoids is the protection of the mastocytes from disintegration and degranulation during some stress. As a result, the liberation of the pre-inflammatory amines is inhibited. On the other hand, the flavonoids obstruct the physical inactivation of noradrenaline. The protection of the vascular integrity is the cumulative effect of these two factors: the prevention of the liberation of the pre-inflammatory amines and the inhibition of the inactivation of the anti-inflammatory amines.

According to van Cauwenberge *et al.* (1969), O-(β-hydroxyethyl)rutin (10 mg/100 g i.p.) inhibits the permeability-increasing action of histamine, serotonin and bradykinin (Evans blue test). The permeability-decreasing effect of the rutin derivative was demonstrated by the chloroform test.

Our own experiments have also confirmed that the permeability increase induced in rats by i.c. administered bradykinin can be avoided by the prior treatment of the experimental animals with O-(β-hydroxyethyl)-rutin or hesperidin methylchalcone. In further experiments we have shown that the contraction caused by bradykinin or serotonin in the isolated uterus of the rat can be inhibited to a significant extent by the above rutin derivative (Gecse *et al.* (1970)).

From among the most recent studies it is worth noting that Felix (1970) was able to inhibit the histamine-induced contraction of the isolated ileum of the guinea pig with O-(β-hydroxyethyl)rutin.

Our knowledge relating to the anti-inflammatory action of bioflavonoids has increased to a significant extent during the last 20 years, and we have come nearer to an understanding of the mechanism of the action. It must be noted, however, that with very few exceptions researchers have carried out, in general, only one

model study for the demonstration of anti-inflammatory action, although as it has been often emphasized, numerous tests must be made to check the effect.

Even today the relation between the anti-inflammatory effect of the bioflavonoids and the biochemical effects has not been elucidated. As is well known, the flavone compounds are polyvalent, that is, they affect several enzymes (hyaluronidase, histidine decarboxylase, xanthinoxidase, succinoxidase, etc.). The effects of the flavonoids on the enzymes is attributed to SH-blocking and the complexing of certain essential metal ions.

According to Samveyan and Khlgatyan (1969), the anti-oedematous action of rutin is due to the fact that it increases the viscosity of hyaluronic acid and inhibits the activity of the membranous brain ATPase.

In the future it seems very important to carry out further biochemical studies: the demonstration of the effect on proteolytic enzymes (e.g. chymotrypsin), the effect stabilizing the lysosome membrane, the possible effect interrupting oxidative phosphorylation, etc.

The solution of the problems we put forward would lead for instance to the solution of such an important question as the recognition of the possible effect of flavonoids on chymotrypsin. (The degranulation of mast cells and the liberation of histamine are attributed to chymotrypsin (Pastan and Almquist (1966).)

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PART TWO

I. EFFECT OF O-(β-HYDROXYETHYL)RUTIN ON THE CHROMATE ION TRANSPORT OF RED BLOOD CELLS

As has already been mentioned, the flavonoids have stood in the forefront of interest since the studies by Armentano *et al.* (1936) of their pharmacological and therapeutic effects. Even today, however, their molecular biological actions are scarcely known. We wish to report below on transport experiments made on red blood cells with an isotope technique using the water-soluble flavonoid compound O-(β-hydroxyethyl)rutin (HR) (Latzkovits, Szentistványi and Gábor (1970)).

Method

The red blood cells of healthy men and women were used in the study. After the blood was taken in citrate, it was centrifuged at 600–800 r.p.m. at 0–2 °C, and the plasma and the white blood cell layer removed. The red blood cells were then washed three times at 0–2 °C with 10 volumes of 0.9% NaCl solution, the centrifugation being carried out at 1,000–1,200 r.p.m.

The cells were suspended in 1 : 4 ratio in a Krebs–Ringer phosphate solution of pH 7.35 not containing glucose; the radioactive concentration was 1 μCi carrier-free $Na_2{}^{51}CrO_4$. The suspension was incubated at 37 °C for 90 minutes with careful, froth-free shaking; samples were taken at 10-minute intervals and immediately cooled to 0 °C. The samples were likewise centrifuged at 0 °C and 0.5 ml of the supernatant solution was used

for activity measurement, performed with a γ-ray sensitive hollow crystal. Since the activity measurements of all the samples were made under identical conditions, the results were given in c.p.m. and this was plotted graphically. The HR was dissolved in Krebs–Ringer phosphate and was used in the study in concentrations of 1–100 μg/ml.

Results and discussion

The results of the transport experiments are shown in Fig. 1. It can be seen from the control experiments that the decrease of chromate concentration in the first 10 minutes is very fast, and then in the following period becomes asymptotic. The effect of HR is already pronounced in the section of the fast decrease, especially when used in high concentrations. The inhibiting effect at low concentration can be strikingly seen in the asymptotic section of the curve.

Relating to the effect of HR, Danon and Elazar (1968) observed a pronounced increase of glucolysis during *in vitro* experiments with red blood cells. Since, however, according to the data of Wittam *et al.* (1964), the regulator of glucolysis in the case of red blood cells is the membrane itself, the possibility is not excluded that the above-mentioned increase of glucolysis is a result of the fact that the point of attack is the membrane. On the basis of such considerations, the CrO_4 ion transport of red blood cells was selected as the model for permeability investigations. According to Kleine and Schmidt (1962), the CrO_4 ion transport is independent of the metabolism and primarily gives information on the membrane function. For this reason glucose was not used in the experiments.

From the results it can be said that HR decreases the red blood cell permeability as regards the CrO_4 ion

transport. These data are in agreement with the studies by Heténi (1968) with a synthetic flavonoid derivative of different structure. He observed that 7-hydroxy-

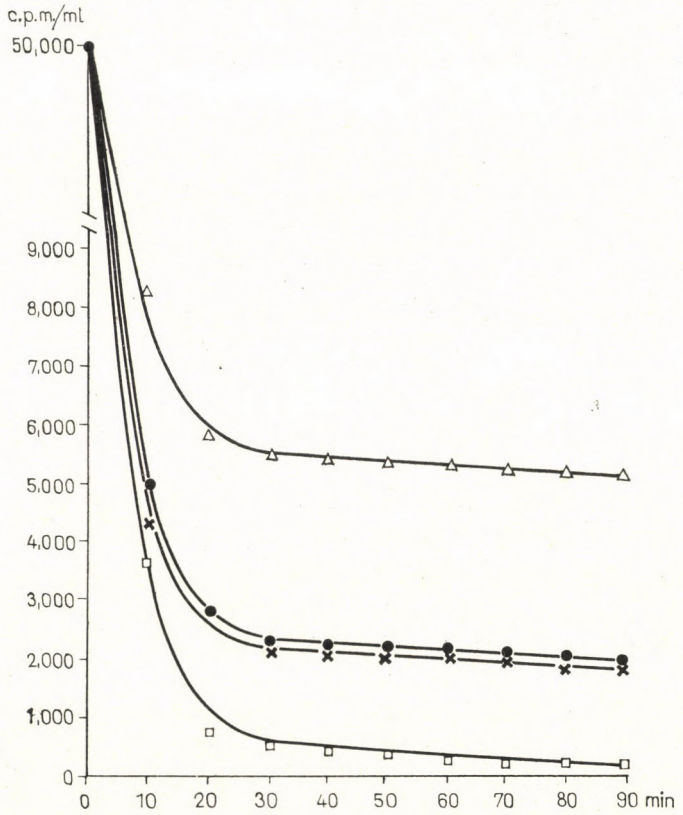

Fig. 1. Decrease of activity of the Krebs–Ringer phosphate solution. Cell : Krebs = 1 : 4. □: Control; ×: 1 μg HR/ml; ●: 10 μg HR/ml; △: 100 μg HR/ml

flavone-β-dimethylaminoethyl ether decreased the potassium loss of red blood cells.

To summarize, the effect of O-(β-hydroxyethyl)rutin on the CrO_4 ion transport of red blood cells was studied by a radioisotope method. The results showed that O-(β-hydroxyethyl)rutin markedly inhibits the CrO_4 ion transport even in a concentration of 1 μg/ml.

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II. DATA ON THE MECHANISM OF THE ANTI-INFLAMMATORY EFFECT OF FLAVONOIDS

In recent years the anti-inflammatory effects of certain flavonoids have been reported in an ever-increasing number of papers. Considering that bradykinin, which plays an important role in inflammation reactions of various aetiology, has come more and more to the centre of interest (Edery and Lewis (1962), Gecse *et al.* (1965, 1969a, b), Lewis (1964), Rocha e Silva and Antonio (1960), Szekeres and Gecse (1967), Webster (1968)), it appeared timely to provide newer data for the influence of flavonoids on this chemical mediator (Gecse, Zsilinszky, Horpácsy and Gábor (1970)).

Method

(*a*) *Studies on the isolated rat uterus.* 0.05 mg of oestradiol was given to each of 30 female R-Amsterdam rats of 180–200 g weight for the artificial accomplishment of oestrus. 20–24 hours following the treatment, the removed uterus was placed in a double-walled organ-bath containing 5 ml de Jalon solution at 30 °C with an atropine content of 10^{-6} g/ml. The temperature was maintained constant with an ultrathermostat.

Synthetic bradykinin (BRS 640 Sandoz) and serotonin creatine sulfate (Sandoz) were used as agonists in the organ-bath.

The contraction produced in the uterine muscle was recorded with a 6× magnification directly with the

side-writing lever on a kymograph, and the extent of the muscle contractions was expressed in mm.

O-(β-Hydroxyethyl)rutin (HR) was used as antagonist in concentrations of 10^{-5} and 10^{-4} g/ml.

(*b*) *Study of the capillary permeability.* Experiments were made on 30 male R-Amsterdam rats of 180–200 g weight.

Evans blue (10 mg/100 g) was administered i.v. to the animals, and then 1.25 or 2.5 microgram bradykinin in 0.05 ml physiological salt solution was injected intracutaneously into the previously depilated dorsal skin. 30 minutes after the bradykinin injection the animals were killed by decapitation. The dorsal skin was removed, cleaned of fat and connective tissue, and dried in a thermostat at 56 °C. The amounts of dye passed out from the blood vessels as a result of the permeability increase were extracted with pyridine by the method of Judach and Willoughby (1962) from about 150 mg of tissue excised from the sites of administration of the physiological salt and bradykinin. The dye was determined with a Beckmann spectrophotometer at 610 nm.

HR, hesperidin methylchalcone (HMC) (Gee Lawson Chemical, Ltd.) and aesculin (Fluka, Buchs) were used as antagonists. 100, 250 or 500 mg/kg HR, 100, 250 or 500 mg/kg HMC, 10 or 50 mg/kg aesculin was administered i.p. 15, 30 or 60 minutes before the i.c. administration of bradykinin.

Results and discussion

(*a*) *Studies on the isolated rat uterus.* Figure 2 shows the dose–effect curve produced on the isolated rat uterus with bradykinin. The contraction caused by the agonist is shown on the ordinate in mm. On the abscissa is the logarithm of the bradykinin dose.

Even in the case of a concentration of HR of 10^{-4} g/ml, an inhibiting effect of approximately 50% was observed; this further increased with increase of the amount of HR.

After the antagonist was washed away, the original contractions could be produced with the agonist on the smooth muscle preparation.

A similar inhibiting effect of HR was observed when serotonin was used as agonist on rat uterus. Our results

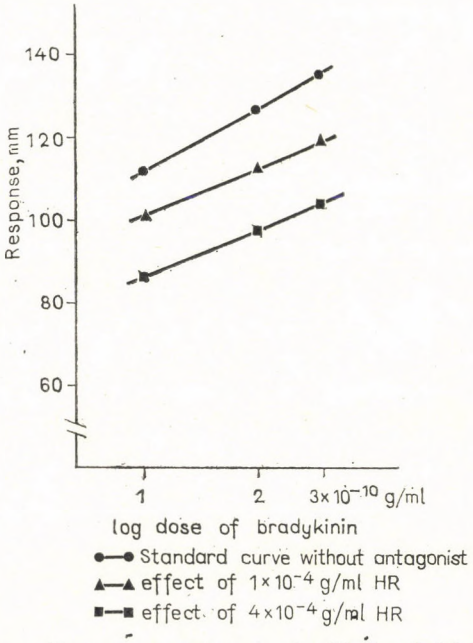

Fig. 2. Dose-effect curve determined with bradykinin in isolated rat uterus. The smooth muscle contraction induced by bradykinin is plotted on the ordinate in mm. The log bradykinin dose appears on the abscissa

are shown in Fig. 3, the method of plotting being the same as in Fig. 2.

A very significant smooth muscle contraction inhibiting effect was produced by 10^{-4} g/ml HR when serotonin was used as the agonist. With the increase of the quantity of antagonist the effect increased.

It must be mentioned that the inhibiting effect shown was observed when the HR had been acting on the muscle preparation for 15 minutes. These values are given in the plots of our results.

(*b*) *Influence on the capillary permeability increasing effect of bradykinin.* The change in the capillary per-

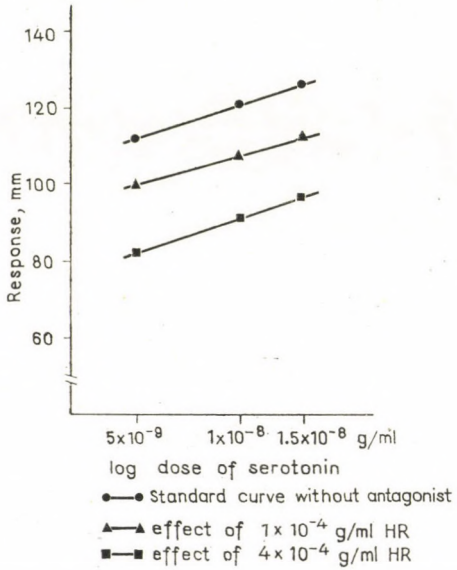

Fig. 3. Dose-effect curve determined with serotonin in isolated rat uterus. The ordinate and abscissa have the same significance as in Fig. 2

meability-increasing effect of bradykinin after a pretreatment with HR is shown in Fig. 4. The amount of Evans blue exuding from the blood vessels is plotted on the ordinate in μg, and the permeability increasing substance is given on the abscissa.

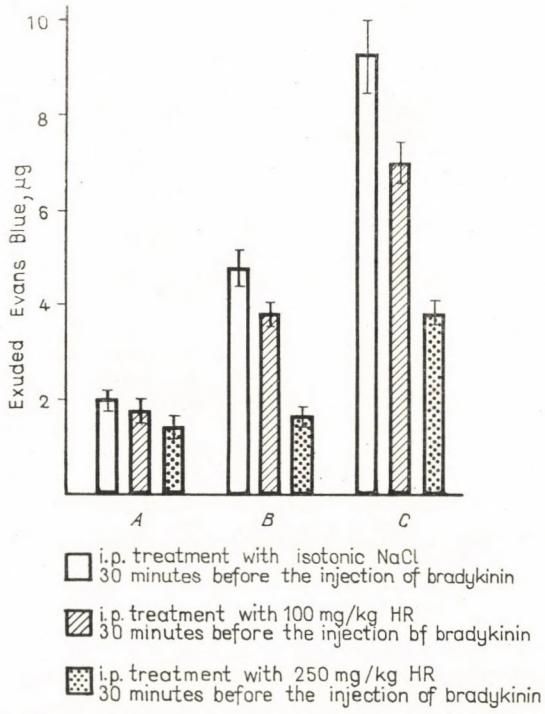

Fig. 4. Effect of HR on the vascular permeability increase induced by bradykinin. The amount of Evans blue exuded as a result of the vascular permeability increase is given on the ordinate in μg. On the abscissa: *A*: injection of physiological salt solution i.c.; *B*: injection of 1.25 μg bradykinin i.c.; *C*: injection of 2.5 μg bradykinin i.c.

As a result of even 100 mg/kg HR, a significant inhibiting effect was observed in the cases of both the smaller and the larger i.c. administered doses of bradykinin. This was very pronounced if the dose of HR was increased to 250 mg/kg.

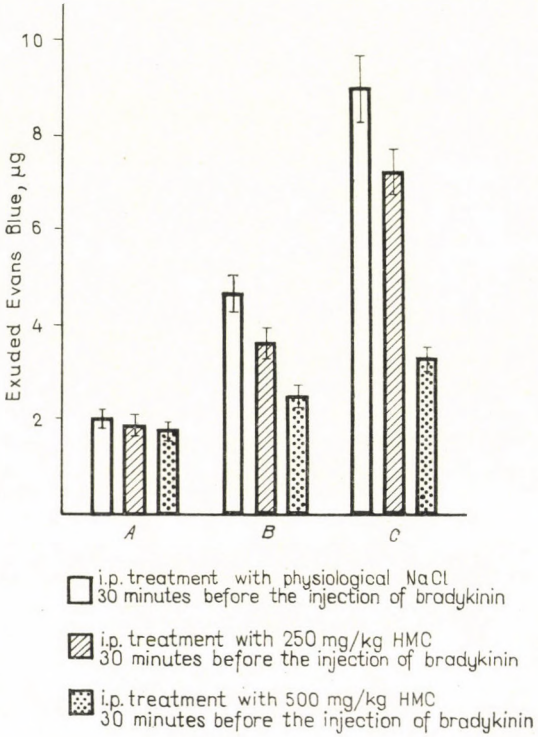

Fig. 5. Effect of HMC on the increased vascular permeability induced by bradykinin. The ordinate and abscissa have the same significance as in Fig. 4. *A*: Effect of physiological salt solution i.c.; *B*: 1.25 μg bradykinin i.c.; *C*: 2.5 μg bradykinin i.c.

The effect of HMC is shown in Fig. 5 with a plot similar to the previous one. As a result of the smaller dose, a significant but not considerable permeability increasing effect was observed, whereas with the larger dose the effect was very considerable.

With the use of the previously mentioned dose and time factors, aesculin was found in this study to be ineffective.

In a discussion of our own results it should be mentioned again here that van Cauwenberge and Franchimont (1968) found that rat paw oedema induced by bradykinin and serotonin is inhibited to a significant extent by HR (30 mg/kg i.p.).

In more recent rat experiments of van Cauwenberge *et al.* (1969) it was shown that after the administration of HR (100 mg/kg i.p.) the time of appearance of the blue stain at the site of i.c. injected histamine (50 μg), serotonin (2.5 μg) or bradykinin (0.5 μg) was significantly longer than in the control animals.

In agreement with our results, Leme and Walaszek (1969) and Chau and Haley (1969) demonstrated the actions antagonizing the smooth muscle contraction induced by bradykinin of several flavonoids, such as apiin, hesperidin, quercetin, rhamnetin and homoeryodictiol, on the isolated ileum of the guinea pig. A significant effect was not found with aesculetin.

In our own experiments, for the development of the optimum effect of HR, it was necessary to wait 15 minutes before administering the chemical mediators (bradykinin and serotonin). This fact raises the possibility that the linkage of HR to the receptor takes place very slowly.

The permeability-increasing effect of bradykinin is known from the studies of Rocha e Silva *et al.* (1949). Today a number of pharmacons are known which antagonize this effect (Cline and Melmon (1966), Gecse *et*

al. (1969a, b), Rocha e Silva and Antonio (1960), Rocha e Silva (1964)). According to our own experiments, the capillary permeability-increasing effect of bradykinin is inhibited to a significant extent by the use of HR and HMC.

Our results make it probable that the above flavonoids can be applied in the treatment of those inflammatory symptoms in which bradykinin or serotonin act as pathogenetic factors.

Thus our studies on the isolated uterus of the rat show that the application of O-(β-hydroxyethyl)rutin in a concentration of 10^{-4} g/ml significantly inhibits smooth muscle contraction induced by both bradykinin and serotonin. The effect is further increased when a higher amount of the rutin derivative is applied. The permeability increase induced in rats by the i.c. administration of bradykinin can be significantly decreased by a preliminary treatment of the experimental animals with the rutin derivative (100–500 mg/kg i.p.) or hesperidin methylchalcone (100–500 mg/kg i.p.).

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III. INFLUENCE OF FLAVONOIDS ON THE DECREASE OF CAPILLARY RESISTANCE IN INFLAMMATION CAUSED BY RED PAPRIKA

(*Capsicum annum* L. Solanaceae)

Our earlier investigations showed that the capillary resistance (CR) decreases in the inflammatory process induced by red paprika (Gábor and Endrényi (1970)).

In the present work we report on the influence of O-(β-hydroxyethyl)rutin (HR) on the CR decrease caused by the strong paprika.

Method

Similarly to our previous studies, the values of CR were determined by the method of Lavollay and Neumann (1949) (see p. 50). To induce the inflammation, a 2×5cm slice cut from red paprika picked in the autumn was affixed with its pungent side to the skin by sticking-plaster to the depilated back of rats, near the spinal column, for 45 minutes. The CR was determined at the site of the paprika slice, before treatment and immediately after the removal of the paprika. An aqueous solution of HR was injected i.p. into the experimental animals immediately prior to the attachment of the paprika slice.

The results were evaluated statistically by the Student 't' test.

Results and discussion

A total of 60 rats of both sexes, weighing 130–170 g, was used in the experiments in 2 groups. Analysis of

the results shown in Table 4 leads to the following findings.

Control group. Of the 20 rats examined, 19 showed a decrease in CR, and in 18 animals the decrease was 10 cmHg or more. In 2 cases, falls of 35 cmHg were observed. The extent of the decrease was significant ($p < 0.001$).

Treated groups. As a result of the i.p. administration of HR in doses of 100 mg/kg, of the 20 rats studied, 11 showed unchanged values of CR after the removal of the paprika slice, and of the other animals 2 even showed an increase of CR. In spite of this, the decrease of CR was still mathematically significant: $0.02 < p < 0.05$.

Of 10 rats pre-treated with doses of HR of 200 mg/kg, only 2 showed a decrease of CR and this was no longer significant: $0.8 < p < 0.9$.

When the rutin derivative was administered to 10 rats in very large doses of 400 mg/kg i.p., a decrease of CR was not observed in a single case; in fact, an increase was observed in 6 animals. The increase of CR was significant: $0.01 < p < 0.02$.

For convenience the results are shown graphically (Fig. 6).

As has already been mentioned, the CR decreasing effect of paprika is attributable to the substance responsible for its burning taste, capsaicin. In an earlier study, the CR on the depilated back of rats treated with capsaicin ointment decreased in every animal. From experiments made to study the mechanism of the effect, we came to the conclusion that the CR decreasing action of capsaicin is mainly due to the liberation of histamine and bradykinin (Gábor and Endrényi (1970)). The experiments of Bracco *et al.* (1954) and our former investigations (Gábor *et al.* (1969)) showed that, in an interesting way, with serotonin the CR is increased.

TABLE 4

Effect of O-(β-hydroxyethyl)rutin (HR) on the decrease of capillary resistance (CR) in inflammation induced by red paprika

No.	Control group		Treated groups					
	CR in cmHg before (*a*) and after (*b*) application of paprika slice		CR in cmHg before (*a*) and after (*b*) treatment		CR in cmHg before (*a*) and after (*b*) treatment			
			100 mg/kg HR i.p.		200 mg/kg HR i.p.		400 mg/kg HR i.p.	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*
1	30	20	40	35	20	20	20	30
2	20	5	10	10	20	20	30	30
3	20	5	30	30	40	40	20	40
4	20	5	25	25	40	45	40	45
5	10	5	20	10	40	50	25	30
6	45	10	45	45	30	35	20	20
7	20	5	10	10	20	25	15	25
8	20	10	25	20	25	15	20	20
9	20	10	35	35	40	30	15	15
10	25	10	30	20	40	40	20	30

11	30	30	30	35			
12	20	10	30	30			
13	30	10	35	35			
14	25	10	40	40			
15	30	10	40	35			
16	25	10	35	40			
17	30	10	20	10			
18	25	10	25	10			
19	40	5	40	40			
20	25	5	30	30			
$\overline{X}$	15.75		2.5		−0.5	−6	
$S_{\overline{X}}$	1.86		1.18		2.03	2.08	
p	$p < 0.001$		$0.02 < p < 0.05$		$0.8 < p < 0.9$	$0.01 < p < 0.02$	

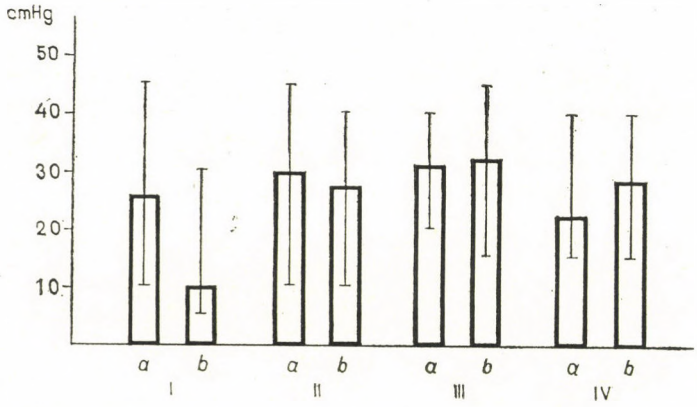

Fig. 6. Inhibition by O-(β-hydroxyethyl)rutin (HR) of the decrease of capillary resistance (CR) induced by red paprika. I *a*: CR before treatment with red paprika; *b*: CR after treatment with red paprika. II *a*: CR before treatment with red paprika + HR (100 mg/kg i.p.); *b*: CR after treatment with red paprika + HR (100 mg/kg i.p.). III *a*: CR before treatment with red paprika + HR (200 mg/kg i.p.); *b*: CR after treatment with red paprika + HR (200 mg/kg i.p.). IV *a*: CR before treatment with red paprika + HR (400 mg/kg i.p.); *b*: CR after treatment with red paprika + HR (400 mg/kg i.p.)

In the present study, a decrease of CR was not observed in 65% and 80% of rats following the i.p. injection of 100 mg/kg and 200 mg/kg of HR, respectively. As a result of the injection of 400 mg/kg of HR, the capillary resistance did not decrease in a single case, and in 60% of the animals it even increased.

As has already been reported earlier, the decrease of CR induced by paprika did not occur in a single rat of those pre-treated with another flavone derivative, hesperidin methylchalcone (250 mg/kg i.p.) (Gábor (1970)).

Thus, as a result of the attachment of a paprika slice to the depilated back of rats for 45 minutes, the capillary resistance at the site of irritation decreases to a significant extent. By pre-treatment of the experimental animals with O-(β-hydroxyethyl)rutin (200–400 mg/kg i.p.) the capillary resistance decreasing action of paprika can be avoided to a significant extent.

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IV. INFLUENCE OF O-(β-HYDROXYETHYL)RUTIN ON ERYTHEMA INDUCED BY UV RADIATION

The anti-inflammatory effects of the various flavonoids have already been dealt with in many papers. Interestingly, however, only one brief work is known (Winder *et al.* (1958)) concerning the action of flavonoids on the erythema induced by ultraviolet radiation (UV erythema), which is one of the most generally applied methods for the study of this effect.

The aim of the present chapter is to report the erythema-decreasing action of the water-soluble O-(β-hydroxyethyl)rutin (HR) (Gábor and Antal (1970)); the method used had been previously developed by us and permitted continual or optionally periodic recording (Antal and Gábor (1970)).

Method

For the details of the method the reader is referred to our earlier publication. Albino guinea pigs of the same strain, weighing 400–500 g, were used in the experiments; 24 hours before the study their backs were depilated. Before the treatment, two 20-mm diameter areas of skin, 10 mm apart on one side of the spinal column, were irradiated for 20 minutes with a 350 W analytical quartz lamp (Type AN-4, Irodagépipari és Finommechanikai Vállalat, Budapest) from a distance of 15 cm (control side). On the following day the same animals received HR (25, 100 or 250 mg/kg s.c.), and 60 minutes later the symmetrical areas on the other

side of the spinal column were irradiated under the same conditions as previously. The increase in intensity of the erythema was followed with a sensing head containing a light source and a photocell connected to a rectifier, and was recorded at 30-minute intervals by a Hellige multiscriptor. The deflection was measured in mm.

Results and discussion

Our studies were carried out at two different times (in January and April), on each occasion on 10 guinea pigs. One group received 25, a second 100, and a third 250 mg/kg HR s.c. (We wish to note here that in the case of the i.p. injection of HR in rats the DL_{50} was 27.16 g/kg, while of 5 guinea pigs treated per os with 45 g/kg HR one died (Radouco-Thomas *et al.* (1965)). Data referring to the toxicity of HR administered s.c. in guinea pigs are not available.)

The deflections recorded on the multiscriptor are given in Tables 5 and 6. The increase of the erythema intensity observed before and after the HR treatment is shown in columns *a* and *b*, respectively.

The difference between the intensities of the skin-reddening on the control side and on the symmetrical side after treatment with 100 or 250 mg/kg HR in the same guinea pigs becomes significant from 2.5 hours after irradiation. The injected dose of 25 mg/kg does not cause a significant change.

For convenience, the results are also plotted graphically (Fig. 7). It can be seen that in the first 1.5 hours after irradiation the curves show a fairly constant increase, but at about 2.5 hours they exhibit a striking separation, the maximum difference being observed after 3–3.5 hours.

TABLE 5

Effect of O-(β-hydroxyethyl)rutin (HR) (100 mg/kg s.c.) on the development of UV erythema in guinea pigs

No.	0 hr		1 hr		1.5 hr		2 hrs		2.5 hrs	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
1	0	0	0	0	0	2	6	6	14	8
2					2	0	7	2	19	6
3	0	0	0	0	3	4	7	8	21	13
4	0	0	0	0	4	0	9	2	16	5
5	0	0	0	0	5	10	10	14	25	20
6	0	0	0	0	6	0	12	2	26	10
7	0	0	0	0	5	4	14	6	38	9
8	0	0	0	0	8	6	14	8	30	11
9	0	0	0	0	11	19	19	13	31	12
10	0	0	0	0	9	6	18	8	33	14
$\bar{X}$	0		0		1.2		4.7		14.5	
S_x	0		0		1.0116		1.5133		2.3393	
p					$0.2 < p < 0.3$		$0.01 < p < 0.02$		$p < 0.001$	

TABLE 5 (*continued*)

No.	3 hrs		3.5 hrs		4 hrs		4.5 hrs		5 hrs	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
1	27	14	30	19	36	24	39	26	34	29
2	30	11	32	17	33	21	41	27	42	30
3	34	18	37	26	40	31	43	37	45	34
4	34	10	40	15	43	22	47	28	45	24
5	30	24	43	29	46	31	47	30	49	34
6	38	17	46	23	48	22	51	24	50	25
7	45	17	47	22	52	20	54	23	55	22
8	46	16	54	22	55	26	57	29	53	32
9	50	20	53	25	56	27	58	31	57	30
10	51	20	55	24	56	27	55	28	57	32
$\overline{X}$	21.8		21.5		21.4		20.9		19.5	
$S_{\bar{x}}$	2.1229		2.5571		2.7455		2.6139		2.7131	
p	$p < 0.001$		$p < 0.001$		$p < 0.001$		$p < 0.001$		$p < 0.001$	

Recorded deflection in mm before (*a*) and after (*b*) treatment with HR

TABLE 6

Effect of O-(β-hydroxyethyl)rutin (HR) (250 mg/kg s.c.) on the development of UV erythema in guinea pigs

No.	0 hr		1 hr		1.5 hrs		2 hrs		2.5 hrs	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
1	0	0	0	0	1	1	3	1	8	7
2	0	0	0	0	2	1	5	3	18	7
3	0	0	0	0	2	2	7	6	23	21
4	0	0	0	0	3	3	8	5	14	11
5	0	0	0	0	6	4	10	9	24	20
6	0	0	0	0	6	4	10	9	22	20
7	0	0	0	0	7	5	12	8	27	20
8	0	0	0	0	6	7	15	10	30	20
9	0	0	0	0	8	9	14	13	32	20
10	0	0	0	0	9	8	15	10	30	16
$\bar{X}$	0		0		0.6		2.5		6.6	
$S_{\bar{x}}$	0		0		0.3712		0.5217		1.5217	
p					$0.1 < p < 0.2$		$p < 0.001$		$0.001 < p < 0.01$	

TABLE 6 (*continued*)

No.	3 hrs		3.5 hrs		4 hrs		4.5 hrs		5 hrs	
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
1	12	10	17	16	18	17	20	20	22	20
2	28	10	35	15	40	19	40	26	41	23
3	30	27	37	30	40	34	43	35	44	33
4	28	17	40	25	43	28	44	30	45	32
5	33	24	40	27	43	36	45	37	45	35
6	37	24	45	27	48	36	50	38	53	40
7	40	23	43	27	53	30	54	33	52	35
8	40	30	50	34	54	34	54	32	55	34
9	45	27	44	30	55	33	56	35	60	36
10	43	22	50	30	60	39	58	45	62	45
$\overline{X}$	12.2		14.0		14.8		14.3		14.6	
$S_{\overline{x}}$	2.0374		1.8607		2.4935		2.3431		1.9732	
p	$p < 0.001$		$p < 0.001$		$p < 0.001$		$p < 0.001$		$p < 0.001$	

Recorded deflection in mm before (*a*) and after (*b*) treatment with HR

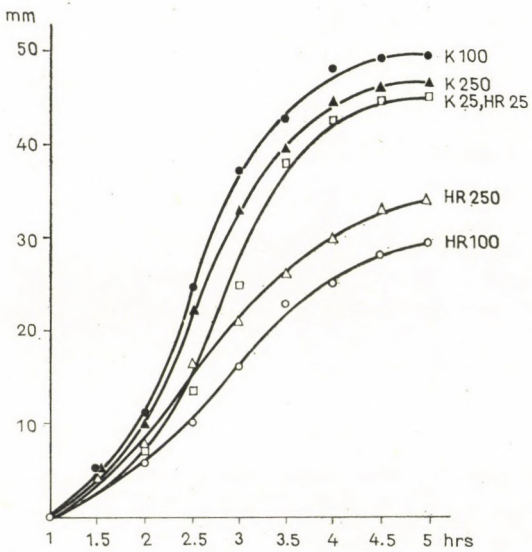

Fig. 7. Effect of O-(β-hydroxyethyl)rutin on erythema induced by UV radiation. (K 25, K 100 and K 250 = the increase of the erythema intensity on the control side before HR treatment. HR 25, HR 100 and HR 250 = increase of the erythema intensity after treatment with 25, 100 or 250 mg/kg HR, respectively)

It should be mentioned in connection with our experiments that to obtain reproducible results, Wilhelmi (1949) likewise irradiated guinea pigs for longish periods of 8, 12 or 14 minutes. He used a high-pressure quartz tube as the source of UV radiation (Hanovia, Model IV; the author did not give the wattage). He found that the irradiation of one side of the animals did not affect the UV light effect produced on the other side on the following day.

Winder *et al.* (1958) used a 500 W Analytical Model quartz lamp (Hanovia) for the 60-second irradiation of

guinea pigs. In agreement with Wilhelmi's method, the estimation of the erythema was made 2 hours after the exposure.

The procedure employed by Görög (1968) agrees with that of Winder *et al.* (1958). He used a 1,000 W mercury vapour lamp, and the irradiation time was 60 seconds. Three states can be distinguished in the appearance of the erythema, and these are evaluated as follows: 0 = erythema not visible, 0.5 = detectable erythema, 1 = pronounced erythematous reaction.

As is well known, in addition to the UV procedure mentioned above, the following 3 methods are used for the measurement of the anti-inflammatory action of a pharmacon: rat paw oedema (Domenjoz (1953)), cotton pellet granuloma (Winder *et al.* (1962), Meier *et al.* (1950)), and the granuloma pouch method described by Selye (1952, 1953). As Sporny (1967) has pointed out, these methods have two main disadvantages: 'In every case the studied substance must be used preventively, while in the practical application we try to promote recovery from more or less developed inflammatory processes with the anti-inflammatory agents. It is also a considerable drawback that apart from the rat paw oedema procedure the course of the process cannot be followed, and only the final state is established.'

The UV erythema method developed earlier by us is suitable not merely for determining the final state, but also permits continuous recording (or at intervals if required) of the increase of the erythema intensity.

From our studies with the 350 W instrument the following statements can be made concerning the erythema-inhibiting action of HR:

(1) The HR treatment does not inhibit the appearance of the erythema.

(2) The increase in the intensity of skin-reddening is slowed down as a result of the treatment, and the degree

of intensity of the erythema observed before the treatment is not attained.

(3) Injected s.c. in a dose of 100 mg/kg, HR leads to a pronounced reduction in the intensity of the erythema. The use of a small dose (25 mg/kg) does not produce a significant change compared with the erythema induced on the control side. An increase of the effect with the increase of the dose was not observed. It should be noted that our experiments were carried out at two different seasons (January and April) and on two separate groups of guinea pigs kept on different diets. The April group received fresh green fodder.

In experiments which were published after our own investigations, Leuschner (1970) found that the use of O-(β-hydroxyethyl)rutin in gel form likewise affects the intensity of the UV erythema to a small extent.

It should finally be mentioned in connection with our studies that in the experiments of Winder *et al.* (1958) (in addition to several other substances) it was observed that the administration of suspended hesperidin or rutin (400 mg/kg) by gastric tube led to an erythema-inhibiting effect. According to their findings the effective dose is higher than 400 mg/kg.

Our results can be summarized as follows. Erythema is induced in the skin of guinea pigs by irradiation for 20 minutes with a 350 W analytical quartz lamp. As a result of the injection s.c. of O-(β-hydroxyethyl)rutin in a dose of 100 mg/kg, the increase of the intensity of the skin-reddening is slowed down and the degree of intensity of the erythema observed before the treatment is not attained. The difference in the intensities of the skin-reddening observed on the control side and on the symmetrical side of the same animals after the HR treatment is significant from 2.5 hours after the irradiation. The application of HR in small doses (25 mg/kg) does not cause a significant change.

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V. EFFECT OF O-(βHYDROXYETHYL)RUTIN ON THE OEDEMA-PREVENTING ACTION OF BUTAZOLIDIN

Numerous steroid and non-steroid anti-inflammatory substances are known today for the moderation of rat paw oedema brought about by various irritants. Among others, phenylbutazone (butazolidin), which is mainly used in combination with aminopyrine, possesses a very intense oedema-inhibiting action. As can be read in Chapter II of Part One of this monograph, many papers have dealt during the last twenty years with the oedema-moderating effect of the flavonoids. In possession of these data we investigated the effect of O-(β-hydroxyethyl)rutin (HR) on the oedema-inhibiting action of butazolidin (Gábor and Iván (1970)).

Method

Oedema was induced in the hind paw of male R-Amsterdam rats, weighing 120–150 g, by the injection under the skin of the paw of 0.1 ml of a solution of serotonin, hyaluronidase, dextran or formaldehyde. The maximum of the serotonin or hyaluronidase oedema was attained 30–45 minutes after the sub-plantar injection, whereas for dextran or formaldehyde oedema it occurred after 90–120 minutes. To measure the degree of the oedema, the foot of the animal was immersed in mercury and the volume change recorded electrically with a Hellige multiscriptor.

The rats were treated with the water-soluble HR (100 mg/kg i.p.) once daily for 3 days, and then 30 minutes

before the production of the oedema. The butazolidin was injected (50, 75, 100, 150 or 200 mg/kg i.p.) 30 minutes before the production of the oedema. The experiments were made in groups of 10; the volume of the rat's right hind paw was determined as reported before and after the administration of the pharmacon and the inflammation-inducing agent, and a comparison was made with a control group treated with 0.9% NaCl solution.

The results were evaluated mathematically with the Student 't' test.

The solutions employed to induce the oedema were:

(*a*) 0.01% serotonin creatinine sulfate (Sandoz, Basel),
(*b*) 6% dextran (Rheomacrodex, Pharmacia Uppsala),
(*c*) 1% formaldehyde,
(*d*) hyaluronidase 150 E (Reanal, Budapest).

Results and discussion

Our experiments were made in three parts. In the first part, before the injection of serotonin, dextran, formaldehyde or hyaluronidase, the rats were treated with 50, 75, 100, and in some cases 150 or 200 mg/kg butazolidin i.p. A measure of the oedema relative to the controls (100%), expressed in %, is given in Table 7.

In the second part of the experiments, the effect on the oedema was studied of HR (100 or 200 mg/kg i.p.) administered before the injection of the above oedema-inducing agents. The two different doses of HR did not lead to a significant difference in inhibition of the oedema (see Table 7).

In the third part of the experiments, on the 4th day of the HR treatment the HR (100 mg/kg i.p.) and butazolidin (50 or 100 mg/kg i.p.) were injected together before the induction of the oedema. As a result of the

TABLE 7

Paw oedema in rats treated with butazolidin and

Treatment	Dose, mg/kg	Oedema	
		Serotonin	
0.9% NaCl	—	100	
Butazolidin	50	84.6 ± 2.64	$p < 0.001$
Butazolidin + HR	50 100	69.6 ± 3.13	
Butazolidin	75	70.5 ± 2.92	$p < 0.001$
Butazolidin + HR	75 100	57.7 ± 3.20	
Butazolidin	100	63.9 ± 1.86	$p < 0.001$
Butazolidin + HR	100 100	47.8 ± 3.43	
HR	100	82.6 ± 2.64	
HR	200	79.8 ± 2.51	

combination of the two drugs, the extent of the oedema induced by serotonin or hyaluronidase decreased significantly, even in the case of the use of 50 mg/kg butazolidin, compared with the oedema in rats pre-treated exclusively with butazolidin. A more moderate difference was observed in the formaldehyde oedema. No difference was found between the effects of butazolidin administered alone and in the above combination on the paw oedema induced by dextran (see Table 7).

The relation between dose and effect is shown in Figs 8 and 9. The percentage inhibition of the rat paw oedema is given on the graphs.

In discussing our results, we mention here only that numerous authors have dealt with the influence of

O-(β-hydroxyethyl)rutin (HR) (expressed in %).

inducers		
Hyaluronidase	Formaldehyde	Dextran
100	100	100
87.5 ± 0.86	85.6 ± 1.83	81.2 ± 2.29
69.6 ± 2.67 $p < 0.001$	75.8 ± 2.64 $p < 0.01$	80.3 ± 2.78
75.0 ± 2.64	—	—
53.8 ± 3.26 $p < 0.001$		
64.3 ± 3.45	68.6 ± 13.88	60.3 ± 1.91
44.0 ± 0.61 $p < 0.001$	62.9 ± 3.58	59.1 ± 2.85
82.4 ± 1.76	88.4 ± 1.45	84.0 ± 3.06
79.8 ± 2.87	83.0 ± 3.11	80.1 ± 3.76

flavonoids on oedemas induced in the hind paw of the mouse and the rat (Gross (1950a, b), Küchle and Wegener (1952), Martin *et al.* (1953), Formanek and Höller (1960), Vogel and Marek (1961), Texl (1963), Vogin and Rossi (1963), van Cauwenberge and Franchimont (1967), Bonta (1969), etc.; *cf.* Part One, Chapter II, pp. 40–44).

The difference between the results of van Cauwenberge and Franchimont (1967) and our own experiments as to the extent of the effect of HR on serotonin oedema is to be sought in the difference of concentration of the serotonin solutions used to induce the oedema.

Information on the mechanism of the effect may be given by the experiments of van Cauwenberge and

Franchimont (1968) which showed that 1 or 2 hours after the i.p. injection of the rutin derivative the ascorbic acid and cholesterin contents of the adrenal glands significantly decrease, while the fluorescing steroid level in the blood plasma significantly increases. In the case of i.m. administration the plasma steroid level increase occurs after 2 and 4 hours, but in the case of oral doses merely after 4 hours. Thus, the mode of action of HR is probably based on the stimulation of the adrenal gland.

Our experiments appear interesting from the point of view that with a possible HR and butazolidin combination the use of aminopyrine would be dispensable. As is well known, the prolonged taking of aminopyrine, especially in elderly females, may give rise to agranulocytosis (Issekutz and Issekutz (1969)). The side-effects

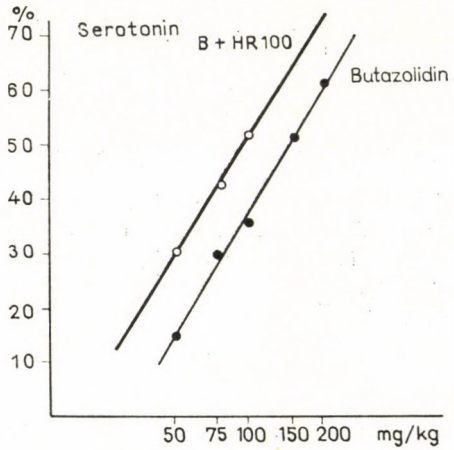

Fig. 8. Effect of butazolidin or of butazolidin (B) + O-(β-hydroxyethyl) rutin (HR) on the paw oedema induced in rats with serotonin (the inhibition is expressed in %)

of butazolidin must likewise be considered (Goodman and Gilman (1970)), whereas up to the present no side-effects have been observed during the use of HR. We consider the importance of our results to lie in the fact that our combination of drugs permits a reduction in the butazolidin dose.

Before the possible practical use of the HR–butazolidin combination, it must naturally be studied in numerous other tests. Our present experiments give an answer exclusively on the influence of HR on the oedema-inhibiting action of butazolidin.

Finally, as a comparison with regard to toxicity, we note that for the i.p. administration of aminopyrine and butazolidin to rats, DL_{50} is 248 and 215 mg/kg, respectively (Hazleton *et al.* (1953)), whereas that for HR is 27.16 g/kg (Radouco-Thomas *et al.* (1965)).

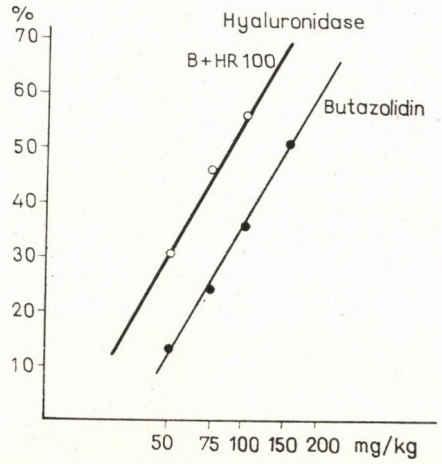

Fig. 9. Effect of butazolidin or of butazolidin (B) + O-(β-hydroxyethyl) rutin (HR) on the paw oedema induced in rats with hyaluronidase (the inhibition is expressed in %)

To summarize, in our experiments, the effect was studied of the water-soluble O-(β-hydroxyethyl)rutin (100 mg/kg i.p.) on the oedema-inhibiting action of butazolidin (50, 75 or 100 mg/kg i.p.) on rat paw oedema. It was found that the oedema induced by serotonin or hyaluronidase was decreased to a significant extent by the joint administration of the two drugs, compared with the oedema in rats treated exclusively with butazolidin. A moderate difference was observed in formaldehyde oedema, but no difference was found between the effects exerted on dextran oedema by butazolidin alone and in the above combination.

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AUTHOR INDEX*

* Numbers in italics refer to pages where the co-authors' names are not separately mentioned.

SUBJECT INDEX

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