THE ACUTE INFLAMMATORY REACTION IN THE RABBIT EAR CHAMBER WITH PARTICULAR REFERENCE TO THE PHENOMENON OF LEUKOCYTIC MIGRATION

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Plates 52 to 55

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A study using time-lapse cinemicroscopy of the healing process in rabbit ear chambers failed to reveal any directional guidance on the leukocytes migrating in the granulation tissue (1). Following the development of a new method for injecting small quantities of test materials into various sites in rabbit ear chambers (2) it became possible to extend observations to the migration of leukocytes during reactions produced by injections of various test materials. The experiments reported below were performed to determine whether antibody-antigen complexes which have been demonstrated to have positive chemotactic properties for leukocytes in vitro (3) exerted any similar influence in vivo.

Materials and Methods

Details regarding the construction and insertion of the ear chambers, and the maintenance of the rabbits during experimental use were as described previously (2). Injections of antibody (Ab), antigen (Ag), or complexes of the two (Ab:Ag) were made into 10 ear chambers which were inserted into either one or both ears of 7 cross-bred half-lop rabbits of either sex with an average weight of 3.0 kg. The antigens used were hen ovalbumin (OA), human serum albumin (HSA), and washed chick erythrocytes (CE). Homologous sera containing specific antibodies to these antigens were obtained from the blood of rabbits that had been immunized by serial injections of these antigens. Antibody-antigen precipitates were prepared in the cases of OA and HSA in the usual manner and were centrifuged and resuspended in saline before injection. Chick red cells were coated with antibody by incubating for 10 min at 37°C in each of 2 changes of heat-inactivated (56°C) antiserum and then washed 3 times before resuspending in saline for injection. In all but the first 2 injections made the test materials were injected either as a 1:1 or 2:1 mixture with 0.05% agar so as to secure a better degree of localization within the ear chamber. In addition acute inflammation was produced in 2 ear chambers in a further 2 rabbits by the injection of turpentine. In one case mineral turpentine and in the other 20% wood turpentine in olive oil was used.

When making an injection into an ear chamber the tip of the cannula was positioned at the apex of the injection notch and the test material was injected according to the method previously described (2). The amount of test material injected in each instance was extremely small having a calculated volume of approximately 0.05 mm^3.

When an animal received injections of antigenic material either complexed with specific

TABLE I

Type of Injection and Degree of Leukocyte Sticking Produced in Each Ear Chamber

Ear chamber		Antiserum (homologous) dil.1:1/0.05% agar	Antigen dil.1:1/0.05% agar	Antibody-antigen complex dil. 1:1/0.05% agar
54	R			Anti-OA:OA (NA) +++ Anti-HSA:HSA (NA) ++
57	L	Anti-OA ±	Day 1* OA 0.2% +	Day 5 Anti-OA:OA +++
	R			Day 4 Anti-OA:OA ++
70	R			Anti-OA:OA ++
71	L		Day 1 OA 0.2% ++	
	R			Day 4 Anti-OA:OA ++
58	L	Anti-CE −	Day 1 CE 30% +	
	R			Day 3 Anti-CE:CE ++

CE, Chick erythrocytes (washed).
HSA, Human serum albumin.
OA, Ovalbumin (hen).
NA, No agar added.
−, No granulocytes sticking to endothelium.
±, Very small numbers of granulocytes sticking to endothelium.
+, Moderate numbers of granulocytes sticking to endothelium.
++, Large numbers of granulocytes sticking to endothelium.
+++ Very large numbers of granulocytes sticking to endothelium and to one another.

* When an animal received more than one injection containing the same antigenic material the first injection is given as day 1 and the time of following injection is shown in appropriate square.

TABLE I—*Concluded*

Ear chamber		Antiserum (homologous) dil.1:1/0.05% agar	Antigen dil.1:1/0.05% agar	Antibody-antigen complex dil. 1:1/0.05% agar
64	L			Day 1 Anti-CE:CE +++
	R	Anti-CE Trauma +++	Day 2 CE 30% +	
68	L			Anti-CE:CE +
71	L			Anti-CE:CE +++

antibody or alone on more than one occasion, whether into two ear chambers or via different notches in the same ear chamber, the maximum time allowed to elapse between the injections was 4 days (Table I).

Cinemicroscopy was generally performed using a high power (× 63) objective corrected for cover slip thickness, but several sequences suitable for subsequent analysis were obtained using the × 40 objective.

The cine camera used was a Vinten Mk. 1 scientific camera (W. Vinten Ltd., London). Filming was performed routinely with a 7½ sec interval between frames at a shutter speed of 1/20 sec on Ilford Pan F. film. Each field chosen was filmed for a period of 1 hr. Supplementary to the cine records 35 mm photomicrographs were made of selected fields in the ear chambers both before and at various intervals after each test injection.

RESULTS

Direct Observations.—The very similar responses obtained with the 3 different Ab:Ag preparations used are shown in Table II. Summarizing the results from the 10 experiments in which Ab:Ag complexes were injected (Table I) the main features were as follows. The response to the injections developed gradually during the first hour after injection. At first the related blood vessels dilated and their rate of blood flow decreased (Figs. 1 and 2). Subsequently the rate of blood flow in the affected vessels increased and became pulsatile, while the vessels remained dilated (Fig. 3). This state of hyperemia was apparent by 2 to 3 hr after injection and subsided by the 6th to 8th hr after injection. Associated with the hyperemia it was possible to detect edema in the ear chambers which produced a characteristic cloudiness of the tissues. When the vascular reaction did not involve the whole area of the ear chamber it was possible to observe a localization of these changes around the injection site. Leukocytes first started to adhere firmly to the endothelium of the venules in the ear chambers about

TABLE II

Time Sequence of the Events Occurring after the Injection of Antibody-Antigen Complexes and Antigens alone Compared to the Effects of a Traumatic Injection Procedure

Nature of injection	Ear chamber	0 to 1 hr	1 to 2 hr	2 to 4 hr	4 to 6 hr	6 to 8 hr	24 hr
Anti-HSA:HSA	54 R		Flow ★ Leuk/s (+)	Flow ↑ Leuk/s (+)	Flow ↓ Leuk/s (++) Leuk/T (+)	— Leuk/s (++) Leuk/T (+)	— Leuk/s (−) Leuk/T (++)
Anti-OA:OA	54 R	Flow ↓ Leuk/s (±)		Flow ↑ Leuk/s (+++) Leuk/T (+)	Flow ↑ Leuk/s (+++) Leuk/T (++)	Flow ↑ Leuk/s (++) Leuk/T (++)	Flow ★ Leuk/s (−) Leuk/T (+++)
Anti-CE:CE	64 L	Flow ↓ Leuk/s (+)	Flow ↓ Leuk/s (++)	Flow ↑ Leuk/s (+++) Leuk/T (+)		Flow ★ Leuk/s (+) Leuk/T (++)	Flow ★ Leuk/s (+) Leuk/T (++)
OA	71 L		Flow ★ Leuk/s (±)	Flow ↑ Leuk/s (++)		Flow ↑ Leuk/s (++)	
CE	64 R		Flow ↑ Leuk/s (+)	Flow ★ Leuk/s (+)		Flow ★ Leuk/s (+)	Flow ↑ Leuk/s (±) Leuk/T (+)
Homologous serum (trauma)	64 R	Flow ↑ Leuk/s (+++)	Flow ↓ Leuk/s (+)	Flow ★ Leuk/s (±) Leuk/T (+)		Flow ★ Leuk/s (±) Leuk/T (+)	

Leuk/s, Leukocyte sticking scored from (−) to (+++) as for Table I.
Leuk/T, Infiltration of tissues by leukocytes scored from moderate (+) to intense (+++).
Flow, Blood flow scored as reduced (↓), normal (★), or increased (↑). Other abbreviations as for Table I.

1 hr after injection. At first the number of leukocytes sticking to the endothelium was small (Fig. 2), but increased considerably over the subsequent 3 to 4 hr. (Figs. 3 and 5). In severe reactions leukocytes could be identified adhering to one another as well as to the endothelium (Fig. 4). By about 6 hr after injection the number of leukocytes sticking to the endothelium of the venules was decreasing. In several ear chambers at this later stage it was noted that leukocyte sticking was occurring also in smaller vessels (postcapillary venules) than those initially involved. The vessels in which leukocyte sticking occurred were the venules into which the blood from the region of the injection site drained (Fig. 3). Where the pattern of venous drainage was such that all blood draining from the region of the injection site was collected into the one venule, or small system of venules, leukocyte sticking could be identified as occurring only on the walls of those vessels (Fig. 3). Frequently the sticking of leukocytes to the endothelium on only one side of the affected venules was observed (Figs. 3 to 5). This asymmetrical sticking of leukocytes occurred on the venule walls on the same side as and downstream of junctions with tributaries draining from the region of the injection site (Figs. 3 to 5). Downstream of such junctions in venules it was observed frequently that the streams of blood that came together at the confluence of the vessels did not mingle but remained separated (Fig. 8).

Following the sticking of leukocytes to the endothelium of venules in every experiment with Ab:Ag complex injections these cells emigrated from the vessels and became very numerous in the paravascular regions (Figs. 4 to 6) Such cells were first identified emigrating from venules 1½ hr after injection of Ab:Ag complex. As the inflammatory process progressed so the paravascular accumulations of leukocytes extended further out from the vessel walls and in places became confluent with similar regions related to neighboring vessels. Where the sticking of leukocytes in vessels was asymmetrical (above) the paravascular concentrations of emigrated leukocytes showed similar asymmetrical distributions (Fig. 6). In general the paravascular collections of leukocytes persisted for 1 to 2 days after injection (Fig. 6) but by the 3rd to 4th day the ear chambers had regained a normal appearance except for the presence of some additional large macrophages (Fig. 7). On only two occasions was a concentration of leukocytes observed at the actual site of injection.

Following the injection of OA or washed CE, which had not been complexed with antibody, an inflammatory reaction occurred (Tables I and II). The reactions however, were generally milder with no initial depression of blood flow and emigration of leukocytes from vessels either did not occur or did so only after considerably longer intervals as compared to the Ab:Ag experiments (Table II). In the experiment where an injection of homologous serum produced a significant reaction (Tables I and II) the manipulations during the injection procedure due to a defect in the construction of the ear chamber caused a good deal of injury to the edge of the ear chamber tissue. The results

of this experiment are shown to illustrate the inflammatory effect of trauma associated with the injection of a material that was otherwise inert in this respect. Within 10 min of the injection being made there was widespread hyperemia and edema and in many venules over the half of the ear chamber related to the injection site there was very severe leukocyte sticking. By 1 hr after the injection both the hyperemia and leukocyte sticking had decreased considerably and by 3½ hr after injection blood flow was normal and leukocyte sticking was minimal.

The acute inflammatory reaction produced by the injection of turpentine was in every respect more severe than those produced by Ab:Ag injections. Immediately following injection the vessels nearest the injection site showed stasis and elsewhere there were small hemorrhages, hyperemia, and edema present in the ear chambers. Associated with these changes was the development of widespread leukocyte sticking in venules. By 3 hr after injection the vascular changes were more generalized in the ear chamber and by 24 hr tissue necrosis was present in the region of the injection site. In the vessels that were still flowing leukocyte sticking was very severe. The necrotizing process continued remorselessly and by 5 days after the injection ⅓ of the ear chamber tissue was necrotic and the surviving ear chamber tissue continued to show severe inflammatory changes.

Time-Lapse Cinemicroscopy.—Fourteen sequences were analyzed from experiments using Ab:Ag covering the time range from 2½ hr to 6½ hr after injection and one additional sequence was obtained at 24 hr after such an injection.

Behavior of Leukocytes Sticking to Endothelium Following Antibody-Antigen Injection.—Large numbers of leukocytes were identified sticking firmly to the endothelium and were seen emigrating through the walls of the venules into the paravascular tissues. A large number of the leukocytes that were firmly adherent to the endothelium moved in an amoeboid manner upon the endothelial lining of the venules. This amoeboid movement occurred both in the direction of the blood flow in the vessel and against it. Of the 76 cells whose amoeboid motion on the endothelium of venules was detected 65% were moving against the direction of the blood flow and 35% were identified moving in the same direction as the blood flow. A leukocyte that was moving in an amoeboid manner either upstream or downstream could, at times, either reverse its direction of migration or commence moving transversely upon the vessel wall. It was possible to establish in certain instances that the amoeboid locomotion of leukocytes upon the endothelium was a preliminary to the cell becoming fixed at a point on the vessel wall and then emigrating into the surrounding tissue. The majority of cells followed, however, either moved out of the field of observation or else ultimately became detached from the vessel wall and were carried off in the blood stream. The average distance moved by 14 leuko-

cytes in this amoeboid manner was 29.5 μ (range 7.6 to 63.0 μ) and the mean velocity (with SE) for this group of cells was 6.25 ± 0.62 μ/min. The maximum rate of amoeboid movement detected for cells moving against the stream was 8.9 μ/min, while that for cells moving with the stream was 8.7 μ/min (Table III).

Behavior of Emigrated Leukocytes after Antibody-Antigen Injection.—The numbers of emigrated leukocytes present in the fields analyzed varied from high (estimated as "scores" of cells) to low (10 cells). In general, fields were chosen where a venule was running more or less directly away from the injection site, so that emigrated cells from each side of the vessel were able to move uninterruptedly either towards or away from the injection site. In each sequence a large proportion of the emigrated leukocytes remained within the observation area during filming, however a definite to and fro traffic of leukocytes did occur at the boundaries of the areas. The method was adopted of making 4 counts of the cells moving into and then 4 counts of the cells moving out of the observation area across each of the boundaries of the field. Cells entering or leaving the field while moving in a direction towards the injection site were scored as positive and in a direction away from the injection site were scored as negative. The score for each boundary was taken as the average resultant of 4 estimations. Of 65 leukocytes entering or leaving the observation areas in directions either towards or away from the injection sites 19% were scored as positive and 81% were scored as negative. In 3 sequences where movements across 2 of the boundaries could be considered neither as towards nor as away from the injection site (that is exactly at right angles to the ex-

TABLE III

Rate of Amoeboid Motion by Leukocytes (μ/Min) in Various Situations during Acute Inflammation

Situation in tissue	Stimulus for inflammation	Rate of migration	
		Mean and SE	Maximum
Adherent to endothelium	Ab:Ag	6.25 ± 0.62 (14)*	8.9
Emigrated into tissue	Ab:Ag	7.4 ± 0.94 (11)	11.8
Emigrated into tissue	Turpentine	7.5 ± 1.3 (10)	9.4

Ab:Ag, Antibody-antigen complex.
* Number of cells in group.

pected directions) 9 cells were counted. This total was made up of the average figures (see above) of 4.5 for each of the "neutral" directions.

The rate of migration of leukocytes in the tissues following Ab:Ag injection was estimated for 11 cells selected from 5 sequences. The mean value with standard error was 7.4 ± 0.94 μ/min and the highest rate of migration found was 11.8 μ/min. (Table III).

Behavior of Emigrated Leukocytes after Turpentine Injection.—Three sequences commencing immediately, 3 hr and 6 hr after injection were analyzed from the experiments using turpentine. Because of the poor microscopic resolution in the severely inflamed tissue due to the edema and the very large number of leukocytes present, it was not possible to analyze in detail the migrations of the leukocytes as in the case of the Ab:Ag experiments. The rates of migration of 10 leukocytes were obtained from the 3 sequences filmed during turpentine inflammation and the mean for these cells, with standard error was 7.5 ± 1.3 μ/min with a maximum rate observed of 9.4 μ/min (Table III).

DISCUSSION

Acute inflammatory reactions within rabbit ear chambers were first described by Clark and Clark (4).

In any procedure designed to demonstrate a relationship between the introduction of a test material into a rabbit ear chamber and the subsequent development of an acute inflammatory reaction the possibility that the inflammatory reaction has been produced merely as the result of trauma associated with the introduction of the test material must be eliminated. In recent experiments using the rotating valve injection chamber repeated injections of pyrogen-free saline were used to "stabilize" the chambers to the injection procedure (5). In the present experiments it was possible to differentiate between the almost immediate development (within 1 to 2 min) of an inflammatory reaction due to trauma to the ear chamber tissue which reached a maximum in about 1 hr and thereafter subsided over the next 2 to 3 hr (Table II) and the acute inflammatory reaction provoked by test material which developed gradually over the first hour following injection, continued to increase in severity until 4 to 6 hr after injection and then declined in severity (Table II). Rapidly developing inflammatory changes that subsided in 2 hr following mild trauma to ear chambers were described by Clark and Clark (4). A similar rapidly developing acute inflammation was described following thermal damage to ear chamber tissue (6), in these experiments, however, the changes produced persisted for about 24 hr, which could well be attributed to the considerably more widespread and severe damage produced by the heated wire. The slowly developing and more prolonged acute inflammatory reactions obtained in the present experiments after the injection of antigenic material either combined with, or without specific antibody (Table II) are similar to

those which developed after ultraviolet irradiation of the ear chamber tissue (7) or followed the injection of the products of in vitro incubation of leukocytes (5) or activated Hageman factor (8). The differences noted in the acute inflammatory reactions produced by antigens alone as compared to those produced by the antigens complexed with specific antibody (Table II), indicate that immune complexes are more effective agents in producing acute inflammation than are simple antigens when injected into nonimmunized animals.

The localization of leukocyte sticking to the venules observed in the present experiments is in agreement with other observations on the acute inflammatory reaction in rabbit ear chambers (4–6) and in other tissues (9–12). The highly asymmetrical distribution of the sticking of leukocytes to the walls of affected venules (Figs. 3 to 5) has been observed previously (6). The "unilateral" sticking of leukocytes observed by these workers also occurred on the sides of the venules that were nearest to the site of the stimulus and because it was the earliest manifestation of the phenomenon of leukocyte sticking and was rapidly replaced (within 15 to 30 min) by a generalized sticking to the entire walls of the affected venules they interpreted these findings as indicating the action of some product of cell damage liberated at the site of injury which diffused into the surrounding tissue and affected first the sides of the venules that were nearest the site of tissue damage and then with continuing diffusion both sides of the venules. However, the transient nature of the "unilateral" or asymmetrical leukocyte sticking observed by Allison et al. (6) was not confirmed in the present experiments where this pattern of leukocyte sticking persisted for times in excess of 5 hr (Fig. 5). In addition the venules in which leukocyte sticking was occurring were only those which were draining the region of the injection site (Fig. 3). Similar localization to the draining venules was observed by Clark and Clark (4) with localized tissue trauma. There is considerable evidence to support the concept both of specific chemical mediators (8, 13, 14) and of less well defined mediators acting on blood vessels in acute inflammatory reactions (7, 15–18). It is postulated herein that the chemical mediator(s) which produce leukocyte sticking on the venule walls do not reach these vessels directly as a result of diffusion, but are first absorbed into the minute blood vessels of the injured region in which they produce no sticking of leukocytes (Fig. 3). Once the blood from these vessels containing the postulated active material(s), comes in contact with the endothelium of the draining venules it produces sticking of leukocytes (Figs. 3 to 5). The existence of mediators showing a marked degree of specificity for venular endothelium is well documented (11, 14). The flow separation that persists downstream of junctions in the vascular system (Fig. 8) would localize the endothelial changes producing leukocyte sticking (19) to that side of the venule into which the tributary containing the specific mediator(s) is draining (Figs. 3 to 5). When a region of tissue damage extended so that tributaries on both sides of a venule

could contain the postulated mediator(s) no unilateral effect on the venular endothelium was observed. A decreasing gradient in the severity of leukocyte sticking was observed downstream of such junctions on the venule walls (Fig. 3). Further evidence of a gradient of adhesiveness on the endothelial lining of affected venules was the difference found in the proportion of amoeboid leukocytes that migrated upstream as opposed to downstream (approximately 2:1), it having been demonstrated that differences in the physical properties of the substrate are of cardinal importance in determining the direction of migration of cells adhering thereto (20–22). Similar values for the rate of migration of leukocytes on endothelium have been published (23) while "random" migration of leukocytes adhering to the endothelium of venules was described by Allison et al. (6).

The majority of emigrated leukocytes tended to remain in the fields studied over the periods of observation with little nett displacement, as described previously by Allison et al. (6). This finding is in accord with the maintenance of the concentrations of emigrated leukocytes in the paravascular regions observed above and by others (5). Of the relatively small numbers of cells entering or leaving the fields the proportion moving in directions away from the injection sites as opposed to those moving towards was 4:1 indicating a centrifugal spread of the cells away from the injection sites. No polarity was demonstrated in the neutral or tangential directions of leukocyte migration. For such an effect to be produced on a population of leukocytes in response to a gradient of some active material diffusing from the region of the injection site it would be necessary to invoke the action of negative chemotaxis (see reference 24 for review). Such a negative chemotactic effect of immune complexes for rabbit leukocytes in vivo would be at variance with the results obtained in vitro which demonstrated a positive chemotactic effect of immune complexes for rabbit leukocytes (3, 25) and demonstrates once again the inability to correlate in vitro demonstrations of chemotactic effects of substances with events that occur in the living animal (for review see reference 24). A factor of major significance in evaluating the results obtained is whether the leukocytes in the tissues can migrate towards or away from the test material with equal facility (26). The present experiments demonstrated that the concentration of leukocytes was highest in relation to the venules that were nearest to the injection site and decreased as their distance from the injection site increased. In this way the emigrated leukocytes formed a population which was most dense in a zone immediately surrounding the test material and which decreased in density as the distance from the test material increased. The greatest mean free path for randomly moving and colliding cells in such a population lies in the direction of decreasing population density that is in a direction away from the region of the stimulus. In this way the slow centrifugal expansion in the population of emigrated leukocytes could be explained without recourse to negative chemotaxis.

The rates of migration of leukocytes in the tissues during acute inflammation were not significantly influenced by the nature of the stimulus producing the inflammation (Table III), and are in agreement with values obtained elsewhere with time-lapse cinemicroscopy (23) and also with values obtained for leukocytes migrating in healing tissue by the same technique (1). It would appear that the figure of about 20 μ/min as an estimated speed for leukocytes migrating in an ear chamber (27) is too high for amoeboid movement and suggests that the few cells observed moving at this speed were not moving in this manner.

SUMMARY

Responses to injections of various materials into rabbit ear chambers were studied by in vivo microscopy. The acute inflammatory responses provoked by injections of antibody-antigen complexes were both quantitatively and qualitatively different from the responses obtained after injections of either homologous sera or the antigens alone. The sticking of leukocytes to endothelium during these responses occurred only in the venules draining the injection sites and was frequently present only on the sides of the venules towards the injection sites. An explanation of this finding was proposed in terms of absorption by the minute vessels related to the injection sites of postulated mediator(s) with specific activity on venular endothelium.

Analysis of the rates and direction of movement of leukocytes during the reactions produced by the antibody-antigen complexes was performed with the aid of time-lapse cinemicroscopy. The leukocytes that were sticking to the venular endothelium frequently exhibited amoeboid locomotion within the vessels. Twice as many of these cells moved against the direction of blood flow as with it. This finding was discussed and an explanation proposed.

A method for detecting a drift in the overall population of emigrated leukocytes within the inflamed tissue was described and revealed that four times as many amoeboid cells moved away from the injection sites as towards them. This result was discussed in the light of the in vitro chemotactic properties of antibody-antigen complexes demonstrated for rabbit leukocytes. An alternative explanation was proposed in terms of variation in the population density of these cells and their random movements and collisions.

The rates of amoeboid movement of leukocytes during the acute inflammatory reactions produced by antibody-antigen complexes were similar to the rates found during turpentine inflammation and were compared to other published values.

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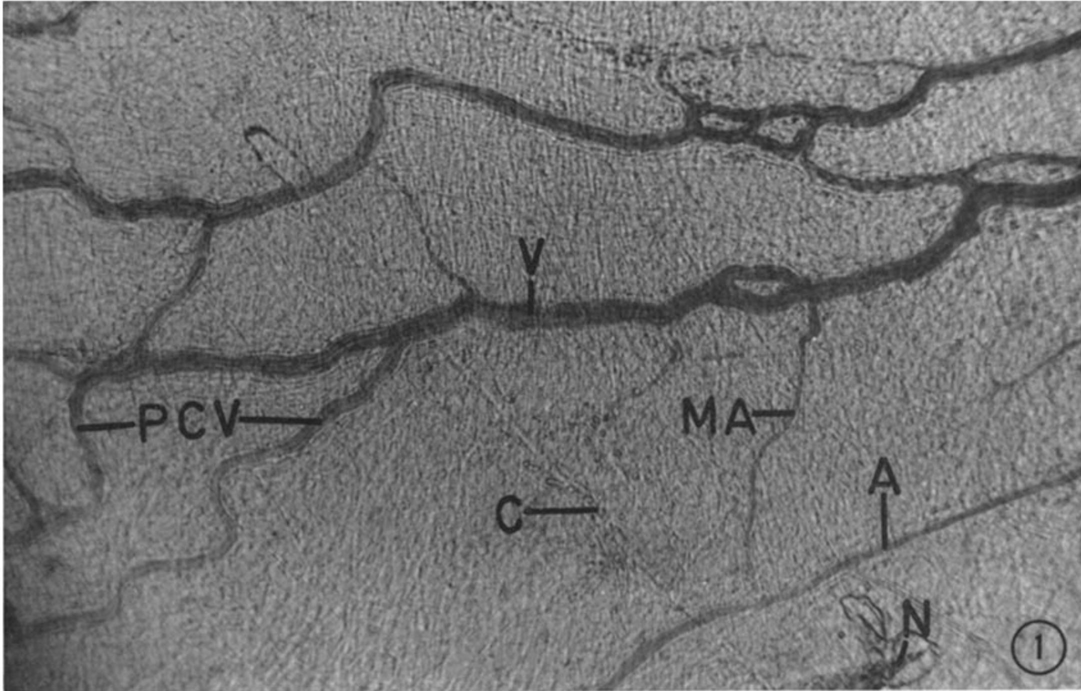
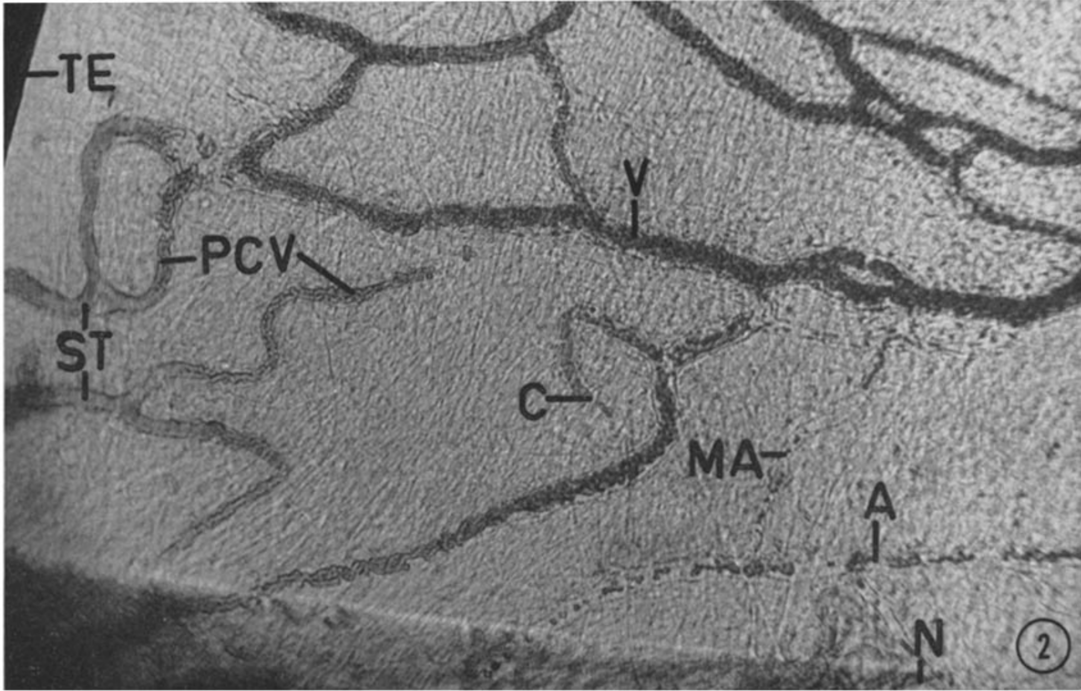
EXPLANATION OF PLATES

Plate 52

Fig. 1. Region of rabbit ear chamber immediately before an injection of Ab:Ag complex was made into the injection notch whose tip is visible at *N*. A venule (*V*) is flowing from the left to the right side of the figure and drains the blood coming from the region of the injection notch via postcapillary venules (*PCV*), and a metarteriole (*MA*), and a small system of capillaries (*C*) which arise from the arteriole (*A*) that enters the chamber near the tip of the injection notch (*N*). × 125.

Fig. 2. Same region as Fig. 1, 1 hr after the injection of Ab:Ag (CE) complex into the notch (*N*). The blood vessels have dilated and blood flow within them has slowed very considerably as shown by the sharp images of the contained blood corpuscles. A small area of vascular stasis (*ST*) is present in the system of postcapillary venules (*PCV*) near the table edge (*TE*). Other labeling as for Fig. 1. × 125.



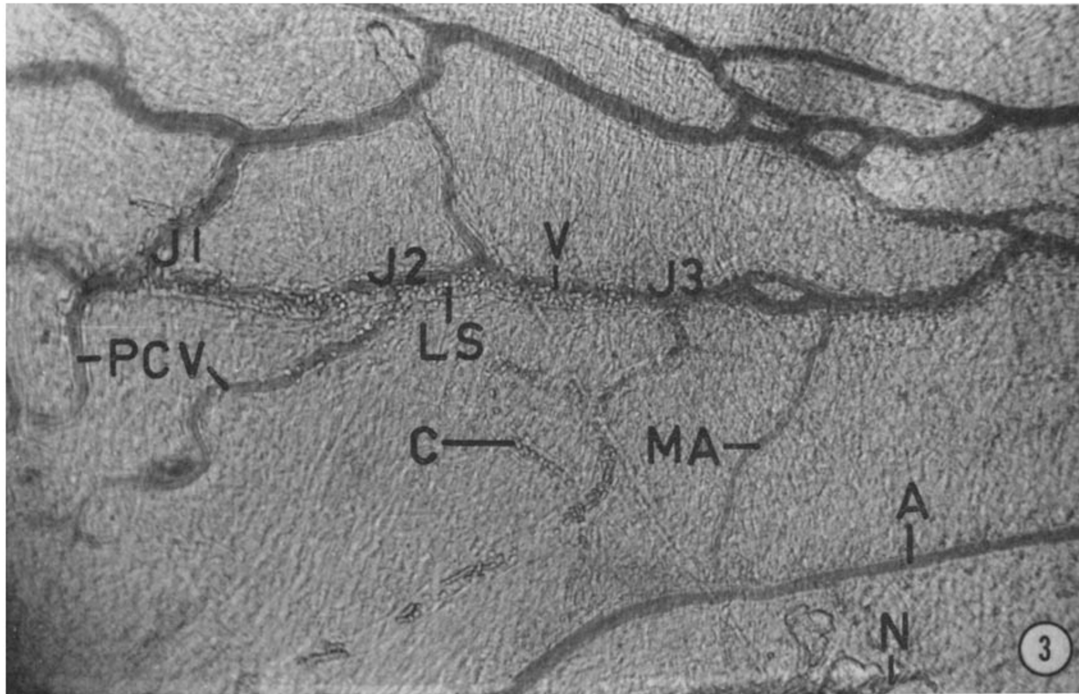


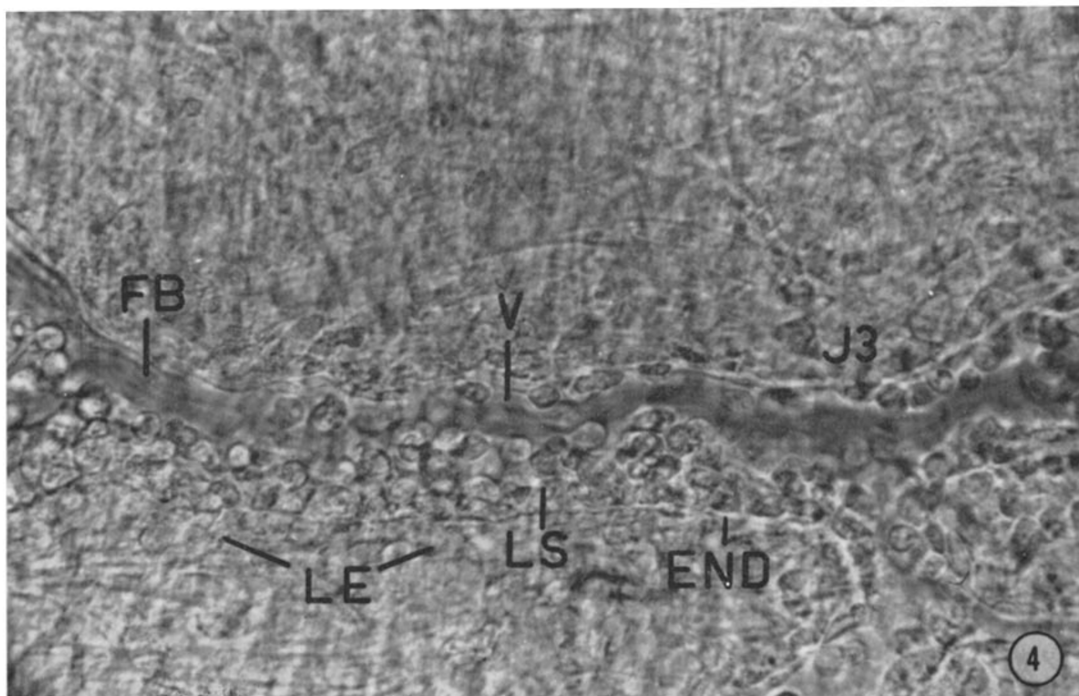
(Cliff: Acute inflammatory reaction and leukocytic migration)

Plate 53

Fig. 3. Same region as Figs. 1 and 2, 3 hr after the injection of Ab:Ag (CE) complex. The blood vessels are dilated compared to Fig. 1 and blood flow has increased considerably in rate as compared to Fig. 2. Leukocyte sticking (*LS*) is occurring in the venule (*V*) and is reasonably symmetrical in the sector *J1* to *J2*. Downstream of the junction *J2* where a postcapillary venule is draining blood from the region of the injection site into the venule (*V*) leukocyte sticking (*LS*) is more marked and highly asymmetrical with large numbers of leukocytes sticking to the venule wall on the side towards the injection notch (*N*). The sector of venule so affected extends almost to the junction (*J3*) but approaching this junction and downstream of it leukocyte sticking is less severe and loses its asymmetrical distribution. Other labeling as for Fig. 1. × 125.

Fig. 4. This is a higher power of part of the venule (*V*) in Fig. 3. The junction *J3* in Fig. 3 is present to the right of the figure. Details of the leukocyte sticking (*LS*) as described in Fig. 3 are more readily recognized at this higher magnification. The small depth of focus of the high power objective produces an optical section through the regions of leukocyte sticking (*LS*) and demonstrates that the leukocytes are sticking 3 to 4 deep to one another as well as to the endothelial lining (*END*) of the venule. The very severe leukocyte sticking has reduced the effective diameter of the vessel in which the flow of blood (*FB*) is occurring to approximately one half. Numerous emigrated leukocytes (*LE*) are present in the paravascular regions. × 500.



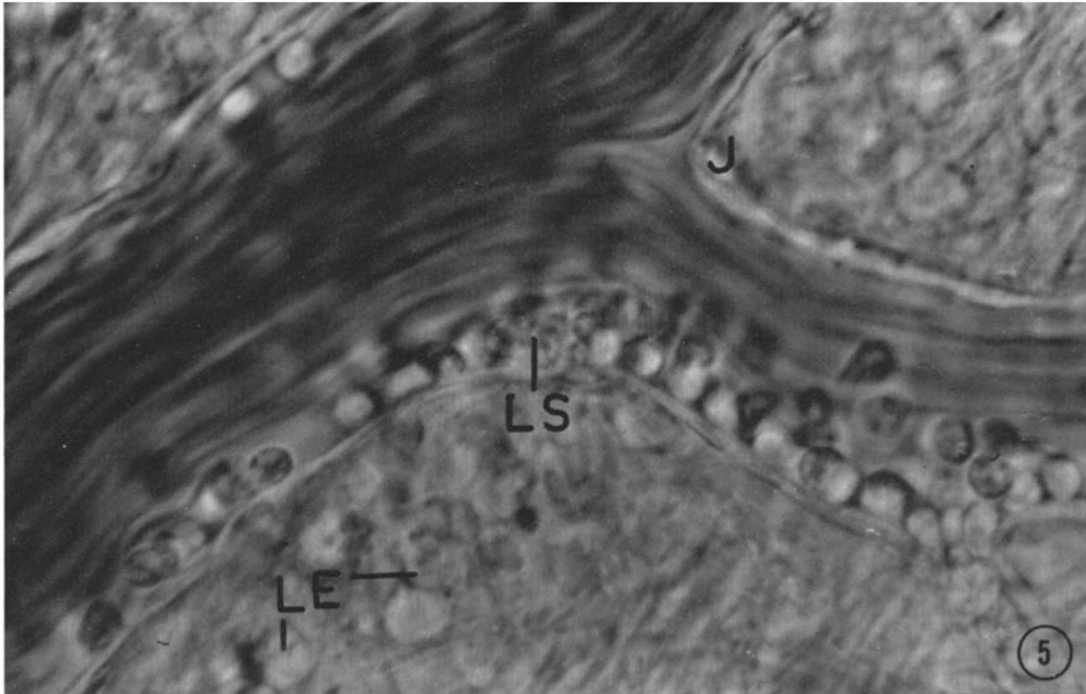


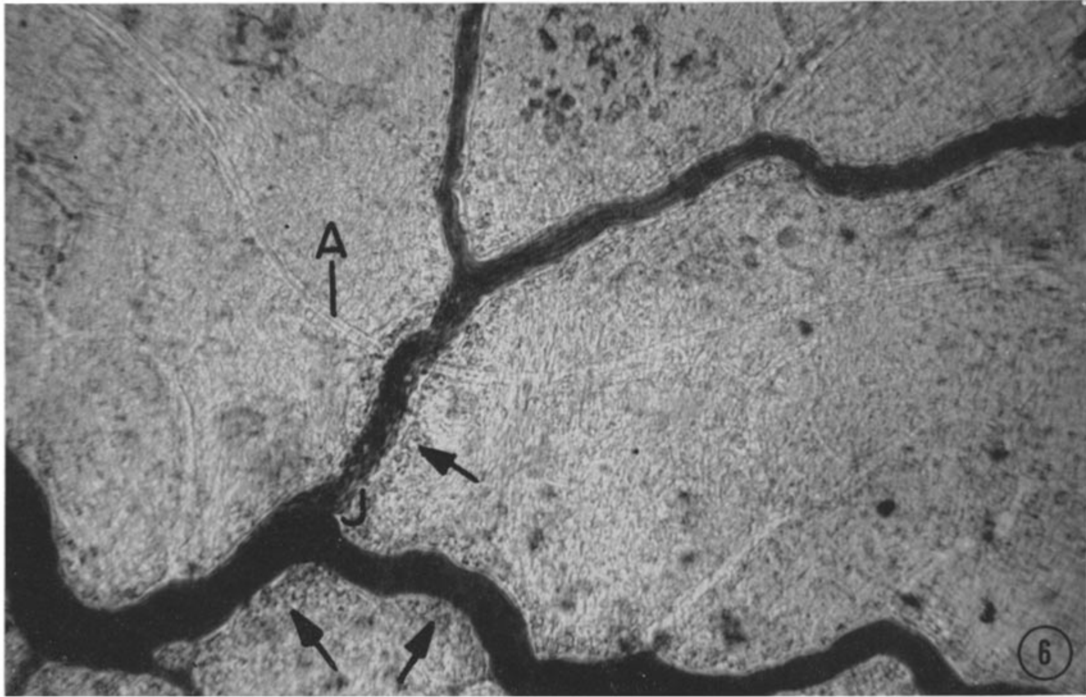
(Cliff: Acute inflammatory reaction and leukocytic migration)

Plate 54

Fig. 5. A junction (*J*) in a large venule system in which the blood is flowing from the top and the right to the lower left of the figure, 5¼ hr after the injection of Ab:Ag (OA) complex into a rabbit ear chamber. The lower limb of the junction is draining from the injection region. Leukocyte sticking (*LS*) is occurring on only one side of this tributary and extends downstream on the wall of the large venule on the same side that the affected tributary enters. Emigrated leukocytes (*LE*) are present in the paravascular region. × 800.

Fig. 6. A low power view of the same region of the rabbit ear chamber as Fig. 5 including the junction (*J*) in the venular system 1 day after the injection of Ab:Ag complex. Paravascular accumulations of leukocytes are present and are most marked (arrows) on the sides of the vessels towards the injection notch. *A*, arteriole. × 125.



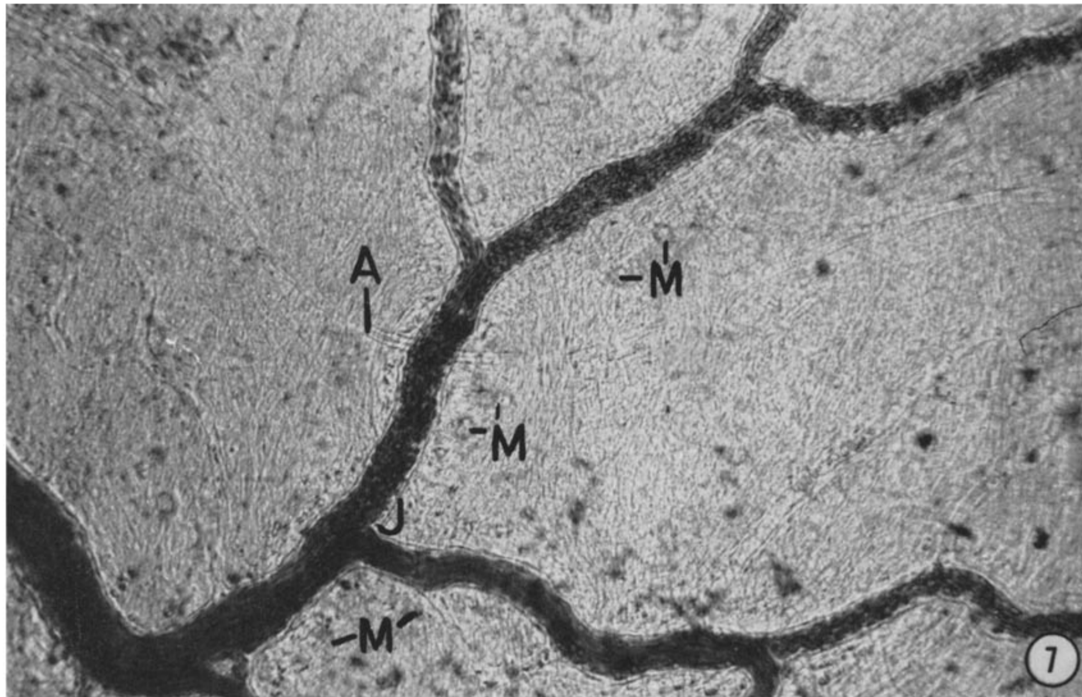


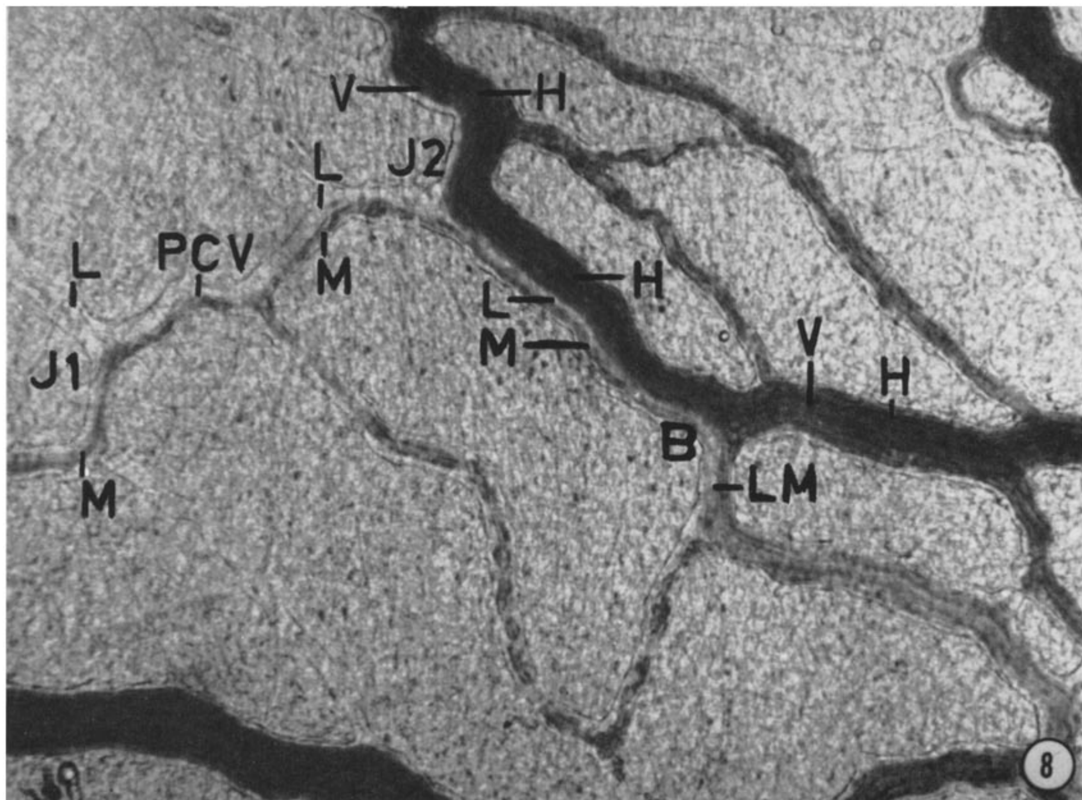
(Cliff: Acute inflammatory reaction and leukocytic migration)

Plate 55

Fig. 7. Same field as Fig. 6, 3 days after injection of Ab:Ag complex. The paravascular accumulations of leukocytes are no longer present and numbers of additional macrophages (*M*) can be identified related to the vessels. Other labeling as for Fig. 6. × 125.

Fig. 8. Venous system in a rabbit ear chamber in which blood is flowing from the upper and left side to the lower and right side of the figure. At *J1* a postcapillary venule (*PCV*) arises by the junction of two vessels the upper one of which has a very low hematocrit (*L*) and the lower one has a moderate hematocrit (*M*). The confluent blood streams by virtue of their differing hematocrits (*L*, *M*) can be seen to remain unmixed throughout the length of this vessel from *J1* to *J2* where it joins a draining venule (*V*). The blood within the venule *V* upstream of the junction *J2* has high hematocrit (*H*) and downstream of this junction the blood stream remains separated into 3 regions of differing hematocrits corresponding to the high value (*H*) of the venule (*V*) upstream of the junction (*J2*) and the regions *L* and *M* corresponding to the low and moderate values within the postcapillary venule (*PCV*). At *B* a branch is given off by the venule *V* and the *L* and *M* streams which have mingled by this stage are diverted into this branch (*LM*), leaving the high hematocrit (*H*) stream to follow the more direct course of the venule *V*. × 185.





(Cliff: Acute inflammatory reaction and leukocytic migration)