THE INFLAMMATORY RESPONSE

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INTRODUCTION

Like most fundamental concepts of pathology, inflammation is difficult to define. In vertebrates it could perhaps be described as the local reaction to injury of the living microcirculation and its associated tissues, in which would be included blood leukocytes and such features of perivascular tissue as mast cells and histiocytes.

Any noxious stimulus can provoke the inflammatory reaction. Where such injury is associated with death of tissue, the reaction will often be found at the periphery of the lesion where the microcirculation survives.

The inflammatory response has attracted renewed interest of recent years with the realization that many diseases exist in which a large portion of the tissue damage results from the inflammatory response itself. In these conditions, the stimulus provoking the reaction, unlike simple bacterial invasion, would be innocuous were it not for its ability to induce an inflammatory reaction. Rheumatic and allergic diseases fall into this category.

Despite these aspects of the problem, it remains true that the most important cause of inflammation is bacterial invasion of the tissues.

The inflammatory response is a series of events rather than a single event and follows a course which is essentially uniform. Different types of injury do, however, lead to variation in the relative intensity and duration of particular aspects of the reaction.

The events of inflammation fall naturally into two broad divisions, involving the fluid and cellular phases of the circulation, respectively. The fluid-phase reaction consists of a transient vasoconstriction followed by sustained dilation of arterioles, capillaries, and venules, during which blood flow is increased and subsequently decreased, and permeability to plasma protein is raised. The cellular response consists of swelling of histiocytes and tissue macrophages and emigration of leukocytes from the vessels, preceded by swelling of vascular endothelial cells and the adhesion to them of leukocytes.

INFLAMMATION DUE TO BACTERIAL INVASION

Following earlier experiments by Rigdon (111), Burke and Miles (19) re-investigated the sequence of events after inoculation into the skin of small doses of various living microorganisms. The bacteria used included *Clostridium welchii*, *Corynebacterium diphtheriae*, *Listeria monocytogenes*, *C. ovis*, *Staphylococcus aureus*, *Pseudomonas pyocyanea*, *Proteus vulgaris*, and *Escherichia coli*.

The vascular response was measured by increased permeability to dye-labeled plasma protein and by adhesion of carbon particles to vascular endothelium. After inoculation, these changes developed very rapidly, reaching a peak within 15 to 20 min; they declined to a low point 1 hr after infection, but then rapidly developed again and reached a new high level at 3 to 4 hr. Thereafter, leakage of labeled protein declined fairly rapidly, reaching normal values at 6 hr. In spite of this return to normal, induration and edema in the injection site persisted, the degree varying directly with the intensity of increased vascular permeability. It seems likely that this persistent edema is due to retention in the tissues of water, electrolyte, and protein, as a result of changes taking place after injury. Much of the fluid could well be intracellular or intrafibrillar.

The data presented by Burke and Miles (19) indicate a biphasic vascular response, the major phase being the delayed component. This pattern was observed in every case except *P. vulgaris*, when large doses of bacteria had to be injected. It seems likely that with heavy inoculations, as in the case of *P. vulgaris*, the initial transient vascular change becomes incorporated into a progressive monophasic response which reaches its peak at about 3 to 6 hr (111). Like persistent induration, necrosis in the inoculation site was found to vary directly with the degree of increased vascular permeability; in this instance, the residual permeability was measured at 6 hr. Thus, tissue death and intensity of inflammatory response were directly proportional.

Infiltration of the inoculated tissues by leukocytes was first observed at 1 hr, although at that time it was very slight. The infiltration progressed steadily in most cases and was still increasing 6 hr after injection; that is, at a time when increased vascular permeability to protein had returned to normal.

A comparison of the time course of vascular changes and leukocytic infiltration after inoculation of bacteria with similar changes after other types of injury (e.g., thermal or chemical) shows that they are very similar (145, 131, 126, 53, 119). It may be presumed, however, that in natural, as opposed to experimental, local infections the interval between infection and inflammation will

be longer if only very few organisms gain entry. The results of Burke and Miles (19) suggest that the threshold dose required to initiate an inflammatory response varies with the organism and that almost any bacteria, however "nonpathogenic," will induce such a response if inoculated in sufficiently large numbers.

Vascular Response to Inflammation

Increased Vascular Permeability

The endothelial wall of capillaries and venules forms a semipermeable barrier that allows free movement of small molecules but normally restricts the passage of plasma protein. A cardinal feature of inflammation is a striking increase in the permeability of these vessels to such protein. It could indeed be said that in structures such as the skin the addition of increased permeability to hyperemia and vascular dilation represents the transition from a "physiological" to a "pathological" response.

The importance of increased vascular permeability has made it a focus for research on vascular changes in inflammation. In effect, it poses two separate problems: the structural and functional changes occurring in the vessel wall, and the intermediary mechanism by which injury brings these changes about.

Methods of Estimating Increased Vascular Permeability

Most of the experimental techniques employed for this purpose have been discussed recently in another review article (128). These methods include measurement of edema or exudate gravimetrically and volumetrically, and observation of the leakage from blood vessels of azo dyes coupled in vivo to blood albumin. Another procedure, used by Wasserman, Loeb, and Mayerson (142), involves the intravenous injection of albumin labeled with I^{131} and determination of its subsequent concentration in the lymph of the thoracic and right ducts, and in the plasma. As vascular permeability increased, so did the concentration of labeled protein in lymph relative to that in plasma. This technique has the advantage of being truly quantitative, and by varying the choice of lymphatic vessel cannulized its usefulness can be extended, e.g., to changes confined to all or part of one limb.

Another advance is the development of a method by Judah and Willoughby (58) in which leakage of protein-bound trypan blue into rat skin as a result of increased vascular permeability is estimated by chemical extraction of the dye and its subsequent colorimetric determination. The technique involves disintegration of the frozen tissue in a percussion mortar followed by extraction with ether and ethanol to remove unwanted lipids, and then extraction of the trypan blue with hot aqueous pyridine.

Structural Aspects of Increased Vascular Permeability

The classical concept of the structure of the capillary wall, as outlined by Chambers and Zweifach (20) and recapitulated recently by Fawcett (31), was from within outward: an endocapillary layer of adsorbed plasma protein, the vascular endothelium separated by substantial quantities of intercellular cement substance, a basement membrane (the existence of which was not universally accepted), and a pericapillary sheath of argyrophil connective tissue fibers.

The classical concept of vascular permeability to plasma protein, elaborated by Chambers and Zweifach (20), suggested that the protein molecules left the vessel by passing not through the endothelial cytoplasm but through the interendothelial substance which was regarded as the "nonliving" constituent of the blood vessel as opposed to the "living" endothelial cell. This passage was normally very slow but became greatly augmented after injury.

There were two main reasons for believing that proteins and indeed many other substances of smaller molecular weight left the vessel by way of the "nonliving" intercellular substance. The first was the extreme rapidity of transcapillary exchange which made it difficult to visualize the endothelial cell surviving such a disturbance of its cytoplasm. The second reason was that the rate of transfer of different molecules across the vessel wall varied inversely with the molecular size, and often with no other factor. This differential speed of passage was demonstrable among the plasma proteins as well as between colloids and crystalloids such as glucose. Although the climate of opinion is now somewhat different from that prevalent when Chambers and Zweifach (20) wrote their review, these two points still have to be explained by any hypothesis that is advanced. Chambers and Zweifach and others were able to find experimental evidence in sup-

port of the importance of intercellular cement in the control of capillary permeability. Thus, after injury, masses of translucent material were seen apparently being discharged from the endothelial surface. In addition, calcium ions were found to be essential to maintain normal semipermeability of vessels in perfused tissues, and it was suggested that the intercellular cement consisted partly of calcium proteinate.

The view that plasma protein left the vessels between the endothelium received powerful support from the experiments and mathematical analysis of Pappenheimer (99) and his colleagues. Pappenheimer came to the conclusion that his findings and those of others could best be explained on the assumption that nonlipoid soluble molecules including plasma proteins left the vessels by diffusion through water-filled channels or pores of fixed dimensions, occupying only about 0.2% of the volume of the wall and quite possibly situated in the intercellular substance. The major importance of Pappenheimer's (99) analysis, however, lies in the concept of molecular sieving that he advanced. He suggested that the facts were most easily explained by assuming that the water-filled pores were of a uniform size, somewhat larger than the biggest protein molecule, i.e., with an effective radius of about 44 A. Molecular sieving, i.e., the passage of smaller proteins such as albumin more rapidly than the larger globulins, was explained by restricted diffusion. This term meant in practice that two forces hampered the passage of plasma proteins through these pores in the capillary wall—viscous drag between the molecule and the walls of the pore, and steric hindrance, i.e., obstruction to the passage of the molecule if it impinges on the margins of the pore before entering. Because of the predominant importance of restricted diffusion, Pappenheimer's (99) hypothesis suggested that the passage of plasma protein would be independent of filtration of water and of filtration pressure.

Ultrastructure of the Normal Capillary and Venule

Pappenheimer's (99) review was published in 1953, and his analysis dominated all thinking from that time until the results of electron microscopy ushered in a new era and enabled workers to re-examine the problem. The first electron microscopist to describe the essential features of the ultrastructure of the normal capillary was Palade (96), whose initial results were published in abstract form. The lack of correlation between the ultramicroscopic appearances and current concepts of vascular semipermeability took a relatively long time to become a major issue. Palade was, however, followed by a number of other electron microscopists, none of whom has disagreed fundamentally with him in the nature of their observations. These workers include Yamada (156), Karrer (59), Kisch (61), Moore and Ruska (89), Policard, Collet, and Pregermain (106), Hampton (44), Alksne (1), Bennett, Luft, and Hampton (12), and Fawcett (31).

It has emerged that capillaries of different tissues or of different parts of the same tissue may vary in some of their features, such as the prominence of the basement membrane or the investing layer of periendothelial cells (12). With one or two exceptions, notably the glomerular capillaries (42, 156), these small vessels have essentially similar characteristics, recently summarized by Fawcett (31). The first point revealed by electron microscopy was that the hypothetical endocapillary layer either does not exist or is not preserved by current techniques, the former seeming more likely since blood cells may be seen virtually in contact with the endothelial surface. The inner surface of the capillary endothelium is seen to be generally smooth, although projections of cytoplasm or "tentacles" may occasionally be observed (61). The endothelial cytoplasm contains a characteristic pattern of intracellular structures including mitochondria, Golgi apparatus, endoplasmic reticulum, granular material, infoldings of the plasma membrane (caveolae intracellulares), fine filaments probably less than 100 A thick (31) possibly indicative of contractile powers, and certain vesicles about which there has been much discussion. These vesicles are between 500 and 700 A in diameter, very variable in number, and distributed mainly along the inner and outer cell membranes, often communicating by way of ostia with the lumen or the extracellular space. The basement membrane has been noted to vary in prominence, to be about 500 to 600 A thick, and to consist of a network of fine filaments in a homogeneous matrix (156). The membrane is relatively electron-dense and stains strongly for 1-2 glycol linkages, indicating a high mucopolysaccharide content. Fawcett (31) pointed out that the basement membrane is preserved by osmium fixation, whereas the ground substance of the surrounding

connective tissue is lost, indicating that the two are not identical. Some observers feel that the features of the basement membrane suggest that it is elaborated by the endothelium or by the other cell types with which it is associated, e.g., epithelium, muscle cell sarcolemma, and Schwann cells.

Perhaps the most immediately important result of electron microscopy of normal small blood vessels (except in the kidney and in one or two other places) is the failure of all investigators to detect any appreciable interendothelial space. The actual distance between the opposing cell membranes is usually between 90 and 150 A (31), and the line of junction is characteristically oblique and interdigitating, with a high degree of overlap of the adjoining cell borders. The electron density of the narrow interendothelial space is relatively high and is similar to that of the basement membrane. The space does not correspond to the argentophilic zones seen in the capillary wall and formerly thought to represent interendothelial cement substance. The argyrophilic bands are in the position of the cytoplasmic condensations that adjoin the interendothelial space.

It is apparent that the extent of the intercellular space seems too small for the role assigned to it by Chambers and Zweifach (20) in vascular permeability to protein and also too small to be the sole site of the water-filled pores postulated by Pappenheimer (99) as the route by which protein leaves the vessel. The diameter of the interendothelial space is in fact only two to three times that of a single pore of the type suggested by Pappenheimer.

The unexpected failure of the anatomical intercellular space to live up to the expectations of physiologists led investigators, especially electron microscopists, to seek alternative pathways for the movement of proteins across the vessel wall. In particular, the vesicles described above have been suggested as part of an active transport mechanism across the endothelial cytoplasm (96, 89), and the hypothetical process has even been given a name, cytopempis (89). Many attempts have been made to follow the distribution in the vessel wall of electron-dense particles within the size range of plasma-protein molecules in an attempt to solve this problem. Substances used to this end have included ferritin, thorium dioxide, and colloidal mercuric sulfide (31, 154). Such particles have been shown to pass specifically through the intercellular junctions of endothelial surfaces in amphibia (31).

Many attempts have been made to demonstrate selective concentration of such particles in the intracytoplasmic vesicles of normal capillary endothelium and, although some particles were seen to be intravesicular, others appeared free in the cytoplasm or in the interendothelial space (31). More recently, Palade (98) and his colleagues have produced convincing evidence of the selective concentration in cytoplasmic vacuoles of such particles, notably colloidal gold and ferritin. It can, however, be argued that the particles are treated by the capillary as "foreign" material and not as isologous protein; there is no doubt that many of the particles are much larger than plasma-protein molecules, being up to 400 A in size.

Two objections to the hypothesis that protein leaves vessels in the vesicles have been raised by Fawcett (31). The first is the distribution of vesicles at both cell borders with none seen in the cell interior. This would mean either that the vesicles move across the cell very rapidly and are seldom seen in transit or that the vesicle disappears on leaving the luminal border and forms again at the outer boundary of the cell. The second objection is the presence of precisely similar vesicles associated with the plasma membrane of mesothelium, fibroblasts, and smooth and cardiac muscle cells and fibers. Indeed, in the arteriolar wall, the smooth muscle cells often contain more vesicles than the vascular endothelium. This wide distribution does not suggest a specific function in transporting colloids from plasma to extracellular space, but rather indicates some more general purpose such as the bulk uptake of fluid for cell metabolism.

The failure to demonstrate intercellular pores has led also to the suggestion that the limiting factor in vascular permeability to protein is the basement membrane (31, 96, 12, 98), the endothelial cytoplasm yielding free access to colloids with or without the proposed vesicular transport system. It has also been suggested that the real barrier to protein lies outside the vessel wall and consists of the ground substance intervening between pericapillary space (containing plasma) and lymphatic space (115). This view postulates free permeability of the capillary wall to plasma protein. There is, however, no real evidence in its favor. The best support for the view that

molecular sieving of proteins and similar molecules occurs beyond the endothelia themselves is found in the glomerulus where the endothelial surface is discontinuous and where particles of ferritin have been seen, electron microscopically, to accumulate between the endothelium and its basement membrane (30). The role of the basement membrane will be discussed below.

Ultrastructure of Abnormally Permeable Capillaries and Venules

Electron microscopy has been applied also to the study of capillaries in a state of increased permeability. Alksne (1) showed that the dermal capillaries of the mouse, after the application of histamine, underwent striking changes. Many, but not all, capillaries exposed to histamine developed complex intracytoplasmic membrane structures giving the endothelial cytoplasm a honeycomb, foamy, or vesicular appearance. The vesicles thus formed were much larger (up to 2,000 A in diameter) than the normal vesicles and differed from the normal vesicles in possessing irregular, indistinct borders that were often contiguous with other vesicles. The interior of the abnormal vesicles appeared less electron-dense than that of their normal counterparts, although Alksne (1) did not comment on this, and differed again in being distributed diffusely through the cytoplasm and not being confined to the region of the cell membrane. From independent study of the published electron micrographs, it seems doubtful whether the normal vesicles increase significantly in number during increased capillary permeability induced by histamine, and the connection between the large abnormal and the smaller normal vesicles also seems dubious.

Histamine-treated capillary endothelium showed complexity of the endoplasmic reticulum and of the Golgi membranes; these signs were taken to confirm the presence of general increased membrane activity. In addition, well-marked infoldings of the cell membrane were seen, sometimes leading by way of wide ostia into channels extending deep into the cell and sometimes even forming a direct connection between lumen and extravascular space. Some channels had branching processes linking up with similar passages. Sometimes the endothelial cytoplasm showed wide fenestrations up to 0.5 μ across.

Similar results were obtained by Moore (88) after compression of the leg of a mouse for 1 to 2 hr. Edema develops in the limb 10 min after the release of the tourniquet, the fluid being rich in plasma protein with a preponderance of albumin, i.e., the picture typical of increased vascular permeability after injury. Moore (88) described the appearance of numerous very large clear vesicles, deep indentations of the luminal border, and deeply penetrating clear channels and fenestrations. Both Alksne (1) and Moore (88) reported no detectable change in the basement membrane or in the interendothelial junctions. Alksne (1) attempted to follow the course of mercuric sulfide particles from the blood stream into the tissues. The colloid was found in the large vesicles, in the perforating channels, and in the normal vesicles; it also apparently accumulated between cell and basement membranes as if the latter structure were an obstacle to its continued passage.

All these observations indicate a profound disturbance of the endothelial cytoplasm during increased capillary permeability. It has been suggested that the numerous large vesicles indicate an acceleration of a normal active transport system for protein. This vesiculation and its associated phenomena such as perforating channels and fenestrae could, however, equally well be the result of increased filtration of fluid. Bennett (11) suggested that membrane flow might be involved in the passage of protein through endothelium, and evidence of increased membrane activity was certainly forthcoming. What is clear is that none of these investigators saw changes in the interendothelial spaces that would suggest increased protein loss by this route.

A very different conclusion, however, emerges from the more recent work of Majno and Palade (98). Palade and his colleagues reported that, using the rat cremaster muscle and stimulating the small vessels therein by injections of histamine and serotonin (5-hydroxytryptamine), the resultant increased vascular permeability was associated with striking changes in the vessel wall, particularly in the venules. In contradistinction to all previous findings, which Palade (98) attributed to unsuitable selection of tissue and time intervals, it was found that increased vascular permeability was associated with unequivocal separation of endothelial cells so that large gaps appeared between them. As a rule, only a portion of the intercellular junction was affected by this change. The perivascular basement membrane consistently appeared intact. When colloidal mercuric sulfide was used as a

marker, the particles were seen to pass through the interendothelial gaps and to accumulate between the cells and the basement membrane, then traversing the latter structure. This accumulation was taken to indicate that the basement membrane was acting as a filter. Another feature was the observation that in histamine- or serotonin-treated vessels there appeared to be fewer particles in the intracellular vesicles than in normal vessels, suggesting a diversion from the vesicles to the interendothelial gap.

These observations indicate that increased vascular permeability is due largely to loss of continuity between the endothelium, as suggested much earlier by Chambers and Zweifach (20). They contrast sharply with the findings of Alksne (1), Moore (88), and others, which suggest that increased vascular permeability is due to augmented transcytoplasmic movement. Palade and Majno observed the changes induced by histamine and serotonin to be present within 3 min and to have disappeared within 10 min. The rapidity of the phenomena seems, therefore, to be the most likely explanation of earlier failures to demonstrate them.

Molecular Sieving

Notwithstanding the question of intercellular as opposed to transcellular transport of protein, it is apparent that in the normal vessel no structures have been seen with the requisite dimensions of the pores postulated by Pappenheimer (99). In capillaries in a state of increased permeability, new channels and vacuoles appear, but these seem too large to cause molecular sieving by a process of restricted diffusion. This latter finding might be of less relevance were it not for the overwhelming evidence that molecular sieving continues to operate even when, as a result of injury, vascular permeability to plasma protein is greatly increased. A commonplace manifestation of such molecular sieving is the high concentration of albumin in inflammatory exudates relative to that in plasma (88, 105).

Following the long-established but relatively crude demonstration of molecular sieving in inflammation, the recent researches of Mayerson (76), Wasserman (142), and their colleagues have placed molecular sieving of proteins and other colloids through both normal and abnormally permeable capillaries on a more accurate basis.

These workers infused dextran or plasma proteins of graded molecular size into the circulation and measured the concentration of the various fractions in the lymph draining the injected portion of the body. They found that the plasma-lymph concentration ratios of the various dextran fractions were directly proportional to their molecular weight (142). It was also found that increasing the plasma volume by an infusion of albumin or dextran led to increased permeability of the capillaries to albumin and dextran. In dogs whose plasma volume had been expanded in this fashion, the plasma-lymph ratios of albumin or dextran fractions decreased in direct proportion to the size of the molecule, i.e., the greater the molecular weight, the greater the relative increase in filtration (121). Shirley et al. (121) interpreted this finding as a stretching of pores in the capillary wall as a result of distension by the increased plasma volume, although clearly there are alternative explanations. Courtice (23), also, using lipids and lipoproteins, has demonstrated molecular sieving after thermal injury.

Mayerson (76), Wasserman (142), and their colleagues have recently extended their observations to the microcirculation of the liver, intestines, and neck, and have demonstrated molecular sieving in all these situations, although in each case the actual permeability of the vessels to a given molecular size was different. Careful analysis of the plasma-lymph concentration curves suggested to them that a "cut-off" effect existed and led them to the conclusion that two sets of pores are present in the capillaries of the dog: small pores allowing the passage of molecules with molecular weights up to 250,000, and larger pores allowing the passage of molecules with molecular weights up to at least 412,000 (76). Earlier, Grotte (40) had come to the somewhat similar conclusion that the capillaries of the leg and neck contain a large number of uniform pores (radius: 30 to 45 A) that normally restrict albumin almost completely, together with a few "leaks" affording no selective restriction of molecules with molecular weights up to at least 300,000 (40).

A firm conclusion concerning the apparent presence of two sizes of pores and the effect of large infusions of colloids on vascular permeability would probably be premature. There is no doubt, however, of the relevance of these results to the study of inflammation, even if they cannot yet be correlated with observations by electron microscopy.

Hyaluronic acid is probably a major constituent of basement membrane, and perivascular ground substance and its molecules, even in dilute solution, form a flexible-chain polymer forming a complicated network. Solutions of hyaluronic acid have recently been shown to reduce the rate of filtration of colloids such as plasma albumin, the filtration rate of solutes declining as their molecular size rises (92). As might be expected, the filtration rate declines also with an increasing concentration of hyaluronic acid, i.e., with the denseness of the network. These findings suggest that hyaluronic acid in basement membrane or perivascular ground substance might be the structural basis of molecular sieving of plasma proteins. If this is so, however, it might have been expected that hyaluronidase would increase capillary permeability to a greater extent than has been observed (128).

It is difficult, also, to reconcile this explanation of molecular sieving with the evidence in favor of pores of definite but variable dimension obtained by Wasserman et al. (142), Grotte (40), and Courtice (23). In spite of the remaining uncertainties, the suggestion that venules and capillaries exhibit, after injury, combination of interendothelial gaps and persistent molecular sieving through the basement membrane goes a long way toward explaining the greatly increased but still selective protein loss which occurs from inflamed vessels.

If, however, other work should lead to the conclusion that molecular sieving may occur as part of a transcytoplasmic transport mechanism in the endothelial cell, a possible model for such a system might be found in gel filtration through cross-linked dextrans (67, 107). This material consists of small grains of a hydrophilic insoluble gel formed by cross-linking the complex polysaccharide, dextran, so that it forms a three-dimensional network of molecular chains. This network is nonionic, and its polar properties are due largely to hydroxyl groups; the greater the degree of cross linkage, the lower the porosity of the gel. When solutes are passed through this substance, large molecules are excluded from the internal water volume of the gel grains, whereas small ones gain entry. The precise dimension of the respective molecules which are taken up or excluded depends upon the degree of cross linkage of the gel. If the endothelial transport mechanism is conceived not in terms of vacuoles but rather as a system of gel grains into which molecules must penetrate to be transported across the cell, it is clear that the smaller plasma proteins could be shifted more readily than the larger ones. In this way, molecular sieving might be achieved by relative exclusion from the transport system of the larger molecules. It is conceivable, too, that pathological changes might lead to alteration in the degree of cross linkage in the gel composing the transport system, with resultant modification of the pattern of molecular sieving.

Basement Membrane in Vascular Permeability

It is apparent that, at the same time electron microscopy has provided massive documentation of the absence of structures corresponding to Pappenheimer's (99) pores, equally weighty evidence has been accumulating confirming the existence of molecular sieving apparently through channels of fixed dimensions.

One possible explanation of this discrepancy is that the basement membrane of the capillary or venule acts as a filter and restricts the passage of the protein molecules (97, 30). The main evidence to support this view comes from the accumulation of ferritin particles, 1 to 2 hr after injection, on the luminal side of the basement membrane (30). This result was obtained, however, in the renal glomerulus where the endothelium is known to be porous to macromolecules and to possess gaps in the continuity of its cytoplasm (156, 42). Most extrarenal capillaries are less porous to protein and do not possess such gaps. In fact, in the tongue and heart, Palade (98) found that micelles of colloidal gold previously injected into the circulation passed through the walls of normal capillaries chiefly within vesicles, but no accumulation of the particles was detected either between the endothelium and the basement membrane or within the latter structure. Thus, study of muscle capillaries in a normal state provides, as yet, no evidence to confirm the role of the basement membrane as a filter.

Observation of capillaries in a state of increased permeability to proteins does, however, lend support to the view that the basement membrane may act as a barrier and molecular sieve. In rats in which the nephrotic syndrome has been induced experimentally, the glomerular capillaries are in a condition of increased permeability. After the injection into the circulation of ferritin, particles of this substance can be seen in electron micrographs to be present within the basement

membrane in quantities much greater than that observed in normal animals (29). Increased accumulation between endothelium and basement membrane was not observed. These experiments suggest that increased permeability in the glomerular capillaries may be due to partial loss of the ability of the basement membrane to restrict the passage of protein.

Study of the passage of particles of colloidal mercuric sulfide through abnormally permeable capillaries and venules in the cremaster muscle reveals a different picture. Here, electron micrographs show massive accumulation of particles between endothelium and basement membrane or within the layers of venule walls. Only a relatively small proportion of particles are seen within the basement membrane. These results could be interpreted as showing that in this instance increased permeability is due to the formation of interendothelial gaps (see above), the basement membrane retaining much of its normal function. Smaller particles (40 to 60 A) may, however, readily pass through the basement membrane.

In the case of the normal or abnormally permeable glomerulus or of the abnormally permeable cremaster vessels, it is difficult to escape the conclusion that molecular sieving is due to the properties of the basement membrane. This is so because proteins seem likely to reach this structure by passing through discontinuities in the endothelial barrier which are too large to lead to selective passage of protein molecules. In the case of normal muscle capillaries where proteins may reach the basement membrane by way of intraendothelial vesicles, the position is less clear. Even here, however, the basement membrane may be the selective filter.

Palade (98) and his colleagues observed no pores of any kind in the basement membrane and concluded that they are either nonexistent or so tortuous as to be invisible. They received the impression that their marker molecules were moving through a yielding gel.

Filtration Pressure and Increased Permeability

Related to the problem of the route by which protein leaves the vessels and the structures which impose molecular sieving is the question of whether increased vascular permeability after injury is due partly to increased filtration pressure or entirely to changes in the vessel wall. If Pappenheimer's (99) analysis were to be accepted in its entirety, increased intravascular pressure should have little effect in raising protein filtration, since the latter was thought to be rate-limited by the factors restricting diffusion. However, the challenge to this analysis posed by electron microscopy and by the evidence favoring "pore-stretching" justifies a re-examination of the problem.

Accepting a limited transfer of plasma protein across the capillary wall but making no assumptions as to the mechanism involved, three factors could contribute to an increase in the protein filtration from the vessels of a part. These are a change in the permeability of the vessel wall, an increase in filtration pressure, or an increase in the blood flow through the part as by opening up of dormant capillary networks (110).

Changes in the vessel wall have been discussed above. Increased loss of protein due to raised filtration pressure has received some consideration lately as a result of work by Haddy (41). Before considering Haddy's results, however, the earlier investigations by Landis and others need recalling.

In 1927, Landis (64) provided experimental proof of Starling's hypothesis, in the frog mesentery, and at the same time showed that raised intracapillary pressure could lead to an increased rate of passage of colloidal dyes from these vessels. Further investigations by the same worker (65) revealed that after the injection of histamine into human skin, a procedure leading to local increased vascular permeability, the intracapillary pressure in the region of the injection site was about 40 mm of Hg in the venous and 50 mm of Hg in the arterial limb. Similar pressures were recorded after injury caused by heat and cold. Later, Landis et al. (66) produced graduated increases in the intraluminal pressure of the small veins of the human forearm by compression of the base of the limb. They found that this procedure led to the passage into the tissues of fluid, the plasma protein content of which varied with the venous pressure. At 60 mm of Hg, the filtrate contained about 0.3% protein; at 80 mm of Hg, about 1.5%; at 90 mm of Hg, about 2.3%; and at 100 mm of Hg, about 2.8%. Increased filtration of protein-free fluid was demonstrable at pressures as low as 20 mm of Hg, the normal pressure in the small veins being about 9 mm of Hg. They repeated also that the edema fluid accumulatng as a result of vena caval obstruction or of the nephrotic syndrome contained

only about 0.2 to 0.4% protein, or less than the protein content of normal lymph.

It would seem from these experiments that the degree of raised intracapillary pressure required to lead to massive filtration of plasma protein is too great for such a mechanism to be held responsible for the accumulation of protein-rich edema in inflammation. Stauffer and Hyman (136), however, have demonstrated that, after acceleration in the human centrifuge, jugular vein pressures may rise to 100 to 190 mm of Hg and that there is an associated loss of fluid from the vessels, the calculated protein content of this fluid being from 2.4 to 8.3 g/100 ml. The capillary pressure in their subjects was thought to be in the region of 160 mm of Hg, or at least twice the critical figure required for loss of protein from the vessels.

Recently, Haddy (41) has shown that the regional edema after local application of histamine is associated with a raised pressure within the very small veins (0.5 mm), edema developing when the figure exceeded 26 mm of Hg for 7 min or more. He also found that, in the presence of a constant blood flow, histamine caused neither edema of the tissues nor elevated pressure in the small veins. Haddy (41) came to the conclusion that histamine raises the pressure in capillaries and venules by causing both arteriolar dilatation and constriction of those veins into which the venules drain. He also concluded that histamine-induced edema could be due entirely to raised capillary pressure.

It would seem from these experiments that some inflammatory edema might result solely from raised filtration pressure, particularly where the protein content of the filtrate is low. To progress beyond this statement, further information is required; namely, the protein content of edema produced under conditions similar to those in Haddy's (41) experiments, more measurements of the intracapillary and intravenular pressures existing in inflamed tissues, and the correlation of changes in lymph-plasma distribution of protein molecules of progressively increasing size (142, 40, 23) with progressive increase in capillary pressure in the region under study. Even if filtration pressure in inflamed capillaries were high enough to lead to the loss of large amounts of plasma protein, this effect might, nevertheless, be an indirect one due to changes in the vessel wall brought about by raised pressure. Thus, venous compression leads to striking ultrastructural changes in the capillary endothelium distal to the block (88). In addition, an elevation of plasma volume has been found to be associated with a marked increase in vascular permeability to colloids (142).

Phagocytic Activity in Stimulated Vascular Endothelium

Several workers have shown that, after a variety of injurious stimuli, vascular endothelium exhibits striking phagocytosis of circulating particles, notably carbon (55, 15, 10). Gözsy and Kato (38) have demonstrated that in the rat, after injection of dextran (a procedure that leads to increased vascular permeability), edema formation and uptake of carbon particles by endothelium are separate phenomena. The increased phagocytic activity of stimulated vascular endothelium bears an obvious resemblance to the similar response of the reticulo-endothelial system in general to appropriate measures, e.g., the injection of various colloidal or particulate substances (123). Whether phagocytosis by vascular endothelium plays an important part in increasing vascular permeability and in the formation of inflammatory exudates is uncertain. The relationship of this phenomenon to the evidence of increased membrane activity and cytoplasmic vacuolation seen in electron micrographs of damaged capillaries (see above) is also obscure.

DIRECT DAMAGE AND CHEMICAL MEDIATORS AS MECHANISMS OF THE INFLAMMATORY RESPONSE

Having dealt with the structural and functional changes that occur in the vascular wall in association with increased permeability to protein after injury, it is appropriate to consider the mechanism whereby injury brings about such changes.

Most workers believe that between injury and vascular reaction there occur a series of chemical reactions, in particular the release or activation of vasoactive substances whose effects are responsible for the changes in the microcirculation characteristic of acute inflammation. The alternative view is that the vascular changes which follow are due largely to direct physical damage to the structure of the vessel wall.

The essential facts which the rival hypotheses must explain could be listed as: (i) the occurrence of inflammatory changes, often reversible, only in living vessels; (ii) the consistent nature and orderly sequence of events shown by the reac-

tion, in spite of the great variety of effective stimuli; (iii) the suppression of the reaction by drugs; and (iv) the existence of a latent period between injury and reaction. Bearing in mind that injury is essentially destructive to living processes, it would seem that the "direct damage" theory might be valid if the essential change were some form of reversible disturbance in the permeability of the vascular endothelial cells such as occurs in the cells of tissue slices exposed in vitro to anoxia or chilling. This implies some cellular component, particularly susceptible to injury, destruction of which would lead to increased vascular permeability while the endothelial cell retains sufficient integrity to maintain other metabolic processes and eventually to recover. This view might explain points i and ii above. It might also explain point iii if all the drugs in question were known to enter cell membranes and exert a protective effect on changes in the membrane permeability. Certainly some antihistamine drugs have this property, but only in doses much higher than those needed for an anti-inflammatory effect. It is, however, difficult to explain a long latent period on the basis of direct physical injury. Depending on the nature of the injury, this latent period takes the form of a delay of 2 to 24 hr between tissue damage and the onset of the major sustained increase in vascular permeability and of leukocyte emigration. The delayed vascular changes usually follow an immediate transient increase in vascular permeability, the two phases being separated by a silent period. These delayed changes have been demonstrated in a number of species after thermal injury (119, 131, 145), X-ray damage (150), chemical irritation (132, 126), and bacterial infection (122, 62). More details of the delayed response will be found in the recent article by Miles (82).

Persuasive evidence in favor of the view that the vascular sequelae of injury are due to endogenous chemical "mediators" comes from the demonstration of endogenous substances with actions on blood vessels similar to those provoked by injury, of their release or activation by such injury, of the demonstration of their presence at the time when they should be exerting their effect, and of their absence when inflammation subsides or has not yet begun. Further support for the hypothesis is provided by the suppression of inflammatory changes by measures apparently specifically antagonistic to a suspected chemical mediator.

These criteria, essentially similar to those proposed many years ago by Dale, have been discussed elsewhere by Spector (129) and Miles and Wilhelm (86). Consideration of the facts given above and of the observations now to be discussed make it clear that the participation of chemical "mediators" is virtually certain.

Endogenous Substances in the Early Inflammatory Reaction

Histamine

The ability of histamine to reproduce the acute vascular changes of injury, its widespread distribution, and the ease with which it is released from its bound form in the body are well-known and have been recently reviewed (128, 101). There is also ample evidence of its release by a wide variety of noxious stimuli including thermal, chemical, and anaphylactic injury (128). Further evidence of the importance of histamine has come from the ability of the antihistamine drugs to suppress the vascular phenomena of anaphylactic reactions, notably urticaria and hay fever.

Attempts to influence the course of experimental inflammation with the aid of antihistamine drugs had been largely unsuccessful until Spector and Willoughby (132) gave mepyramine (Neo-Antergan) to rats in doses small enough to be specifically antagonistic only to histamine and found that this treatment delayed the onset of increased vascular permeability after chemical injury by 1 to 2 hr. Moreover, a very similar result was achieved by depleting the rats of bodily histamine prior to injury with the aid of repeated injections of the histamine liberator, compound 48/80. Together with analysis of inflammatory exudates showing the presence of histamine in the first 30 min after such chemical insult (128) but not at later times, these results indicate that endogenous histamine is responsible for the initiation of the vascular changes in this form of injury, other mechanisms being then required to maintain these changes. The antihistamine drugs have now been used in thermal injury in rats (131) and very thoroughly in many other species (145). In all cases, their administration suppressed the earliest but not the subsequent increase in vascular permeability, and the conclusion has been reached that the role of histamine

is to initiate but not to sustain the vascular sequelae of tissue damage. Furthermore, the technique of depletion of bodily histamine has been employed prior to local injection of microorganisms in rats with the result that the onset of vascular changes was considerably delayed. Thus, in this bacterial inflammation, too, local release of histamine appears to initiate alterations in vascular flow and permeability (120).

Since the role of histamine is confined to the earliest stages of inflammation, it follows that, viewing the course of the reaction as a whole, histamine plays a minor role. This is apparent from the failure of antihistamine drugs to modify the inflammatory response as measured some hours after the introduction of bacteria into the rat peritoneal cavity (122). Varieties of injury may well differ in the relative importance of histamine in the ensuing reaction; in the rat, experimental bacterial peritonitis seems to cause little histamine release (122). In other experimental systems, the failure of antihistamine measures to modify the inflammatory response may be misleading. Thus, in passive cutaneous anaphylaxis, the vascular reaction is explosive and has run its course in 20 min. Because events are compressed into a short space of time, any release of histamine would almost certainly coincide with activation of other vasoactive substances, e.g., plasma kinins (see below). It is not surprising, therefore, that antihistamine drugs failed to diminish increased vascular permeability in passive cutaneous anaphylaxis (17).

This interpretation of the failure of antihistamines to influence passive cutaneous anaphylaxis is supported by experiments on X-ray injury in the rat (150). This procedure leads to increased vascular permeability in the intestine of the irradiated animals, not apparent until 24 hr after injury and maximal at 72 to 96 hr. The inflammation thus follows a slow course and is preceded by the latent period characteristic of irradiation injury. Administration of antihistamine drugs at the time of exposure delays the onset of increased capillary permeability in the bowel until 48 hr after injury, the vascular changes thereafter being as severe as in control irradiated animals. Biological assay of the damaged gut reveals that histamine release occurs in the initial 24 hr but not subsequently. There is, thus, a close correspondence between the results of administering antihistamine drugs in thermal, chemical, and irradiation injury in the rat; in each case, only the initial phase of the vascular reaction is suppressed, although the duration of this phase varies greatly, being 20 min, 1 hr, and 24 hr, respectively.

All the above results are consistent with a role for histamine, however transitory, in the inflammatory response. Much of the evidence in favor of this view could, however, be challenged from another quarter. It has been known for a long time that some, at least, of the antihistamine drugs, especially in high dosage, will inhibit the increased vascular permeability caused by substances other than histamine (43). The significance of this observation has been increased by the more recent demonstration that the antihistamine drugs produce striking effects on cell surfaces and biological membranes in general. Thus, they greatly reduce the adhesiveness of damaged platelets, owing to an effect at the surface of the cells or their containers (91). Antihistamine drugs also prevent the accumulation of water by damaged tissue slices (72) and by isolated mitochondria (57), again presumably owing to an action at cell or subcellular membranes. This action could be of a biochemical nature, possibly involving interference with phosphoprotein turnover (57). It could also be associated with the ability of antihistamine drugs in common with other weakly basic, amphipathic compounds to penetrate lipid monolayers in vitro and to introduce positively charged groups into such films, since the lecithin-cholesterol monolayers could be taken as a model for the cell membrane (7).

It is unlikely that this considerable body of evidence concerning the ability of antihistamine compounds to influence the properties of surfaces is entirely irrelevant to the action of these drugs in suppressing increased vascular permeability. On the other hand, even if this is so, it could well be true that histamine, too, exerts its vascular effects by affecting the properties of cell surfaces, presumably in a direction opposite to that of the antihistamine drugs. Thus, there is no real reason to challenge the view that in suitable concentration these drugs are specific antagonists of histamine. This would be particularly likely if histamine were found to influence cell surfaces at some point which was especially sensitive to a blocking action of the antihistamine drugs. Unpublished experiments by Spector and Wil-

loughby have shown that for a large number of antihistamine drugs there is a wide difference between the minimal dose that will inhibit the effect of histamine on capillary permeability and the corresponding dose that will have a similar effect on other vasoactive drugs such as serotonin, the polypeptide substance P, and permeability-increasing globulins. There is no doubt, therefore, that, whatever their mode of action, these compounds are, to a greater or lesser degree, specific antagonists of histamine. It is also beyond dispute that the suppression of inflammatory changes, e.g., in turpentine-induced pleurisy or thermal injury, can be achieved by doses that are effective only against histamine and that this suppression is demonstrable only in the early stages of the reaction during the period of histamine release.

Mechanism of Histamine Release

An account of the controversy concerning the mechanism of histamine release was presented in a previous paper (128). Recent work, mainly by Uvnäs (140) and his colleagues, has concentrated on the mast cell, which is the probable source of much of the histamine liberated by injury in many species (112). Uvnäs (140), working on rat mast cells isolated and in situ, has concluded that release is due to the lytic action on the mast cell membrane of an enzyme normally situated on the membrane. This enzyme is activated by chemical histamine liberators such as compound 48/80, extracts from jellyfish and other biological sources, and antigen-antibody reactions. Activation is thought by Uvnäs (140) to lead to rapid degranulation of mast cells and release of histamine. The enzyme is believed to be a phospholipase containing essential NH_2 and SH groups. The evidence for these conclusions is that of 35 enzymes only phospholipase A degranulated mast cells; that the actions of this enzyme and of antigens, biological extracts, and compound 48/80 were all blocked by similar inhibitors, notably agents that inactivate NH_2 and SH groups; and that extracts of dog mast cells will degranulate rat mast cells, the effect being prevented by agents that block NH_2 or SH groups. The process appears to be independent of oxygen, but it may be that glycolysis provides sufficient energy for the reaction to take place. The hypothesis of Uvnäs (140) and his colleagues is consistent with most of the observed facts, although histamine release from isolated cell particles seems to be governed by other factors (140, 87). There is also histochemical evidence of the existence on the mast cell membrane of an esterase (37), the properties of which would be compatible with those of the enzyme postulated by Uvnäs (140). The possible mechanisms whereby injury might lead to the activation of such enzymes will be dealt with later.

Serotonin

Serotonin is a vasoconstrictor, low concentrations of which in some species, notably the rat, induce increased vascular permeability. Serotonin is present in many tissues, including brain and intestine and platelets, and, in the rat at least, occurs in the mast cells (128). In many types of injury, there is a release of serotonin which parallels that of histamine, and, in the rat, some chemical histamine liberators release serotonin too. Although serotonin is present in inflammatory exudates up to 1 hr after injury and absent from samples taken at later times, specific inhibitors of the substance fail to influence the vascular changes leading to the accumulation of such exudates (128). On the other hand, the edema provoked in the rat by injection of egg white and dextran is considerably lessened as a result of administration of these compounds (43, 100). The serotonin antagonist BOL 148 (bromolysergic acid diethylamide tartrate) has been reported also to have some effect on passive cutaneous anaphylaxis in the rat, but the significance of this result is doubtful (17). Nevertheless, it seems that release of serotonin is likely to be important in certain specialized vascular reactions provoked in the rat by injection of egg white or dextran. The failure of serotonin antagonists to diminish the vascular reaction to chemical and thermal injury (128, 130) need not necessarily exclude its participation, since similar inhibitors fail to influence the syndrome associated with argentaffinoma and thought to be due to excessive production of serotonin. Depletion of histamine and serotonin by repeated injections of compound 48/80 leads to a longer delay in the onset of vascular changes in the inflamed rat pleura than does dosage with antihistamine drugs; this observation could be regarded as an indication of a complementary role for serotonin in the early phase of the inflammatory reaction. Similarly, in irradiation injury of the rat intestine, there is profound depletion of the serotonin content of the bowel wall, most

marked between 24 and 48 hr, whereas the similar loss of histamine occurs mainly in the initial 24 hr (150). The effect of prior repeated injections of compound 48/80 could not be tried in this experimental system, since the substance does not deplete the intestinal wall of serotonin.

The above time relationships suggest that if serotonin contributes to the vascular changes of injury in the rat it does so by augmenting and temporarily sustaining the similar effect of histamine. A comparable action of serotonin in species other than rat or mouse seems doubtful, however, since in other species it does not increase vascular permeability to a significant extent (124).

ENDOGENOUS MECHANISMS POSSIBLY RESPONSIBLE FOR THE DELAYED VASCULAR CHANGES OF INJURY

It is apparent from the experimental evidence that the release of histamine, and perhaps in some circumstances of serotonin, can account for only the initial immediate phase of the vascular changes of inflammation and that some other explanation must be sought for the subsequent delayed onset of sustained vasodilatation and increased vascular permeability. This situation was envisaged by Krogh (63), who postulated that tissue damage leads to the release both of a diffusible, readily destroyed substance akin to histamine and of a less diffusible, less readily inactivated principle which he called H-colloid. The claim of certain endogenous compounds to fulfill this latter role will now be discussed.

Polypeptides

The effect of peptides in causing vasodilatation, particularly in connection with the phenomenon of "peptone shock" has been known for a long time (104). The more detailed study of the possible role of these substances in the local response to injury began with the observation of Menkin (79, 81) that tryptic digests of albumin cause increased vascular permeability on injection into the skin of living animals. Menkin found also that his active principle induced emigration of leukocytes from blood vessels and, as a result, named it "leukotaxine." Menkin thought that a similar substance was responsible for the ability of inflammatory exudates to increase vascular permeability (79), but, for reasons given elsewhere (128), this conclusion seems less certain. In addition, his belief that his extraction procedures had produced a pure crystalline end product is now known to be incorrect (128). Nevertheless, all subsequent work has confirmed that certain polypeptides derived from many sources have an effect on vascular permeability (128). Their action on leukocyte emigration will be discussed later.

Investigation of the role of polypeptides in inflammation was greatly advanced by the discovery that such substances could be formed in blood plasma subjected to a variety of procedures in vitro. These peptides are known collectively as plasma kinins and have the common property of inducing a slow contraction of the plain muscle of the isolated intestine and uterus, of dilating arterioles and increasing vascular permeability, of lowering the blood pressure, and of causing pain on application to a blister base. In addition to the kinins formed in plasma, peptides with similar pharmacological properties have been found to exist elsewhere, e.g., in wasp venom (49) and in the intestinal wall and brain, this latter compound being known as substance P (103). Other biologically active peptides with less relevance to inflammation were discussed in a previous review (128). Once formed, plasma kinins are rapidly destroyed by further contact with plasma, due presumably to the action of peptidases. They are destroyed also by chymotrypsin and are dialyzable.

The subject of plasma kinins has been recently reviewed (70), but briefly it may be said that plasma of many species, including man, contains a substrate in the serum globulins, often in the α_2 fraction, from which the active peptides are formed. If trypsin or snake venom is allowed to act on whole serum or on the α_2 globulin fraction, an active kinin is formed, the peptide being called bradykinin by virtue of its slow-contracting properties (113). Bradykinin has now been purified, and it has been confirmed that the pure substance possesses all the properties of kinins listed above (28). Bradykinin contains nine amino acid residues in its molecule, a fact that corresponds with earlier results based on end-group analyses of impure peptide preparations (125).

Plasma kinin can be formed from plasma not only by the action of trypsin and snake venom, but also by the actions of at least two enzymes present in the blood. The first of these is plasmin, the blood fibrinolysin (68); this enzyme, however,

forms kinins relatively slowly, compared both with its action on fibrin and with other kinin-releasing enzymes (14). The other blood kinin-forming enzyme is kallikrein. This substance, like plasma, is present normally in an inactive form but may be prepared from serum in the active state by casein adsorption followed by fractionation with organic solvents (14). Kallikrein obtained in this fashion is very potent in forming kinins from serum. It also, unlike plasmin, causes increased vascular permeability on injection into skin, due presumably to formation of kinins in vivo (14). The kinin formed by the action of kallikrein is known as kallidin, and there is reason to believe that it is different from bradykinin in its chemical constitution.

In addition to kinin formation by the action of certain enzymes on plasma proteins, similar peptides are formed when plasma from a number of species is diluted with saline (116) or brought into contact with glass (4). Thus, plasma so treated causes a slow contraction of plain muscle (116), gives rise to pain on application to a blister base (4), and increases capillary permeability. With the exception of the vascular effects (see below), these properties acquired by plasma are very short-lived, due presumably to destruction of the kinins by peptidases. Because of the similarity of their actions to those of bradykinin, plasma kinins released by dilution or glass contact or by injury in vivo (see below) are often known collectively as bradykinin, although this term should perhaps be reserved for the kinin formed from plasma by the action of trypsin or snake venom.

Serum kallikrein appears to be the most potent kinin-forming enzyme in the body (4), and it has been suggested that the formation of plasma kinins by dilution or contact with glass is due to activation of this enzyme. This suggestion is an attractive one, but only further investigation can establish its validity. The inhibition of kallikrein activity in vitro by salicylates (53), antiesterases (143), and other substances (53) might provide one approach to the problem.

Permeability-Increasing Globulins (PF/DIL)

It has already been stated that dilution with saline gives plasma the property of increasing vascular permeability. This phenomenon was observed by Miles and his colleagues (85) who went on to demonstrate that the active principle has a potency comparable with impure preparations of bradykinin (146, 147) and resides in the serum globulins, the particular fraction varying with the species (82). Serum contains also a specific inhibitor of this permeability-increasing globulin (146, 147). The active substance was found to exist in an inert form and to be activated by dilution, for which reason it was called PF/DIL. Contact with glass may also be necessary for activation to occur. In some species, these globulins are not activated by dilution, e.g., in the rabbit, cat, dog, horse, and ox, although this does occur in man, rat, guinea pig, mouse, and baboon (82). For this reason, the terms globulin permeability factors (globulin PF) or permeability-increasing globulins are preferable to PF/DIL. In spite of differences between species, there is enough evidence to suggest that globulin PF is a general feature of vertebrate plasma (82). Its mode of activation remains obscure, but it seems possible that both alteration of the globulin molecule and removal of specific inhibitors are needed (82, 127).

Activation of globulin PF by dilution, contact with glass or other charged surfaces (82), or with antigen-antibody precipitates (25) or organic solvents resembles the similar activation of serum kallikrein (144), the formation of kinins (4, 116), and the early stages of blood clotting (74). It is very tempting to accept the suggestion that globulin PF is identical with the kinin-forming enzyme of plasma, which in turn might be identical with serum kallikrein. Unfortunately, guinea pig globulin PF, although regarded by Miles (82) and his colleagues as an enzyme, does not appear to possess significant kinin-forming activity. Guinea pig globulin PF does have the capacity to split synthetic esters (9) but, as even the best preparation available is far from pure, this observation may not be significant. Similarly, human globulin PF will form kinins from suitable substrates but, because of its relative impurity, the same objection applies. Indeed, as Miles (82) pointed out, until they have been more highly purified it cannot be fully accepted that all the actions of the various kallikreins are due to formation of kinins.

The activation of globulin PF by dilution of plasma with saline is much more effective in glass than in polyethylene vessels, and Spector (127) and Margolis (75) have suggested independently that the function of dilution is to slow the destruction of the factor activated by glass contact. Margolis (75) has investigated the mechanism whereby contact with a glass surface gives human plasma the ability to increase vascular permea-

bility (75). It is clear from what has been said that this property could be due to activated kallikrein or globulin PF, or to plasma kinin.

From his experiments, Margolis (75) concluded that this reaction requires the adsorption onto glass of Hageman factor, a substance required for the clotting of blood in vitro (108), followed by interaction with two substances, components A and B, both of which can be exhausted after contact with glass. Plasma exhausted of component B and then activated by glass contact will not give rise to plasma kinin. It will, however, increase vascular permeability.

The simplest interpretation of these results is that formation of plasma kinins and activation of globulin PF are different steps in the same sequence of events and that the permeability-increasing activity of activated plasma is not likely to be due to kinins but probably results from the presence of globulin PF. The suggestion of Margolis (75) that globulin PF is identical with the kinin-forming enzyme (kininogenase, substance A, kallikrein) is more doubtful and has already been discussed. It is only too obvious from this discussion that the relationship of kinins, kinin-forming enzymes, kallikrein, and globulin PF remains obscure.

Inhibitors

The confusion concerning the identities of kinins, kinin-forming enzymes, and globulin PF is reflected in the actions of specific inhibitors of these substances. Such inhibition has been demonstrated in the case of various kallikrein preparations, kinin-forming enzymes activated in plasma, and globulin PF.

The natural inhibitor of globulin PF in serum has already been discussed. A similar inhibitor of blood kallikrein also exists, and has been characterized as thermolabile, basic, partially dialyzable, and destroyed in peptidases; the susbtance is thought to be a basic polypeptide (35). Similar kallikrein inhibitors exist in the tissues.

Serum also contains antiplasmin, an inhibitor of plasmin (54). Apart from its effect on plasmin, antiplasmin inhibits plasma kallikrein (143), but not kallikrein prepared from exocrine glands, glandular secretions, and urine (69).

Soya bean trypsin inhibitor (SBTI) is an antiprotease and antiesterase with important properties in relation to the substances under discussion. SBTI inhibits plasmin and plasma kallikrein (143) but not kallikrein from other sources (69); this fact, coupled with the similar action of antiplasmin, will, if confirmed, throw serious doubt on the relationship between blood kallikrein and kallikrein from exocrine glands and their secretions and urine. SBTI also inhibits the activation by dilution of kinin-forming enzymes in plasma (68, 116), prevents the activation by dilution of globulin PF in guinea pig and rat plasma (146, 148), and diminishes the effect on vascular permeability of isolated globulin PF from guinea pig and rabbit (146, 148).

Diisopropylfluorophosphonate (DFP), like SBTI, is an antiprotease and antiesterase. This substance inhibits the activation by dilution of PF/DIL and also the activity of isolated globulin PF from many species (82). In the rat, the globulin PF in plasma can be activated not only by dilution but also more powerfully by incubation with minced tissues (127); DFP inhibits both types of activation (134). Particularly in the case of activation by dilution, it was found necessary to add the DFP as soon as possible to the whole blood, since the effect of the inhibitor was much less pronounced if it was added after separation of the serum (134). DFP has been shown also to inhibit the action of blood kallikrein on synthetic esters (143).

Salicylate, too, has been shown to antagonize the kinin-forming properties of diluted serum and of salivary kallikrein in vitro (94). When added to rat blood prior to clotting and separation of the serum with subsequent dilution with saline, or to serum or resolving inflammatory exudate before incubation with minced tissues, salicylate greatly lessens activation of globulin PF (132). Quinine has similar actions (134). The actions of salicylate, however, are too numerous and poorly understood to draw many conclusions from its effects on globulin PF. The inhibitory effects of DFP and SBTI, however, do suggest that kinin formation and activation of globulin PF involve the action of esterases or proteases.

ROLE OF POLYPEPTIDES, PLASMA KININS, KININ-FORMING ENZYMES, AND GLOBULIN PF IN INFLAMMATION

Peptides Other Than Kinins

The presence in inflammatory exudates and especially in pus of protein breakdown products has been known for a long time. Modern investigation virtually began with Menkin's (79, 81)

demonstration that 24- to 48-hr-old pleural exudates, induced by injection of turpentine, contained a factor that increased vascular permeability to circulating dye and that induced emigration of leukocytes. This substance, "leukotaxine," was thought to be a polypeptide but for reasons given elsewhere this conclusion is debatable (128). Later work confirmed the presence of a comparable substance in similar exudates in goats (24). Here again, some evidence favored a peptide. Neither investigation, however, excluded the possibility that only a small proportion of the vascular activity in the exudate was due to the presence of polypeptides. In fact, a more recent investigation of the problem, using turpentine-induced pleural exudates in rats, showed that the great majority of such activity was nondialyzable and resided in the mixed globulin protein fraction. The exudate did contain some peptide nitrogen but this remained constant from 1 to 24 hr, whereas the permeability-increasing activity of the globulins rose and fell in parallel with the inflammatory changes in the pleural vessels (126). This experiment therefore provided little evidence that freely dialyzable peptides of the "leukotaxine" type play a part in inflammation.

Kinins

Plasma kinins such as bradykinin are characterized by their rapid destruction in plasma. It cannot, therefore, be expected that the experiments just described would necessarily suffice to demonstrate their presence. On the other hand, successful attempts to show the existence of kinins at the appropriate time after injury have been few in number. Beraldo (13) showed that increased plasma kinin activity sometimes appeared in the circulation of dogs after peptone or anaphylactic shock, but that the phenomenon was not constant. This finding has been confirmed and extended recently (18). Roche e Silva and Rosenthal (114) have demonstrated plasma kinin activity in rat tissue after thermal injury. This experiment, however, was performed by injecting saline into an air pouch whose wall was formed by the injured skin. It is, therefore, open to the objection that the kinin activity may have been a consequence of dilution of exuded plasma by the injected saline. Elsewhere, Miles (82) has reported a failure to detect kinin activity during the inflammatory response of rabbits to infection. In a nonpathological context, the appearance of plasma kinin has been demonstrated in saline perfusates of the human forearm after heating (34), and this has been interpreted as indicating a role for plasma kinin in functional hyperemia. As Lewis points out (70), however, this result might be explicable on the basis of activation of plasma by the dilution consequent on saline perfusion. The increased quantity of plasma kinin found after heating could be attributed to an additional supply of substrate and enzyme provided by the augmented blood flow.

Kinin-Forming Enzymes

The precursors of these enzymes are present in blood, interstitial fluid, and substances which activate them and are found in many tissues (70). If the enzymes play a role in inflammation, it might be expected that their presence in active form could be demonstrated after injury. There is, in fact, a great deal of evidence that proteolytic enzymes of various types are activated by injury (70, 128). In the specific instance of kinin-forming enzymes or kallikrein, however, experimental demonstration of such activation is scanty. The best evidence yet available of their importance in the body concerns a physiological rather than a pathological role. Thus, Hilton and Lewis (47) demonstrated greatly increased activity of a kinin-forming enzyme in saline perfusates of cat salivary gland after stimulation of the glandular secretion by a number of nervous and humoral methods. No significant number of kinins were detected in the perfusate, and the authors concluded that the kinin-forming enzyme was activated in the interstitial fluid, the kinins being formed and then rapidly destroyed in this compartment (70). The presence of the kinin-forming enzyme in the perfusate was attributed to increased vascular permeability induced in the gland by perfusion (70). In his discussion of these results, Lewis (70) indicated that the absence of kinins from the perfusate excludes dilution of plasma by perfusion fluid as a cause of the increased kinin-forming activity. It could, however, be argued that the failure to detect formed kinins [as compared with results on the human forearm (34)] was due to a particularly rapid rate of inactivation of these peptides, the more stable kinin-forming enzymes remaining demonstrable. If the kinin-forming enzyme in the cat salivary gland (possibly salivary kallikrein) were in fact activated by dilution, the increased amounts found after glandular stimula-

tion could perhaps be due to additional supplies of precursor or activator being made available as a result of hyperemia.

Most other attempts to demonstrate a role for kinin-forming enzymes in hyperemia and inflammation have been unsuccessful. Hilton and Lewis (48) were unable to demonstrate activity of the system during vasodilation in the skeletal muscle of the tongue, and Miles has reported failure to demonstrate such enzymes in infective inflammation in the rabbit (129). Similarly, Spector and Willoughby (135) did not find kinin-forming activity in inflammatory exudates collected from the rat pleural cavity from 30 min to 24 hr after injection of turpentine.

From the experimental data now available, it has therefore to be concluded that, in spite of the powerful effects of plasma kinins and the enzymes which form them on vascular caliber and permeability, their role in inflammation remains to be proven.

Globulin PF

Miles and his colleagues were unable to demonstrate activated globulin PF in the circulation of guinea pigs subjected to local injury (147) but, in view of the localized nature of the inflammatory response, this result was not unexpected. Changes in activity of globulin PF in inflammation have, however, been demonstrated by Spector (126) in turpentine-induced pleurisy in the rat. In these experiments, active globulin PF was found in pleural exudates collected at times when pleural vascular permeability to protein was at its height, i.e., 1 to 8 hr. As the inflammation subsided and the permeability of the pleural capillaries reverted to normal (12 to 24 hr), the globulin PF in the exudate returned to its normal inactive state. The activation of the globulin PF was found to be due at least in part to reduced activity of its specific inhibitor. These results have been criticized on the grounds that the presence of globulin PF in the exudate could have been the result rather than a cause of increased vascular permeability (82). Such a view, however, does not account for the transformation of inactive precursor to active globulin PF followed by reversion to a completely inactive state, all in the space of 12 hr, and in parallel with the rise and fall of vascular permeability in the tissue from which the exudate arose (126). Turpentine could have caused activation of globulin PF in the formed exudate, but even so it is hard to see how a similar reaction could have failed to occur within the pleura too, and equally difficult to conceive how such activation in the vicinity of the pleural capillaries and venules could have failed to influence the permeability of these vessels. It is of interest that workers in Miles's (82) laboratory have recently found small amounts of active globulin PF in bacterial inflammation in the rabbit, kinin-forming activity not being demonstrated.

Current work on turpentine-induced pleurisy (135) reveals that the exudates contain two substances that cause a slow contraction of isolated plain muscle. One of these substances has little demonstrable effect on blood vessels, but the other appears closely related, in its time of appearance and disappearance and in its properties, to the globulin PF of the exudates. Thus, it is possible that some of the permeability-enhancing effect of these exudates might be due to this slow-contracting substance. Globulin PF of serum or plasma does not contract isolated plain muscle preparations. The effect of 1- to 6-hr pleural exudates on vascular permeability could therefore be due to the presence both of globulin PF and this unidentified slow-contracting substance. If globulin PF is regarded as a type of kallikrein or kinin-forming enzyme, the slow-contracting substance could be considered as some form of kinin formed by the action of globulin PF.

The inhibitory effect of DFP and salicylate on the activation of globulin PF in vitro has been described above. Spector and Willoughby (132) have shown that prior administration of salicylate suppresses the increased vascular permeability present in the rat pleura 1 to 6 hr after intrapleural injection of turpentine. The period of vascular permeability susceptible to salicylate occurs subsequent to that earlier phase of vascular change which is suppressed by small doses of antihistamine drugs and develops whether or not the "histamine phase" is so suppressed. The vascular changes inhibited by salicylate undoubtedly constitute the major portion of the inflammatory response, as opposed to the minor role of the "histamine phase," and comprise what Miles (82) termed the delayed vascular response. A similar effect of salicylate and also of quinine and related compounds has been observed in thermal injury in the rat (134). Administration of DFP, too, suppresses the delayed increase in vascular permeability seen in rats after thermal injury to the skin and turpentine-

induced pleurisy (131, 134). In the case of thermal injury, a single injection of DFP was found to inhibit increased vascular permeability, even when given 36 hr before injury (134). Another group of workers have obtained less impressive results with DFP in thermal injury, but this discrepancy may be due to variation between samples of DFP. This interpretation is supported by the striking results obtained by Willoughby (150) after the administration of DFP [from the source used by Spector and Willoughby (132)] to irradiated rats. Willoughby (150) found that a single injection of DFP suppressed the delayed increased vascular permeability developing in the intestine for as long as 72 hr after irradiation. This effect was obtained when the DFP was given both before and 24 hr after irradiation. Apart from its diminution of vascular permeability, DFP led to a striking reduction of the mortality consequent on intestinal damage and also of the associated blood-stained diarrhea (151). These results do not support the view expressed elsewhere (145) that DFP is necessarily too toxic for its anti-inflammatory effects to be considered valid.

The real objection to interpreting the actions of DFP, salicylate, and quinine as evidence in favor of the participation of globulin PF or kinin-forming enzymes in inflammation lies in their possession of other effects. Thus, apart from inhibiting a wide range of enzymes and causing a number of metabolic disturbances, these compounds lead to a general suppression of vascular reactivity, so that after their administration the increased vascular permeability induced by intradermal injections of histamine, serotonin, globulin PF, or polypeptides is much diminished (134). Salicylate, moreover, diminishes the action of some of these antagonists on smooth muscle in vivo and in vitro (94). Finally, it has to be mentioned that SBTI, which in vitro inhibits both globulin PF and kinin-forming enzymes, consistently fails to lessen the vascular changes of inflammation (82, 145).

Other Possible Endogenous Mechanisms Responsible for the Vascular Changes of Injury

Polymorphonuclear Leukocytes and Lactic Acid

Many investigators have noted that the delayed increase in vascular permeability seen in many types of tissue injury (see above) coincides with the onset of leukocytic emigration from the affected vessels (82). This phenomenon has been observed in the Arthus reaction (138, 50) and in bacterial infections (19). Moreover, if the numbers of circulating polymorphonuclear leukocytes were reduced by injection of antileukocyte serum (51) or administration of nitrogen mustard (138, 50, 95), the edema and leakage of circulating dye resulting from local injury by antigen-antibody union (138, 50, 51) or chemical irritation (95) were considerably reduced. These results suggested that disintegration of polymorphs at the site of injury might yield a substance that contributed to the maintenance of altered vascular permeability. Unpublished observations of Hurley and Spector show that saline extracts of rat leukocytes cause edema and dye leakage on intradermal injection, but that their effect is less than that of comparable extracts of other cell types. In the guinea pig, extracts of granulocytes have no permeability-enhancing effect, and may even be inhibitory (82). Partly because of this latter observation, it has been suggested that granulocytes might increase capillary permeability indirectly by virtue of the free lactic acid which accumulations of these cells produce, a view supported by the observation that 90 μg of lactic acid will cause maximal vasodilation in 1 g of guinea pig skin (82). This degree of activity is not unduly high. It has, however, been shown that rabbit skin inflamed and prepared for the Schwartzman phenomenon by injection of meningococcal toxin can produce up to 2.5 mg of lactic acid per hr per g of skin (wet wt) and that this excess production is due to increased aerobic glycolysis (139). Normal skin produced up to 0.5 mg of lactic acid per hr per g of skin (wet wt). Skin prepared for the Schwartzman reaction was found to contain up to 2 mg of lactic acid per g of skin (wet wt) as compared with a maximal figure of 0.5 mg in normal skin (139). These figures certainly indicate increased local lactic acid production in this type of skin injury, and it has been claimed that a slow, prolonged increase in vascular permeability can be induced by concentrations of lactic acid comparable to those found at the site of delayed hypersensitivity reactions (137). Apparently successful attempts to link the increased aerobic glycolysis seen in skin prepared for the Schwartzman reaction with infiltration of the skin with leukocytes have been made by parallel inhibition of the two phenomena by administration of nitrogen mustard (138). On

the other hand, other types of tissue insult cause intense leukocytic infiltration, but no demonstrable increase in aerobic glycolysis or lactic acid accumulation (139).

As stated above, the simultaneous development of granulocytic infiltration and delayed increased vascular permeability has led to the suggestion, supported by the results of induced leukopenia, that polymorphs are a cause of the delayed vascular response. In the rat, however, agranulocytosis induced with nitrogen mustard or busulfan (Myleran) fails to lessen the inflammatory edema consequent on thermal injury (52). In this animal, increased vascular permeability returns to normal by 6 hr after thermal injury (52, 145), whereas leukocytic emigration is not fully developed until this time and maximal leukocytic infiltration is not reached until some time later (53). Also, in the rat, injections of saline or histamine give rise to a delayed leukocytic emigration not apparent until several hours have elapsed, and this emigration is not accompanied by any demonstrable increase in vascular permeability (53).

Much of the evidence in favor of the view that polymorph leukocytes contribute to altered vascular permeability after injury comes from the inhibition of this phenomenon, which is observed when neutropenia is induced in injured rabbits or guinea pigs by dosage with nitrogen mustard (138, 50, 51, 95, 22). Other results, however, throw serious doubt on this interpretation of these experiments. Thus, in the Arthus reaction, Humphrey (51) showed that guinea pigs rendered granulocytopenic as a result of the administration of antileukocyte serum did not show the diminished vascular permeability exhibited by animals treated with nitrogen mustard. More recently, Johnstone and Howard (56) have rendered animals deficient in neutrophils to a similar degree by means of either nitrogen mustard or whole-body irradiation. They found that after nitrogen mustard the vascular changes of the Schwartzman reaction were greatly suppressed, whereas after whole-body irradiation the response was unimpaired.

These results suggest that nitrogen mustard depresses vascular permeability by an action unrelated to its effect on granulocytes. Nevertheless, Humphrey (51) and Page and Good (95) have observed that neutropenia from causes other than nitrogen mustard is associated with a reduction in the edema and induration, if not of the increased vascular permeability resulting from tissue damage.

These findings probably indicate that the accumulation of leukocytes in injured tissues has little influence on vascular permeability but does contribute to the retention of water at the site of inflammation. Such an effect might be produced by blockage of lymphatics or by an uptake of water by the granulocytes themselves and could augment other factors such as osmotic forces tending to cause water accumulation in inflamed areas.

Local Inactivation by Injury of Endogenous Vasoconstrictor Substances

Discussion up to this point has been concerned largely with the release of substances that produce the characteristic vascular changes of inflammation. It is, however, theoretically possible that such changes could be brought about also by the local inactivation of substances, e.g., epinephrine (adrenaline), whose presence would otherwise lead to vasoconstriction and reduced vascular permeability; in fact, evidence that such a mechanism contributes to the development of the inflammatory response has recently been obtained.

The vasoconstrictor amines, epinephrine, norepinephrine (noradrenaline), and serotonin are destroyed in the body partly by catechol-*o*-methyl transferase and partly by monoamine oxidase (5). The administration of a competitive inhibitor of catechol-*o*-methyl transferase (pyrogallol) was found to have no effect on the reaction to thermal injury (133). Similar administration to the rat of one of a number of inhibitors of monoamine oxidase, however, led to a striking diminution in the enhanced vascular permeability consequent on such injury (133). This inhibitory effect was reversed by concomitant administration of substances antagonistic to epinephrine, notably dibenamine, and was potentiated by bretylium tosylate, a compound that enhances the effect of "free" or circulating epinephrine. The anti-inflammatory effect of salicylate or DFP (see above) and quinine was not reversed by giving dibenamine. The inhibitory effect of monoamine oxidase inhibitors on the reaction to thermal injury could be reproduced by injections of epinephrine but not of norepinephrine or serotonin. The epinephrine metabolites nor-

metanephrine and 3-methoxy-4-hydroxymandelic acid were also without effect (153). Results similar to those obtained after thermal injury were observed in the inflammatory reaction after intrapleural injection of turpentine. In addition, striking suppression of increased vascular permeability after administration of monoamine oxidase inhibitors has been found in the inflammation of the rat intestine that develops after abdominal irradiation (152).

These results suggest that in addition to the effect of endogenous substances that enhance vascular permeability, the response of blood vessels to injury, at least in the rat, is due partly to local inactivation, apparently by monoamine oxidase, of locally released epinephrine. The inhibitory efficacy of monoamine oxidase inhibitors as compared with the lack of effect of inhibitors of catechol-*o*-methyl transferase could be explained by the suggestion that the latter enzyme regulates the general level of monoamines in the tissues, whereas monoamine oxidase deals with any sudden excess concentration.

Fibrin Deposition and the Vascular Response to Injury

Jancso (55) has recently suggested that increased vascular permeability after injury is in some way a consequence of fibrin formation on the luminal border of the small vessels. This hypothesis originated in the observation of Jancso that, after injury, colloidal silver adheres to the walls of small vessels, especially venules, and is incorporated into the cytoplasm of the vascular endothelium. Jancso considered that this might be due to trapping of the colloidal particles by fibrin and went on to show that administration of certain anticoagulant drugs, notably derivatives of the rare earths, e.g., neodymium (thrombodym) and sodium polyanetholesulfonate (liquoid), diminished this effect and also lessened edema after many types of injury. Jancso found also that rats rendered afibrinogenemic by injections of thrombin and of trimethylpolyanthium iodide showed similar suppression.

There are, however, certain difficulties in accepting Jancso's (55) interpretation of his results. Thus the powerful anticoagulant, heparin, had no effect on edema or on the adsorption of colloidal silver. Again, some of the compounds used may have led to circulatory collapse. A more serious objection is inherent in the indirect nature of the evidence, since all the inhibitory compounds almost certainly possess powerful biological effects not directly related to blood coagulation. In particular, they seem likely to be highly reactive surfactants with a large number and high density of charged radicals. Substances of this type would be expected to inhibit the adsorption of colloidal particles onto cell surfaces and also to interfere with the alterations in electrical charge and chemical configuration at the surface of the endothelial cell that are a probable feature of the vascular response to injury. Indeed, these surface-active properties are likely to be the source of their anticoagulant activities. In any case, there are many similarities between the mechanism of blood clotting and that of the formation of vasoactive substances. There are further dangers in the assumption that colloidal silver particles behave similarly to plasma-protein molecules. If, after injury, increased passage of protein into the tissues depended upon transport through the endothelial cytoplasm, prior adsorption to the cell surface might be a necessary precursor. The latest observations of Palade (98), however, suggest that protein loss occurs through interendothelial gaps. Even if protein adsorption were a necessary prelude to increased vascular permeability, there are many mechanisms other than fibrin deposition whereby this might be achieved, e.g., charge reversal or calcium bridging. Finally, if Jancso's (55) view is correct, it might be expected that high-power electron micrographs of injured venules would reveal a fibrin layer such as he postulates. This has not yet been the case (98, 1, 88, 149, 73, 33). Other types of investigation based on the induction of hypofibrinogenemia, too, have failed to find evidence involving fibrin or fibrinogen in the properties acquired by inflamed blood vessels (2, 3). In spite of this lack of supporting evidence and in spite of the difficulty in attributing increased vascular permeability to fibrin deposition, if it could be shown that damaged vascular endothelium acquired an adsorbed monolayer of protein, this would certainly facilitate the explanation of the phenomenon of leukocyte adhesion to inflamed venules, since carbonyl groups on the protein might cross-link with the leukocyte surface (see below). With regard to increased vascular permeability, it should be recorded that a view opposite to

Jancso's has been propounded, i.e., that blood coagulability is a requisite for the maintenance of normal vascular impermeability. This conclusion is based on the appearance of increased vascular permeability in rats after intramuscular injection of dicoumarol (155).

Activation by Injury of Enzymes in the Vessel Wall

This mechanism for bringing about the vascular changes of injury remains hypothetical. If it were operative, it might be hoped that future histochemical investigations would reveal activation of esterases, proteases, mucopolysaccharides, or other enzymes in the vessel wall as a sequel to injury.

Augmented Histamine Synthesis

Yet another possible explanation of the delayed, sustained local vascular response to injury has been provided by Schayer (117, 118). In these investigations, it was found that a variety of insults, including chemical irritation and injection of endotoxin, led to a local increase in activity of the histamine-synthesizing enzyme histidine dicarboxylase. This increase was of the order of 500% and reached its peak about 6 hr after injury, corresponding roughly with the development of the delayed increase in vascular permeability seen in inflammation (118). It is clear that such an additional synthesis of histamine (as opposed to release of histamine from existing stores) could in theory account for the delayed vascular changes. Since, however, these changes are unaffected by all but very large and therefore nonspecific doses of antihistamine drugs, the acceptance of the hypothesis depends upon the assumption that this newly formed histamine is unsusceptible to amounts of these drugs that apparently suppress completely the effects of released pre-existing histamine. In view of the prevailing uncertainty about the mode of action of the antihistamine compounds, this objection seems by no means insuperable. In the light of Schayer's (117, 118) findings, it would be of considerable interest to observe the effect of inhibitors of histidine dicarboxylase on the inflammatory reaction. It does seem possible, however, that the increased histidine dicarboxylase activity represents emergency resynthesis of histamine to replace that lost in the explosive release immediately after injury.

MODE OF ACTION OF SUBSTANCES THAT INCREASE VASCULAR PERMEABILITY

Because of lack of knowledge, there is little that can be written on this topic. It could, however, be argued that a possible indication is provided by the general ability of substances that increase vascular permeability to contract smooth muscle, e.g., in gut or uterus. It seems unlikely that the effect of these compounds on the microcirculation is due entirely to an action on smooth muscle in the vessel wall leading to arteriolar dilatation and venous constriction (see above). It would appear, therefore, that an effect on the vascular endothelium (including structures such as the basement membrane) has to be assumed. The nature of any suggestions concerning this effect depends largely on the ultimate decisions of the electron microscopists regarding the changes that take place in the vessel wall after injury and, in particular, whether or not interendothelial gaps appear. If, as now seems likely, such spaces do in fact develop, one could speculate that they are a result of endothelial contraction due to shortening of contractile elements in the endothelial cytoplasm akin to those in plain muscle cells. Alternatively, it could be postulated that stimulation of endothelium leads, as in plain muscle, to loss of intracellular potassium and entry of sodium and water. This would lead to endothelial swelling and perhaps to loss of endothelial continuity.

If further research fails to confirm the existence of intercellular defects in inflamed vessels, attention might then be focused on the possibility that permeability-increasing substances lead to accelerated transcytoplasmic transport, perhaps by diverting energy derived from ATP to this particular channel.

If, finally, future observations indicate that the essential changes of injury occur in the basement membrane or even in the perivascular ground substance, it would seem more likely that permeability factors exert an essentially physical effect in rendering these structures more porous to the passage of large molecules.

Link Between Injury and Activation of Endogenous Vasoactive Systems

Possible "trigger" mechanisms. Whatever the precise mechanisms of vascular reactivity to injury and whatever the differences between species that seem likely to exist, because of the

consistent nature of the response and the multiplicity of effective noxious stimuli, it seems inescapable that injury and the subsequent vascular reaction must be linked by some form of "trigger" mechanism. Such a mechanism must be capable of discriminating between "injurious" and physiological stimulation, and of reacting to a very wide variety of chemical or physical insults.

These requirements seem best met by postulating some form of alarm system in the tissues distinguished by its extreme sensitivity to unfavorable environmental changes. This could take the form of a protein, highly susceptible to denaturation. Such a protein might in normal tissues act as a natural inhibitor of enzyme systems which on activation as a result of denaturation of their inhibitor contribute to the inflammatory response. A model for this hypothesis could exist in the scheme of inhibitor and lytic enzyme on the cell surface that Uvnäs (140) has proposed as the system controlling the release of histamine from mast cells or in the globulin PF-inhibitor system of plasma and extracellular fluid. A model of a different kind may be found in the lysozymes of liver cells (26), particles containing a number of enzymes and characterized by extreme fragility so that they rupture under conditions in which other cell structures continue to function normally. In this instance, the ultrasensitive component is a particularly simple boundary membrane. It is not impossible that potential mediators of increased vascular permeability, e.g., kinin-forming enzymes, exist in similar fragile containers in the vicinity of blood vessels or even in the blood itself.

Leukocyte Emigration

A cardinal feature of the inflammatory response is the emigration of leukocytes from small blood vessels into the injured tissues and their accumulation at this site. The emigration is preceded by the movement of leukocytes from the center to the periphery of the blood stream and by the adhesion of the cells to the luminal surface of the vascular endothelium (32). Emigration on a small scale may be apparent within a few minutes of injury, but the major migration does not usually commence until 1 to 6 hr have elapsed. Initially, neutrophil polymorphonuclears predominate in the exudate but, as the stimulus subsides, these are eventually replaced by monocytes and lymphocytes; the mononuclear cells then rapidly undergo transformation to macrophages and histiocytes.

There are at least four mechanisms whereby emigration of leukocytes might be brought about. These are chemotaxis (i.e., a positive directional response to a chemical stimulus), the changes in the vessel wall which lead to increased permeability to protein, changes in the vessel wall other than those associated with increased permeability to protein, and changes in the leukocytes themselves leading to their migration.

There are again several possibilities concerning the route taken by leukocytes in their passage through the vessel wall. Thus, the cells might pass through interendothelial gaps, they might invaginate the endothelial cytoplasm and push it aside, or they might even enter the endothelial cytoplasm and become enclosed by it, like a particle undergoing phagocytosis. In all three cases, the polymorph must presumably eventually penetrate the vessel's basement membrane to reach the extravascular tissues.

Route of Leukocyte Emigration

Until recently, it was assumed that leukocytes left the vessels by passing through gaps between the endothelial cells. Current electron-micrograph studies, however, have made possible a much more detailed study of the phenomena. Florey and his collaborators (33) have studied leukocyte migration after injury in both rabbit and rat (73). They found that the leukocytes penetrate the endothelium of the vessel wall by thrusting out pseudopodia and that this passage sometimes and possibly always occurs at interendothelial junctions, although an appearance of penetrating the endothelial cytoplasm is often given. Having passed the endothelial barrier, the leukocytes are then arrested for a variable period by the basement membrane, perivascular collagen fibers, or periendothelial cells and sometimes move parallel to the endothelium until they find a site in the perivascular sheath through which they can enter the surrounding connective tissue.

A similar electron-microscopic study has been performed by Williamson and Grisham (149) in the vessels of the dog pancreas. In this organ, injured endothelium develops numerous cytoplasmic processes which project into the lumen and appear to enmesh leukocytes; the blood cells seemingly become completely enveloped by endothe-

lial cytoplasm. In spite of this, some at least of the leukocytes were said to occupy an interendothelial position in the vessel wall and presumably to leave the vessel by this route. These workers also observed a delay in penetration of the perivascular sheath and found that, as the leukocytes lay between endothelium and basement membrane, a new layer of the latter structure was formed between the leukocyte and the endothelial cell. The new layer was continuous with the old, outermost layer which then disappeared, allowing the leukocyte to enter the connective tissue.

Adhesion of Leukocytes to Vascular Endothelium

It is clear that leukocyte emigration depends, first, upon adhesion of white cell and endothelium and, second, on pseudopod formation by the leukocyte and sometimes by the endothelial cell. It is not impossible that, once adhesion of leukocyte and endothelium has occurred, the natural mobility of the leukocyte would lead to at least some degree of emigration. Observations on living vessels and the delay which ensues between injury and onset of adhesiveness strongly indicate that the essential change occurs in the endothelial cell (39).

Most suggestions as to the nature of leukocyte-endothelial adhesion have invoked the deposition on the endothelial surface of some coagulum with the properties of a glue, capable of trapping any cell with which it comes into contact. Electron micrographs of inflamed venules have failed, however, to reveal the presence of such material (73, 33, 149). Moreover, this hypothesis fails to explain the relative immunity from adhesion exhibited by certain blood cells, notably small lymphocytes and erythrocytes.

It seems rather more likely that the phenomenon of leukocyte adhesion to endothelium is explicable in terms of the electrochemical forces operative at cell surfaces. The nature of the endothelial surface is unknown although it is probably rich in negatively charged carboxyl groups. The surface charge of the polymorph leukocyte, however, is thought to be due to a predominance of carboxyl radicals with no basic groups at physiological pH, whereas that of the small lymphocyte is thought to be due chiefly to phosphatide groups of the cephalin type. Similarly, the surface charge of the erythrocyte is attributed largely to singly charged phosphate groups or to the sialic acid type of carboxyl radical. Bangham and Pethica (6) have suggested that the ability of polymorphs to adhere to adjacent surfaces is due to cross linkage with other carboxyl groups by means of bridging with calcium ions or with plasma proteins or both. Conversely, the relative lack of adhesiveness of lymphocytes and red cells could be due to phosphate and phosphatide surface groups cross-linking poorly with proteins or calcium ions. In this connection, it is of interest that Garvin (36) has reported that the adhesion of polymorphs (but not lymphocytes) to glass columns in vitro is dependent upon the presence of divalent cations, magnesium being at least as important as calcium. There are, however, other factors capable of bringing about the adhesion of leukocytes and endothelium. London-Van der Waal's forces might be expected to do so, were it not for the generality of these forces as compared with the great differences in adhesiveness between different cells. Since most cells are alike in electron densities and are composed chiefly of light atoms similar to those of the suspending medium, if Van der Waal's forces were important, cell adhesiveness would depend largely on cell size. This is not the case.

A force which seems more suitable to the task of causing cell adhesion is hydrogen bonding between the carbonyl radicals of the cell surface and of plasma protein or between a carbonyl and an amino group. The strength of an individual hydrogen bond may not be very high, but the total strength of many such bonds is much greater. This hypothesis has the merit that such bonding is dependent upon the chemical nature of the cell surface, thus making observed variations in adhesiveness between cell types explicable.

Closely related to the question of the forces that may bind leukocytes to vascular endothelium is the problem of how such forces are brought into play by injury. It is possible that damage leads to a great increase in the number of reactive groups at the endothelial cell surface, perhaps owing to a reorientation within the molecules composing the membrane.

The problem may also be approached by considering the forces which normally keep leukocytes and endothelium apart and how these forces might be reduced in inflammation. Since leuko-

cytes and endothelium both have negatively charged surfaces at physiological pH, it is reasonable to follow Bangham and Pethica (6) in applying to this problem theoretical concepts based on the repulsion of particles of lyophobic colloids as a result of the diffuse electrical double layer at their surfaces. The theory postulates that the cells are normally prevented from adhering by mutual repulsion by the like, negative charges of the two cell surfaces. For spherical particles (or cells) the free energy of repulsion due to this double layer interaction is given by:

$$Vr = \frac{E\alpha\psi_0^2 \log}{2}(1 + exp - KH)$$

where E is the dialectric constant of the interparticle medium, α is the radius of the particles, ψ is the potential of the diffuse double layer, K the Debye-Hückel reciprocal thickness of the double layer, and H the smallest distance between the particles.

This equation could be applied to all biological particles with a radius greater than 0.1 μ, although the value of Vr given will be maximal since the equation does not take into account the spongelike nature of some charged cell surfaces. In isotonic media, K^{-1} is about 8 A.

It is apparent that, other things being equal, the force of repulsion will vary directly with the radius of the particle or cell. It is also clear that, because of the rapid decline of the repulsion force with distance, the operative repulsion force will be largely determined by those parts of the approaching cells or particles that are closest together. These considerations led Bangham and Pethica (6) to suggest that the formation of pseudopodia whose radius of curvature was much smaller than that of the whole cell would enable a cell to penetrate the electrical field of another and thus come close enough (5 A) for calcium bridging to occur. It is worth recalling that electron microscopy has shown pseudopod formation in both polymorphs and endothelium after injury. It is possible that, in addition to pseudopodia proper, minor undulations of cell surfaces, as seen in the electron micrograph, might serve a similar purpose. It has been shown that, in accordance with the views on the importance of radius of curvature on cell adhesion, the agglutination of antibody-sensitized erythrocytes occurs in an end-to-end fashion, the cells adhering at the rim of the disc and the planes of the successive discs being at right angles.

It is suggested, then, that adhesion of leukocytes and endothelium may be brought about by a reduction in the energy of repulsion between the two cell surfaces due to reduction in their radius of curvature as a result of pseudopod formation, the cells then adhering as a result of cross linking with calcium or magnesium ions with or without the intervention of plasma protein-molecules as additional ligands. It has, however, to be recognized that many forms of attachment other than those involving charged surface groups are possible.

Chemotaxis and Leukocyte Emigration

Chemotaxis of leukocytes, especially granulocytes, has received much attention from past investigators. Harris (46) reviewed the extensive literature and concluded that much earlier work was invalid because of inadequate techniques. Using the criterion of migration in straight lines to the test object, Harris (45) found that colonies of living *S. albus*, *Salmonella typhi*, *C. diphtheriae*, and *Mycobacterium tuberculosis* were unequivocally equally chemotactic to granulocytes. *S. aureus* and *Streptococcus pyogenes* were not chemotactic, due presumably to a toxic action on the whole cells. *Bacillus anthracis* and *Klebsiella pneumoniae* were neither chemotactic nor toxic to leukocytes. No killed microorganisms were found to be chemotactic nor were any minced tissues, although starch grains possessed this property.

Although it is widely assumed that granulocytic infiltration in local bacterial infections is due to chemotaxis, if Harris's criterion of chemotaxis is accepted, it is apparent that there is little correlation between the chemotactic powers of microorganisms and their ability to induce granulocyte accumulations in vivo. Thus, the typhoid and tuberculosis organisms are distinguished by their lack of effect on the emigration of granulocytes in the body. In addition, inflammation due to the *S. aureus*, *S. pyogenes*, or the anthrax and *K. pneumoniae* organisms is characterized by accumulation of granulocytes.

These discrepancies may be explicable on the basis of differences between conditions in vivo and in vitro. On the other hand, the massive focal accumulations of polymorphs seen in many bacterial infections could equally well be due to

random (as opposed to directed) migration, followed by immobilization of the leukocytes by the organisms due, for example, to a toxic action insufficiently strong to prevent the initial emigration. It is difficult to see how this problem can be settled until techniques similar to those used by Harris can be applied to a preparation of living tissues.

The work of Meier and Schär (77) suggests that the chemotactic properties of microorganisms might be due to their content of polysaccharide or lipopolysaccharide, since they found such chemical compounds to be chemotactic to polymorphs. These observations do not seem to accord well with the finding of Harris that clumps of killed *S. albus* are not chemotactic to granulocytes. The discrepancy could be explained either by the use of different methods of demonstrating chemotaxis or by the polysaccharides within the killed organisms being inaccessible to the leukocytes.

Finally, although many investigations purporting to show that various tissue fluids or extracts were chemotactic to leukocytes did not (by Harris's criteria) do so, it may well be that some of these experiments nevertheless demonstrated the ability of these fluids or extracts to accelerate the random movement of leukocytes. In the inflammatory response, an effect of this nature would be as important as true chemotaxis.

Leukocyte Emigration as a Result of Increased Vascular Permeability to Protein

Whereas chemotaxis can be unequivocally demonstrated only in vitro, leukocytic emigration from blood vessels can be shown only in vivo. That the phenomenon occurs is easily seen in preparations of living tissues; its detailed nature and its occurrence in response to specific stimuli are usually demonstrated by histological methods with a light or electron microscope.

The procedure whereby a substance is injected intradermally and the injection site examined histologically some time later for evidence of an effect in promoting leukocyte emigration was first used seriously by Menkin in demonstrating the properties of his "leukotaxine" preparation (80). This procedure has been subjected to criticism based partly on the variability in the response to a given stimulus and partly on the possibility of intermediary reactions between stimulus and response. Both these objections are valid, but careful standardized technique can do much to minimize their importance. In addition, a good deal of the existing confusion can be resolved by consideration of the importance of time factors. Thus, it has been shown that single injections of sterile pyrogen-free saline and of other solutions will lead to significant leukocytic emigration at the injection site, provided that some hours are allowed to elapse between injection and histological examination (53). A similarly delayed but much more intense reaction follows single injections of proteins. This easily elicited, delayed leukocytic emigration and the absence of such a response in the initial 2 hr after injection does much to explain the discrepancies in the published reports of the effect in vivo of various substances on the movement of white cells.

On the assumption that a well-marked degree of leukocytic migration from vessels within 45 min of injection of a test substance may be significant, it would appear that all compounds that increase capillary permeability will also induce diapedesis of leukocytes. The action of Menkin's "leukotaxine" preparations (131) appears to fall into this category. In some cases, however, the dose required is so high as to throw doubt on the meaning of the result. Thus, in the guinea pig the amount of histamine needed for an effect on leukocytes is 50 to 100 times that producing a strong increase in vascular permeability (146), and a similar situation exists with regard to bradykinin (71), globulin PF (146), and peptide mixtures derived from fibrin (125).

In addition, the action on leukocyte emigration of even the highest doses of vascular permeability factors are very weak relative to the similar effect of injury, even allowing for prolonged stimulation in the latter instance. The impression gained from study of skin sections injected 30 to 60 min previously with large doses of substances that increase vascular permeability is that the release or activation of such substances by injury might just conceivably account for the mild leukocytic migration observed in the initial hour or so of such an inflammatory reaction. It seems unlikely, however, that they could be responsible for the considerable accumulation of white cells seen in most types of injury. It is possible to argue that in bacterial infections chemotaxis augments the effect of substances that increase vascular permeability. There is, however, enough evidence to make it clear that in other

types of tissue damage additional factors must be operative.

Leukocyte Emigration Due to Specific Changes in Leukocytes or Blood Vessels

The view that leukocytic emigration in inflammation might be both nonchemotactic and not directly related to increased vascular permeability has received little attention. Thus, in Harris's otherwise definitive review (46), the possibility does not appear to be mentioned, not surprisingly since there was no evidence for the existence of such a mechanism. Until recently, it was generally assumed that any substance causing leukocytic emigration in vivo, in excess of any effect that might be predicted because of an action on vascular permeability, owed its special properties to chemotaxis. Harris (46), however, was able to convince most investigators that damaged tissues by themselves were not chemotactic, and was able to quote evidence for the random nature of leukocyte migration in sterile injury. He was, therefore, left with the conclusion that in the latter circumstances the emigration of white cells was a consequence of the changes in the vessel wall associated with increased vascular permeability to protein.

However, it soon became apparent that considerable discrepancies existed between the ability of various extracts or chemical substances to increase vascular permeability and their effect on leukocyte emigration. Thus, extracts of estrogenized mouse uterus have much the same effect on permeability as comparable extracts of other tissues but possess a much greater activity in promoting migration of white cells (130). Moreover, it has recently been shown that intradermal injections of sterile, pyrogen-free isotonic saline lead to significant delayed leukocyte emigration although the injection causes no detectable edema or leakage of circulating trypan blue (53). Similarly, after injections of histamine, there is a comparable diapedesis of white cells, beginning at about 3 hr, although leakage of circulating dye ceases 20 to 45 min after the injection (53). Again, injections of homologous serum or heterologous plasma proteins produce intense delayed leukocyte accumulation but immediate, weak, and transient increased vascular permeability to protein (53). Thermal injury in the rat is followed by increased vascular permeability to protein and leukocytic emigration. The vascular permeability has reverted to normal 6 hr after injury (53, 145), whereas the major leukocytic emigration is not yet at its peak (53). This delay in the appearance of large-scale migration of white cells seems unlikely to be due to any essential sluggishness of the process, since one or two leukocytes can often be seen to leave the vessels within a few minutes of injury.

Because sterile minced tissues do not appear to be chemotactic to leukocytes and because of lack of correlation between increased vascular permeability to protein and the emigration of white cells, it seems necessary to postulate the existence of a specific endogenous mechanism other than chemotaxis for inducing leukocyte emigration. Thus, injury might lead to an increase in the pseudopod activity of the white cells (due, for example, to a metabolic effect), thus facilitating adhesion to the endothelium (see above) and accelerating their movement, so that the cells migrate through the vessel wall in large numbers. Alternatively, there might be an effect on the vessel wall, increasing the activity of the endothelium, attacking the integrity of the basement membrane, opening up gaps at the interendothelial junctions, or merely rendering the endothelial surface "adhesive" (or nonrepulsive) toward leukocytes.

At the moment, there is insufficient evidence to justify a detailed consideration of the relative merits of the various possibilities. On general grounds, it would seem more likely that the essential change occurs in the vessel wall. On the other hand, Ketchel and Favour (60) have shown that plasma fractions have variable effects on the rate of leukocyte migration in vitro. Since it seems unlikely that true chemotaxis was involved in these experiments, their results suggest that suitable plasma constituents might accelerate leukocyte migration in vivo by a direct action on the white cells.

Because of the ease with which delayed local white cell infiltration occurs after injection of biological fluids, it is difficult to assess the validity of past attempts to identify substances with specific powers of inducing leukocyte migration in vivo. Hurley and Spector (52) have, however, made a fresh effort to do so in the rat; they found that of saline extracts prepared from many different tissues only that obtained from granulocytes themselves fulfilled the criterion of a specific leukocyte migration-promoting sub-

stance. This criterion was the induction of striking local white cell emigration within 40 min of intradermal injection of 0.1 ml of the test solution. A similar property was, however, acquired by serum after its incubation with certain tissues, notably liver. It was suggested that the principle existed as a precursor in plasma susceptible to activation by a substance in certain cells, e.g., liver and cardiac muscle, and that granulocytes contained either the activated substance or both activator and precursor (the latter possibly in adsorbed plasma). In this connection, it was found that activation of serum could be achieved by incubation with amounts of granulocytes much lower than that required of other tissues. The leukocyte emigration factor was found to be nondialyzable, heat-labile, and destroyed by trypsin, properties which suggest that it is a protein. Hurley and Spector suggested that, after injury, plasma extravasated owing to increased vascular permeability acquired the property of inducing leukocyte emigration as a result of contact with damaged tissue cells or with granulocytes damaged directly by injury or as a consequence of adhesion to inflamed capillaries.

The separate identities of the factors in the extracts and incubation mixtures causing increased vascular permeability and leukocyte emigration were shown by the lack of correlation in the relative potencies of the various biological fluids. Their separation was also indicated by the results of other ether-ethanol protein precipitation, virtually all the permeability activity residing in one fraction, and most of the material promoting leukocyte emigration in another. If plasma protein left the vessels by passing through the endothelial cytoplasm, whereas leukocytes passed between the endothelium, it might well be expected that the two phenomena could be controlled by different endogenous substances. The results would be explicable, too, if it were shown that leukocyte emigration was independent of structural changes in the vessel wall. If, however, the passage of both protein and leukocytes through inflamed vessels is due primarily to loss of endothelial continuity, the position would be less clear. A detailed study of the ultrastructural changes in the walls of small vessels at short time intervals from 3 min to 6 hr after injury might help to resolve these problems.

Considering the three possible mechanisms of leukocyte emigration after injury, i.e., chemotaxis, increased vascular permeability to protein, and specific changes in vessel or leukocytes, it seems likely that all three may play a role. Thus, the rapidly developing but slight diapedesis of white cells seen immediately after injury could well be a result of the changes in the vessel wall leading to increased permeability to protein. The much more extensive migration that develops later could, in the case of bacterial infection, be due to chemotaxis, and, in nonbacterial injury, delayed leukocytic migration could follow the operation of a mechanism such as that outlined above. In every case, it seems likely that an essential preliminary to migration would be a change, probably in the endothelial cell surface, leading to loss of the normal mutual repulsion between leukocyte and endothelium.

Development of Mononuclear Cell Predominance in Inflammatory Exudates

In the early stages of an inflammatory response, the cellular exudate is dominated by neutrophil polymorphonuclear leukocytes. As the reaction subsides, mononuclear leukocytes replace polymorphs as the preponderant cell type. The duration of the phase of polymorph dominance depends largely on the cause of the inflammatory reaction. In bacterial infections associated with pus formation, neutrophils remain in the majority until resolution or organization finally supervenes. Where such infections are of long duration, the picture tends to vary from one part of the affected tissues to another with polymorph collections in places and mononuclear infiltrates elsewhere. Other areas may show the process of organization in all stages from granulation tissue to avascular bundles of dense collagen.

In other bacterial infections, e.g., tuberculosis or typhoid fever, polymorphs are virtually absent from the cellular response seen in established lesions, the picture being dominated by mononuclear cells often of specialized types. It has, however, been established, at least in the case of tuberculosis (14) and brucellosis (16), that the very earliest stages of infection are accompanied by an inflammatory exudate in which neutrophils exercise a transient predominance.

It seems, then, that the replacement of polymorphs in the exudate by mononuclear cells is a

polymorphs. In fixed preparations of inflamed tissues in the rat, cells corresponding to the changed polymorphs described by Clark et al. (21) can undoubtedly be seen. A few of these cells might be mistaken for monocytes, but the majority are obviously degenerate with clear eosinophilic cytoplasm and a dense, flattened, eccentric nucleus. In either case, cells of this type form only a small minority of the mononuclear cells of the exudate, produced in this instance by intradermal injection of fibrinogen or dextran sulfate (102). Since Clark et al. (21) themselves considered the changed polymorphs to be degenerate, it seems very unlikely that these cells could undergo subsequent transformation to the relatively long-lived macrophages and macrophage derivatives of the older inflammatory exudates. The simulation of mononuclear cells by degenerate polymorphs as a possible cause of mononuclear cell predominance in exudates has been considered at some length because there is ample evidence that such mononuclears are pre-eminently hematogenous and equally weighty lack of direct evidence for the selective emigration of monocytes or lymphocytes subsequent to polymorph emigration.

In spite of the absence of proof, this latter hypothesis has often been accepted for want of a better one. However, evidence indicating the validity of the view that polymorphs and monocytes emigrate concurrently, the former then disappearing and the latter remaining, has recently been obtained by Paz and Spector (102). In this investigation, a cellular inflammatory exudate was provoked by the injection of various macromolecular substances, notably fibrinogen and dextran sulfate. Using standardized techniques and fixed sections, differential counts of polymorphs and mononuclear cells were performed around skin vessels at various times after injection of different doses. A parallel investigation was performed on smears from peritoneal exudates induced by similar means. Phagocytosis of the injected macromolecules led to characteristic changes in the exuded hematogenous mononuclear cells, enabling their subsequent fate to be followed.

Observations showed that polymorphs and mononuclear cells always left the blood vessels concurrently and that there was no detectable selective migration of any cell type. However, polymorphs left the vessels faster than did the mononuclear cells, so that a dominance of polymorphs was soon established in the tissues. Once emigration ceased, however, the polymorphs disappeared due to disintegration and movement away from the blood vessels. (Large numbers were arrested in hair follicles or in the epidermis.) The mononuclears, on the other hand, remained in the vicinity of the vessels and suffered transformation to macrophages and thence to special cell types whose nature depended on the stimulus. Thus, after injection of lipids they became altered to epithelioid cells. Examination of large numbers of preparations made it clear that the precursors of the great majority of such macrophages and macrophage derivatives were the hematogenous mononuclears that left the vessels during the same period as did the polymorphs. Another feature of the results was the demonstration of repeated fresh waves of emigration of both polymorphs and mononuclears. It was also found that the smaller the injected dose, the sooner did emigration cease and the sooner did mononuclear cells dominate the exudate.

Paz and Spector (102) concluded that where polymorph dominance persists, as in pyogenic infections, it is due, as suggested by Harris (46), to sustained active emigration due to the intensity of the stimulus but also to immobilization of polymorphs at the site of injury. Conversely, responses where mononuclear cells predominate from the early stages were thought to be due to a weak emigratory stimulus, coupled with immobilization of mononuclears, the polymorphs escaping unimpaired or suffering destruction. It was suggested that long-lasting mononuclear exudates, e.g., in tuberculosis, were due to such a process, augmented by repeated fresh waves of emigration and local proliferation of exuded or tissue cells.

Lymphocytic Accumulation in Inflammatory Exudates

Collections of lymphocytes, especially small lymphocytes, are a characteristic feature of long-standing inflammatory infiltrates. Thus, they are seen constantly at the periphery of tubercles and other granulomatous lesions. Apart from these dense accumulations, they are also found as constitutents of the predominantly mononuclear cell exudates of inflammatory responses which, although of several days or weeks duration, are not as chronic as those of

constant feature of the inflammatory response. The mononuclear cells under discussion include blood monocytes, large, medium, and small lymphocytes, macrophages, histiocytes, specialized derivatives such as epithelioid or giant cells, and plasma cells. Fibroblasts and new blood vessels constitute a separate problem which will not be discussed here. A special feature of some mononuclear cell infiltrations are accumulations of small lymphocytes often around blood vessels or at the periphery of localized lesions such as tubercles.

The mononuclear cells of inflammatory tissues could in theory be derived from either blood or tissue cells, the controversy having been reviewed by Ebert and Florey (27). In living preparations, however, intensive study has failed to reveal any transformation of connective tissue or endothelial cells into any form of mononuclear leukocyte (21, 27). Proliferation of tissue macrophages might account for mononuclear cell preponderance but, although such tissue cells have been observed to increase in size soon after injury, mitotic activity is not a feature (102).

There is, on the other hand, a great deal of evidence to suggest that the mononuclear cells of inflamed tissues are hematogenous. Thus, as already stated, many resemble blood monocytes, especially in the early stages. Moreover, the transformation of hematogenous monocytes and lymphocytes to typical inflammatory macrophages by successive stages of cytoplasmic increase and nuclear change has been observed by many investigators using a variety of techniques (27, 62, 109). In addition, blood monocytes have been labeled intravitally, the tagged cells then being observed in inflammatory exudates and their progressive transformation to macrophages and histiocytes followed (27).

Assuming, as seems likely, that most of the mononuclear cell exudate of inflammation is derived from blood leukocytes, there remain three possible explanations of their accumulation. The most usual view is that there is a selective emigration of monocytes and lymphocytes from injured vessles which takes place after the similar diapedesis of granulocytes has ceased. A number of reasons for this selective migration have been suggested, e.g., pH changes, but lack of evidence has prevented their acceptance. Similarly, the observation that monocytes respond chemotactically to the same stimuli as do polymorphs (46) makes an explanation based on specific chemotaxis difficult to accept. On the other hand, the use of smear techniques for sampling inflammatory exudates has failed to show significant numbers of mononuclear cells until a considerable time after injury (109). This would seem to support the hypothesis of subsequent selective migration. Such preparations, however, can trap only a limited number of cells. They therefore give information only about the proportion of mononuclear to polymorph leukocytes in the exudate, not about the absolute number of mononuclears at the relevant times. Moreover, direct microscopic observation of living inflamed tissues has not revealed a secondary migration of blood mononuclear cells (21). On the contrary, where investigators have looked for them, monocytes have been observed in inflammatory exudates (8) and emigrating from injured vessels (73) at the very earliest times after injury.

In view of the importance of the blood monocyte in the development of the mononuclear cell inflammatory exudate, these observations suggest the possibility, advanced for example by Harris (45), that polymorphs and monocytes leave the vessels over the same period of time, but that the extravasated polymorphs subsequently disappear whereas the monocytes remain in the tissues.

The third possibility is based on the findings of Clark, Clark, and Rex (21) on inflammation in the tail of the living tadpole. These workers followed the fate of individual polymorphs after their passage through the vessel wall and observed that many changed into round cells with clear cytoplasm and an ellipsoid, horse-shoe shaped, or small, dark, rounded nucleus, usually at one end of the cell. They considered that these were degenerate forms of polymorphs and that most of the mononuclear predominance of late inflammatory exudates was due to this change in the exuded polymorphs. This hypothesis has not found general acceptance for a number of reasons. Thus, it fails to accord with the observation that significant numbers of monocytes can be seen emigrating through the vessel wall at various times after injury. Moreover, the examination of consecutive smears of inflamed tissues shows the progressive transformation of monocytes and apparently lymphocytes to macrophages (109). The nuclear characteristics of these cells rule out the possibility that they are changed

most infective granulomata. Lymphocyte collections, often perivascular, are also frequently observed in healing tissues where all other cellular reaction has apparently ceased and as the only type of exudation in certain mild inflammatory reactions, e.g., due to viral invasion or an immunological reaction.

Lymphocytes outnumber monocytes in the peripheral blood when mobile and in the tissues are, if anything, somewhat faster than the latter type of cell (46). If, therefore, lymphocytes and monocytes emigrated from vessels indifferently, lymphocytes should in theory predominate in early mononuclear cellular exudates. This is not the case in fact, and monocytes usually outnumber lymphocytes in such exudates.

It would seem, therefore, that lymphocytes are a case apart, and this is borne out by their inability to respond to chemotactic stimuli (46). It is known also that the surface of lymphocytes differs from that of polymorphs (and probably of monocytes), and Harris (46) has pointed out that, whereas polymorphs and monocytes adhere to any wettable surface, lymphocytes do not, nor are they phagocytic. The results of Bangham and Pethica, showing powerful charge reversal by uranyl ions (6), provide a strong indication that the negative charge of lymphocytes is due to a preponderance at the cell surface of phosphatide groups of the cephalin type. Such a surface will form calcium bridges and adsorb protein very poorly compared with the surface of the polymorph. In addition, the tendency of lymphocytes to remain immobile (46) and their scanty cytoplasm would probably make pseudopod formation difficult and thus hinder the cell from forming areas of lowered radius of curvature. For reasons given above, this should make it hard for lymphocytes to penetrate the electrical field of opposing cell surfaces, e.g., of vascular endothelium, and thus to approach sufficiently close to form calcium or other types of bridge, upon which adhesion might depend.

Harris (46) noted that lymphocytic accumulations might be due to a specific change in vessels leading to selective emigration, to immobilization of lymphocytes in the process of circulating through the tissues, or to migration from lymphatics. Clark et al. (21) suggested that degenerate polymorphs account for many of the foci apparently composed of lymphocytes, but their evidence on this point is not altogether convincing. Although under some circumstances all these mechanisms may operate, observation suggests that, once lymphocytes can be induced to adhere to vascular endothelium, emigration and local accumulation might occur without the necessity for further changes. The special properties of the lymphocyte surface make it likely that the cell will stick to endothelium only if a mechanism independent of suitable charged surface groups is available. One of the most likely of such alternative systems is the existence of specific antigen-antibody binding. Thus, lymphocytes bearing antibody at their surface might well adhere to and migrate through endothelium on whose surface the appropriate antigen is present. Since lymphocytes carry the antibodies causing a wide variety of delayed hypersensitivity and homograft and heterograft reactions and since such reactions are characterized by lymphocyte accumulations, the above hypothesis would appear all the more plausible. A recent investigation has in fact shown that labeled sensitized lymphocytes accumulate at the site of injection of the appropriate antigen while the initial inflammatory leucocyte emigration is subsiding, whereas nonsensitized labeled lymphocytes failed to do so (93). Lymphocyte accumulations in infective granulomata could also be attributed to antigen-antibody reactions, and those found in scars could be associated with the large amounts of protein synthesized locally in healing tissues, perhaps owing to a special affinity of the protein molecules for the lymphocyte surface.

Inflammation as a Defense Against Bacteria

There are many classical experiments concerning the role of antibodies, leukocytes, bodily secretions, and nonspecific endogenous bacteriostatics in the defense of the body against bacterial infection. Much of this work can now be found in standard texts and will not be discussed here.

The defensive function of the inflammatory response *per se* has been scrutinized recently by Miles and his colleagues (84). Injections of epinephrine and "liquoid" (sodium polyethanol sulfonate) and the induction of dehydration shock were found to enhance the infections produced by intradermal injection of nine different bacteria. The degree of infection was

determined by measurement of the size of the resultant area of induration and erythema at 24 hr, its time of maximal development. After infection-enhancing treatment, the dose of bacteria required to produce a standard 24-hr lesion was found to be reduced by a factor of up to 100,000, the degree varying with the particular combination of organism and enhancer. The ability of the different measures to facilitate infection was observed to be confined to the first few hours after inoculation and was not seen after 5 hr. The degree of enhancement for any particular organism was found to correlate with the susceptibility of the organism to the bactericidal action of the blood in vitro. Miles and his colleagues concluded that defense against primary bacterial lodgments in skin is probably provided by nonspecific endogenous bactericidal substances and that this defense fails to operate in the absence of adequate circulation through the affected region. The enhancing action of liquoid was attributed to direct interference with the natural bactericidal mechanism, that of epinephrine to vasoconstriction, and that of shock to deficient peripheral circulation. On the other hand, if endogenous bactericidal substances were not to be invoked, the effect of epinephrine and shock could be attributed simply to deficient blood flow or to suppression of an inflammatory reaction dependent on such blood flow. Because of increased vascular permeability to protein and diapedesis of leukocytes, the inflammatory reaction might facilitate access of plasma bactericidal substances to the bacteria, and the presence of leukocytes could augment the action of such substances.

Burke and Miles (19) investigated this point further and found that primary inoculation of many types of bacteria was followed by a biphasic increase in vascular permeability. The first phase was of immediate onset and no longer present 1 hr after injection. The second phase began at 1 hr and was maximal at 3 to 4 hr. Leukocytic infiltration was first detectable at 1 hr and was maximal at 6 hr. Burke and Miles noted that the period of maximal bacterial destruction, as indicated by previous experiments in which the effects of infection were enhanced (84), did not correlate with the height of the inflammatory vascular response. Because of this and because they suspected that the initial phase of increased vascular permeability was due to factors of nonbacterial origin in the inoculation medium, they concluded that the inflammatory reaction played little or no part in the defense of the body against a primary lodgment of bacteria in the skin. They suggested instead that all that may be required is an adequate normal circulation to enable the nonspecific bactericidal substance in the blood to reach the inoculation site.

It may be, however, that some of the initial transient increase in vascular permeability was due directly to the organism, and that although weak it allowed sufficient blood bactericidal substance to enter the tissues to deal with the invading organisms and thus to be a necessary part of bodily defense. Moreover, the pattern of vascular permeability change may be different in natural infections, so that more time is available for the inflammatory response to develop. Moreover, from some of Burke and Miles's own results it would seem that intradermal injection of large numbers of bacteria cause a progressive increase in vascular permeability, well-marked at 15 min and powerful at 2 hr. In this instance, there is no period of relatively normal permeability around 1 hr, and the phase of maximal local bacterial destruction corresponds in time with the development of maximal vascular permeability to protein. However, even if inflammation plays little part in dealing with a small initial lodgment of bacteria, the delayed increase in vascular permeability and emigration of leukocytes could hardly fail to be important in defending the body against the organisms that survive the initial few hours in the tissues, in view of the well-known role of leukocytes and antibodies in this defense.

Miles and Miles (83) have shed some light on the possible defensive role of coagulation in lymphatics as a means of preventing bacterial spread, i.e., the "fibrin barrier" of Menkin (78). After inoculation of organisms, such coagulation was found to occur in 24-hr-old lesions, but only in areas of necrosis, and was not detectable at 5 hr. This process seems, therefore, to be incapable of contributing to the localization and destruction of the initial bacterial lodgment. These results have, therefore, to be added to the considerable body of evidence indicating the relative unimportance of lymphatic obstruction in the localization of bacteria in the tissues.

Pain in Inflammation

It is not proposed to discuss this topic in detail. It can, however, be said that, apart from direct stimulation of sensory nerve endings by injury and by pressure of exudate, pain in inflammation could result from the action of substances which dilate arterioles and increase vascular permeability, since all such compounds cause pain of varying intensity on intradermal injection or application to a blister base (4). It is also possible that nucleic acid derivatives may play a part, since xanthosine and xanthine in minute quantities cause intense pain in man (90).

Summary and Future Outlook

The summary most appropriate to this review article is probably a statement of the topics that seem most in need of further elucidation. A detailed study of the ultrastructural changes in the tissues involved in all stages of the inflammatory response would certainly appear to be called for. The mechanisms responsible for the delayed phase of the vascular reaction to injury also require much further study. Again, the interrelationships of kallikrein, kinins, kinin-forming enzymes, and globulin-permeability factors badly need to be resolved. Other problems that urgently require investigation are the nature and mode of action of substances with a specific action on leukocyte emigration and the changes which occur at the surfaces of the cells involved in the inflammatory response. The possibility that the individual injured vascular endothelial cell might show reversible changes in permeability akin to those observed in the cells of liver and kidney also seems to justify some thought. As is often the case, however, the formulation of problems is easier than their solution.

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