THESIS

THE ACTION OF VITAMIN P

by

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HISTORY OF VITAMIN P

The investigations which led to the discovery of vitamin P were undertaken by Armentano, Szent-Gyorgyi and co-workers[1] to determine why purified ascorbic acid, in contrast to the crude product, was a therapeutic failure in vascular purpura. Since earlier work on plants had shown that ascorbic acid was coupled with an essential flavone, the experimenters proceeded to isolate this substance. From about 200 kg of lemons or 70 L of lemon juice, approximately 2 g of flavone material could be obtained, which because of its yellow color and origin was named citrin.

Citrin was tested on scorbutic guinea pigs and animals so treated were said to outlive the controls, though for only a brief period. These experiments were hampered by the difficulty of preparing a diet completely free of flavones. In addition, as these substances tended to have a natural distribution similar to vitamin C, the flavone-free diets were also deficient in vitamin C. Guinea pigs maintained on such diets developed scurvy, the hemorrhagic features of which made it impossible to delineate capillary changes due solely to lack of flavones.

The experiments were therefore shifted to clinical material. Increased capillary permeability and fragility were found in seventeen patients. Three had vascular purpura which responded favorably to injections of citrin. In one case, daily doses of 300 mg ascorbic acid given

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prior to the citrin, failed to alter the capillary or clinical picture. Seven were cases of thrombocytopenic purpura of which only four had abnormal capillary findings. In two, citrin produced a partial improvement in the capillary tests, but not in the overall picture. Similarly, in seven cases of inflammatory disease and one of myxedema, the capillary tests improved, but there was no alteration in the general course of the disease. In two cases of diabetes mellitus, citrin had no effect on the low capillary resistance.

On the basis of these clinical results and the admittedly inconclusive animal experiments, it was believed that the flavone fraction was an essential rather than a purely pharmacological substance. It was therefore labelled vitamin P to signify its role in the regulation of capillary permeability.

Subsequently the animal experiments were elaborated. It was claimed that scorbutic guinea pigs treated with citrin[3], outlived the controls (44 vs 23 days), lost less weight (23 vs 117 g), and showed a remarkable reduction in the severity of hemorrhages in the intestines, muscles and joints. Other signs of scurvy, such as looseness of teeth, swelling of joints, and fragility of bones, were present to the same degree in both groups. The purified flavones, hesperedin and demethylated hesperedin were said to yield equally good results, but quercitrin was inactive[4]. These guinea pig experiments were repeated by Zilva[5,6], Moll[7], Hiramatsu[8], and Detrick and co-workers[9], and in no instance could the alleged benefits of vitamin P be reduplicated. Indeed, one

of the original group of workers, Szent-Györgyi[10] found himself in the same predicament. Bentsah and Szent-Györgyi[11] then qualified their observations and stated that vitamin P required the presence of a trace of ascoribc acid which in itself was insufficient to modify the scorbutic process.

The confusing situation was clarified by Zacho,[12] who applied to guinea pigs, the capillary fragility test which Armentano et al[1], had used in the clinic. He circumvented the difficulty of producing a diet lacking only in vitamin P, simply by enriching the flavone-free diet with pure ascorbic acid. By this means, a pure vitamin P deficiency state which was not complicated by scurvy was produced in guinea pigs. The only abnormality which could be detected in guinea pigs on this diet was an increased capillary fragility. This became normal if citrin was given. If the scorbutic state was first produced and then treated with pure ascorbic acid, the signs of scurvy resolved, but the capillary resistance remained low until vitamin P was added to the diet. These findings have been confirmed in guinea pigs by Sevin,[13] Parrot[14] and others[15], in the rat, by Rusznyak and Benko,[16,17] and in humans by Scarborough.[18,19] Rats are able to synthesize ascorbic acid and thus when placed on scorbutic diets they do not readily develop scurvy. Rusnyak[2] and Benko found however that they do acquire a decreased capillary resistance which responds to citrin but not to ascorbic acid. Similar findings were made with pure flavones such as hesperedin, hesperetin, quercitrin, eriodictyol and rhamnetin.

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Although according to the majority of workers,[12-19] vitamins C and P appeared to have separate and distinct functions, a recent attempt has been made to prove that this is not the case. This, in effect, amounts to a revival of the original concept of Armentano et al.[1] This latest trend arose out of a report by Lavollay and Sevestre[20] in which it was claimed that synthetic ascorbic acid raised the capillary resistance of normal as well as P deficient scorbutic guinea pigs. Parrot[21-23] maintained that ascorbic acid increased capillary resistance as long as some vitamin P was present. In the complete absence of this vitamin, ascorbic acid was found to have no capillary effect. The requirement for ascorbic acid was claimed to vary inversely as the body store of vitamin P. Parrot concluded that the function of vitamin P was to spare ascorbic acid by sensitizing the tissues to it. He attributed the findings of Lavollay and Sevestre[20] to the fact that their so-called vitamin P-free diet, in common with most other scorbutic diets, actually contained traces of this vitamin. Animals maintained on such diets suffered from only partial depletion of their stores of vitamin P and hence when given ascorbic acid, responded by an increase in capillary resistance.

Several inconsistencies in this new schema are apparent. In the first place, other investigators[12-19] using similar diets have never been able to show that ascorbic acid modified capillary resistance. Secondly, the response of normal animals to ascorbic acid frequently was only

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temporary even though the vitamin was continued.[20] Thirdly, the failure of vitamin P to prolong the life of scorbutic guinea pigs,[5-10] a finding which Parrot[22] himself reported, is hardly indicative of a sparing action on ascorbic acid. Finally, since P deficient guinea pigs receiving only small quantities of ascorbic acid do not develop evidence of vitamin C insufficiency, it is obvious that contrary to Parrot's claim, the demand for vitamin C in P deficiency is not substantially increased. In Zacho's[12] experiments, P deficient animals received only 1.0 mg ascorbic acid daily and on this relatively small dosage progressed normally aside from their low capillary resistance. Thus the majority of the experimental observations are not in accord with Parrot's concept that the function of vitamin P is to spare vitamin C.

The bulk of the evidence and the most consistent findings[5-10,12-9] favor the view that vitamin P is a separate entity whose function is in no way related to vitamin C. The finding by the majority of workers that ascorbic acid fails to raise the capillary resistance of a vitamin P deficient animal clearly shows that vitamin C cannot substitute for vitamin P. Likewise, the inability of vitamin P to influence the course of scurvy unquestionably shows that these two vitamins have distinct individual functions. Scurvy, as it occurs naturally, represents a combined deficiency of these similarly distributed vitamins. However, pure scurvy is not accompanied by excessive fragility of the capillaries, just as pure vitamin P deficiency presents none of the signs of scurvy.

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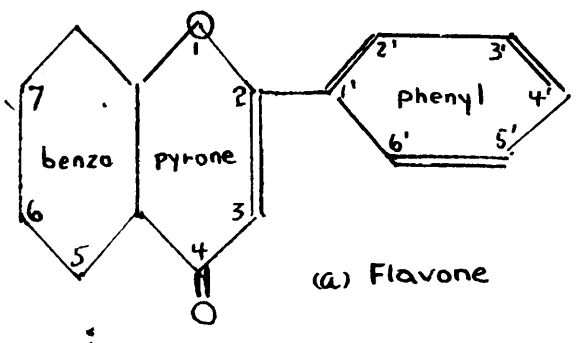
THE CHEMISTRY OF VITAMIN P

The flavones are a widely distributed group of plant dyestuffs. They have as a common nucleus, the 2-phenyl-y-benzopyrone structure which Kostanecki[24] called flavone proper (a).



(a) Flavone

When a hydroxyl group is attached to C3 as in rutin (b), the new structure is called a flavonol. Flavanones such as hesperedin (c), are characterized by saturation of the double bond between C2 and C3. These three variations involve the pyrone portion of the molecule. For the sake of brevity, derivatives of the three types of structure are collectively referred to as flavones.

The exact identity of the active element in citrin has been the subject of considerable dispute. The first pure fractions to be isolated from citrin were hesperedin and its demethylated glycoside, eriodictin.[25] The presence of these two flavanones was confirmed in spectrographic analyses of citrin by Lajos and Gerendas[26] and by Robeznieks.[27] The latter also discovered the presence of a flavonol similar in behaviour to quercitrin. A fourth substance, limonin, was isolated by Higby[28] and was found to be inactive. In vitamin P deficient rats, hesperedin, eriodictin and quercitrin were all found to be active by Rusznyak and Benko.[17] Higby[28] has shown that hesperedin is ordinarily insoluble and inactive. When converted to its chalcone it acquires solubility, activity and lowers the blood

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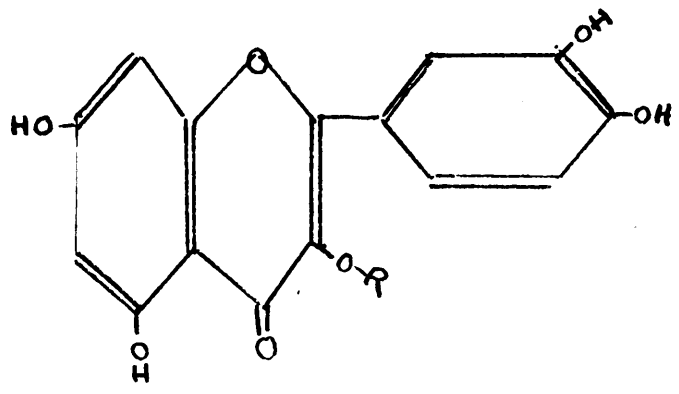
pressure. The chalcone is formed by splitting open the pyrone ring. The reaction takes place in alkaline media and reclosure occurs in the presence of acids. Reclosure is prevented by methylation so that hesperedin methyl chalcone is a stable product. The findings of Higby[28] and the response to hesperedin obtained by Griffith,[29] Scarborough[18,19] and others,[17,109] refute the statement by Shanno[30] that hesperedin has proven to be inactive. His suggestion that rutin is the active fraction seems improbable since unlike citrin, which is soluble and can be administered i/v, rutin is insoluble in water and can be given to humans only by mouth.

An additional series of vitamin P compounds having a modified flavone nucleus has been studied by Parrot, Lavollay et al.[31-33] Here the ketone radicle on C4 is replaced by two hydrogen atoms. When so changed, the pyrone ring is known as a pyranne and the whole molecule, a flavanne. This series is represented by the catechins. It has been said[32] that 0.001 mg of d'epicatechin i/p raised the capillary resistance in guinea pigs from the normal level of 20 cm Hg, up to 26 and 29 cm Hg at 1½ and 24 hours respectively. This substance was judged to be at least five hundred times as active as citrin.

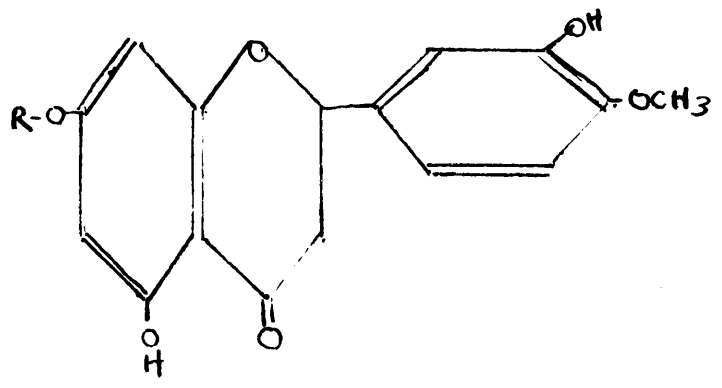
Lavollay[34,35] and Sevestre[34] discovered still another type of active compound, the coumarins. In this group there is no phenyl ring. The double bond in the pyrone ring is located between C1 and C2 and ketone radicles are attached to C3 and C4. Representative of this group are esculin and its aglycone, esculetol. Esculin was found to be as potent as d'epicatechin.

The i/p injection of 0.001 mg markedly increased the capillary resistance of normal and scorbutic guinea pigs. Single 20.0 mg oral doses doubled the capillary strength of normal humans at 24 hours and an elevated level persisted for over a week. In view of its moderate solubility and its marked activity when given by mouth, esculin deserves much greater attention than it has received. Rutin which is currently the type of vitamin P in favor, is ordinarily insoluble. No figures are available as to its effect on normal people, but in pathologic states, a satisfactory response may not be obtained until months or even a year of treatment have elapsed.[151]

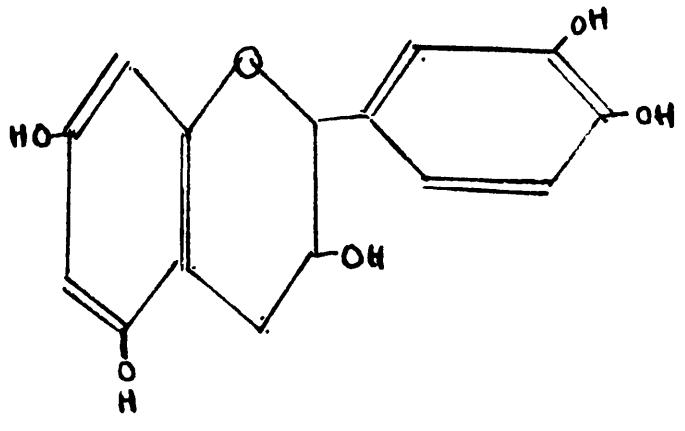
Lavollay[35] has proposed that the phenyl-y-benzo pyrone derivatives (flavones) be named vitamin P1 and the alpha-benzo-pyrone group be named vitamin P2.



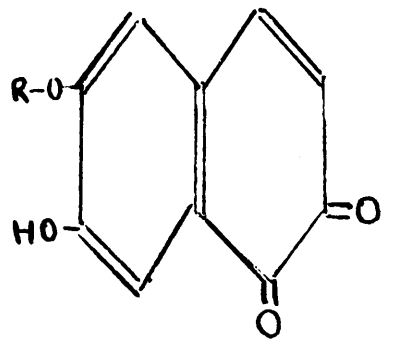
(b) Rutin



(c) Hesperedin



(d) Catechin



(e) Esculin

R = sugar residue

METHODS OF IDENTIFYING VITAMIN P

There are as yet few biochemical methods available for the detection of specific vitamin P substances. When catechins are oxidized to cyanidol, a deep purple color appears, suitable for colorimetric procedures[36]. This oxidation reaction is said to be specific for catechins but it fails to distinguish between different types of catechins. Most flavones react with boric acid to yield highly colored fluorescent solutions. This principle has been adapted by Glazko et al.[37] for the estimation of the concentration of rutin and quercetin solutions. Measurement of the intensity of fluorescence was found to yield more sensitive results than colorimetric procedures. This test is still in its infancy and has yet to be tested on solutions of animal origin. It too, is not specific for any one flavone. Other procedures such as spectrographic analyses require specialized management which is generally unavailable. As a result, studies on absorption, retention and excretion of vitamin P have been limited to a few observations on the urinary excretion of epicatechin.[38] This deficiency has considerably retarded progress in the field of vitamin P studies.

Investigators have been forced to rely on biological tests which yield little in the way of quantitative information. Usually, guinea pigs are made P deficient after the method of Zacho.[12] Compounds which can prevent or cure the increased capillary fragility are said to have vitamin P activity. A serious drawback is the questionable reliability of the suction method of measuring capillary resistance.

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Unfortunately this is the only technique that can be applied to small animals.

Another test has been introduced by Majovski and co-workers[39] in which mice, subjected to a sudden reduction in atmospheric pressure, developed pulmonary hemorrhages. Various types of vitamin P were said to reduce the severity of the bleeding but Kibrick and Goldfarb[40] could not substantiate these claims.

Parrot and Galmiche[33] have shown that vitamin P reduces the bleeding time of normal guinea pigs and have suggested that this observation be utilized as a test for vitamin P activity. The ability of vitamin P to increase the resistance of erythrocytes to hypotonic saline has also been suggested as the basis for a test.[41]

In summary, specific biochemical tests to determine the identity and concentration of vitamin P substances are few and their worthiness remains to be proved. Biological tests actually in use yield no quantitative data and the accuracy of the results is highly debatable.

PHARMACOLOGICAL PROPERTIES OF VITAMIN P

Biologists were aware of the existence of flavones for many years and several pharmacological studies were carried out prior to the realization of their vitamin nature. According to Armentano[42], Akamatsu[43] and Fukuda[44] had shown that rutin, quercetin, myricetin, etc. raised the blood pressure and cardiac output of the frog, stimulated capillary contractions and caused a diuresis. Jeney and von Czimmer[45] obtained similar results with quercitrin and quercetin and these substances were able to restore frog hearts poisoned by chloroform, urethane or lactic acid. In dogs and cats, Armentano[42] found that most of the flavones either lowered the blood pressure and heart rate or elicited no response. In the former group were quercitrin, quercetin, citrin, naringenin and rhamnetin, whereas hesperedin, hesperetin and eriodictyol fell into the latter class. The depressor response was not abolished by atropinization or vagotomy and since a cardiac factor was ruled out, it was attributed to a direct vasodilating effect on the peripheral vasculature. A curious finding was that in about 60% of dogs, but not in cats, tachyphylaxis developed,so that repeated injection of the same or even different flavone (quercitrin excepted) failed to elicit a hypotensive response. In the summer months, blood pressure changes were less marked or even absent.

The effect of various types of vitamin P on capillary vasomotion has been studied by Haley, Clarke and Geisman[46], using the rat mesoappendix preparation of Chambers and Zweifach[47]. All drugs were given in 0.1 cc

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of Locke's solution. Saturated solutions of the insoluble flavones, rutin and hesperedin had no effect. Sodium acid succinate derivatives of these two flavones which are soluble and the methyl chalcone of hesperedin in doses up to 100 gamma, also failed to stimulate capillary contractions. Esculin and adrenochrome in similar dosage were also inactive. The sodium acid pthalates of rutin and hesperedin were slightly active. The catechins in doses as small as 0.0001 gamma were highly active vaso-constrictors.

In summary, these findings indicate that different types of vitamin P have a direct action on the vaso-motor system. Certain of the flavones appear to be arteriolar dilators, whereas the catechins are highly active capillary constrictors.

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THEORIES ON THE MECHANISM OF VITAMIN P

1) PRECAPILLARY TONUS THEORY

In 1941 Lavollay and Neumann[48] set up a working hypothesis which was based on the belief that capillary permeability was governed by alterations in the tonus of precapillary vessels. Since adrenalin was the physiological agent which increased precapillary tonus, they suggested that substances which increased capillary resistance did so by preventing the destruction of adrenalin. By a photoelectric method, they found that the spontaneous in vitro oxidation of adrenalin to adrenochrome was inhibited by an orange extract and by several flavones. These and numerous other flavones including rutin, prolonged the action of adrenalin on the guinea pig colon and seminal vesicles.[49] It was claimed that none of them had a direct action on these viscera. These observations convinced Lavollay[49] that the hypothesis was correct.

2) ADRENOCHROME THEORY

After the injection of adrenalin[50] into guinea pigs and humans, a latent period of ten to thirty minutes expired before capillary resistance began to rise. The maximum was reached in about an hour and an elevated level persisted for several days. In view of the delayed and protracted nature of the vitamin response, in contrast to the immediate and transitory physiological actions of adrenalin, it was concluded that the vitaminic features were not contributed by adrenalin itself but by some initial product of its metabolism such as adrenochrome.

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It was subsequently shown that non-pressor derivatives of adrenalin increased capillary resistance in scorbutic and normal guinea pigs and in humans. The adrenalin vasomotor theory was therefore abandoned.

3) THE CATECHIN-VASOMOTION THEORY

Recently Haley and co-workers[46] revived capillary vasomotion as the mechanism by which highly active capillary constrictors, such as the catechins produced their vitamin P effect.

CRITICISM

Even before this theory was first postulated, Brewer[54] had shown that adrenalin caused an immediate drop in capillary resistance which was maintained during the period of capillary constriction. Parrot and Lavollay[50] discarded this theory when they found that the vitamin P effect of adrenalin was delayed and protracted and did not correspond to the period of increased capillary vasomotion. Finally, Haley et al.[46] found purified d'catechin to be a highly active capillary constrictor, whereas Lavollay, Parrot, and Sevestre[32] had previously shown that this substance was devoid of vitamin P activity. The capillary vasomotion theory is therefore no more acceptable now than when first put forth.

4) ADRENOCHROME HISTAMINE THEORY

The adrenochrome theory was expanded by Parrot[53] so as to include histamine as the chemical antagonist of vitamin P. This was based on Franke's[56] observation that histamine caused increased capillary fragility in humans.

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This new concept appeared to be supported by their own findings that antihistaminics (2876 RP and 2339 RP) increased capillary resistance. The increased capillary permeability and fragility in vitamin P deficiency were therefore ascribed to a relative increase in the level of histamine.[57] When guinea pigs were placed on a scorbutic diet for 15 days, they developed a hypersensitivity to histamine. Where normally 8 mg/kg histamine i/p was required for an LD50 effect, in P deficient animals only 2.5 mg/kg histamine yielded the same mortality rate. Both vitamin P and ascorbic acid were said to be able to restore the histamine sensitivity to normal levels. In normal animals with normal capillary strength, vitamin P failed to increase the tolerance to histamine. It failed to suppress the effect of histamine on the blood pressure of the cat, or on the movements of the guinea pig colon. It was therefore concluded that the action of vitamin P then was not to directly neutralize histamine, but to react with the tissues so as to prevent histamine from increasing their permeability. Thus in vitamin P deficiency, the capillary dysfunction was said to be due to an increase in the sensitivity of the tissues to histamine.[57]

CRITICISM

There are ample reasons to justify rejection of the histamine aspect of this theory. The work of Chambers and Zweifach[56] casts doubt on the role of histamine as a physiologic factor in capillary permeability. They found that histamine increased capillary permeability only when the dose was sufficient to produce visible endothelial damage.

According to Parrot[57], the permeability of capillaries depended on their susceptibility to the action of histamine. When vitamin P was deficient, the susceptibility to histamine was increased and therefore capillary permeability was increased. Parrot[57] stated that in normal animals, the susceptibility of the tissues to histamine was not affected by the administration of vitamin P. Since capillary resistance was said to reflect the degree to which its tissues resist the action of histamine, and since in normal animals resistance to histamine could not be increased by vitamin P, it would be logical to conclude that vitamin P could not increase capillary resistance in normal animals. That this is not the case has been shown by Parrot[31] himself and others.[13,14] The histamine theory is therefore not in accord with experimental observations.

As to the adrenochrome itself, there is no direct evidence to support the concept that vitamin P increases capillary resistance by inhibiting the oxidation of this substance. As will be shown in the next section, vitamin P can increase the resistance of tissue without the intervention of adrenochrome.

5) THE DIRECT ACTION THEORY

A theory commendable for its simplicity has been proposed by Lenaz and Albori[59]. It was based on their findings of the reduction of colloid hydrophilia by citrin. Blocks of 4% agar were prepared of similar shape and size. A portion of these were immersed in control buffer solutions, the balance in citrin buffer solution. At regular intervals

up to sixteen hours, blocks were withdrawn, carefully wiped dry and weighed. For any period of immersion, the blocks in the citrin solution absorbed considerably less fluid than those in the control medium. The results were interpreted according to the postulate of Gellhorn[60], that colloid hydrophilia was a function of colloid permeability. Since citrin decreased the hydrophilia of the agar blocks, it was concluded that it had done so by reducing the permeability of this colloidal material. Some support of this theory is derived from a recent finding of Parrot and Gabe[41], that esculin, rutin and epicatechin increased the resistance of washed red blood cells to hypotonic saline. Since washed erythrocytes outside of the circulation do not have access to new supplies of adrenochrome, one cannot ascribe the increased resistance of the erythrocytes to a higher concentration of this metabolite. A sirect action of the vitamins on the colloidal erythrocyte membrane seems much more likely.

CONCLUSION

It is apparent that little if anything is known about the metabolism of vitamin P. These substances are administered and the end results observed. There is a vast gap in knowledge as to what transpires between these two points. The few theories that have been proposed have done little to improve this situation.

SITE OF ACTION OF VITAMIN P

Although the exact site of vitamin P action is not known, certain observations and deductions suggest it is the pericapillary sheath. In humans, Scarborough[19] has shown that pure scurvy is characterized by normal capillary resistance, and by large ecchymotic hemorrhages. Pure P-avitaminosis, on the other hand, is associated with decreased capillary resistance and with small petechial hemorrhages. Vitamin P does not alter the effects of vitamin C deficiency and vice-versa. It therefore seems reasonable to conclude that these two vitamins act on different parts of the capillary wall.

The capillary wallhas been described as being composed of three layers, an external pericapillary sheath formed by a condensation of connective tissue, a single layer of endothelial cells bound together by a cement substance which these cells elaborate and an internal or endocapillary layer consisting of plasma protein which is adsorbed onto the cement substance.[58] Since ascorbic acid is regarded as the element responsible for the integrity of the endothelial cells and cement substance, this layer may be excluded from the list of possible sites of vitamin P activity. This leaves the endocapillary layer and the adventitial sheath. The former was first proposed by Danielli[61] to indicate a film of serum proteins, which by adhesion to the pores in capillary walls, reduced the range of molecules which could permeate through. This theory was put forth to

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account for the observation that the addition of small amounts of serum proteins to perfusates of low osmotic pressure resulted in a decrease in oedema formation out of proportion to the increment in colloid osmotic pressure. It was based on the finding that certain ranges of collodion membranes ordinarily freely permeable to gum acacia, became impermeable when proteins were added to the solution being dialysed. This stratum has apparently been stained with Evans blue and visualized by Chambers and Zweifach. Although it may be of significance in disorders of protein metabolism, the extremely smallsize of capillary pores makes it highly doubtful that dysfunction confined to this layer could permit the formation of petechial hemorrhages seen in human vitamin P deficiency.

By exclusion then, it appears that vitamin P should act on the precapillary sheath. Some support for this assumption is derived from the observations that weakening of the pericapillary sheath by hyaluronidase or by degenerative change, is accompanied by the development of petechial hemorrhages. These are the same type that develop in vitamin P deficiency in contrast to the large ecchymotic type which appear in pure scurvy.

In summary, there is indirect evidence which suggests that vitamin P regulates capillary fragility and permeability through an action on the pericapillary sheath.

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CRITERIA FOR EMPLOYMENT OF THE TERM "VITAMIN P"

Correct use of the term vitamin P is handicapped by insufficient knowledge of the mechanisms by which the various compounds described as having vitamin P activity effect an increase in capillary resistance. Factors other than vitamin P certainly play a role in the regulation of capillary permeability and fragility. A hormonal influence is indicated by a sharp drop in resistance which occurs premenstrually and to a lesser degree at the mid-interval period.[54] These cyclic changes suggest that a decrease in oestrogen output towards ovulation and menstruation may be responsible for the reduction in capillary resistance. Schweppe et al.[65] have reported the case of a post menopausal female with an increased capillary fragility and spontaneous bleeding who was placed on rutin therapy without improvement. Rutin was discontinued and oestrogens were administered. The capillary resistance then became normal and bleeding stopped.

It has been claimed that adrenal cortical extract and corticosterone increase the resistance of capillaries against the actions of hyaluronidase[66] and leukotaxine.[66,67] In vitamin P deficiency, the thyroid[68,69] and the adrenal cortex[70] have been described as hyperplastic. Restitution to a resting state could be brought about with vitamin P but not with ascorbic acid. It is evident then that hormonal factors play a role in the regulation of capillary permeability and fragility but the reactions involved

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are still obscure. According to Brewer[54], the injection of adrenalin caused an immediate drop in capillary resistance, whereas Parrot and Lavollay[50] have shown that a delayed response occurred in the opposite direction. It is to be expected then that numerous factors such as emotional states, which affect adrenalin output, will have a considerable influence on the functional integrity of capillaries.

It is clear that regulation of capillary permeability and fragility is a highly complex and still poorly understood problem. Changes in either direction may be accomplished by following any one of several physiological pathways. Since the many factors which increase capillary resistance do not achieve this result by the same process and since, as was pointed out in the case of rutin and oestrogen[65], these various pathways need not be interchangeable, there is no justification for considering vitamin P activity as synonymous with increased capillary resistance.

The term vitamin P should be restricted to those substances, which when eliminated from the diet lead to an increased capillary permeability and fragility, capable of being reversed upon restoration of the missing factors. Specifically, this would include the naturally occurring benzopyrones and pyrannes such as hesperedin, epicatechin, esculin and rutin, etc. Other capillary active compounds should be considered strictly as pharmacological agents.

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MEASUREMENT OF CAPILLARY RESISTANCE AND PERMEABILITY

As almost all studies on vitamin Pdepend on detection of alterations in capillary resistance and permeability, the commonly employed tests used to measure these functions will be briefly reviewed and evaluated.

The terms, capillary fragility, strength or resistance, are used to indicate the extent to which cutaneous capillaries rupture when subjected to external suction or increased intracapillary pressure. In the suction or negative pressure test which Hecht[7] introduced in 1906, a transparent cup, usually about 2.0 cm in diameter, is applied to a specific area on the skin, such as the antecubital fossa. The pressure in the cup is then reduced in stages until the first petechia appears and the result is expressed as the critical petechial pressure. An alternative method is to reduce the pressure to a given level for a specific period of time, and the number of petechiae so produced is referred to as the petechial count or petechial index.[73,74] European workers have favored the suction technique in contrast to American investigators, who consider it to be subject to great error.[75] With this method, O'Hara and Hauch found that multiple tests conducted on individuals at any one sitting, yielded results which varied considerably in contiguous areas of skin. In repeated tests carried out on the same area of skin at different times, the results have also fluctuated widely. Similar discrepancies found by Beaser,[76] Rudy and

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Seligman[76], led them to conclude that the suction method was entirely unreliable. Several factors contribute to the inaccuracy of this method. The area of skin tested is so restricted that the findings are not necessarily representative. Since the end point in the critical pressure method is reached when the first petechia appears, only the weakest capillary in the field is tested. This gives no indication of the strength of the average capillary. In animals, the hair must be removed by shaving or chemical epilation so that the effects of bleeding and trauma must be taken into account. Scarborough[77] has shown that after spontaneous bleeding, or the administration of blood by any route, capillary resistance rises within 12 hours by as much as 100%, and an increased level persists for 2 - 4 days. Ungar[78] has shown that a similar effect follows trauma. Although these factors undoubtedly contribute in part to the contradictory findings in studies of vitamin P, the full extent of their significance has not yet been determined.

In the positive pressure tests which originated with Rumpel[79] in 1909, the intracapillary pressure is increased by a cuff placed on the upper arm. The exact pressure, the time factor, and the area in which petechiae are counted vary considerably from author to author. It is thus difficult to compare the results of different investigators. In the Wright-Lilienfeld[80] test, the pressure level was set at midway between systole and diastole. The capillaries of hypertensives are subjected to higher internal pressure than those in individuals with normal blood pressure. The

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result of this choice of occluding pressure is indicated in the findings of Beaser et al.[76] Two different tests were carried out simultaneously on twelve hypertensive diabetics. On the arm that the Wright-Lilienfeld[80] technique was used, all showed positive reactions. On the other arm, the pressure was adjusted to what it would be in a normotensive person (100 mm Hg) and in only five were the tests positive. It is obvious that with this method the incidence of positive tests varies as the height of the patient's blood pressure. The Göthlin[81] test, by using the same cuff pressure in all cases, avoids this objection. Here, the cuff is inflated to 35 mm Hg for fifteen minutes and the petechiae are counted in circles 6 cm in diameter in both anticubital fossae. If the findings are not clearly negative (0-2 petechiae) or positive (6 or more petechiae) the test is repeated after one hour, at 50 mm Hg.

The positive pressure results are generally considered to be more accurate than those elicited with suction techniques. The main objections are that they are time consuming, especially when performed in two stages and they are unable to revealthe presence of supernormal capillary resistance.

In addition to changes in disease states, physiological variations in capillary resistance also occur. In females, there is a marked decline premenstrually and to a lesser extent at the mid-interval period.[54] Testing in females should obviously be avoided at these times.

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The age factor has a bearing but the normal range for different periods of life has not been worked out. Capillary resistance is high at birth and slowly declines thereafter. It has also been stated that the capillaries become more fragile as the day wears on.[82]

In contrast to the widespread use of fragility tests, measurement of capillary permeability has been performed by only a few investigators in vitamin P studies. The Landis method was used by Armentano et al.[1], and by Morii.[63] A cuff on one armwas inflated to 40 mm Hg for 30 minutes. Blood samples were then withdrawn from both arms. By comparing the haematocrit and plasma protein readings, the relative volume of the transudate and its protein content were determined. Normally, a haemoconcentration of 3-10% took place and the filtrate was free of plasma proteins. Griffith and Lindauer[84] have used McMaster's[85] method in which the dye patent blue was injected intradermally and on entering the lymphatics, formed colored streamers. Capillary permeability was guaged on the rate and extent of streamer formation. The validity of this method is based on the assumption that lymphatic flow in the absence of oedema or lymphatic obstruction varies directly with the rate of loss of fluid from capillaries.[168]

In summary, capillary resistance may be measured by negative or positive pressure methods. The latter are considered more reliable and of them, the Gothlin[81] test is the most popular. Measurements of capillary permeability

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have been carried out by comparatively few workers on limited aspects of this field and therefore the dependability of the methods cannot be evaluated.

THE SIGNIFICANCE AND VITAMIN P THERAPY OF INCREASED CAPILLARY FRAGILITY

INTRODUCTION

Increased capillary fragility has been found in many diseases. Its significance varies according to the other pathological changes with which it is associated. It may be a link in a chain of events which threaten the survival of the organism. At times, the detrimental end result has been prevented by strengthening the capillary link with vitamin P. On other occasions, the capillary dysfunction appeared to be of no major importance and its correction in no way improved the disease process of which it was a part.

ALLERGIC STATES

The first investigator to measure capillary strength in allergic diseases was Jersild.[86] In a patient with Henoch-Schonlein's purpura, both the suction and Gothlin tests indicated a considerable increase in capillary fragility. This was accompanied by widespread hemorrhagic phenomena and typical signs of allergic involvement. Ascorbic acid was given without effect but the capillary and clinical pictures dramatically improved when citrin was administered. Relapse and remission followed respectively on withdrawal and reinstitution of citrin therapy. Schwager[87] has made similar findings in a case treated with rutin. Two cases of allergic purpura with abnormal petechial indices were reported by Kugelmass.[88] Both responded favorably to vitamin P. In a

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preliminary note Madison and Pohle[89] have intimated that rutin exerts some beneficial effect on this type of purpura. However, Davis,[90] without giving details, stated that in his experience, vitamin P was of no value in this disease.

Of one hundred allergic children, Rappaport and Klein[91] found increased capillary fragility in forty-nine. Twelve of these whose tests were consistently abnormal, were given calcium eriodictate, and capillary resistance became normal in all twelve cases. Unfortunately, no mention was made of effects, if any, on the associated clinical manifestations. In the guinea pig, protection against fatal serum anaphylaxis by hesperedin has been claimed by Hiramatsu.[92] Raiman, Later, and Necheles,[93] have made similar observations with rutin. However, in a report of preliminary studies, Wilson et al.[94] could not demonstrate anti-anaphylactic action with rutin.

Reports on antihistamine effects of vitamin P are also insufficient to permit conclusions to be drawn. Parrot and Richet,[57] and Wilson et al.,[94] found that flavones did not inhibit the action of histamine on the blood pressure of cats and on intestinal movements of guinea pigs. Raiman and co-workers,[93] found rutin unable to prevent histamine shock in guinea pigs. Wilson and associates,[94] subjected a series of guinea pigs to a preliminary vitamin P deficient diet and then found rutin exerted a protective influence against an LD50 dose of histamine. It is probable that the vitamin P

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deficient diet sensitized the guinea pigs to histamine and the effects of rutin may have been merely the restoration of this hypersensitivity to normal levels. The only definite instance in which vitamin P inhibited the action of histamine appeared in a paper published by Ambrose and De Eds.[95] They found that rutin delayed the appearance of intravenously injected trypan blue in histamine induced skin wheals.

In summary, most investigators have found that allergic diseases are accompanied by an increased capillary fragility which responds to vitamin P. Improvement in the associated clinical condition has been noted in several instances but due to incompleteness of the reports and the possibility of spontaneous remissions, final judgement must be deferred until more information is available. The significance of the decreased capillary resistance is unknown as adequate studies correlating this finding with the degree of activity of the allergic process, have not as yet been made.

SPONTANEOUS HEMORRHAGIC DIATHESES

Although reduction in platelets is usually considered to be the factor which leads to bleeding in thrombocytopenic purpura, Roskam[96] in 1921, showed that variations of bleeding time in this condition depended on a vascular factor as well as the platelet count. Bedson[97], and later Elliott and Whipple[98] have shown experimentally that thrombocytopenia alone was not accompanied by spontaneous

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bleeding and this appeared only when capillary damage was present as well. The latter authors also noted that following spenectomy for this disease, the capillary resistance returned to normal before the platelet count rose. The arrest of bleeding paralleled the restoration of capillary strength rather than the increase in platelets. According to Jones and Tocantins[72], and Madison and Squier,[99] the capillary factor is of prime importance in all types of purpura and the platelet reduction is of secondary significance. The latter workers have also shown that capillary resistance is decreased in leukemia, myeloma, aplastic anemia and in thrombocytopenic and non-thrombocytopenic purpura.[100] Armentano et al.[1] found that capillary fragility was not always increased in thrombocytopenic purpura and in patients whose increased fragility did respond to citrin, improvement in the bleeding failed to occur. It appears then that a third factor operates in this disease. Although Leader[101] could find no morphological alterations in capillaries of children with hemorrhagic diatheses, McFarlane[102] has described structural changes in capillaries of adults suffering from vascular and thrombocytopenic purpura. In normal individuals, closure of capillaries was an important factor in arresting capillary bleeding, whereas in the above conditions, post-traumatic capillary closure failed to occur. It would be of interest to know if this capillary contractility returned after splenectomy but no mention was made of this.

Citrin was used by Armentano et al.,[1] and by Lajos[103]

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in vascular purpura and was said to exert a favorable influence.[104] Davis used unspecified types of vitamin P in purpura simplex and found it to be of no value.[1] Citrin, hesperedin,[105] calcium eriodictate[105] and rutin[30] have all been used in thrombocytopenic purpura with disappointing results. This type of therapy was given for about two weeks. The catechins should be tried in this latter disease since, in addition to increasing capillary resistance, they markedly stimulate capillary contractility. Rutin has successfully controlled nasal hemorrhages in hereditary hemorrhagic telangiectasia.[106-108] In one case, the development of new telangiectasia was said to be inhibited.[107]

In summary, capillary factors, in addition to platelet reduction, are of importance in the genesis of bleeding in spontaneous hemorrhagic diatheses. The low capillary resistance appears to be accompanied by a failure of capillary contractility.[102] The failure of vitamin P to arrest bleeding in thrombocytopenic purpura may be related to the fact that the types so far used do not stimulate capillary contractions.

TOXIC PURPURA

In rabbits subjected to large doses of mapharsen, hesperedin methyl chalcone[109] was said to reduce the incidence of hemorrhagic complications. Hesperedin has also been said to accelerate recovery from vascular purpura due to heavy metal toxicity.[110, 111]

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RADIATION DISEASE

In 1931, Shouse, Warren and Whipple[112] described the syndrome of radiation disease which appeared in dogs following roentgen radiation of their entire skeletons. The most important factor in the cause of death was said to be extensive hemorrhage which appeared during the last day of life. Bleeding was attributed to a marked reduction in platelets. A capillary factor was considered unlikely due to the occurrence of hemorrhage in shielded areas. However, it has since been shown that radiation can produce an increased capillary fragility,[113] and the significance of the platelet reduction has been discounted by Allen and Jacobson[114] who found that the onset of bleeding following radiation did not necessarily coincide with the appearance of thrombocytopenia. More recently the blood of radiated dogs has been shown to contain a substance whose effect on the clotting mechanism was identical to that of heparin.[114] The hypocoagulability of such blood was restored to normal by specific antiheparins such as toluidine blue and protamine. The addition of the hypocoagulable blood to normal blood caused a coagulation defect in the latter which indicated the presence of an anticoagulant in the former. Because of its above mentioned properties, this anticoagulant was considered to be heparin.

Mathewson[115] was the first to use vitamin P for the control of radiation hemorrhage. A patient receiving X-radiation by spray technique for multiple myeloma, developed severe bilateral retinal hemorrhages. Following vitamin P therapy (Permidin T.N.) the retinal bleeding

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stopped as did hemorrhages from the nose and bladder, which antedated the X-ray therapy. Bleeding recurred when vitamin P was withdrawn and stopped again when the vitamin therapy was resumed. Mathewson stressed the importance of the capillary factor in X-ray therapy and suggested the prophylactic use of vitamin P to reduce the danger of hemorrhage from this type of treatment. Experimental confirmation of this suggestion has been brought forth by Reckers and Field[116]. Fifty dogs were subjected to a single exposure of X-radiation of 350 r. Twenty-five served as controls and of these, sixteen (64%) died in from 13 to 30 days. All sixteen as well as three of the survivors showed widespread hemmorhagic phenomena. The other twenty-five dogs were treated with rutin orally, 50 mg t.i.d. beginning one week before radiation. Of this treated group, only three (12%) died. All the survivors of the rutinized group as well as one of the three who died, were relatively free of petechiae and ecchymoses during the post-radiation period and when autopsied at 40-60 days. In addition, several rutinized dogs survived a severe leukopenia lasting from 10-14 days. The authors stated that in their previous experience, spontaneous recovery from severe radiation leukopenia of this duration was extremely rare.

Following the explosion of atomic bombs, three types of energy are liberated, blast, heat radiation and ionizing radiation[117]. The latter is said to induce pathological changes identical to those resulting from overdosage of X-radiation. At Hiroshima and Nagasaki[118] the exposed persons

who survived the blast and heat effects, developed an illness similar to that which Shouse and co-workers[112] and Reckers et al.[116] had produced in dogs with overdosage of X-rays. All the formed blood elements were reduced, mostly due to destruction of bone marrow. Terminally, petechiae appeared in the skin and blood oozed from the body orifices.[119] At autopsy, hemorrhages were found inthe tissues and viscera including the brain, meninges, kidneys and gastro-intestinal tract.[120] In written accounts,[119,120] the reduction in platelets was considered to be the cause of the bleeding, but in a subsequent verbal report, Warren[121] mentioned the importance of hyperheparinaemia. Of the 125,000[120] deaths resulting from the atomic explosions, it has been estimated that from 8 to 20% were due exclusively to radiation effects.[117] In view of the importance of hemorrhage and leukopenic sepsis[119-121] as causes of death in these cases, the ability of rutin to inhibit radiation-induced bleeding and death from leukopenia,[116] is of obvious significance. In addition, it has been shown that rutin accelerates the recovery of tissues burned by overdosage of X-ray.[122] Beck and Meissner[119] found that the degenerated marrows of some of the survivors retained a regenerative capacity and they stated that if advantage had been taken of this feature, the death rate from ionizing radiation would have been much lower.

In summary, the chief causes of death following excessive ionizing radiation are hemorrhage and leukopenia. Experimental evidence indicates that the bleeding is due to an increase in circulating heparin coupled with a

decreased capillary resistance. Experimental results point to a favorable influence by rutin on the development of hemorrhage and the complications of leukopenia. These findings indicate the possible prophylactic value of vitamin P in individuals subjected to ionizing radiation for therapeutic or other purposes.

DICOUMAROL THERAPY

McLean and Bramble[123] used rutin therapy to prevent hemorrhages in patients receiving dicoumarol. Their dosage of the anticoagulant was adjusted to yield a 50% drop in prothrombin activity. Since none of their controls developed hemorrhages, possible anti-hemorrhagic effects of rutin could not be determined. There is only a shred of evidence available at present to indicate the possibility of such a haemostatic effect. Shanno[30] reported a case in whom pulmonary hemorrhages of obscure origin recurred three to four times weekly for three months. The only abnormal findings were a slightly positive petechial index and a prolonged prothrombin time. Rutin was given and after three weeks the bleeding stopped and no recurrences took place during a six month follow up period.

It is not known whether a hypocoagulability of the blood is itself sufficient to produce hemorrhage. Wright and Prandoni[124] have shown that the administration of dicourmal, even when it leads to hemorrhage, is not accompanied by a decrease in capillary resistance.

In view of the widespread use of dicoumarol particularly when therapy is prolonged to prevent recurrence

of coronary thrombosis, investigations into the possible haemostatic effect of vitamin P in dicoumarol poisoning are indicated.

DEGENERATIVE VASCULAR DISEASE

For a considerable time, it has been known that newly formed capillaries developed about atheromatous plaques in sclerotic arteries, whereas they were absent in the intima of normal vessels.[125-128] Intramural hemorrhages[129] were also found in such vessels but the relationship between the two was not appreciated until Boyd[130] suggested that the hemorrhages developed as a result of inflammatory degeneration of the intimal capillaries. This was in contrast to previous observers who interpreted them as being due to the influx of blood from the arterial lumen through cracks in the intimal lining.[131,132] Boyd[130] attributed the overlying thrombus, which all had observed, to the hemorrhagic bulging and disruption of the intimal surface. On studying the local changes in coronary and cerebral thromboses by serial sections, Paterson[133-136] and Wartman[137] confirmed the sequence of intimal capillary rupture, intramural hemorrhage, and vascular thrombosis. Paterson found, however, that the capillary rupture was not due to inflammatory necrosis. On tracing the course of the intimal channels, he found many of them opened directly into the lumen of the artery. He thus implicated a high intracapillary pressure as one of the reasons for their rupture. Two other factors stressed by him, were the poor external support afforded the capillaries by the soft atheromatous tissues and the presence of an increased

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capillary fragility, due possibly to a vitamin C deficiency.[136] The significance of these intimal channels is apparent from the studies of Leary.[127] In normal arteries and in early arteriosclerosis, intimal capillaries were absent, but in 120 cases of coronary sclerosis, all advanced lesions were vascularized. Similar findings were made by Horn and Finklestein.[128] It is apparent that as arteriosclerosis progresses, there develops as part of the picture, changes which are the prelude to vascular thrombosis. It is obvious then, that if rupture of the intimal capillaries could be prevented, the incidence of acute coronary and cerebral thrombosis would be greatly reduced.

Studies on capillary fragility and vitamin P therapy have not been made in coronary thrombosis, or specifically on the thrombotic type of stroke. However the preceding studies in vascular thrombosis and the suggestion by Paterson[135] that a similar mechanism might operate in cerebral hemorrhage, prompted Griffith and Lindauer to investigate the importance of the capillary element in hypertension.[138] Prior to their study, numerous workers had found a high incidence of increased capillary fragility[139,140] in this disease. The significance of the associated capillary state was first pointed out by Levrat.[140] In hypertensives he found that vascular and cardiorenal accidents occurred seven times more frequently in those whose capillaries were excessively fragile. In a series of 1200 hypertensives, Griffith and Lindauer[84] found

increased capillary fragility and/or permeability in 30% (360 cases). In this group 9% gave a history of paralytic stroke and 9% showed stigmata of retinal hemorrhages. Of the 70% whose capillary tests were normal, the complications each had occurred in only 2%. Similar impressions were gained by Schweppe et al.[65], but they published no details.

The correlation between hypertensive vascular accidents and increased capillary fragility becomes understandable when the vascular alterations in this disease are studied. In 1872 Gull and Sutton[141] described thickening of arterioles and capillaries by a hyalin fibrous material in cases of "chronic Bright's Disease". In some cases where this vascular degeneration had occurred, the kidneys were found to be healthy but the heart was hypertrophied. The latter were undoubtedly cases of hypertensive nephrosclerosis which at that time had not been differentiated from the primary renal nephritides. In 1926, Volterra[142] found similar changes in hypertensives and arteriosclerotics. The pericapillary sheaths, which normally were composed of fine anastomosing argyrophilic fibres, had become thickened, collagenized and hyalinized and no longer blackened with silver stains. Through these altered channels, extravasations of red cells had occurred, indicative of the increased porosity of the capillary walls. These findings were extended by Gorev and Smirnova-Zamkova[143]. In both human and experimental hypertension, they described the initial change as a hardening and thickening of the argyrophilic pericapillary membrane. Later on, a stage of softening

occurred in whichthe argyrophilic substance underwent collagenous transformation. Similarly, early fibrotic changes developed in arteriosclerosis. It was stressed that these degenerative changes were not confined to the pericapillary sheaths but involved the entire system of ground substance including that present in the adventitia and media of arteries. In six of seven hypertensives with decreased capillary resistance, Schweppe et al.[65] found advanced retinal degeneration and moderate to severe renal impairment. Kidney biopsies from two of these revealed diffuse[144] arteriolar and arteriol sclerosis. Lack and co-workers have observed morphological alterations in capillaries and arterioles in the bulbar conjunctiva of living patients which they regarded as specific for hypertension. Diapedeses and plasma leakage occurred from these damaged vessels. From allthese observations it is clear that structural changes in the capillaries are associated with degenerative changes in the remainder of the vascular tree. Thus the significance of increased capillary fragility in hypertension is that it is an index of retrograde changes in the whole vascular apparatus.

In 1944, Griffith and Lindauer[138] first reported on the use of vitamin P in hypertensive capillary fragility. More recently they gave an account of rutin therapy in 360 cases.[84] In 75% the capillary tests became normal and among these, cerebral and retinal hemorrhage each occurred in 1.5%. The failure of capillary strength to improve in 25% was attributed to discontinuation of rutin or to

insufficient doseage. In this group, apoplexy and retinal hemorrhage each occurred in 9%. The observation period averaged sixteen months. The authors felt that rutin, when it restored the capillary strength to normal, reduced the likelihood of these vascular complications. Improvement in hypertensive capillary fragility by rutin was also recorded by Shanno[30] and by Zfass.[145] Other investigators claim that rutin was of no value in their experience, but in view of the small size of their series (7 and 22 cases resp.) and the short period of observation (2 and 6 months resp.) their findings bear less weight thanthose of Griffith and Lindauer.[84] Although a capillary mechanism has been implicated in thrombotic lesions, it has never been clearly demonstrated as a factor in cerebral hemorrhage. It has been shown that the ground substance degeneration is a diffuse process which involves the arteries as wellas the capillaries.[143] This factor, aggravated by eroding atheromatous plaques undoubtedly allows the entire thickness of the artery wall to give way. In view of the ability of rutin to prevent cerebral hemorrhages, it appears that the action of rutin is not confined to capillaries, but that it acts at least on arteries as well.

In summary, the presence of an increased fragility in hypertension reflects a diffuse degeneration of the vascular tree. This is corroborated by the much higher incidence of vascular accidents when capillary strength is reduced. Cerebral hemorrhage is due to vascular rupture and the prevention of this event by rutin probably reflects its

strengthening effect on the diffuse system of ground substance. There is also some evidence that hesperedin and rutin prevent or restore the decrease in capillary resistance which follow thiocyanate administration.[84,115]

DIABETES MELLITUS

All observers are agreed on the high incidence of increased capillary fragility in diabetes mellitus, and when retinitis is present, the figure approaches 100%.[105, 147-151] The early appearance of degenerative disease in this condition is well known. However, as Wagener[148] has pointed out, some other factor must be present since diabetic retinitis is a separate entity distinct from the retinal changes in arteriosclerosis. On direct visualization of nail beds, Leader[101] observed increased capillary tortuosity in two diabetic children whose disease was present for a considerable time. With the same technique, Davis[152] noted the spontaneous occurrence of microscopic capillary hemorrhages in four of twenty-six cases.

In the paper which announced the discovery of vitamin P,[1] it was reported that citrin failed to raise the low capillary resistance of two diabetics and this has been the experience since then.[105, 148] Hesperedin, calcium eriodictate,[105] lemon juice[105] and rutin[150,151] have all been used with rather disappointing results.[151] Rodriguez and Root used rutin up to 180 mg daily in fourteen severe and six moderately severe cases of diabetic retinitis. After 8 months of therapy, five had regained normal capillary resistance, five had improved tests, and in ten, capillary fragility

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did not change. In one case, capillary fragility became normal only after 12 months of rutin treatment. Although improvement in the visual fields and retinal pictures were not forthcoming, fresh hemorrhages did not occur in those who regained a normal capillary resistance. As these authors have stated, vitamin P has been used usually in advanced cases of retinitis and better results might have been obtained in earlier phases of the disease. Recently, Schneider, Lewis and McCullagh[153] found alterations in plasma proteins in diabetics with poor dietary control. The albumin was decreased and the globulin increased. If retinitis was present, the changes were more marked. In four of six cases of retinitis who were placed on a relatively high protein diet, the retinal picture improved and the plasma albumin level rose. In the two who failed to improve, the plasma albumin did not increase. These findings suggest that a dysfunction of the protein endocapillary layer may be of importance in the development of retinitis.

In summary, in diabetis mellitus, there is a high incidence of positive capillary tests, particularly when retinitis is also present. Both the capillary and retinal pictures have responded poorly to vitamin P therapy. The retinitis is undoubtedly associated with factors other than atherosclerosis. Recent findings of alterations in plasma proteins raise the possibility that a disorder of the endocapillary layer may be causally related to the retinitis.

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FEBRILE AND INFECTIOUS DISEASES

The cause of increased capillary fragility in fibrile and infectious diseases is unknown. Leader[101] could detect no morphological changes by capillary microscopy. Allergic sensitization may be a factor in conditions such as tuberculosis. In those infections due to hyaluronidase-producing organism, the entrance of the enzyme into the circulation could conceivably increase capillary fragility by dissolution of pericapillary sheaths. In fevers, the requirement for all vitamins is increased so that a relative vitamin P deficiency might exist under these circumstances. Armentano et al.,[1] and Morii[83] have shown that vitamin P improves the capillary status in these conditions without affecting the course of the disease.

ALBUMINURIC DISEASES

Dunn[154] states that Cohnheim originated the idea that damage to peripheral capillaries was important in the development of oedema. Some support for this concept is afforded by the findings of Göthlin[81] that capillary resistance is decreased in albuminuric conditions. Although the cause of this change is unknown two possibilities are present. Glomerulonephritis and its variant, lipoid nephrosis, are believed by many to develop on the basis of an acquired sensitivity to streptococcal toxin and as was previously shown, capillary fragility is increased in allergic conditions.[86-89, 91] Thus an increased capillary fragility and permeability of an allergic nature may explain the early

formation of oedema before plasma proteins are significantly reduced. The loss of albumin through visibly damaged glomeruli might also contribute to the capillary damage by disrupting the endocapillary layer.

Armentano et al. used citrin in one case of subacute nephritis with no appreciable change.[103] Lajos claimed that citrin was of value in the treatment of hemorrhagic nephritides of different origin,[155] but Decker was unable to substantiate this claim.

NERVOUS AND MENTAL DISEASE

It is rather surprising that in this field where so many morphological capillary studies have been made, very little investigation has been done as to possible changes in capillary fragility and permeability.

In a series of 1047 schizophrenics, Olkon[156] found by capillary microscopy, that bizarre morphological alterations were present which progressed as the disease advanced. The importance of these capillary changes depends on whether or not they are causally related to the mental changes. Olkon remarked on their similarity in certain respects to the capillaries seen in arteriosclerosis. In excited patients, the frequent occurrence of capillary hemorrhages was noted. The probability of similar petechial hemorrhages in the brain substance attaches a considerable significance to these capillary alterations.

In epilepsy, structural capillary abnormalities have been found,[157] particularly in the cryptogenic and deteriorated types.[158] The ease with which seizures are

induced by forcing fluids, suggests that an increased capillary permeability associated with the morphological alterations may be one of the factors which initiate epileptic seizures.

In migraine,[157] which is genetically related to epilepsy,[151] capillary structural abnormalities similar to those in cryptogenic epilepsy, were found in twenty of thirty-seven patients. During migraine attacks, Redisch and Pelzer[160] have shown that an increased capillary permeability occurs as indicated by blurring and indistinctness of the capillary outline.[161] These changes were absent during the headache free interval. The observation of these functional capillary alterations in the nail fold and oral mucous membrane capillaries indicates that they are of a widespread distribution and are not secondary to the exaggerated arterial pulsations which are felt only in the cranial vessels. The significance of functional capillary abnormalities in this disease is evident from the close relationship between the onset of attacks with conditions which are associated with a decreased capillary resistance. The attacks commonly occur at the menstrual period, at which time Brewer[54] has shown there is a sharp drop in capillary resistance. Redisch and Pelzer[160] found blurring of the capillaries in normal women at the menstrual period so that it appears that an increased capillary permeability also develops normally at this time. Migraine attacks have many features of allergic disorders and as several authors[8-89,41] have shown,

allergic conditions are accompanied by a decreased capillary resistance. Finally, migrainous episodes are frequently precipitated by emotional upsets. As Brewer[54] has shown, adrenalin causes an immediate decrease in capillary resistance and it is more than likely that the outpouring of adrenalin during such emotional crises causes a decrease in capillary resistance. These findings suggest the possibility that a decrease in capillary resistance may be one of the factors which lead to the autonomic discharges responsible for the characteristic symptomatology of migraine. Warter and co-workers[162] have shown that capillary resistance decreased under emotional stress and the capillary change could be prevented by hesperedin.

In summary, schizophrenia, cryptogenic epilepsy and migraine are associated with structural capillary abnormalities. In each case, associated changes in fragility and permeability may be one of the factors which determine the course of the disease. It is suggested that stabilization of this link may arrest the disease process, just as strengthening of the capillaries in radiation disease by rutin prevented the development of the hemorrhagic phenomena.

EXPERIMENTAL SECTION

INTRODUCTION

When vitamin P was discovered, its function was said to be the regulation of the permeability and fragility of capillaries. Since then all the work which has been done in this field has been focused on capillaries and the possibility that this vitamin might be concerned with the regulation of permeability of other tissues, has received scant attention.

A broader aspect of vitamin P activity was suggested to the author by the findings of Griffith and Lindauer[84], that rutin prevented cerebral hemorrhage in hypertensive patients with increased capillary fragility. Although their study was stimulated by Paterson's[135] suggestion that intimal capillary rupture might lead to cerebral hemorrhage, a capillary role has never been clearly demonstrated in this type of vascular accident. It is more likely that cerebral hemorrhage is due to direct rupture of the whole thickness of the artery wall as a consequence of arteriosclerotic weakening combined with increased arterial blood pressure. If such is the case, it points to an action of rutin on the entire vascular wall rather than to an effect limited to capillaries. Evidence has been presented which indicates that the pericapillary membrane is the site of action of vitamin P on the capillary wall. Gorev and Smirnova-Zamkova[143] have shown that the pericapillary sheath, the media

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and externa of arteries and the connective tissue in general, all have in common, argyrophilic ground substance. It therefore appeared that the strengthening effect of rutin on the capillaries and arteries might be due to a stabilizing effect on ground substance.

In seeking an explanation for the decreased capillary resistance in vitamin P deficiency, the possibility of weakening of ground substance by an increased hyaluronidase activity was considered. This enzyme (s) has been shown to increase capillary fragility.[5] According to Duran-Reynals,[16] small amounts of hyaluronidase are present in most if not all tissues. This is in accord with the suggestion that the action of vitamin P was not confined to capillary walls. The possibility therefore suggested itself that this diffusely distributed enzyme was a factor which enhanced the permeability of ground substance in capillaries and elsewhere, and its overactivity was prevented by vitamin P. A working hypothesis was therefore set up to the effect that the state of the permeability of ground substance in general was the result of the interaction of vitamin P and hyaluronidase.

PROCEDURE

To test out the above hypothesis, it was planned to determine the effects of rutin on the inhibition of mucin clot formation by hyaluronidase. As rutin is insoluble in water, a solution was prepared in propylene glycol. When the enzyme solution was added to the rutin solution, the rutin crystallized out. This occurred even when distilled water was added to the rutin solution

Alkaline solutions of rutin were not tried because the above test requires the presence of an acid medium. At this time, water soluble types of vitamin P were not readily available and the in vitro experiments were therefore abandoned and animal experiments were begun.

A.

THE EFFECT OF RUTIN ON THE DIFFUSION OF HYALURONIDASE AND SALINE

Procedure

In this experiment, forty-four male albino rats were used, weighing from 250-400 grams. Ten animals were given rutin intraperitoneally 3.5 hours before the hyaluronidase. The dose was 200 mg dissolved in 1 cc propylene glycol. Ten were given 1 cc propylene glycol, but no rutin. Fourteen were not pretreated. The hyaluronidase was prepared in a 2% solution in 0.9% saline. The dry powder assayed 75 T.R.U. per mg and was purchased from the Tremond Company, New York. The freshly prepared enzyme solution was gently mixed with an equal volume of doubly filtered Higgins india ink and 0.1 cc of the final solution was injected intradermally into the right flank of all animals. Into the left flank 0.1 cc of a solution of equal volumes of ink and saline was injected intradermally. An attempt was made to make all injections at the same point in each animal to avoid regional differences in spreading. This point was midway between the upper and lower extremities and 4 cm from the mid-dorsal line. Twenty-two hours later, the rats were killed with ether and skinned. The outlines of the black

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areas on the inner surface of the skins were traced out and transposed onto cellophane paper. The enclosed areas were then measured with a planimeter. The results are presented in tabular form and analysed statistically. The method of analysis of variance was used throughout since it is the most rigid criterion of significance. In Table 1, Student's t test was used to compare the effects of propylene glycol when used alone as a control and when used as the rutin solvent.

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TABLE 1

EFFECT OF RUTIN ON SPREADING ACTIVITY OF HYALURONIDASE AND SALINE†

Controls		Propylene Glycol		Rutin in Prop.Glyc.	
Ink-Sal.	Ink-Hy'ase	Ink-Sal.	Ink-Hy'ase	Ink-Sal.	Ink-Hy'ase
0.14	1.20	0.35	1.14	0.11	0.40
0.28	1.10	0.21	0.87	0.12	0.22
0.44	1.41	0.25	0.84	0.12	0.35
0.22	1.20	0.24	0.45	0.07	0.38
0.27	2.00	0.49	0.90	0.10	0.39
0.25	2.50	0.25	1.28	0.13	0.40
0.22	2.52	0.30	0.96	0.14	0.30
0.30	1.92	0.28	0.98	0.14	0.43
0.28	2.20	0.33	0.85	0.13	0.33
0.28	1.00	0.32	0.80		
0.35	1.20				
0.10	1.30				
0.17	0.59				
0.20	1.05				
$\bar{X}$ 0.25	1.51	0.302	0.907	0.117	0.355
$S_{\bar{X}}$ 0.030	0.196	0.041	0.118	0.016	0.085

Mean Differences in Ink-Hyaluronidase Data ("t"-test)

$\bar{X}$(control) - $\bar{X}$(propylene glycol) = $0.603^{*} \pm 0.228$

$\bar{X}$(propylene glycol) - $\bar{X}$(rutin propylene glycol) = $0.552^{**} \pm 0.145$

Mean Differences in Ink-Saline Data

$\bar{X}$(control) - $\bar{X}$(propylene glycol) = 0.052 ± 0.050

$\bar{X}$(propylene glycol) - $\bar{X}$(rutin propylene glycol) = $0.185^{***} \pm 0.043$

Analysis of Variance

	F(observed)	F(0.01 signif.)
Ink-Hyaluronidase	19.2***	5.39
Ink-Saline	16.3***	5.39

* significant
** highly significant
*** very highly significant

† All figures in this and subsequent tables are in square inches.

COMMENT

From the data it is clear that the administration of rutin results in a marked diminution of the spreading activity of hyaluronidase solution and a reduction in the diffusion of saline. From the analysis of variance, it is evident that the differences in spread are due to rutin treatment and not to variations in response within each group of animals. Propylene glycol also reduced the spreading activity of hyaluronidase and the further reduction in spreading activity on the addition of rutin was highly significant. The administration of propylene glycol with or without rutin was accompanied by a slight degree of shock and it is possible that the resulting liberation of adrenalin accounts for the propylene glycol component of the inhibitory effect. Favilli[164] and Homburger[165] have shown that adrenalin depresses the spreading activity of the enzyme.

It was of course impossible to conclude from these results whether the inhibition of hyaluronidase was a direct action on the enzyme or whether it was secondary to an increased resistance of the tissues. To clarify this point the effect of rutin on the diffusion of a non-vital spreading factor was determined.

B.

EFFECT OF RUTIN ONTHE DIFFUSION OF AZOPROTEIN

PROCEDURE

P-diazobenzene sulfonic acid was prepared according to the directions of Claude[166] and it was coupled with horse serum to yield azoprotein. The spreading activity of this substance extends over several hours, in contrast to the activity of

hyaluronidase, most of which is over in one hour. Rutin 1.3 g was dissolved in 20 cc 0.2N sodium hydroxide. This yields a 6.5% solution which is close to the saturation point. The ph of this solution was measured in a Beckman ph meter and found to be 9.4 To the 20 cc of this solution 1.25 cc N hydrochloric acid was added at which point the solute precipitated out to form a smooth creamy emulsion. The ph of this was 8.4 A control solution of sodium hydroxide of ph 8.4 was prepared. The spreading solution consisted of three parts azoprotein to one part doubly filtered india ink. The control solution was three parts saline to one part ink. Intradermal injections were made in the flanks as previously but this time 0.2 cc of each solution was used. If an appreciable amount of the solution leaked out onto the skin surface, or if the needle was too deeply placed so that part of the injection was subcutaneous, the animals were immediately killed and discarded. The test animals were male albino rats weighing between 275 and 325 g. Eighteen served as controls. Eighteen were given 1.0 cc of the rutin suspension i/p and eighteen were given 1.0 cc of the control sodium hydroxide i/p solution four hours before the inks were injected. Three rats from the first group, five from the second, and one from the third group were discarded for reasons previously stated. The injection of the rutin and control solutions caused no apparent discomfort or shock. Twenty hours after the intradermal injections, the animals were killed and skinned and the inked areas measured as before. The results are given in Table 2 and were analysed by the method of

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analysis of variance. Where differences were less than 15% they were considered to be insignificant and were therefore not analysed.

TABLE 2

EFFECT OF RUTIN ON DIFFUSION OF AZOPROTEIN AND SALINE

Controls		Control NaOH		Rutin in NaOH	
Azoprot. ink	Saline ink	Azoprot. ink	Saline ink	Azoprot. ink	Saline ink
3.86	0.60	3.40	0.80	1.90	0.30
2.60	0.80	3.40	0.70	1.80	0.40
4.00	0.53	3.20	0.64	4.00	0.50
3.50	0.70	3.85	0.55	1.76	0.40
3.50	0.60	3.00	0.65	2.63	0.45
3.05	0.60	2.35	0.40	1.93	0.30
3.83	0.75	4.00	0.60	2.57	0.43
2.72	0.60	2.96	0.70	1.65	0.44
2.94	0.70	3.45	0.65	1.84	0.45
4.20	0.68	4.30	0.70	2.34	0.43
3.02	0.69	4.20	0.50	2.07	0.30
2.91	0.58	3.77	0.64	1.50	0.40
3.35	0.83	3.41	0.60	1.92	0.30
3.20	0.70			1.62	0.23
4.93	0.70			1.60	0.33
				2.72	0.43
ΣX 51.61	10.01	45.29	8.13	1.82	0.44
				35.67	6.53
N 15		13		17	
$\bar{X}$ 3.44	0.67	3.48	0.62	2.10	0.38

Variance Ratios Control cf. Rutin

	Azoprotein	Saline
F (observed)	51.66***	95.0***
F (.01)	7.56	7.56

***very highly significant.

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RESULTS

Inspection of the mean areas of spread reveals that the control sodium hydroxide solution failed to reduce the diffusion of azoprotein or saline. A solution of rutin in sodium hydroxide on the other hand, markedly inhibited the spread of both.

DISCUSSION

In view of the non-vital nature of azoprotein, the effect of rutin on its spread cannot be attributed to enzymatic inactivation. Although inactivation by a specific chemical reaction between the two is possible, the fact that the diffusion of the inert saline-ink mixture was also reduced, favors the view that rutin caused a non-specific increase in the resistance of connective tissue. Since hyaluronidase is still able to spread through isolated dead skin,[167] its diffusion, at least in part, must be independent of the circulation and must occur directly through the ground substance of the connective tissue. It appears then, that the action of rutin is to increase the resistance of ground substance against permeation by inert substances as well as active spreading factors.

CONCLUSIONS

The findings fail to support the hypothesis that rutin directly inhibits hyaluronidase. Rather it appears that both these substances have direct but diametrically opposite actions on the permeability of ground substance. The net result is a balance between the opposing effects rather than between the substances themselves.

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C.

EFFECTS OF CATECHIN, HESPEREDIN AND ESCULIN ON CONNECTIVE TISSUE PERMEABILITY

In order to determine if the effects of rutin on ground substance were representative of vitamin P. the experiments were repeated with catechin, hesperedin methyl chalcone and esculin. These types of vitamin P, especially the first two, are water soluble and were given i/p in aqueous solution. Three groups each of 20 female albino rats weighing between 225 and 300 g were used. The rats of one group were each given 10 mg of catechin in 0.25 cc water. The second group were given 100 mg hesperedin in 0.25 cc water. The third group served as controls and were given 0.25 cc plain water i/p. One hour later intradermal injections of inked hyaluronidase and saline were made as before. The injection mass was 0.2 cc in each case and the quantity of hyaluronidase was the same as before i.e. 2.0 mg per dose. The animals were killed 20 hours later and the skins prepared as usual.

OBSERVATIONS

On inspection there was no significant difference inthe spreads between the three groups so that measurements were not made. Since the experiments on the effect of catechins on capillary resistance were made in guinea pigs, the above experiment was repeated in part on these animals.

PROCEDURE

Twenty-four male and female guinea pigs weighing from 250-400 g were divided into two groups so as to be

[illegible]merized mixture containing 33% d'epicatechin

equally distributed according to weight and sex. This time 5.0 mg of catechin was injected i/p and this was repeated in six hours. The intradermal solutions were injected forty minutes after the second dose of catechin. The animals were killed 20 hours later and the spread of the inked solutions observed.

OBSERVATIONS

On inspection there seemed to be a slight reduction in the spread of hyaluronidase. On measurement this difference was found to be 12% which arbitrarily and statistically was insignificant.

TABLE 3

EFFECT OF CATECHIN ON THE DIFFUSION OF HYALURONIDASE AND SALINE IN GUINEA-PIGS

Controls		Catechin	
hyaluronidase ink	saline ink	hyaluronidase ink	saline ink
3.87	1.40	2.93	1.55
4.40	1.20	2.60	1.00
1.90	0.43	3.00	0.80
3.00	1.22	2.80	0.64
3.15	0.73	2.70	0.81
1.98	0.78	3.12	1.10
2.25	0.82	2.30	0.82
2.30	1.00	2.32	1.00
2.97	1.00	2.10	0.88
2.07	1.05	2.20	0.85
3.16	0.92	2.34	0.96
		1.78	1.00
Σx 30.19	11.41	31.05	10.55
$\bar{x}$ 2.52	0.95	2.82	0.96

VARIANCE RATIO

Catechin cf. control

	F(.05)	F(observed)
ink-hyaluronidase	4.32	1.43

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CONCLUSION

An epimerized mixture of catechins in single 10 mg doses inrats and in two 5 mg doses in guinea pigs, and hesperedin methyl chalcone in single 100 mg doses in rats failed to reduce the diffusion of hyaluronidase or saline. The catechin mixture was used in small doses because it was claimed to be the most potent type of vitamin P. It is possible that larger doses might have been effective.

PROCEDURE

In view of this experience with catechin, it was decided to give esculin over a period of days before its possible effect on ground substance was tested. Short supplies prevented extended administration of hesperedin and catechin. Five mg esculin in 1.0 cc water was injected i/p daily for six days, into thirty-six of a group of seventy-two hooded male rats weighing between 150-200 g. This concentration of esculin was slightly above its saturation point so that a small amount of the material was in suspension. On the 6th day, the group was divided into two. One group received intradermal injections of hyaluronidase and of saline, the other group, azoprotein and saline. The hyaluronidase in this series assayed 100 T.R.U. /mg of which 2.0 mg was present in each 0.2 cc injection. The azoprotein solution was similar to that used previously. The untreated thirty-six rats were also divided into two groups to serve as hyaluronidase and azoserum controls. Seven rats were rejected and killed immediately because of poorly placed intradermal injections. The remainder were killed twenty hours after the intradermal

injections, and the areas of spread were measured as usual. The results are presented in Table 4 with an analysis of the variance. The saline injections in both control series were treated as one group.

RESULTS

Esculin given in 6 daily doses each of 5.0 mg inhibited the diffusion of azoprotein and saline to a degree which was statistically highly significant. The effect on the spread of hyaluronidase though not as marked, was still significant.

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TABLE 4

EFFECT OF ESCULIN ON DIFFUSION OF HYALURONIDASE, AZOPROTEIN AND SALINE

Controls		Esculin	
Hy'ase	Saline	Hy'ase	Saline
2.38	0.53	1.40	0.30
2.45	0.45	1.78	0.57
2.74	0.58	2.00	0.50
1.55	0.40	3.52	0.55
2.70	0.47	2.50	0.48
2.66	0.50	1.70	0.40
1.82	0.63	1.28	0.50
2.74	0.80	1.37	0.43
1.68	0.53	1.56	0.59
1.60	0.53	1.10	0.49
1.87	0.50	1.63	0.47
1.20	0.43	1.70	0.44
2.55	0.75	1.70	0.49
2.44	0.74	1.23	0.30
1.90	0.48	1.42	0.63
2.66	0.60	1.73	0.40
2.15	0.76	1.51	0.41
3.00	0.59	1.32	0.42
$\bar{X}$ 2.23		1.69	0.46
Azoprot.	Saline	Azoprot.	S aline
3.14	0.50	3.10	0.58
2.39	0.45	2.53	0.50
3.12	0.70	2.35	0.46
3.40	0.46	2.92	0.54
2.33	0.50	2.80	0.55
3.72	0.50	2.20	0.40
2.17	0.64	2.30	0.33
3.37	0.53	2.24	0.20
2.68	0.54	2.39	0.62
3.70	0.55	1.84	0.42
2.80	0.50	1.90	0.46
3.15	0.60	1.92	0.42
2.55	0.40	2.18	0.48
2.53	0.47	2.60	0.55
3.40	0.56		
$\bar{X}$ 2.96	0.55	2.38	0.47

VARIANCE RATIO

Esculin cf. control		
	Hy'ase	Ink
F obs.	4.48*	8.40**
F.05	4.13	4.06

VARIANCE RATIO

Esculin cf. control	
Diazo	
F obs.	12.32**
F.01	7.68

EFFECTS OF VITAMIN P DEFICIENCY ON PERMEABILITY OF GROUND SUBSTANCE

In the experiments listed so far, the various types of vitamin P were administered to animals on normal diets* and the results could be interpreted as pharmacological rather than vitaminic. It was therefore decided to determine if the state of tissue permeability was altered in vitamin P deficient rats. It was decided to use the diet which Parrot[23] recommended, because of his warning that ordinary scorbutic diets such as the Sherman-La-Mer-Campbell diet which contained white oats, were not completely free of vitamin P. Unfortunately, grey oats, one of the major constituents, was not available locally and this method could not be used. As an alternative procedure, advantage was taken of Parrot's[169] finding that turnips contained a substance with the properties of antivitamin P. Two hundred gamma of an alcoholic extract of turnip produced a drop in capillary resistance which could be prevented by 20 gamma of catechin. The turnip factor accelerated the invitro oxidation of ascorbic acid whereas vitamin P substances inhibited this reaction. The action of the turnip factor was therefore diametrically opposed to that of vitamin P both in vivo and in vitro and it was therefore called antivitamin P.

Procedure

In view of the above findings, forty-five of a group of sixty male white albino rats weighing from 180-210 g

* Purina Fox chow for rats

* Various greens for guinea pigs

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were placed on a diet of turnips and water ad lib. for 14 days. The remaining fifteen rats served as normal diet* controls. By the 12th day, eight of the turnip group and one of the control group had died. At this time, the turnip group was divided into three sub-groups. One sub-group was given 50 mg ascorbic acid i/p on the 12th and 13th days. Another was given 5.0 mg esculin i/p on the same days and the third sub-group was not treated. On the 14th day intradermal injections of inked azoprotein and saline were made in all animals and several were rejected because the injections were too superficial or too deep. The correctly injected animals were killed 20 hours later and the areas of spread measured. The results are recorded in Table 5 with an analysis of the variance.

RESULTS

The most striking finding was the marked increase in diffusion of both azoprotein and saline in the rats on the turnip diet. With the amounts of esculin used, the increased spread of the azoprotein was moderately but significantly reduced. Esculin had no significant effect on the enhanced diffusion of saline. Ascorbic acid failed to inhibit the increased spread of both azoserum and saline.

CONCLUSIONS

Rats maintained on a turnip diet for 14 days acquired a marked increase in the permeability of their

* Purina Fox chow.

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ground substance. Small doses of esculin partially offset this change but ascorbic acid had no effect. The failure of relatively large doses of ascorbic acid to increase the resistance of the ground substance is in accord with the findings of most investigators[12-19], that vitamin C does not increase capillary resistance. In view of the findings of Parrot[169], previously discussed, it is suggested that the increased permeability of the ground substance is due to a deficiency of vitamin P induced by ingestion of turnip antivitamin P factor.

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TABLE 5

EFFECT OF TURNIP ANTIVITAMIN P FACTOR ON DIFFUSION OF AZOPROTEIN AND SALINE

Regular Diet		Turnip Diet	
Azoserum	Saline	Azoserum	Saline
4.30	0.85	4.50	1.15
4.00	0.60	5.66	1.02
3.66	0.38	5.00	1.67
3.64	0.55	5.90	1.20
2.50	0.62	4.27	0.80
3.89	0.66	4.80	1.00
2.63	0.40	3.92	1.50
3.10	0.40	6.50	1.57
2.70	0.37	5.08	1.27
3.89	0.70		
2.25	0.44		
36.56	5.97	45.63	11.18
$\bar{X}$ 3.32	0.54	5.07	1.24

Turnip Diet+Esculin		Turnip Diet+Ascorbic Acid	
Azoserum	Saline	Azoserum	Saline
3.20	1.11	5.23	0.90
4.70	0.97	4.04	1.04
3.16	1.55	4.30	1.04
5.24	1.05	4.30	1.95
5.18	0.75	4.57	1.15
4.60	1.04	4.80	1.00
4.04	0.87	5.05	1.35
4.50	0.50	4.10	1.08
4.13	1.05	3.67	1.14
4.03	1.20		
3.80	0.90		
46.58	10.99	40.06	10.65
$\bar{X}$ 4.23	1.00	4.45	1.18

VARIANCE RATIOS

Turnip Diet cf. Regular Diet		
	Azoprotein	Saline
F (obs)	25.6***	47.3***
F (0.05)	4.41	4.41

Turnip Diet+Esculin cf. Turnip Diet alone		
F(obs)	5.8*	3.82
F(0.05)	4.41	4.41

Turnip Diet+Ascorbic acid cf. Turnip diet alone		
F(obs)	3.67	---
F(.05)	4.49	---

GENERAL SUMMARY

A working hypothesis was set up in which the permeability and resistance of ground substance in capillaries and in connective tissue elsewhere, were considered to be the result of the interaction of vitamin P and hyaluronidase. When it was found that rutin inhibited the spread of a non-vital spreading agent, azoprotein, as well as the diffusion of inert saline, it was concluded that rutin had no direct antihyaluronidase activity but that it acted on the ground substance so as to cause a non-specific increase in its resistance. The findings with rutin were reduplicated with esculin, but not with catechin or hesperedin methyl chalcone. A probable vitamin P deficient state was produced by submitting rats to a turnip diet which was said to contain an antivitamin P factor. In such animals there was a marked increase in the permeability of ground substance which could be reduced partially by esculin but not by ascorbic acid. It is felt that these findings indicate that vitamin P is a factor in the regulation of the permeability not only of capillary gound substance, but of connective tissue ground substance in general. Since the pericapillary sheath is commonly regarded as a condensation of ordinary connective tissue, these findings support the concept that vitamin P regulates capillary permeability and resistance through an influence on the pericapillary membrane.

Abundant evidence has been reviewed by Duran-Reynals [163] which indicated that the susceptibility of the organism to toxic

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and infectious agents varied directly as the degree of permeability of the connective tissues. In addition, the spread of certain malignant tumors appeared to be facilitated by autoproduction of hyaluronidase. Duff[170] has clearly shown that in experimental rabbit arteriosclerosis, an alteration in the intimal ground substance preceded the deposition of lipoids. These observations raise the possibility that by controlling the ground substance factor, the spread of infections and malignancies and the development of degenerative vascular disease may be inhibited. The finding that vitamin P increases the resistance of ground substance therefore assumes an importance whose extent remains to be determined by future experimentation.

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