SUBSTANCES WHICH AFFECT CAPILLARY PERMEABILITY

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I. INTRODUCTION

The capillary wall is a barrier freely permeable to water and electrolytes but only very slightly permeable to protein. The term "increased capillary permeability" therefore refers normally to an alteration in the capillary wall leading to an accelerated rate of passage of plasma proteins into the tissues. It is used in this sense in the present review. Increased capillary permeability is one of the cardinal features of acute inflammation, *i.e.*, the local reaction of living tissue to injury. It is in this and in other pathological states, *e.g.*, proteinuria, and in relation to the mode of action of vaso-active compounds that the importance of the phenomenon lies.

For the purpose of this review substances which increase capillary permeability are defined as those compounds which exert this effect in reasonably low concentrations and without causing apparent tissue destruction. Thus chemical irritants of all types have been excluded from consideration, as well as concentrated salt solutions.

The following compounds have been considered to fall within the scope of this article—histamine and substances which release it; 5-hydroxytryptamine and substances which release it; certain peptides and peptide mixtures, certain proteins, notably serum globulins, and finally, oestrogens and relaxin. Although this list is reasonably complete at the moment, there is no reason to suppose that other examples do not remain to be discovered.

Of the compounds which suppress increased capillary permeability, those which act by antagonising specific substances, for example, the antihistamine drugs, have been discussed together with the permeability-increasing factors the action of which they oppose; others whose mode of action is still obscure have been considered separately.

II. THE MECHANISM OF INCREASED CAPILLARY PERMEABILITY WITH SPECIAL REFERENCE TO STRUCTURAL CONSIDERATIONS

The relationship of the structure of the capillary wall to alterations in its permeability has been reviewed by Chambers and Zweifach (21). Passage of proteins across the wall could occur theoretically through the endothelial cells or through the intercellular substance. Chambers and Zweifach concluded that all the available evidence indicated passage through the intercellular substance. The decade which has passed since their article appeared has not produced any convincing evidence to refute their conclusion.

The speed with which proteins leave permeable capillaries suggests that they do so by passing between the endothelium rather than through the cells. It is unlikely, although not impossible, that endothelial cells could survive the rapid and sustained passage of large amounts of plasma protein through their cytoplasm. It is true that the renal tubular epithelium transports plasma proteins from the glomerular filtrate to the blood but this process is relatively slow and is moreover inhibited by noxious stimuli rather than initiated by them (107).

The speed with which water passes through the normal capillary wall suggests that this substance too may leave the vessel between the endothelial cells. Wilbrandt (153) has shown that under comparable conditions of pressure, area and time, the capillary wall is 100 times more permeable to water than are leucocytes, red blood corpuscles, fibroblasts, amoebae and arbacia eggs.

Chambers and Zweifach (21) stressed also that the chief factor determining the rates of passage of different types of molecules through the capillary wall was the size of the molecule. They concluded that a selective passage of this nature could occur only through "non-living" constituents of the wall and that cell transport mechanisms could not be involved.

It might be supposed that electron microscopy would have led to notable advances in our knowledge of structural and functional relationships in capillary walls, but this is not yet the case. Karrer (71) studied the ultrastructure of capillaries in mouse lung and commented on the apparent absence of any anatomical gaps between the endothelial cells, on the presence of very numerous tiny vacuoles in their cytoplasm and on the fine prolongations of cytoplasm which help to maintain a continuous layer of endothelium. It cannot be denied that the vacuoles might be anatomical evidence of transport mechanisms of great rapidity but there is no other evidence to support such a view.

Evidence of anatomical changes in capillary walls during increased permeability has been provided by direct observation (21, 47). Chambers and Zweifach found softening and stickiness of intercellular cement always to precede increased permeability to large molecules. Microtrauma applied to the vicinity of capillaries in frog mesentery was followed by the sloughing into the blood stream of a substance that may have been intercellular cement. Chambers and Zweifach concluded that vascular endothelium may continually replace the intercellular substance. Their conclusion may be related to the recent suggestion that such endothelium synthesises mucopolysaccharide in large quantities (27). McGovern's observations on injury to endothelial surfaces (84) indicating physico-chemical

changes in intercellular polysaccharides are possibly relevant to the findings of Chambers and Zweifach.

Another anatomical change associated with increased capillary permeability was observed to follow local application of hypertonic mannitol and sucrose solutions, *i.e.*, a contraction of endothelial cells with development of apparent spaces between them through which passed red blood corpuscles. It is possible (47) that the apparent swelling of vascular endothelium in inflammation is related to this observation.

Chambers and Zweifach (21) also considered the maintenance by the normal capillary wall of a state of semipermeability. Danielli (29) has advanced the hypothesis, which is difficult to prove or disprove, that certain plasma proteins might be adsorbed on to and within the intercellular material to form an endocapillary layer whose presence might be essential to maintain semipermeability. It has been suggested that the endocapillary layer and even the interendothelial substance itself have certain similarities to fibrin and that heparin prevents their formation (3). It is not yet possible to form an opinion on this hypothesis but there is no evidence that the injection of heparin into the skin leads to increased capillary permeability (135).

Our understanding of the passage of large molecules through capillary walls has been improved by Pappenheimer's review of the subject (100). Pappenheimer's analysis led him to the conclusion that non-lipoid-soluble molecules leave the capillaries by passing through water-filled channels in the capillary wall; that these channels occupy only a very small proportion of the capillary wall (about 0.2%), and that they may well be confined to the interendothelial substance as suggested by Chambers and Zweifach. Pappenheimer concluded also that the diameter of these channels is probably similar throughout the capillary and of an effective pore radius of 44 Å, probably varying from the capillaries of one tissue to that of another. In addition, he suggested that selective passage based on molecular size is governed by "restricted diffusion". This means that although the channel is larger than the molecule passing through it the molecule's passage is nevertheless hindered by at least two forces, first viscous drag as seen between a sedimenting particle and the walls of its container, and second, steric hindrance. Steric hindrance implies that diffusing molecules can enter a pore only if they do not strike the edges of the pore. Applied to the current conception of increased capillary permeability, Pappenheimer's views suggest that, in this condition, either the system of channels disappears or the channels enlarge or the forces which restrict diffusion are in abeyance. In particular, alterations in charge might occur at the pores.

Pappenheimer also pointed out that if the filtration of water from the capillary were greatly reduced, then even restricted diffusion of protein might keep pace with passage of water so that the concentration of protein on both sides of the capillary wall would be similar. It is uncertain whether filtration of water ever falls to such a low value *in vivo* but, if present, such a state of affairs might be hard to distinguish from a condition of increased capillary permeability. On the other hand, the oedema fluid which might accumulate as a result of cessation of

vasomotion in the precapillary sphincters coupled with dilatation of the terminal arterioles (21), although it might contribute to inflammatory swelling, should contain only a low concentration of plasma protein.

An observation which may be important but the significance of which is still obscure is that the porosity of capillaries to colloidal dyes is highest at their venous end (116). Chambers and Zweifach (21) confirmed this but then found that on reversal of the direction of blood flow, the former arterial end was the more permeable to dyes and the former venous end of the capillary the less permeable. They suggested that the increased porosity was due to a factor in the venous blood. It seems clear that attempts to correlate theoretical analyses such as Pappenheimer's with the observed facts of increased capillary permeability are desirable. At the moment, however, it must be admitted that almost nothing is known of the intimate anatomical, physiological and biochemical changes initiated in capillary walls by exogenous and endogenous substances which increase capillary permeability.

III. DEMONSTRATION OF INCREASED CAPILLARY PERMEABILITY

Increased permeability of capillaries in inflammation manifests itself by the formation of a fluid exudate containing 1–6 g of plasma protein per 100 ml. Demonstration of the phenomenon experimentally can occasionally be achieved by direct measurement of plasma protein concentration in the extravascular compartment, *e.g.*, in blister fluid. More often, however, recourse has to be made to labelling the plasma protein in some way that facilitates observation of its passage through the vessel walls.

1. Direct microscopic observation. Chambers and Zweifach (20, 21) as well as others (47) have observed the passage of dye bound to plasma proteins through capillary walls damaged by microtrauma. The protein-dye complex was not seen to leave undamaged vessels. Permeability to protein did not appear until the microtrauma reached a certain level of severity. Milder damage led only to accumulation of red corpuscles, platelets and leucocytes against the vessel wall. Direct and therefore convincing observations of this kind have been purely qualitative and attempts to study vaso-active compounds by such techniques have been few in number. Zweifach has, however, demonstrated that histamine increases capillary permeability in rat mesentery (159), although only in high concentration. It would probably be profitable to revive the technique in order to study the effects of other compounds that increase capillary permeability.

2. Perfusion. The development of oedema in perfused tissues, *e.g.*, frog mesentery or rat hind quarters, is usually taken to indicate the onset of increased capillary permeability. By varying the constituents of the perfusate some knowledge can be obtained of their role in maintaining the condition of the vessel walls. Thus the omission of calcium and a fall in pH lead to oedema (20). The effect of various colloids in maintaining normal capillary permeability has also been studied by this technique (21, 29). The chief merit of perfusion experiments is that they provide one of the few ways of determining which compounds may be necessary to maintain the normal semipermeability of vessel walls.

3. Leakage of protein-bound dye into skin. Reference has already been made to the use of protein-dye complexes in the mesentery. The technique now to be described, however, is the most widely used in the study of the effect of substances on capillary permeability. It is generally thought to have been originated by Ramsdell (106) and was revived by Menkin (90, 92). The principle is to inject an animal with one of a number of dyes, usually trypan blue, Evans blue or pontamine blue. This dye becomes attached to circulating plasma albumin to form a stable dye-protein complex (108). The test substance is then injected in small volume into the shaved or depilated dorsal or ventral skin. If the substance increases capillary permeability the bleb so raised soon becomes coloured with the protein-bound dye as it leaks out of the permeable vessels in the injection site. Areas of inflammation show similar dye-staining (110). The method is always at least roughly quantitative as falling dilutions of a substance can be injected into the same animal until, below the lowest effective dilution, no local leakage of dye occurs.

The test has been used on rabbits, mice, guinea pigs, rats and even man (140). The use of the guinea pig for this purpose has been explored fully by Miles and Miles (93). These workers showed the area of dye leakage in trunk skin to vary directly in linear fashion with the log of the dose of histamine, "leucotaxine" and compound 48/80 (a mixture of polymers prepared by condensing N-methyl-homoanisylamine with formaldehyde) when these were injected intradermally in a constant volume. The increase in capillary permeability caused by these compounds was found to commence 3 to 5 min after injection and to have ceased 10 to 15 min after injection. From 30 min to 4 hr after injection the capillaries at the site of inoculation were immune or refractory to further doses of the compound and sometimes to other permeability-increasing compounds.

Deep anaesthesia, shock, chilling and raising the air temperature to 37°C were found to reduce the leakage of circulating dye caused by the test compounds, possibly because of a lowering of blood pressure or of changes in the capillary wall, as yet obscure, which rendered the vessels immune to further stimuli.

The abdominal skin of albino rats has also been used extensively (133-139). Rats show a linear relationship between log dose of test compound and area of cutaneous dye leakage, similar to that demonstrated in guinea pigs (132). In addition, rats are easily available and probably less susceptible to variations due to anaesthesia and temperature.

Rabbits have also been used widely (90, 106) and they, too, respond in linear fashion as guinea pigs (132). Mouse skin, however, is too thin to give good results. It is now generally realised that results obtained in one species cannot be assumed to apply to other species without experimental confirmation (132).

A similar method of estimating the effect of substances on capillary permeability, especially in guinea pigs, is to inject the test compound intravenously together with the dye. Leakage of dye then occurs into the skin in various parts of the body wherever the compound under investigation is able to exert its effect. No attempt has yet been made to make this technique quantitative, although the rate of disappearance of the dye from the circulation has been so used (19).

4. Observations on the paws of rats. Another method now employed is to inject the test substance into the dorsum of the feet in rats (117). If capillary permeability is increased, oedema results; this can be estimated by eye, by weight, by increase in diameter or by content of water, or even measured plethysmographically (19, 24, 57, 103, 117, 157). If dye is injected into the circulation the skin of the oedematous area becomes dye-stained. Parratt and West (103) believe that visual judgment permits estimation of the degree of swelling as accurately as any other means. The reviewer has used the method and finds it roughly quantitative but does not agree that it provides an accurate assessment of increased capillary permeability.

Gözsy and Kato (52) have advanced the view that oedema and local accumulation of circulating protein-bound dye are due to different mechanisms. They observed intracutaneous injection of histamine to cause dye leakage which was inhibited by the administration of mepyramine, whereas inunction of histamine into the skin led to oedema which was not antagonised by mepyramine. To produce this effect, however, it was necessary to prolong the inunction for at least six minutes. In addition, the resultant oedema was slow to develop and lasted for several hours. Its characteristics suggest, therefore, that it was due not to the application of histamine but to activation of other mechanisms by local trauma. There is no reason to expect oedema of this nature to be susceptible to mepyramine (Neo-Antergan, 2[(2-dimethylaminoethyl)(*p*-methoxybenzyl)-amino]pyridine). Failure of the oedematous areas to show dye staining might be due to poor affinity of the subcutaneous tissues for the protein-dye complex even though this may have been leaving the blood vessels in large quantities (see below).

Gözsy and Kato observed also that the accumulation of circulating dye in the skin of the periphery following systemic injection of the histamine liberator, compound 48/80, was inhibited by administration of mepyramine; the associated subcutaneous oedema was not so inhibited. This difference could, however, be of a quantitative nature or be due to the liberation in the subcutaneous tissue of substances other than histamine, or result from differences in the susceptibility to mepyramine of endogenous histamine liberated from skin and subcutaneous tissue respectively. The reviewer, working with Willoughby on thermal injury, has found leakage of dye to be a reliable guide to increased capillary permeability in the skin. In the subcutaneous tissue, however, increased capillary permeability was best estimated by measurement of oedema, and the oedematous tissue often showed less dye staining than did the skin, probably due once again to a relatively low affinity of the subcutaneous tissue for the dye. It is clear, therefore, that the anatomical site of increased capillary permeability may determine whether leakage of dye can be demonstrated, and whether dye leakage and oedema can be prevented by compounds such as the antihistamine drugs.

In general, there seems little reason to doubt that the passage of protein-bound dyes does in fact reflect increased capillary permeability.

5. Other techniques. Another method, first used by Landis, consists in measuring rates of filtration of water and protein from capillaries during venous congestion caused by compression of the base of a limb (59, 75). The advantage of the

method is that it is potentially fully quantitative, but it has not been sufficiently used for its limitations to be assessed.

Direct estimation of protein concentration in inflammatory exudates, *e.g.*, in blister fluid, has been used as a measure of increased capillary permeability. The technique has been employed chiefly to estimate the ability of drugs such as cortisone to depress increased capillary permeability caused by inflammatory stimuli. Measurement of the volume of exudates can also be used for this purpose under certain conditions (139).

The passage of plasma albumin labelled with radio-active iodine into an inflammatory exudate has been employed to follow the rise and fall of increased capillary permeability in inflammation (134). Although possessing the advantage of being truly quantitative such techniques are confined to a limited number of examples of true inflammation and they have not yet been applied to the study of compounds that increase capillary permeability. It is possible that refinements of surface-counting of radioactivity over very small areas might make this possible. There is no doubt that a need exists for a method that is capable of estimating such permeability-increasing compounds and which is sufficiently accurate to be submitted to statistical analysis.

IV. SUBSTANCES WHICH INCREASE CAPILLARY PERMEABILITY

A. Histamine

1. The action of histamine on capillaries. Histamine causes oedema and leakage of both circulating protein and protein-bound dye into the tissues in many species including man (50). Reference has already been made to the work of Miles and Miles on quantitative aspects of this action in guinea pig skin. Considerable differences exist in the sensitivity of capillaries of different species to histamine. Thus in guinea pig skin histamine causes leakage of circulating protein-bound dye at a concentration of 1 μg/ml, in rabbit skin at 5 μg/ml (62, 132), and in rat skin at about 25 μg/ml (132, 137).

The mode of action of histamine on capillary permeability remains obscure. The compound causes swelling of capillary endothelium when injected *in vivo* and Zweifach (159), on the basis of his studies on rat mesoappendix, has suggested that it leads to contraction of endothelial cells and widening of the spaces between them. After local application of high concentrations of histamine he has observed red corpuscles to pass through the vessel wall between the contracted endothelium. The possibility that increased capillary permeability follows shrinkage of endothelium will be discussed later.

By virtue of its ability to dilate the terminal arterioles, histamine might cause oedema without altering the properties of the capillary wall. Even though normal vasomotion be maintained by the precapillary sphincters, such vasodilatation might be followed by increased intravascular hydrostatic pressure and by a net outward filtration of fluid (21). Clinical and experimental studies show, however, that oedema fluid formed solely as a result of raised intravascular hydrostatic pressure has a much lower protein content than that formed as a result of increased capillary permeability (59, 75).

2. The binding and distribution of histamine in the body. Feldberg has recently reviewed the very wide distribution of histamine in the tissues of animals and plants (42). He stressed also the equally wide variations in histamine content between different tissues and species. An important example of species difference is found in platelets. Humphrey and Jaques (64) have listed the platelets of different species in order of their histamine content, rabbit platelets having most histamine and those of man and rat having least.

Recent work on the distribution of histamine has been much concerned with the mast cells. It is now apparent, largely as a result of the work of Riley, that these cells are a major source or storehouse of bodily histamine. The evidence for this has been recently summarised (49, 111) and therefore will not be presented here. It seems likely that much of the histamine found in intracellular particles after their separation from disrupted cells (88, 122) originates from within mast cell granules.

3. The release of histamine from tissues by exogenous compounds. A large part of this subject has been reviewed recently by Paton (104). Histamine release is achieved by many types of chemical substance including trypsin and other proteolytic enzymes (113), surface-active compounds such as the detergent Tween 20 (74), lysolecithin, snake venom (43), some basic peptides and proteins, *e.g.*, polymixin, licheniformin and peptone (18, 44, 89), certain large molecular compounds including dextran, ovomucoid and polyvinyl pyrrolidone (56, 104), and bile salts (104, 113, 118). In addition, there is the large group of basic substances of known structure, usually termed histamine liberators, the best known of which is compound 48/80 (79, 104).

Some histamine releasers do not fall easily into any category. Thus the nucleoside inosine releases histamine only in rats, whereas the nucleoside xanthosine does so only in man (137). Other naturally occurring releasers have not yet been fully characterised (67).

4. The mechanism of histamine release by exogenous compounds. This topic, which has been reviewed recently by Paton (104) and Uvnäs (147a), is the subject of controversy. It is generally agreed, however, that four broad mechanisms are possible: by the splitting of an hypothetical peptide bond linking histamine to a protein, by leakage of histamine through a cellular or subcellular membrane rendered permeable or destroyed, by release, due to an ion exchange reaction, from combination with an acidic intracellular substance or by rupture of a polar linkage with protein or lipoprotein. Details of the controversy will be found elsewhere (54, 85, 86, 88, 95, 104, 111, 113, 147).

Some recent experiments by Hogberg and Uvnäs (147a) may prove important in elucidating the mechanism of histamine release. These workers found compound 48/80 to release histamine from incubated rat mast cells *in vitro*. This release was prevented by various enzyme inhibitors and also irreversibly prevented by heating the mast cells to 50°C prior to addition of compound 48/80. Histamine release from mast cells was also achieved by a tertiary amine related to compound 48/80. Release of histamine by this tertiary amine was prevented by the presence of 1,3-diphosphoimidazole (DPI) which is known to inactivate

enzymes such as lecithinase A or hyaluronidase, which possess essential amino groups, by phosphorylating these groups. The tertiary amine was chosen because, unlike compound 48/80, it does not itself suffer such phosphorylation. Nevertheless, there is no reason to suppose that the mechanism of histamine release by the tertiary amine differs from that of compound 48/80.

It was concluded from these results that a lytic enzyme with an essential amino group exists on the surface of mast cells and that compound 48/80 may act by removing a naturally occurring inhibitor of this enzyme, which then attacks the cell membrane and liberates histamine. The theory is supported by the observation that the addition of phosphoamidase (which removes phosphate from phosphorylated amino groups) reversed the action of DPI and allowed the tertiary amine related to compound 48/80 to release histamine as before. Although this hypothesis is satisfactory for whole cells it cannot, however, be applied without modification to the action of compound 48/80 on suspensions of intracellular particles (88, 122), since metabolic inhibitors may actually potentiate the action of this substance on these preparations.

Little evidence exists as to the mode of action of long chain compounds as histamine releasers. The occasional histamine release caused in man by dextran may be due to natural hypersensitivity and a resultant antigen-antibody reaction (69). However, there is as yet no reason to suppose that the group in general acts in this fashion.

Depletion experiments show that repeated administration of dextran and ovomucoid to rats leads eventually to a failure of histamine release when these compounds are injected. Animals thus treated nevertheless continue to release histamine in response to administration of compound 48/80. On the other hand, rats depleted of their histamine by repeated injections of compound 48/80 cease to release histamine on injection of dextran and egg white (56, 103). It has been suggested on the basis of these results that histamine release by compound 48/80 on the one hand and by dextran and ovomucoid on the other are due to different mechanisms. The differences observed could, however, be of a quantitative nature. Nevertheless, there is no reason to suppose that all chemical releasers act in a similar fashion.

5. The evidence that endogenous histamine causes increased capillary permeability in tissue injury. a. Evidence of histamine release. Lewis was the first to draw systematic conclusions from the analogy between the actions of histamine and the effect of mild injury on skin capillaries, and to postulate local liberation of a histamine-like substance by such injury (83). Following his observations numerous workers found evidence to support the hypothesis that histamine itself is released after burns, chemical injury and bacterial invasion (30, 126, 158). Some of these conclusions were based on changes, not always easy to interpret, in the concentration of tissue histamine following injury. More reliable evidence has come from the demonstration of abnormal amounts of histamine in blood and in perfusates of tissues *in situ*. It has been demonstrated recently that histamine is present in the very early inflammatory exudate of pleurisy induced in rats by injection of turpentine, but is no longer detectable 60 min after injury (138).

The release of histamine by anaphylactic stimuli is very fully documented. It was first shown to be released into the blood of sensitised dogs (33, 114) and guinea pigs (22) after injection of the appropriate antigen. Histamine is also released after the addition of antigen to isolated sensitised human (122, 123) and guinea pig tissues (7, 121). Perfused tissues including skin, ear, liver and lung also liberate histamine under these conditions (45, 119, 124). Histamine is released also from the sensitised white cells and platelets of rabbits, guinea pigs and man by the presence of antigen (23, 32, 72). Release *in vivo* by anaphylaxis has been unequivocally demonstrated in man only by an increased excretion of histamine in the urine (2).

b. Inhibition and depletion studies. The demonstration of histamine release after injury is not in itself sufficient evidence that histamine causes the increased capillary permeability that may follow such injury.

Ideally, the action of antihistamine drugs should prevent the appearance of increased capillary permeability where this is due to local release of histamine. Unfortunately, the effects of endogenous histamine are not invariably suppressed by these compounds (28, 66).

In addition, all the antihistaminics are not completely specific in their action. Thus promethazine hydrochloride (Phenergan) inhibits the leakage of circulating dye caused by several substances unrelated to histamine (58, 137).

On the other hand, mepyramine maleate (Anthisan) in moderate doses, does seem to antagonise effectively only histamine (58, 137). Thus the urticarial wheals which are seen in patients suffering from drug or food allergy are abolished completely by mepyramine (200–300 mg) given over a period of 24 hours (5). Until such doses of mepyramine are shown to have an action on capillary permeability not dependent on antagonism of histamine, this result must be taken as confirmation of the role of histamine in this form of urticaria.

The question of the role of endogenous histamine in the causation of increased capillary permeability has recently been studied by repeated injections of a powerful histamine liberator, such as compound 48/80 (46). An animal so treated then practically fails to respond to further injections of histamine liberators or to other histamine-releasing stimuli. In the rat, such treatment renders the skin capillaries immune also to the action of 5-hydroxytryptamine releasers. The depletion technique might be a desirable adjunct to the antihistamine drugs but has not yet been sufficiently widely used for its usefulness and limitations to be assessed.

Oedema and leakage of circulating dye, due to increased capillary permeability in the skin, occur in animals showing those anaphylactic phenomena known as the Arthus and Shwartzman reactions and passive cutaneous anaphylaxis. Here, union of antigen and antibody probably occurs abruptly in the immediate vicinity of the affected capillaries and the reaction is not transient as in urticaria but prolonged and progressive. All attempts to modify the reaction significantly with antihistamine drugs in rabbits, rats and guinea pigs have been unsuccessful (16, 130). As has been said, however, endogenous histamine is not always antagonised by the antihistamine drugs. In addition, local application of histamine to living

rabbit mesentery fails to reproduce those changes in the vascular endothelium which follow when antigen is applied to the sensitised preparation (78, 80). However, local application of histamine may not always reproduce the effects of endogenous histamine.

Similarly, although such results are not conclusive, administration of antihistamine drugs has failed to diminish oedema or leakage of circulating dye following cutaneous thermal burning in man, guinea pig and rabbits (126, 150). In rabbits, however, there appeared to be some reduction in the erythema and vasodilatation in those burnt animals pretreated with an antihistaminic agent (150). A similar result with reduction in erythema but not of oedema was observed after intracutaneous injections of turpentine (150).

On the other hand, evidence has been obtained recently that the initial, immediate increase in capillary permeability that follows intrapleural injection of turpentine in rats may be due to local release of histamine. Thus the amount of exudate obtained 30 to 60 min after the injury was reduced from a mean of 1.0 ml to a mean of 0.1 ml in those animals pretreated with mepyramine or promethazine (1 mg/kg), or previously depleted of bodily histamine with compound 48/80 or with polymixin. This effect on volume was accompanied by a similar reduction in the quantity of protein-bound trypan blue passing into the pleural cavity from the circulation. Specific antagonists of 5-hydroxytryptamine were without effect and salicylate, too, failed to reduce the volume of the one-hour exudates (139). This apparent demonstration of the role of histamine in turpentine pleurisy in the rat contrasts with the failure of such attempts in other types of experimental inflammation. However, differences undoubtedly exist between species, tissues and types of injury with regard to the susceptibility of endogenous histamine and to the amount of histamine liberated. In other cases, lack of success might be due to failure to appreciate certain temporal aspects of the inflammatory reaction which will be discussed at the close of this review.

6. The mechanism of histamine release in anaphylaxis and other types of injury. In general, little attention has been paid to the mechanism of histamine release by tissue injury such as burning or trauma. The exceptions to this statement are anaphylaxis and antigen-antibody reactions.

Histamine release due to chemical liberators and that due to antigen-antibody combination show both similarities and differences. Thus a single competitive inhibitor will antagonise both processes (88). On the other hand, chemical releasers will act on subcellular particles whereas anaphylaxis will act only on whole cells (122). Paradoxically, chemical releasers will not liberate histamine from rabbit platelets whereas anaphylaxis will do so (65). Again, Mongar and Schild have shown that ch ical release is potentiated by metabolic inibitors and anaerobiosis whereas a hylactic release is prevented by these means (96, 122). Nevertheless, whether the release mechanisms are similar or not, depletion of tissue histamine by repeated injections of compound 48/80 prevents subsequent histamine release by anaphylaxis (56).

Ungar's (147) indirect evidence for the role of proteolysis in histamine release from guinea pig tissues by anaphylaxis is, on the whole, unconvincing. There does

seem, however, to be definite evidence of activation of protease in dog blood during anaphylaxis (11, 112, 114). On the other hand, McIntire states that soya bean trypsin inhibitor and antifibrinolysin do not inhibit anaphylactic release in rabbit blood, that there is no correlation between histamine release and activation of proteases or of coagulation mechanisms in rabbit blood during anaphylaxis *in vivo* and *in vitro*, and that streptokinase activates rabbit blood protease *in vivo* without causing histamine release. He states that in the guinea pig fibrinolysin does not release histamine from the perfused lung and that fibrinolysin does not initiate anaphylaxis *in vivo* (86, 87). There is no reason to challenge these findings. Moreover, other workers have failed to confirm Ungar's basic observation that serum protease is activated in anaphylaxis (68). On the other hand, Humphrey has found that antigen will release histamine from sensitised rabbit platelets only if both plasma and calcium are present. He has stated also that hyperimmune serum will prevent this release and also the release due to trypsin (65) and further, that if the antigen-antibody precipitate is allowed to form before addition of the supernatant plasma no histamine is released from the platelets. To explain his results in the light of those of McIntire and others, Humphrey suggested a transient activation of proteolysis with the possible formation of surface-active peptides capable of rendering platelets permeable to histamine. The transience of protease activation has in fact been stressed by Ungar (146). It seems quite clear, however, that whatever protease is concerned it is not plasma fibrinolysin or plasmin (96). There is little evidence concerning the role of ion exchange reactions or rupture of polar linkages in histamine release due to anaphylaxis and tissue damage.

Dale has stated his belief, based admittedly largely on speculation, that antigen-antibody reactions alter the permeability of cell membranes and it is possible that such a phenomenon could lead to release of histamine from platelets or mast cells, perhaps by a mechanism akin to that proposed by Hogberg and Uvnäs (147a). It is known that anaphylaxis leads to degranulation of mast cells (49, 111) with apparent dissolution of the cell membrane, but the significance of this phenomenon is obscure.

In summary it can be said only that tissue injury might liberate histamine either by direct damage to cells containing this substance or by releasing or causing to be formed compounds which do so. Numerous enzymic and non-enzymic reactions might be implicated in this process.

B. 5-Hydroxytryptamine (5-HT, Serotonin, Enteramine)

The relationship of this substance to capillary permeability has only recently been discovered but its other pharmacological properties have been known for a considerable time.

1. The effect of 5-hydroxytryptamine on capillary permeability. 5-Hydroxytryptamine was not known to affect capillary permeability until Rowley and Benditt (10, 117) showed that on injection in microgram amounts it caused immediate oedema of rats paws. It has been shown in rat skin that 5-HT causes vasoconstriction at concentrations above 50 μg/ml but increases capillary permeability to circulating protein-bound dye below this concentration (138). Parratt and

West (101, 102, 103) were able to confirm the effect of 5-HT on rats paws and demonstrated abolition of the oedema by 2-bromolysergic acid diethylamide bitartrate (BOL 148), a specific antagonist of 5-HT.

Sparrow and Wilhelm (132) have shown recently that the ability of 5-HT to cause leakage of circulating dye may be confined virtually to the rat. Thus this action in the rabbit was almost non-existent and in the guinea pig only very weak, whereas in the skin of rats 5-HT was fifty to one hundred times more potent than histamine in causing leakage of circulating dye.

2. The release of 5-hydroxytryptamine in the body. Bhattacharya and Lewis (13) found that the histamine liberators compound 48/80, propamidine (4,4'-diamidinophenoxy propane) and morphine released considerable quantities of 5-HT from the perfused hind quarters of the rat, most of it coming from the skin and much of it from the skin of the feet. Morphine actually released more 5-HT than histamine from this preparation wheras compound 48/80 did the reverse. Compound 48/80 failed to liberate 5-HT from the perfused tissues of cats, dogs and rabbits.

Rowley and Benditt (117), using a different approach, observed that the oedema of rats' paws following local injection of the histamine liberators compound 48/80, dextran, ovomucoid and testicular extract was largely abolished by pretreatment with N,N-dibenzyl-β-chloroethylamine (dibenamine), an antagonist of 5-HT. A combination of dibenamine and an antihistaminic completely abolished the oedema. They concluded that most of the oedema produced by these histamine liberators in the rat was in fact due to local release of 5-HT, a minor part being played by histamine, and that the proportion of liberated 5-HT to histamine was 1:24. It will be recalled that the rat is much more responsive to 5-HT than to histamine. Parratt and West (102, 103) have confirmed the major role played by 5-HT in the oedema produced by these compounds in rats. They did so with the aid of BOL 148, a more specific antagonist of 5-HT than dibenamine. There is no doubt that these chemical releasers liberate both histamine and 5-HT in the ventral skin and dorsum of the foot in the rat. In guinea pig skin, too, it seems that compound 48/80 may liberate both histamine and 5-HT (132) although here the latter is a minor constituent.

With regard to natural as opposed to exogenous liberators of 5-HT, Toh (144) has described a substance present in the kidney and gastric mucosa of the dog, capable of releasing 5-HT from platelets. The action of this substance was inhibited by the chelating agent ethylenediamine tetraacetic acid (Versene), but its nature is unknown.

3. The mode of action of 5-hydroxytryptamine on capillaries. Most investigators have assumed that 5-HT increases capillary permeability by a direct action on the vessel wall. Sparrow and Wilhelm, however, have shown that although 5-HT is merely a weak histamine liberator when perfused through rat hind quarters, it releases more histamine than compound 48/80 when incubated with rat skin *in vitro* (132). This latter effect, however, seems to be essentially an acceleration of a spontaneous process of diffusion and may not be comparable to histamine release *in vivo*.

In addition these workers found the action of 5-HT to be diminished by mepy-

ramine. To be effective, however, mepyramine had to be injected intradermally together with the test dose of 5-HT and in relatively high concentration (132). It is known that systemic mepyramine, in doses which completely suppress the ability of histamine to cause leakage of circulating dye, has no effect on the similar action of 5-HT (132, 137, 139). Sparrow and Wilhelm found also the actions of 5-HT and compound 48/80 on skin capillaries to be equally, and rather weakly, antagonised by local mepyramine, and concluded that both these substances increase capillary permeability in part by release of histamine. Their results, however, seem equally compatible with the considerable body of evidence that compound 48/80 acts in rat skin largely by release of 5-HT (103, 117) and that the modest inhibition of 5-HT obtained with locally injected mepyramine was due to factors other than the specific antihistaminic properties of this drug. Other antihistamine drugs, *e.g.*, promethazine have such non-specific actions and it is likely that in very high local concentration mepyramine shares them.

These experiments raise the question of how to judge the histamine-releasing powers of substances that increase capillary permeability. Although negative results would not necessarily be conclusive it is reasonable to expect that the action of compounds that increase capillary permeability by causing histamine release should be blocked by measures which antagonise specifically the comparable action of both histamine itself and of known histamine liberators such as compound 48/80. Systemic mepyramine in doses of 1–5 mg/kg in rat and guinea pig often provides such a measure.

Direct measurement of histamine release, too, should provide essential evidence. Substances whose histamine-liberating properties are beyond doubt all release histamine from preparations of tissue *in situ*, for example, perfused skin flaps or rat hind quarters. Histamine release which can be demonstrated only *in vitro* is less convincing; the ultimate test is histamine release *in vivo*. At the moment it is not certain which of the various pharmacological tests for histamine-releasing activity is the best guide to events *in vivo*.

4. The release of 5-hydroxytryptamine by anaphylaxis and other types of tissue injury. The study of the relationship of 5-HT to capillary permeability is of recent origin. As a result little has been published as yet concerning its role in inflammation and anaphylaxis. No inference about this role can be made solely from the observation that histamine liberators also release 5-HT in the rat.

There is evidence of release of 5-HT from sensitised platelets of rabbits, dogs and guinea pigs on addition of antigen in the presence of plasma (65). In addition, Waalkes *et al.* (149) have found 5-HT to appear in the circulation of rabbits during hypersensitivity reactions to horse serum and egg albumin. The 5-HT concentration was maximal 1 min after stimulation and normal 5 min later. The peak value was only 0.7 μg/ml and it must be remembered that the skin capillaries of the rabbit are completely insensitive to concentrations of this order (132). The significance of the finding is therefore dubious. 5-Hydroxytryptamine has been found in pleural exudates withdrawn 30 min after injection of turpentine into the pleural space of rats (136). Even though the rat is highly sensitive to 5-HT the finding may be of little significance since selective inhibition studies

indicated that 5-HT contributed little or nothing to the development of increased capillary permeability in the inflamed pleura (139). On the other hand, in cutaneous inflammation induced by antigen-antibody reaction in the rat (the Arthus reaction) 5-HT may play such a role since the phenomenon is diminished by the administration of BOL 148 (17).

In summary, it seems at the moment that the importance of 5-HT as a "mediator" of altered vascular permeability in inflammation is likely to be confined to certain tissues, perhaps only the skin, of the rat and possibly of the mouse.

C. Peptides

1. The action of peptides on capillary permeability. a. Enzymic digests of proteins. The first convincing demonstration in recent times, apart from studies with "peptone" (44), that certain peptides increase capillary permeability was probably provided by Menkin (92), one of the pioneers of the chemical approach to inflammation. He showed that blood albumin, following its partial digestion by trypsin, caused leakage of circulating dye into the skin. Menkin achieved a partial purification of the active principle by extraction with acetone and pyridine and, together with an extract obtained from inflammatory exudate also with the aid of these solvents, it was designated as "leucotaxine". It now seems probable that Menkin's extraction procedure was essentially a deproteinisation with acetone, the pyridine acting as a buffer and helping to prevent adsorption of active peptides on the floccules of precipitated protein. Duthie and Chain (35) although unable to substantiate the claim that Menkin's preparation was relatively pure and crystalline, were nevertheless able to prepare an active peptide by digesting fibrin with pepsin and to purify it partially by specific adsorption. Cullumbine and Rydon (26) obtained peptides that caused leakage of circulating dye by digesting several proteins with various proteases, including skin protease. The reviewer (133) has shown that all such activity in a peptic digest of fibrin was precipitated with ammonium sulphate after prior deproteinisation, and that it migrated to the cathode on electrodialysis at pH 3 to pH 8; the activity was shared between several subfractions whose average chain length varied from 8 to 14 amino acids and that fractions with an average chain length of less than 5 amino acid residues failed to cause leakage of circulating dye. Crystalline pancreatic trypsin inhibitor, a peptide with a molecular weight of 6,000, was also found to cause leakage of circulating dye on injection into skin (133). After these various investigations it seems clear that the ability to increase capillary permeability was a property shared by a large number of different peptides.

b. Some special peptide preparations. In addition to the work described above certain peptide preparations have been subjected to a more intensive pharmacological investigation. Although studied chiefly for their action on smooth muscle in gut and uterus, many such preparations have an action on capillary permeability. Peptides in this group include bradykinin (62, 112), kallidin (61, 62, 152), substance P (41, 105), substance U (12), pepsitensin, pepsitocin and pepsanurin (25), kinin (62) and angiotonin (hypertensin) (98, 99).

In the skin of guinea pigs, bradykinin and kallidin cause leakage of circulating

dye in concentrations similar to those required of histamine, while kinin is even more powerful (62). Similarly, substance P is active in this test in concentrations of 2.5 μg/ml. Substance U and pepsitocin were less active and angiotonin was the weakest of all.

Rats are less sensitive than guinea pigs to these peptides, just as they are less sensitive to histamine. Thus substance P causes leakage of circulating protein-bound dye into the skin of rats in a concentration of 10–20 μg/ml whereas substance U and pepsitocin are less active and angiotonin is inert. Since all these preparations, except angiotonin, probably contain inactive impurities it is not possible to form firm conclusions about their potencies relative to each other or to other compounds.

2. The relationship between peptides that increase capillary permeability. Certain features are common to the peptides listed above. They behave as cations on electrophoresis, are inactivated by chymotrypsin and other peptidases and have solubility characteristics in common. Many of them share an origin in the α_2-globulin fraction of serum. Pharmacologically, they tend to stimulate smooth muscle of the uterus and intestine of various species, to be hypotensive in the dog, cat or rabbit, and to cause pain on application to a blister base.

There is some evidence that, despite different modes of preparation, some of the peptides derived from blood serum may be identical. Thus kallidin and bradykinin could not be differentiated either chemically or pharmacologically (62). The sources of kallidin and bradykinin in the α_2-globulin of serum show many similarities, but also differences and it has been suggested that they are different parts of the same molecule which may also be the precursor of hypertensin (148). Pharmacological differences between some of these peptides do exist, but since all the preparations are impure, arguments founded on this basis have a limited value. On the other hand, active peptides have been derived from sources differing as widely as wasp venom and dog urine. Moreover, even if apparently identical in broad pharmacological and chemical properties it does not follow that two peptides are identical in structure. Nevertheless, until they are fully characterised it seems more profitable to stress the similarities within the group rather than the differences.

3. The mode of action of peptides on capillary permeability. a. Direct action on capillaries. Hypotheses bearing on the mode of action of these peptides are few in number and facts are almost non-existent. It has been suggested that surface-active compounds with free amino groups might compete with and replace certain constituents of the hypothetical endocapillary layer. In this location the peptides might form an ultra-thin layer and not be able to maintain the semi-permeability of the interendothelial substance and leakage of protein might result (29). It also seems possible that basic peptides might form compounds with acidic tissue constituents such as heparin, hyaluronic acid or chondroitin sulphuric acid whose presence in acid form might be essential for capillary semipermeability. However, all these suggestions are hypothetical and it is possible to speculate on this topic almost indefinitely.

b. Histamine release by peptides. Peptone shock after the injection of complex

mixtures of proteins and peptides is associated with release of histamine into the circulation (44). Peptic digests of fibrin have been thought to increase capillary permeability by releasing histamine, because they reduced the histamine equivalent of cat skin (31) and because their ability to cause leakage of circulating dye in guinea pig skin was diminished by intravenously injected mepyramine maleate (93). However, in the experiments on cat skin the peptide solutions probably contained known histamine releasers such as basic amino acids. The results based on the action of mepyramine are more convincing, but even here only a modest diminution in the action of the peptides was obtained, suggesting that histamine release might account only for part of their effect on capillaries.

In the rat, peptide preparations in low concentration caused leakage of circulating dye and failed to release significant quantities of histamine on perfusion through the hind quarters (137). Similarly, the ability of substance P and other peptides to cause leakage of circulating dye in rat skin was not inhibited by mepyramine (139) when the drug was administered systemically in moderate doses. Moreover, in the guinea pig, the peptide kinin behaved in a different fashion from known histamine liberators such as compound 48/80 (62).

In contradistinction to the doubtful and weak histamine-releasing properties of the peptides so far discussed, certain basic peptides are powerful histamine liberators (89). Some basic amino acids share this action (38). However, the ability to cause massive histamine release is clearly not a general property of peptides which increase capillary permeability and their mode of action must therefore lie elsewhere.

4. The evidence that peptides cause increased capillary permeability in inflammation. Several reports exist in the older literature of the presence in pus of protein breakdown products such as "peptone". Menkin (90, 92) found that pleural exudates in dogs induced by turpentine contained a principle that caused leakage of circulating dye in rabbit skin; this principle he believed to be a peptide. It seems possible that some of the activity in these pleural exudates was due to protein. Thus, published drawings (90) of the effect of the non-dialysable residue suggest a central vasoconstriction with a narrow zone of increased capillary permeability at the periphery of the injection site. This appearance is often produced by high concentrations of compounds that increase capillary permeability. There is also the possibility that some of the activity of the dialysate was due to ammonium sulphate present in the reaction mixture. Since some of the parent exudates had little activity it may also be that, as in serum, chemical manipulation led to activation of permeability-increasing globulins (94).

Cullumbine and Rydon (26) and Spector (133) found permeability-increasing activity in 24-hour old inflammatory exudates but were able to add little to its characterisation as a peptide. In recent work by Spector (134) inflammatory pleural exudates in rats were studied from 1 to 24 hr after injury. Little activity upon capillary permeability due to peptides was present in these exudates. In addition, such activity did not wax and wane with the rise and fall of capillary permeability in the pleura.

The failure to obtain direct evidence for the participation of peptides in in-

flammation contrasts with their ability to cause leakage of circulating protein-bound dye into the skin of experimental animals. If active peptides enjoyed only a transient existence in inflamed tissues this discrepancy could be resolved. In fact there is evidence that peptides of the bradykinin type are formed in serum from many species by dilution of the serum (120) or by bringing the serum into contact with glass (4). Such peptides exist only for a matter of minutes before their inactivation probably by serum peptidases. Transient formation of a substance with bradykinin-like properties has also been described in the plasma of dogs subjected to anaphylactic and peptone shock (11), phenomena sometimes associated with increased capillary permeability.

The formation of peptides which increase capillary permeability seems likely to be associated with proteolytic activity, but as has been seen, evidence for the development of such activity is incomplete and conflicting. Many of the discrepancies in the literature might be explained if the activated enzymes, like the peptides themselves, were short-lived and rapidly inactivated. Such a scheme has been put forward by Mongar and Schild and also by Ungar to explain the changes that follow antigen-antibody combination *in vivo* (96, 145, 146). Active peptides might also be released without the aid of proteolysis. The peptides could, for example, exist bound to some other tissue constituent by an amide linkage and require the severance of only one bond for their activation. A mechanism of this nature would accord well with the almost instantaneous appearance of bradykinin-like activity in suitably treated plasma (4, 120). If peptides do play a role in causing increased capillary permeability in inflammation it seems more likely that they are formed as a result of activation of a specific enzyme-substrate system, rather than from a general destruction of tissues and resultant proteolysis.

D. Proteins

1. Tissue extracts. It has been known for some time that certain aqueous tissue extracts cause leakage of circulating protein-bound dye when injected into the skin of animals and that this activity is associated with the protein fraction of such extracts (97, 109, 135). The effect of aqueous tissue extracts on capillary permeability is probably due to activation of plasma globulins of the type described below. The substance described by Menkin under the name of "Exudin" may also be a member of this group of globulins (92).

2. Plasma globulins. One of the major recent contributions to the field has been the discovery by Miles and Wilhelm and their colleagues (94, 155, 156) that guinea pig serum acquired, on dilution, the ability to cause increased capillary permeability as measured by leakage of dye into the skin of guinea pigs. Fractionation of the serum with ether also activated this permeability factor, called "PF/dil." Soya bean trypsin inhibitor and certain sulphated dextrans prevented activation of the serum by dilution. The substance was antagonised by an inhibitor present in the α_1-globulin fraction of serum. PF/dil. itself is in the α_2-globulin fraction of serum and none of its activity is dialysable. When assayed in guinea pig skin it was as potent as histamine on a weight basis and two thousand times more potent on a basis of presumed molarity. All mammalian sera so

far examined appear to contain something akin to PF/dil. although significant species differences exist. Thus rabbit serum is not activated by dilution and in man and rabbit activity appears to reside in the β-globulin fraction (154).

The reviewer has studied activation of the permeability-increasing globulins of rat plasma in relation to their possible role in inflammation (135). Inactive globulins from resolving pleural exudate caused leakage of circulating dye following their incubation with isolated lung mitochondria; this activation did not develop in the presence of heparin and citrate. Similarly, globulins prepared from heparinised rat plasma remained inactive in the ether fractionation procedure. Thus the inactive precursor of the permeability factor can be separated from the blood. In addition, plasma prepared in citrate in polythene containers failed on dilution to cause dye leakage. However, the relationship of these results to possible mechanisms of activation *in vivo* remains uncertain.

The mode of action of plasma globulins. Miles and Wilhelm considered that PF/dil. in guinea pig serum was a protease, largely on the basis of its inhibition by soya bean trypsin inhibitor (155). No proteolytic action of the active fraction has, however, been demonstrated. The permeability-increasing globulin itself has no action on smooth muscle although it is hypotensive, nor does it liberate histamine in guinea pig or rat (137, 155). It is not a constituent of complement.

It is worth recalling that plasma globulins, especially the α_2-globulin that contains PF/dil., are a potent source of substrates for the formation of pharmacologically active peptides rather than of proteases (148). No substance can be said truly to increase capillary permeability until it has been tested by injection into the tissues of a living animal. It seems just possible that injection of activated globulins *in vivo* provides a substrate for the action of tissue or plasma proteases or other enzymes which then split off peptides responsible for the altered capillary permeability. The failure to demonstrate such peptides, *e.g.*, in mixtures of globulins and isolated mitochondria (135), could be due to their rapid destruction by peptidases. The view that globulins such as PF/dil. are substrates for the formation of active peptides is compatible with the failure of such globulins to stimulate smooth muscle. The high degree of activity of guinea pig PF/dil. is in favour of its enzymic nature. However, the activity of some peptides on capillary permeability in the guinea pig may be also of a high order (62). Moreover, in other species such as the rat the activity of permeability-increasing globulins is much lower than in the guinea pig (134, 137). It is equally possible that activated globulins exert a non-proteolytic effect directly on the capillary wall without the formation of intermediate compounds, or that the active fractions contain both substrate and enzyme.

3. Anaphylatoxin. Following an observation of Bordet a great amount of work was directed to showing that sera from guinea pigs and rats developed toxic properties after incubation with agar, inulin, starch and many other substances (34). Rocha e Silva reinvestigated the phenomenon and showed that rat and guinea pig serum, after treatment with agar or other polysaccharides, caused a striking release of histamine from guinea pig tissues (113, 115). The active principle described by the older workers and by Rocha e Silva is generally known as ana-

phylatoxin. It appears to be significantly active only in the guinea pig, although rat serum is the richest source. It is a heat-labile protein which has not been separated in any one serum protein fraction. Many of the earlier results were probably due to a variety of compounds. Rocha e Silva's anaphylatoxin has more claims to be considered a single substance although this is far from certain. Interest lies in the methods that prevent anaphylatoxin formation. Thus agar fails to activate rat serum at 0°C, in the presence of agents that bind calcium, such as citrate, or of high concentrations of heparin (115). These conditions are similar to those which prevent release of histamine from sensitised tissues by antigen (95). They also resemble the conditions that prevent activation of the globulin permeability factor in serum (135, 156) and that prevent activation of the first component of complement (see below). These facts suggest that a variety of reactions, all related in some degree to injury, might be set in train by a common mechanism. However, since anaphylatoxin is formed only in rat and guinea pig serum and releases significant amounts of histamine only in the guinea pig, its importance, like the comparable action of horse serum on the cat (45), is doubtful. In addition, any assessment of its action on capillaries would have to be disentangled from that of other vaso-active substances formed simultaneously.

4. Complement. Bier *et al.* (14) found that depletion of serum complement in the rat by repeated injections of antigen led to a significant inhibition of the leakage of circulating dye that normally followed injection of appropriate antibody into the skin of animals whose circulation contained antigen (passive cutaneous anaphylaxis). The authors suggested that complement fixation was an essential step in the capillary changes. It is interesting that sera from species which do not fix guinea pig complement also fail to produce passive cutaneous anaphylaxis. In addition, calcium ion is required for both complement fixation and the release of histamine from sensitised cells by antigen. The ability of antigen to stimulate sensitised guinea pig tissue in the absence of plasma (122) might argue against the participation of complement. Humphrey, however, has pointed out that complement or other proteins could be firmly adsorbed on to the washed tissues.

There is now considerable evidence that activation and fixation of complement, *e.g.*, by antigen-antibody reactions, is associated with development of esterase activity (76, 81, 82). Esterases might split off active permeability factors from their inactive precursors, activate other enzyme systems, *e.g.*, proteases, or attack the membranes of platelets and mast cells, to liberate histamine and other active compounds. Streptokinase-treated plasma protease has been reported to activate the first component of complement, an essential step in complement fixation (76). Although it is too early to evaluate this observation, the finding is interesting in view of the possibility that transient activation of protease may occur as a result of antigen-antibody combination, or other injury. However, the role of complement in increased capillary permeability is far from established.

5. Kallikrein. This substance is a non-diffusible protein of molecular weight 48000 found in urine, pancreas, pancreatic juice, serum, salivary gland and saliva (48, 152). It is an enzyme, presumably a protease, that forms kallidin (see above) from α_2-globulin. It seems possible that kallikrein may serve as a model for the

group of hypothetical enzymes which, after injury, catalyse the formation of peptides capable of increasing capillary permeability. Although as yet uncharacterised, the "bradykinin-releasing enzyme" which is activated in serum when this is diluted or brought into contact with glass (4, 120) may be akin to kallikrein in this respect. Some of the properties of kallikrein resemble those of permeability-increasing serum globulins and provide some support for the view that these globulins are enzymes (see above).

6. Vasodepressor material (VDM, Ferritin). This substance has been described by Shorr (127) and has been identified as the iron-containing protein ferritin. One of its actions is to reduce the response of metarterioles and precapillary sphincters to topical adrenaline (epinephrine), but it has not yet been shown to increase capillary permeability.

7. Clostridial toxins. Elder and Miles (37) have found that subnecrotising doses of partially purified filtrates of cultures of clostridia cause leakage of circulating dye in guinea pig skin. The remarkable feature of this action is its prolonged nature, preceded by a latent period after the injection of the toxins into the skin. In the case of *Cl. oedematiens* the latent period is 6 hr, and increased capillary permeability can be demonstrated up to 96 hr. The action of *Cl. oedematiens* toxin is in sharp contrast to that of all other compounds discussed in the present review which increase capillary permeability, since all these exert an almost immediate effect which disappears by two hours and often sooner (93). The latent period observed may be due to toxins causing either a slow formation of active compounds or a slow and prolonged inhibition of some vital process in the capillary wall.

8. Hyaluronidase. The spreading effect of this substance is well known. Several reports have appeared indicating that hyaluronidase causes oedema and leakage of circulating dye. Some of these reports were based on the effect of tissue extracts rich in the enzyme but also containing other substances which increase capillary permeability (117). In other cases histamine release appeared to be the cause of increased capillary permeability (39). Other experiments utilised indirect and unsatisfactory methods for measuring capillary permeability (9). On the other hand, perfusion studies (21) and observations on leakage of circulating dye in rabbits (142) have failed to demonstrate increased capillary permeability attributable to hyaluronidase activity. The reviewer has found purified hyaluronidase in rat skin to cause leakage of circulating trypan blue at a concentration of 1 mg/ml but not below this level. The balance of evidence therefore seems to be against a significant action of hyaluronidase on capillary permeability. It has recently been reported, against expectation, that hyaluronidase from different sources fails to soften intercellular cement substance (40).

9. The evidence that proteins cause increased capillary permeability in inflammation. Wilhelm *et al.* (156) studied the PF/dil. activity of guinea pig serum and found the dilution effect to be unchanged in acute anaphylaxis, shock due to compound 48/80, acute streptococcal infection, radiation sickness and after the administration of cortisone. These results do not, however, rule out local activation of the globulins.

The reviewer has studied the development of inflammatory exudate in rats following intrapleural injection of turpentine (134). Increased capillary permeability in the pleura was measured by the passage of labelled plasma albumin which reached a peak between 3 and 6 hr after injury. Simultaneously the globulin fraction of the exudate developed the ability to cause leakage of circulating dye into the skin of rats. As the permeability of pleural capillaries returned to normal, the globulin fraction ceased to cause leakage of circulating dye in rat skin. This inactivity was at least partly due to the increased preponderance of a specific inhibitor, also a globulin (134). Reference has already been made to the formation of the permeability-increasing globulin from its inactive precursor (135). These experiments seem to provide some evidence that a system of globulins plays a part in the mediation of increased capillary permeability induced in the pleura of the rat by chemical injury. It is to be hoped that further evidence for or against their participation in inflammation will be forthcoming.

E. Oestrogens and Relaxin

1. Oestrogens. The uterus of animals of many species is known to undergo changes resembling those of acute inflammation, when oestrogen is administered to the animal. These changes include vascular dilatation and engorgement, leucocyte emigration and increased capillary permeability (136). In ovariectomised mice, oedema and leakage of circulating dye develop in the uterus within a few hours of the administration of oestrogen and are maximal at twenty-four hours (136). Ostrogens fail to induce oedema or leakage of circulating dye in any tissue other than uterus (136). The hormones therefore may be presumed to act by way of some specialised mechanism, but the nature of this mechanism is quite obscure. Oestrogens also cause a striking increase in cellular permeability in the uterus (143) which may be related to their effect on capillaries.

2. Relaxin. Relaxin is a substance of uncertain nature that induces relaxation of the pelvic ligaments as occurs in pregnancy (1, 55). Amongst other sources, it is found in the urine of pregnant rabbits and the placenta of the sheep. Storey (140a) has studied its action in the mouse and has suggested that the relaxation effect is due in part to increased capillary permeability in the ligaments with resultant oedema and unwinding of collagen spirals. There is as yet insufficient evidence to accept this hypothesis, although Storey's evidence is suggestive.

V. SOME GENERAL PROPERTIES OF SUBSTANCES WHICH INCREASE CAPILLARY PERMEABILITY

The properties of substances known to increase capillary permeability have been discussed individually. These substances exhibit certain common properties. Thus with the exception of compounds known to affect permeability by means of local histamine release, they are all derived from amino acids. Yet amino acids themselves, apart from those which release histamine, are apparently without significant effect (133).

A second property common to substances which increase capillary permeability is the presence of free amino groups.

Finally, under suitable conditions, practically all substances which increase capillary permeability cause the contraction of smooth muscle preparations. Because of this action it is tempting to speculate as to whether these substances act on capillaries by causing contraction of the endothelial cells. Cells of the vascular endothelium can be seen to assume a globular form in inflammation and after the application of compounds which increase capillary permeability, not only in fixed histological sections but also in living tissues (20, 36, 47, 80). A morphological change of this nature could, of course, be due equally well to passive swelling of the endothelium. In either case it seems possible that actions of this kind on vascular endothelium might modify a system of water-filled channels in the capillary wall through which proteins are thought to pass (100).

VI. SOME COMPOUNDS WHICH INHIBIT INCREASED CAPILLARY PERMEABILITY

A. Calcium Ion

The older literature contains references to the role of calcium in maintaining capillary semipermeability. Calcium salts are still used therapeutically to this end. Chambers and Zweifach (20) found the calcium ion to be essential if oedema was to be prevented in mesentery subjected to perfusion. Similarly, after the injection of calcium-binding agents, rat skin shows an immediate leakage of circulating protein-bound dye. The calcium-binding agent ceases to exert this effect if sufficient ionised calcium is added to the test solution (135). Apart from its direct effect on vascular integrity, calcium ion may be an essential, or at least an accelerating, factor in some reactions leading to the formation of substances which increase capillary permeability (96, 115, 135). In guinea pig skin calcium ion may itself cause leakage of circulating dye (155) in apparent contradistinction to its effect in the rat. In short, this electrolyte seems to play a complex role in the matter of capillary permeability.

B. Adrenal Cortical Hormones

The adrenal corticosteroids, cortisone and hydrocortisone, decrease the fluid exudate seen in rheumatic arthritis and allergic rhinitis and urticaria. Their therapeutic properties are reviewed in most modern textbooks. In experimental inflammation the hormones have a significant inhibitory action on the development of granulation tissue. However, the adrenal corticoids do not appear to diminish the oedema and leakage of circulating dye induced by a variety of insults, *e.g.*, thermal burns (126), turpentine abscess in the mouse (131), wounds and foreign body implants in the rat, and fractures, *B. coli* filtrates and vaccinia virus in rabbits (77). On the other hand, cortisone greatly reduces the oedema and leakage of circulating dye that occur in the active and passive Arthus reaction and in local sensitisation in rabbits (51, 63, 128), and it generally suppresses the reaction of sensitised capillary endothelium to antigen (36).

Cortisone also partially suppresses the leakage of circulating dye caused by intracutaneous injection of certain peptides and of histamine (6). One of the earliest observations of this nature was made by Menkin (91). Adrenal corticosteroids are not generally thought to possess specific antihistaminic properties.

Nevertheless, adrenalectomy potentiates the systemic effect of the histamine releasers dextran and ovomucoid and the potentiation can be largely abolished by administering cortisone (56). In addition, cortisone is believed to inhibit the resynthesis or binding of histamine after depletion of histamine by repeated injections of compound 48/80 (56). Histaminase, too, is said to be depleted in the cat by adrenalectomy and the depletion to be prevented or reversed by the administration of cortisone (70). The significance of these observations is, however, uncertain.

Two hypotheses exist as to the mode of action of cortisone and hydrocortisone on capillary permeability: (a) that they prevent antigen-antibody combination from exerting its effects on the capillary wall, whether this be direct or indirect (as by releasing chemical mediators); or (b), that the hormones cause a general depression of the reactivity of the capillary wall to stimuli which increase permeability (3), but that not all such stimuli are significantly affected. The available evidence favours the view that there is some truth in both these hypotheses.

C. Salicylate

Salicylate has been widely used for many years to suppress the exudative phenomena of rheumatic fever. The drug reduces pain and swelling in the affected joints and this effect is almost certainly due in part to a diminution of increased capillary permeability in the tissues. Clinically, its action is practically specific for rheumatic inflammation. It does not, for example, diminish the swelling of gonococcal arthritis (141).

Capillary permeability increased by experimental injury responds to salicylate in a fashion similar to cortisone. Salicylate inhibits the oedema and leakage of circulating dye seen in the passive Arthus reaction and similar phenomena in the rabbit and guinea pig (130). Salicylate also inhibits leucocyte emigration in the rat cornea after trauma (151).

Salicylate diminishes the leakage of circulating dye induced by intradermal injections of histamine in the rabbit and rat and of 5-HT and serum globulins in the rat (73, 139, 142). In the rat, the effect of salicylate on this action of histamine and 5-HT is incomplete: the leakage of dye under salicylate is slow to develop and reaches about 50% of its usual intensity (139). On the other hand, the action of globulins is completely suppressed by administration of salicylate.

Salicylate also inhibits the release of histamine from sensitised rabbit blood cells by antigen and that from guinea pig ileum by agar-activated serum ("anaphylatoxin") (53). This action of salicylate is not strong enough to be of great significance. The drug does not interfere with the action of histamine on guinea pig ileum (53).

Salicylate probably possesses several distinct properties in relation to capillary permeability. It appears to have a specific antirheumatic action; it also causes a general, although usually incomplete, depression of the reactivity of capillaries to substances which increase capillary permeability. In addition, salicylate prevents the activation of permeability-increasing globulins *in vitro*, probably by direct combination with one or more of the proteins involved. Salicylate also sup-

presses completely the ability of such globulins to cause oedema and leakage of circulating dye in rat skin.

There is some evidence that the antirheumatic activity of salicylate may be partly independent of its action on increased capillary permeability induced by other types of injury. Thus Willoughby and the author found several di- and trihydroxy derivatives of benzoic acid less potent than salicylate in suppressing the formation of pleural exudates induced in rats by the injection of turpentine. In contradistinction, many of these compounds were alleged to be many times more potent than salicylate in suppressing the manifestations of rheumatic fever. However, the results are not conclusive because of possible species differences and because the antirheumatic powers of these compounds have yet to be confirmed.

The beneficial effect of salicylate in rheumatic inflammation was formerly attributed to its ability to suppress formation of antibodies (141). Lack of correlation between this property and the therapeutic action of salicylate, together with its effectiveness in the passive Arthus reaction, where preformed antibodies are injected, have combined to render this view unacceptable. However, the possibility remains that salicylate might interfere with the action of the antigen-antibody complex on capillaries. More recently it was suggested, on the basis of similarities with the action of cortisone, that salicylate might act by stimulating the adrenal cortex. However, although the action of salicylate may be prevented by previous adrenalectomy and the drug may cause adrenal changes resembling those produced by administration of adrenocorticotropin, the balance of evidence is against such a mode of action of salicylate (8).

Administration of salicylate to normal rats leads to hyperglycaemia while hypoglycaemia occurs in adrenalectomised rats (129). It was proposed that salicylate suppresses increased capillary permeability by stimulating the adrenal medulla to produce adrenalin, thereby increasing vascular tone. This theory has its attraction but lacks convincing support, nor is it certain that excess of circulating adrenalin would diminish the increased capillary permeability caused by even mild and transient inflammatory stimuli.

Another theory of the mode of action of salicylate is based on the inhibition of the proteolytic activity of pepsin (151) and fibrinolysin (145). This property may be related to the inhibition by salicylate of the activation *in vitro* of permeability-increasing globulins. It may also be related to the suppression by salicylate of the increased capillary permeability caused by these globulins. However, more convincing experimental support is required for protease activation in inflammation and its inhibition by salicylate before this hypothesis can be accepted.

VII. THE CONSECUTIVE OPERATION, FOLLOWING INJURY, OF COMPOUNDS WHICH INCREASE CAPILLARY PERMEABILITY

Reference has already been made to experiments in which measures believed to be antagonistic specifically to histamine prevented the onset of increased capillary permeability in pleurisy induced in rats with the aid of turpentine. In spite of almost total suppression of thirty- to sixty-minute exudates it was found that even repeated injections of antihistamine drugs and histamine liberators failed

to prevent the development of exudates four hours after injury (139). These results, coupled with pharmacological study of the exudates, suggested that some mechanism other than histamine release might become effective after an initial liberation of this substance. The findings also suggested that the second mechanism was not dependent for its activation on the successful operation of the phase susceptible to antihistamine measures.

Confirmatory evidence came from the use of salicylate (139). In rats treated with this drug alone, pleural exudates showed a modest reduction in volume 30 min after injury, probably due to non-specific action on capillaries or to partial inhibition of histamine release. From 60 min after injury, however, the inflammatory exudates did not differ in volume or protein content from those of control animals. It will be recalled that antihistamine measures almost abolished the sixty-minute exudate.

However, when a similar dose of salicylate was given to rats pretreated with mepyramine or depleted of histamine with compound 48/80, and turpentine then injected intrapleurally, the formation of exudate up to 6 hr after injury was suppressed almost totally. Thus prior abolition of that phase of increased capillary permeability susceptible to mepyramine revealed the ability of salicylate to prevent the development of the subsequent stage of increased capillary permeability. Since salicylate inhibits both the *in vitro* activation of permeability-increasing globulins and their action on capillaries, and since such activated globulins are demonstrable in turpentine-induced pleural exudates (134) it seems possible that the second phase of increased capillary permeability is due to these proteins.

The concept that endogenous compounds which increase capillary permeability might exert their effects in successive phases gains some support from experiments with certain sensitised muscles (15, 43, 60, 122). When antigen is added to such preparations there is an immediate contraction due to liberation of histamine, and this contraction is abolished by mepyramine. Almost at once, however, another substance which has none of the properties of histamine is released causing a slow, sustained contraction of the muscle which is not abolished by mepyramine. Thus, at first sight treatment of the preparation with mepyramine fails to alter its contraction. On close scrutiny, however, the contraction can be seen to be delayed in its onset and to lack its initial peak. The existence of this second phase in anaphylactic contraction of smooth muscle helps, together with the theory of intrinsic and extrinsic histamine, to explain the failure of antihistamines in bronchospasm due to anaphylaxis (28).

The immediate nature of the histamine release following antigen-antibody combination and its transience have recently been emphasised by experiments *in vivo* in which the concentration of blood histamine reached a peak only one min after challenge and fell to normal within 5 min (149). In turpentine pleurisy, too, no histamine is demonstrable in the exudate after 30 to 60 min after injury (138). It is clear that histamine could not sustain increased capillary permeability for long and that other factors come into operation to do so. Studies of thermal burns show clearly that delayed oedema develops in two distinct phases which also is consistent with the consecutive operation of chemical mediators (125).

Mongar and Schild, as a result of exhaustive studies on the inhibition of hista-

mine release in anaphylaxis, have concluded that antigen-antibody combination and possibly other injurious stimuli cause a transient activation of enzymes which simultaneously liberate histamine and initiate other mechanisms able to stimulate smooth muscle (96). Results obtained with salicylate in pleurisy induced by turpentine suggest that in this system, too, activation of these other mechanisms may occur at about the same time as histamine release (139).

The consecutive action in inflammation of endogenous substances which increase capillary permeability has much to commend it as an overall working hypothesis consistent with most of the known facts. It is clear, however, that other interpretations of these facts are possible and that differences between species, tissues and types of injury must be expected.

VIII. CONCLUSIONS

At least three important tasks face investigators in the field covered by this review. The first is the elucidation of that partly hypothetical system of proteins or peptides which may sustain increased capillary permeability in inflammation. The second is the investigation of the structural and metabolic changes in the capillary wall which lead to increased permeability. The third is the establishment of the physical or chemical mechanisms whereby compounds which increase capillary permeability exert their effect. It cannot be said that the attainment of any of these objectives is in sight.

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