STUDIES ON THE PATHOGENESIS OF ACUTE INFLAMMATION

I. THE INFLAMMATORY REACTION TO THERMAL INJURY AS OBSERVED IN THE RABBIT EAR CHAMBER*

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(Received for publication, July 26, 1955)

The principal cellular events which constitute the phenomena of inflammation have been clearly described in the classical works of Addison (1), Waller (2), Cohnheim (3), and Metchnikoff (4). More recent observations have been made with refined techniques by Clark and Clark (5, 6), Florey *et al.* (7), and Zweifach (8). In spite of these and many other detailed investigations, knowledge of the subject remains essentially descriptive. The underlying biochemical and biophysical reactions have not been clearly defined.

The experiments reported in this and the following paper concern the anti-inflammatory properties of cortisone as they pertain to the mechanisms of acute inflammation. Preliminary exploration of this problem revealed the necessity of designing a special method for producing an acute inflammatory reaction which could be made highly reproducible and could be visualized directly *in vivo*. A detailed account of the technique finally adopted and the response which it regularly evoked is presented in this report. The paper that follows relates to the ways in which the inflammatory reaction was found to be affected by adrenocortical hormone.

Methods

The rabbit ear chamber originated by Sandison (9) and subsequently improved by Ahern *et al.* (10) was modified, as previously described (11), to provide better resolution at high magnification. In addition, a heat conductor of platinum wire (0.43 mm. in diameter and 5.5 mm. in length) was fixed in the center of the observation table of the chamber as shown in Text-fig. 1. The wire extended from the inner surface of the table to the outside, where a protruding 2 mm. segment was bent at a 90° angle to prevent inward displacement. Also the plexiglass ring supporting the glass coverslip of the chamber was reduced in thickness

* These studies were supported by a grant from the Life Insurance Medical Fund.

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from 2.5 to 1.5 mm. in order to facilitate use of the 97 $\times$ oil immersion objective. Details of the method used for installing the chamber are published elsewhere (11).¹

The tissue over the table of the ear chamber was burned by heat applied to the platinum conductor from an electric pencil² previously warmed for 10 minutes to a constant temperature. Application of the electric pencil for 15 seconds to the angulated tip of the platinum wire was found to inflict a suitable injury to the tissue. Damage by heat radiating from the body of the pencil was prevented by shielding the base of the chamber with a small sheet of asbestos.

Adult male rabbits of Flemish New Zealand stock weighing 3.0 to 3.5 kg. were used. No ear chamber was employed for more than one experiment. The chambers were not used until the blood vessels were fully mature and the tissue was clear of cellular debris. Each

TEXT-FIG. 1. Schematic diagram of a rabbit ear chamber equipped with a platinum wire.

animal was placed in a standard rabbit box for 6 hours on each of 3 days preceding the experiment. The box was equipped with blinders (Fig. 1). On the day of the experiment the animal was put in the box 1 hour before the start of the study to permit time for stabilization. All studies were made without anesthesia. They were performed in a quiet room with subdued lighting. The room temperature was not allowed to vary more than 2° from a mean of 80°F.

A special frame supported the microscope above and parallel to the rabbit box, with the stage facing downward as shown in Fig. 1. The ear of the rabbit was raised without tension to the level of the microscope, and the ear chamber was clamped in place on a modified plastic stage. Uninterrupted observations were made for periods of 6 to 8 hours and further studies were performed on a day to day basis. No rabbit was kept in the box for more than 9 consecutive hours. Food and water were withheld during each period of study.

Observations were recorded by means of both still photographs and motion pictures.

¹ The syncrobin used in the postoperative treatment of the rabbits was generously supplied by Schenley Laboratories, Inc., Lawrenceburg, Indiana.

² Ungar Electric Pencil, No. 217, Ungar Electric Tools, Inc., Venice, California.

RESULTS

When the experimental conditions were rigorously controlled, as outlined above, the response to thermal injury was found to be remarkably uniform. Its principal features were as follows:—

Circulatory Phenomena.—Following the thermal injury the circulation became permanently occluded in the small segment of tissue immediately surrounding the platinum wire (Figs. 2 and 3). In the outlying tissue where the insult was less intense, blood flow became reestablished after a brief initial period of vasoconstriction, and there followed the typical response of acute inflammation (Figs. 4 to 28).

During the first few minutes after the application of heat the entire chamber was rendered ischemic by intense vasoconstriction. After approximately 5 minutes the circulation was gradually restored as the arteriolar constriction subsided. In becoming reestablished the blood flow by-passed the permanently damaged center of the chamber by collateral channels, in some instances by direct arteriovenous communications (Fig. 6). Within 30 minutes after injury arteriolar dilatation was noted and the total volume of blood flow in the chamber became greater than before injury. Dilatation of the arterioles continued to increase with time and after 24 hours caused engorgement and accelerated flow in the capillary and venous beds (Figs. 4 and 5). Increase in transmission of the arteriolar pulsations to the capillary and venous systems imparted to the tissue an active pulsatile motion. As will be discussed presently, these circulatory changes play an important role in the pathogenesis of the edema and hemorrhage, which characterize the full blown inflammatory response.

The Development of Edema and Stasis.—Within 10 minutes after injury, the red cells in the surrounding vessels began to agglutinate and form rouleaux (Fig. 7). Also they were seen to stick to the endothelium (Figs. 17 and 22). As illustrated in Fig. 7, the erythrocytes continued to accumulate during the first 4 to 6 hours until active circulation in the vessel had ceased altogether. A refractile red color and lack of cellular detail identified the vessels in which circulatory stasis had occurred (Fig. 7, right). Since platelet-fibrin thrombi did not form until relatively late, occlusion by stasis was often reversible in the early stages of the reaction. The semifluid blood was occasionally forced back into the circulation, but the condition usually recurred on the arrival of new red cells. Eventually the stasis often became permanent as the result of thrombus formation (Fig. 8).

Stasis developed because of plasma loss apparently resulting from endothelial injury (12). The perivascular connective tissue appeared increasingly dense owing to the accumulation of edema fluid. After several hours structural details became obscured. In addition to the accumulation of interstitial edema fluid changes in the state of the ground substance may have contributed to the distortion of the connective tissue (13).

Globule Formation.—Numerous intra- and extravascular globular bodies of unknown identity and significance were found in the lesions produced by heat (see Fig. 9). Although usually about the size of a red cell, they not uncommonly exceeded by several times the diameter of a leucocyte. They were devoid of an internal structure, were of variable shape, and were highly refractile and elastic. They frequently appeared to stick to adjacent structures. Occasionally they were seen in the perivascular spaces and on the tissue surfaces, but in the main they occurred within the static capillaries and venules.

Formation of these globular bodies ceased within 1 hour after injury, well before the time of visible fibrin formation. Initially it was thought that they resulted from the effect of heat on the plasma proteins or on plasma or tissue fat. Microscopic sections, however, stained with hematoxylin and eosin and with oil red O failed to establish their composition. The intravenous administration of a fat emulsion stained with Sudan black also failed to result in their taking up the stain.³ Their nature, therefore, remains obscure.

Postinjury hemorrhage.—A bright ring of hemorrhage visible to the naked eye 24 hours after injury was a striking feature of the inflamed ear chamber. Petechial hemorrhages rarely occurred at the time of insult and when present were neither extensive nor progressive. Significant hemorrhage was usually first noted 3 to 6 hours after injury and developed primarily about vessels in the peripheral half of the static zone (see Figs. 10 and 11). It was of greatest intensity about arterioles and venules and showed no specific predilection for points of vascular bifurcation.⁴ Since vascular rhexis did not occur, the red cells apparently reached the perivascular space by means of diapedesis (see below). Hemorrhage was enhanced by the increased intravascular hydrostatic pressure and by the accentuated pulsations, both of which resulted from the arteriolar vasodilatation. Eventually the dense erythrocytic infiltration obscured the tissue and cellular detail where stasis was maximum (Figs. 11 and 18).

Leucocytic Sticking.—Because of the rapidity of the blood flow the circulating leucocytes before injury appeared to be relatively few in number. Those that were visible could be seen to roll freely along the vascular endothelial surfaces at the periphery of the axial stream. Immediately after injury their number seemed to increase because of their tendency to drag on the endothelium. Within a few minutes small numbers of white cells became securely stuck to the vessel walls as shown in Figs. 13 and 14. At the same time a less impressive degree of erythrocytic and platelet sticking developed (Figs. 17, and 21 to 24). By the

³ The fat emulsion was obtained through the kindness of Dr. H. C. Meng, Department of Physiology, Vanderbilt University School of Medicine, Nashville.

⁴ Akers (14) has noted in hamsters treated with heparin that post-traumatic hemorrhage occurs most frequently at the points of junction of postcapillary venules. No such localization was noted in the present experiments.

end of 15 to 30 minutes the leucocytic reaction had become marked. At first it was only unilateral, that is on the endothelial surface nearest to the area of injury (Fig. 14). Later as the reaction advanced the entire endothelial surface of the vessel became covered with adhering leucocytes (Figs. 15 and 17). During the 1st hour of the inflammatory response much of the cellular sticking occurred near the inner margin of the lesion in vessels which in time became static. Gradually the sticking extended peripherally to involve other vessels, most prominently the veins draining the area of injury. Sticking in veins usually exceeded that in capillaries since the venous circulation contained a greater number of leucocytes (5). Arteriolar sticking was infrequent except where the velocity of flow was drastically reduced by the injury. Maximal leucocytic sticking began to subside within 6 to 9 hours and after 24 hours only a minimal reaction was found at the edge of the lesion.

Three points in regard to the sticking of leucocytes deserve particular emphasis. First, vasodilatation does not always precede the sticking of leucocytes since white cell adherence was observed to occur during vasoconstriction (Figs. 12 and 13). Secondly, during the course of the inflammatory reaction leucocytes were frequently seen to stick to one another, indicating that the increased adhesiveness characteristic of the inflammatory response is not limited to the endothelium (Fig. 16). Thirdly, it was repeatedly noted that leucocytes dislodged into the circulation from localized areas of sticky endothelium did not adhere to the walls of the undamaged vessels into which they escaped. Thus leucocytes, although capable of becoming sticky, do not attach themselves to the surfaces of uninjured endothelium.

Diapedesis.—The emigration of white cells into the perivascular tissue began soon after the onset of intravascular sticking. Emigration paralleled in general the intensity of leucocytic sticking. The diapedesis was at first more noticeable in vessels near the lesion than in those located peripherally. This difference may have been due to a gradient of endothelial injury extending outward from the original site of maximal stimulus. In time, however, many of the vessels near the lesion became static and thereafter the scene of the most intensive diapedesis shifted further to the periphery. Despite their lack of flow, however, the static vessels continued as important secondary sites of diapedesis, since many intravascular white cells migrated down their channels and thence entered the lesion. Again, as in the case of the sticking reaction, the leucocytic diapedesis had largely subsided by the end of 24 hours. It tended to remain longest at the periphery of the lesion.

Before passing through the walls of the blood vessels, the intravascular leucocytes first became adherent to the endothelium and then began migrating at random over its surface (Fig. 17). It was not possible in advance to predict the precise locus of cellular penetration, since at no time were there visible endothelial stomata. Once the cell had found a suitable spot for penetration, the

process of diapedesis began. It usually required 3 to 12 minutes for completion (Figs. 19 to 24). After the cell had passed through the vessel wall, a defect in the endothelium seemed to exist, for not infrequently one or more additional cells would follow exactly the same route to the perivascular space.⁵

The diapedesis of erythrocytes appeared to be a passive process. It was not prominent until stasis and vasodilatation had become marked. Red cells, however, emigrated in small numbers during early inflammation, particularly when they became trapped in the endothelial defects left by emigrating leucocytes (Fig. 22). Occasionally, thus trapped, a cell would be swept back into the circulation by a sudden change of intravascular pressure, or as is shown in Figs. 23 and 24, it might break into two parts leaving the extruded portion behind in the perivascular tissue.

Accumulation of Leucocytic Exudate.—There was no evidence that a positive chemotactic force directed the migration of leucocytes once they had entered the perivascular connective tissue. Their motion appeared to be random, although frequently they seemed to follow the route of least resistance between fibrous bands in the connective tissue (Figs. 25 and 26). In this way they were directed by chance toward, away from, or parallel to the injured area. Their motion was not influenced by the movement of edema fluid or by the tissue pulsations produced by vasodilatation. In spite of their random movement, however, they eventually became concentrated about the site of injury. Within 6 hours a considerable number of leucocytes had congregated in the static area of the lesion. After 12 hours the cells had moved into the central ischemic area, and 24 hours after injury, the tissue of the ischemic area was densely packed with motile leucocytes (Figs. 27 and 28). It is of interest that during the total 24 hour period of observation, perivascular histiocytes appeared not to be involved in the reaction. They neither formed daughter cells, nor did they migrate toward the site of injury.

The lesion at 24 hours, as seen in Fig. 18, presented a characteristic microscopic picture in which four zones were visible:—

1. Immediately surrounding the platinum wire was an ischemic zone containing few extravasated erythrocytes but many motile leucocytes. Histologic sections of the ear chamber stained with hematoxylin and eosin revealed in this central area fragmented connective tissue and many inflammatory cells, predominantly polymorphonuclear leucocytes.

2. Adjacent to the inner ischemic zone was a band of stasis in which the blood vessels were filled with bright red cords of agglutinated erythrocytes. Dense perivascular accumulations of edema and red cells, particularly in the outer

⁵ Zweifach has pointed out that leucocytes are detained in the perivascular fibrous sheath for several minutes after emigration (8). We have found this to be the case in most instances. Occasionally, however, white cells appear to migrate directly into the connective tissue without pausing in the perivascular sheath.

half of the area, obscured tissue detail and made estimation of the leucocytic content difficult.

3. A less distinct zone was discernible about the outer edge of the static area where inflammation continued at a reduced rate as evidenced by vasodilatation and minimal leucocytic sticking. Extravascular erythrocytes and motile white cells were present in moderate numbers.

4. The peripheral tissue in the chamber, with its intensely dilated, pulsating vascular bed surrounding the lesion, comprised the 4th zone. Little evidence of edema or cellular exudation was to be found in this region.

DISCUSSION

The principal difficulty encountered in using the rabbit ear chamber to study acute inflammation concerned the problem of delivering to the tissue a single controllable stimulus. Attempts to introduce either bacteria or irritating chemicals into the chamber invariably led to extraneous trauma which affected the early cellular response. In studying acute inflammation particularly, extraneous trauma must be avoided, since its effect upon the tissues reaches a peak at approximately the same time as the reaction to be studied. In the case of more chronic inflammatory reactions, which do not become maximal for many hours (15), the exclusion of early trauma is less crucial. The technique used in the present studies was specifically designed to eliminate mechanical injury to the tissues. That the objective was finally attained is indicated by the uniformity of the response which resulted from the thermal stimulus.

Of the various cellular phenomena involved in acute inflammation none is more fascinating to observe *in vivo* than the early sticking of leucocytes to the vascular endothelium. It begins only after a latent period of 10 to 15 minutes. The factors which account for its occurrence are at present unknown. Speculations relating to the cause of leucocytic sticking have included the role of vasodilatation (16), postulated changes in cellular surface charges brought on by "currents of injury" (17), and the deposit of a gelatinous precipitate upon the surface of injured endothelium (8). Since, as reported in the next paper (18), the most striking effect of cortisone in acute inflammation is upon this sticking reaction, its genesis is worthy of particular consideration.

In his classical description of inflammation, Cohnheim pointed out that the initial phase of the inflammatory reaction is characterized by vasodilatation (3). The importance of this early vascular response has been repeatedly stressed by experimental pathologists. In fact, it has been given so much emphasis in textbooks that the implication has arisen that vasodilatation in some way causes leucocytic sticking. It might be imagined that stretching of the vessel wall would lead to changes in the state of the endothelial lining which in turn might cause leucocytes to become adherent to it. The observations made in the present study, however, indicate that this explanation is not tenable. Con-

tinuous visualization of individual blood vessels following application of the thermal stimulus have repeatedly revealed that leucocytes may stick in large numbers to the walls of blood vessels which at no time during the experiment exhibit an increase in caliber. In fact sticking is not uncommonly seen in vessels which have become constricted rather than dilated. It appears certain, therefore, that leucocytic sticking must be caused by some event other than mere vasodilatation. This conclusion, as will be discussed in the next report (18), is of particular significance in view of the fact that cortisone acts as a potent vasoconstrictor (15).

The attractive hypothesis that leucocytic sticking is due to changes in cellular surface charges is at present unproven. Currents of injury arising from sites of damage in living tissues have been extensively studied by physiologists. Abramson (19) has postulated that such injury currents may indirectly affect the behavior of intravascular leucocytes in such a way as to cause them to undergo diapedesis. Sawyer more recently has demonstrated (17) that aortic endothelium which is normally electronegative becomes electropositive following injury. This observation is in keeping with Abramson's theory, since the net surface charge of circulating leucocytes is electronegative (19). However, it must be emphasized that Sawyer found the change in endothelial charge to be an immediate sequel to trauma, whereas the sticking of leucocytes does not begin, even in acute inflammation, until after 10 to 15 minutes. It seems unlikely, therefore, that the injury potential *per se* is responsible for leucocytic sticking. On the other hand, there is available at present no conclusive experimental evidence to disprove the general thesis that changes in cellular surface charges play a role in this important phenomenon.

The third theory advanced to explain the leucocytic sticking, which characterizes the early stages of acute inflammation, is based primarily upon the observations of Zweifach (8). Following microtrauma to capillaries in the mesentery of anesthetized rats, he has observed the formation of an intravascular gelatinous precipitate which appears to impart an increased adhesiveness to the surface of the endothelium. This viscous material also affects the surfaces of the cellular elements of the blood, particularly the leucocytes. According to Zweifach, the "gelatinous precipitate appears as a consequence of chemical and mechanical tissue trauma, and not as a consequence of reduced blood flow or stasis. . . . There is not sufficient evidence to indicate whether this substance is elaborated by the cell,⁶ or appears in response to substances liberated from the cell which interact with the plasma (8)." In support of the second of these two alternatives, however, there is already much suggestive evidence that fibrinogen may be involved in the process. To begin with, thrombosis is a common feature of the inflammatory response, particularly when the stimulus

⁶ Injured endothelial cell.

is relatively severe (20). Secondly, microscopic gelatinous "clots" simulating those described by Zweifach, and not sufficiently extensive to cause thrombosis, have been repeatedly observed in bacteriemic states during which leucocytic sticking is a prominent feature (11). Thirdly, heparin will prevent the formation of the gelatinous precipitate and at the same time prevent the sticking of leucocytes to the vessel walls. This last observation, just reported by Zweifach (8), we have also made by means of the rabbit ear chamber technique used in the present experiments.⁷

Because of these findings it is perhaps justifiable to assume that the clotting mechanism of the blood may be intimately involved in the sticking of leucocytes to the vascular endothelium. The possibility that partially polymerized fibrin (23) becomes deposited on cellular surfaces during the early stages of inflammation is at least plausible. In support of such a concept is the observation that the sticking reaction involves platelets and erythrocytes as well as the leucocytes and the endothelium. Experiments designed to test this hypothesis further are now in progress.

The manner in which tissue damage causes the inflammatory response in relatively distant vessels has also been intensively studied. There is much evidence to indicate that the reaction results from the diffusion of chemical products from the site of injury to the vessel involved (24-26). Such diffusion might be expected to cause the reaction first to occur on the side of the vessel facing the site of injury. The unilateral sticking of leucocytes described in the present experiments confirms this expectation. Among the chemical substances which diffuse from the site of injury, products of proteolysis appear to be of special significance. Histamine, for example, derived from the hydrolysis of tissue proteins is known to be capable of causing the typical vascular response of acute inflammation (26). Other amino acids and polypeptides likewise appear to be involved (27). Whether proteolytic enzymes released from injured cells also diffuse to the outlying vessels remains to be determined. In this connection, Miles and Wilhelm have recently described in the serum of guinea pigs an enzymatic "permeability factor" which may be blocked by known inhibitors to proteolytic enzymes (28). And lastly it may be mentioned that certain proteolytic enzymes are known to be capable of initiating blood coagulation (29).

The relation of leucocytic sticking to the diapedesis of leucocytes has been repeatedly described (5) and has been amply confirmed by the present studies. Leucocytes always become adherent to the endothelium and migrate about on

⁷ Two recently discovered facts relating to the Shwartzman reaction may also have a bearing upon the possible role of fibrinogen in inflammation. First, the acute inflammatory response characteristic of the localized Shwartzman reaction can be prevented by pretreatment with heparin (21), and secondly occurrence of the generalized Shwartzman reaction is accompanied by a precipitous decrease in circulating fibrinogen (22).

its surface before penetrating the walls of the vessels. Thus adherence to the vascular lining constitutes an essential step in the process of diapedesis. It follows that prevention of leucocytic sticking will likewise block the extravascular accumulation of leucocytes.

The observations made in the present study, as well as those reported by Zweifach (8), indicate that diapedesis of erythrocytes is a purely passive process and depends upon a positive gradient of pressure in the direction of the extravascular space. In contrast, the diapedesis of leucocytes appears to depend primarily upon cellular motility. The sole dependence of diapedesis upon leucocytic motility has been questioned by Miles (30) because of the observation that emigration is diminished during the stasis of shock. Leucocytes have been observed, however, both by us and by Zweifach (8) to migrate from vessels in which blood flow has stopped altogether. It would seem apparent, therefore, that an elevated hydrostatic pressure is not a prerequisite for leucocytic diapedesis.

The factors which cause the leucocytes, once they have emerged from the blood vessels, to accumulate about the primary site of injury are at present obscure. Continuous observation of individual cells reveals that their motion is largely random. Positive chemotaxis of the type known to occur under certain conditions *in vitro* (31) has not been observed. Its absence is in keeping with the recent finding of Harris that extracts of sterile tissue do not exert a positive chemotactic effect upon motile leucocytes (32). Yet the resultant movement of the cells must be toward the original lesion as indicated by their eventual accumulation about its site. Whether environmental factors in the tissues closest to the lesion tend to prevent cells from leaving once they have entered the area of injury is a matter of conjecture. It is of interest that Harris, in reviewing the problem of chemotaxis (33), cites unpublished investigations of Sanders in which he failed to observe attraction of leucocytes to areas of thermal injury produced in the rabbit ear-chamber. The failure of leucocytes to accumulate at the sites of injury in Sanders' experiments may have been due to the fact that he used a less intense thermal stimulus than was employed in the present study. At any rate the behavior of the leucocytes noted in the present experiments was similar to that known to occur in most acute inflammatory lesions (6, 7).

Finally brief mention should be made of the peculiar globular bodies noted in and about the static vessels of the thermal lesions. Although to our knowledge, these have not previously been seen *in vivo*, Moritz *et al.* (34) have observed that the plasma from severely burned dogs often appears turbid because of the presence of "agglomerates of protein" which are demonstrable in wet smears of the blood. Kabat and Levine (35) have concluded that these masses play a role in the death which often follows severe burns. They found histologic evidence of the material, "presumably fibrinogen," in the pulmonary capillaries of animals dying of thermal injury. The origin and significance of these

masses and their possible relation to those observed in the present experiments are unknown.

SUMMARY

A special adaptation of the rabbit ear chamber has been devised to study *in vivo*, under high magnification, the acute inflammatory reaction to thermal injury. Systematic observations of the cellular response have led to the following conclusions.

1. Contrary to the commonly accepted view, vasodilatation does not always precede the adherence of leucocytes to vascular endothelium.
2. The fact that leucocytes often adhere to one another as well as to the endothelium indicates that the increased adhesiveness characteristic of the early stages of inflammation is not limited to the surfaces of the endothelial cells.
3. The sharing of erythrocytes and platelets in this increased stickiness suggests that a "plasma factor" is involved. There is indirect but as yet inconclusive evidence that the plasma factor may concern the clotting mechanism of the blood.
4. The adherence of leucocytes to the endothelium is usually first noted on the side of the vessel closest to the site of injury. This previously undescribed phenomenon of "unilateral sticking" is in keeping with the concept that the vascular reaction is caused by products of cellular damage which diffuse to the vessel from the site of injury.
5. Leucocytes always become adherent to the endothelium before penetrating the vessel wall. They often migrate about for some time on the endothelial surface before undergoing diapedesis.
6. Although no definite stomata are at any time visible in the endothelium, penetrating leucocytes may leave behind temporary defects through which other leucocytes and even erythrocytes may pass.
7. The diapedesis of leucocytes appears to depend primarily upon cellular motility. It may occur in static vessels where there is presumably little if any hydrostatic pressure.
8. The diapedesis of erythrocytes, on the other hand, is a passive process depending upon intravascular pressure. Its occurrence is greatly exaggerated in areas in which intravascular pressure becomes elevated. Such elevations occur as the result of proximal arteriolar dilatation and distal occlusion of vessels.
9. Once they have reached the extravascular tissues the leucocytes move about more or less at random, apparently uninfluenced by any compelling chemotactic force. Their resultant migration, however, is toward the site of injury around which they eventually tend to congregate.
10. The histiocytes normally present in the connective tissue appear to play no role in the type of acute inflammatory reaction produced in these experiments.

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EXPLANATION OF PLATES

Hoffman Laboratories, Inc., New York, generously processed all 35 mm. film using the special fine grain developer, PRA. Photographic prints were made by Mr. K. Cramer Lewis, Department of Illustration, Washington University School of Medicine.

PLATE 75

FIG. 1. Photograph of microscopic equipment.

FIG. 2. Ear chamber before injury. The black shadow in center of field is due to platinum wire. Note that blood vessels run over face of wire. $\times 40$.

FIG. 3. Same ear chamber 5 minutes after thermal injury. The circulation of the tissue adjacent to the platinum wire has become occluded. The patent vessels in the surrounding tissue are clearly visible. $\times 40$.

FIG. 4. Arteriole (*A*), capillary (*C*), and venule (*V*) in periphery of an uninjured ear chamber. Note their calibers. $\times 65$.

FIG. 5. Same area 24 hours after injury. Arteriolar (*A*) dilatation and engorgement of the capillaries (*C*) and venules (*V*) are apparent. $\times 66$.

PLATE 76

FIG. 6. A vascular shunt in which blood flow by-passes lesion located at upper right. Note that the distal arteriole (*DA*) is devoid of flow. Blood is running from proximal arteriole (*PA*) to venule (*V*) where leucocytic sticking is marked. $\times 250$.

FIG. 7. Static venule at margin of lesion 2 hours after injury. The damaged tissue is to the right. Erythrocytic rouleaux formation (*R*) is seen at left. Cellular detail is lost in densely packed blood at right. $\times 250$.

FIG. 8. Arteriolar stasis 24 hours after injury. The thrombus (*T*) lining the endothelium (*E*) partially occludes the lumen. Platelets (*P*) are seen imbedded in thrombus. Static blood (*B*) is visible in center. $\times 540$.

FIG. 9. Static venule containing globules (*G*) 2 hours after thermal injury. Note variation in size and lack of internal structure of globules. $\times 250$.

FIG. 10. Hemorrhage (arrow) about static vessels 3 hours after injury. The area of injury is to the right where the platinum wire is barely visible. $\times 65$.

FIG. 11. Same field 24 hours after injury. Note intensification of hemorrhage in outer half of static zone. $\times 65$.

FIG. 12. A small venule before injury. Note diameter and lack of leucocytic sticking. $\times 250$.

FIG. 13. Same vein 15 minutes after injury. Sticking of leucocytes (*L*) has occurred in the absence of vasodilatation. $\times 250$.

PLATE 77

FIG. 14. Unilateral sticking of white blood cells 30 minutes after injury. The leucocytes (*L*) are adhering to the endothelial surface nearest the area of injury which is located below the vessel. $\times 250$.

FIG. 15. 1 hour after insult the leucocytic sticking has become generalized. $\times 250$.

FIG. 16. An example of intercellular sticking of leucocytes. The "white cell thrombus" contains no platelets but is adherent to the vessel wall. Most of the cells are sticking to each other and have no direct contact with the endothelium. $\times 540$.

FIG. 17. Intravascular migration of leucocytes on endothelial surface prior to diapedesis. Note that motile cells (*ML*) have lost their usual globular shape (*L*). One erythrocyte (*E*) can also be seen sticking to endothelium. $\times 250$.

FIG. 18. Four zones of 24 hour lesion. See text for identification. $\times 40$.

PLATE 78

FIGS. 19 to 24. Diapedesis of leucocytes. Total sequence approximately 15 minutes. First leucocyte (L_1) can be seen to pass through vessel wall near top of field. Most of cell is in extravascular tissue by end of sequence (Fig. 24). Second leucocyte (L_2) passes through vessel wall more rapidly (see Figs. 19 through 21). Red cell (R) then becomes trapped in same endothelial defect through which leucocyte has passed. Extravascular portion of red cell (R) eventually breaks off and is left in perivascular space. Note endothelial sticking of platelets (P), particularly in Figs. 21 and 24. $\times 540$.

FIGS. 25 and 26. Extravascular migration of leucocytes. The white cells (a) and (b) are seen to migrate along strands of connective tissue toward stationary cell (c). Area of injury is at upper right. $\times 540$.

FIG. 27. Tissue of central area of ear chamber before injury. Note connective tissue is devoid of inflammatory cells. $\times 250$.

FIG. 28. Dense extravascular accumulation of leucocytes in same area 24 hours after injury. Granular appearance of background is due to the presence of the motile granulocytes. $\times 250$.