The effect of Histamine and Serotonin on Rat Mesenteric Blood Vessels In Vivo

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HISTAMINE AND SEROTONIN, mediators of increased vascular permeability, cause gaps to appear between the endothelial cells of venules, the capillaries being relatively spared.[1,2] In seeking an explanation for this phenomenon, Rowley,[3] having noted that histamine, serotonin, bradykinin, and 48/80 injected into rat skin caused a local constriction of veins, proposed that the separation of the venular endothelial cells which leads to increased permeability is due to an increase in the intravenular pressure consequent on an obstruction to flow caused by the venous constriction; in other words, venous constriction leads to damming up of blood in venules which thereby become so distended that eventually they leak by literally bursting at the seams. The sparing of capillaries was explained in terms of Laplace's law applied to cylindrical vessels: $T = Pr$, where T is the intramural tension or tangential stretching force tending to separate endothelial cells from one another, P is the outward distending pressure (intraluminal pressure less the surrounding tissue pressure), and r is the vessel radius. Thus, according to Rowley's hypothesis, the tendency for venular rather than capillary endothelial cells to separate at any given intraluminal pressure is explained by the greater tangential stretching force in the walls of the vessels of larger radius.

In the present work we have set out to reappraise Rowley's [3] hypothesis. To this end we have investigated the effects of histamine and serotonin on the caliber, rates of blood flow in, and permeability of, blood vessels in the rat mesentery, using an in-vivo direct observational and cinephotomicrographic approach. In general, our findings support those of Majno and his collaborators [4,5] who studied the blood vessels of the rat and rabbit cremaster muscle. We have found that,

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following the application of histamine or serotonin, venular leakage occurs in the absence of venous constriction or slowing of blood flow and without dilation of the leaking vessels.

Materials and Methods

Male and female rats of the Sprague-Dawley strain weighing 150–200 g. were used. Animals were anesthetized with sodium pentobarbitone (Nembutal, Abbott Laboratories Pty. Ltd.), 3.0 mg/100 g body weight, injected subcutaneously 40 min prior to laparotomy.

In each experiment, a loop of distal ileum was withdrawn from the peritoneal cavity through a short midline incision in the shaved ventral abdominal skin. After finding a suitable vascularized area, the mesentery was kept moistened with Ringer's solution while the rat was laid on its left side and placed on a laminated wood and cork board shaped to fit on and be controlled by the microscope's mechanical stage. The extracted loop of ileum was then carefully pinned out over an elevated 1.5 in. diameter disc of Perspex located in a recessed hole in the wood and cork board; in this way the mesentery could be transilluminated by the substage condenser and examined with the microscope (Fig 1). In some experiments the networks of minute vessels in the mesenteric windows were covered with a coverslip of polystyrene sheet (Polyflex, Plax Corporation, Hartford, Conn) cut to an appropriate shape with scissors.

To demonstrate pathologic leakiness in small vessels, we followed the technique of Majno, Palade and Schoefl.[2] Colloidal carbon black (Batch C11/1431a, Gunther-Wagner, Pelikan Werke, Hannover, Germany), in a dose of 0.1 ml/100 g body weight, was injected into the rat's lateral tail vein. Deposits of carbon appeared between and outside the vascular endothelial cells at the points of leakage.

For testing the effects of histamine and serotonin, the rat was given intravenous colloidal carbon and the preparation was observed for 5 min to detect the presence of pre-existing vascular leaks. Having ascertained the suitability of the preparation, volumes of from 0.02 to 0.04 ml of the test solution were added to the tissue using a Mantoux syringe and fine gauge needle. In some experiments 0.04 ml of the solution was added dropwise directly to the exposed major ileal vessels. In other experiments, where the responses of the small blood vessels under the coverslip were to be studied, 0.02 ml of the test solution was placed on the mesentery at the edge of the coverslip which, being of low density, promptly rose and allowed the added fluid to run beneath by capillary attraction. In this way, the solution came into contact only with the small blood vessels beneath the coverslip, the major ileal vessels being spared.

The test solutions used were histamine acid phosphate (British Drug Houses Ltd.), 2 mg/ml, and serotonin creatinine sulfate (Upjohn Co.), 0.01 mg/ml, both made up in Ringer's solution just prior to each experiment.

Microscopic observations were made with a Leitz Ortholux microscope. Vascular changes were recorded cinephotomicrographically on 16-mm high-contrast document copying film (Agfa Agepan FF) using an Arriflex 16S movie camera. This camera was fitted with a DOM animation/time-lapse camera motor and, to facilitate accurate focusing during filming, an aerial-image focus/viewfinder and periscopic viewfinder attachment. Sequences were filmed at rates of from four frames per second to one frame per 5 seconds. Processed negative films were used to make photographic prints from single frames and to provide movie prints of whole sequences. These movie sequences were analyzed in detail with the help of a motion analysis movie projector (L-W Photo-Optical Data Analyzer 224A, L-W Photo Inc., Van Nuys, Calif.

Results

Vascular Pattern in Rat Mesentery

The ileal branches of the superior mesenteric artery radiate from the mesenteric root toward the ileum, where they divide into two branches which join with branches from neighboring ileal arteries to form arcades close to the mesenteric border of the ileum (Fig 2). The gradually tapering ileal arteries measure approximately 0.25 mm in diameter and are closely accompanied by somewhat larger caliber veins (approximately 0.75 mm in diameter) and, occasionally, by lymphatic vessels. Although these arteries and veins are surrounded by a narrow sheath of fatty tissue, they can be observed microscopically and changes in their calibers recorded photographically. Between these major vascular bundles, the mesentery appears as clear "windows" lined on either surface by a flattened mesothelium and containing connective tissue cells, a meshwork of collagen bundles, and in some areas, networks of small blood vessels linked to the ileal arteries and veins. The size and pattern of these networks vary, but they are always supplied by arteriolar branches from an ileal artery which connect, either through a network of capillaries or through short arteriovenous anastomotic channels, to venules which empty into an ileal vein. It is noteworthy that when the connection is by way of a capillary network, the transition in size from capillary to venule is very gradual and no sharp distinction can be made between the two types of vessel.

Control Experiments

In preliminary experiments it was found that with Ringer's solution alone applied to the mesentery there was usually no carbon labeling of vessel walls following the intravenous injection of colloidal carbon. When such labeling occurred, it was evident within 2–3 min. For this reason, as mentioned above, intravenous colloidal carbon was given 5 min before the test solution was applied to the mesentery so that preparations which showed prior carbon labeling of vessels could be discarded.

Histamine

Effects on Vascular Caliber and Blood Flow

When the histamine solution (2 mg/ml) was added to the major ileal arteries and veins, no measurable change in vascular caliber or rate of blood flow occurred (Fig 3 and 4). When the histamine solution was applied to the networks of small blood vessels within the mesenteric windows, there was no consistent change in the caliber

of arterioles, capillaries, or venules. In most instances they appeared to remain constant (Fig 5 and 6), in which case there was no discernible change in the rate of blood flow. In some instances, however, there was a just discernible dilatation of arterioles with a consequent increase in the rate of blood flow and a moderate dilatation of capillaries and venules. In no instance was dilatation of venules accompanied by a slowing of blood flow.

Effect on Vascular Permeability

Following the application of the histamine solution to the mesentery at the edge of the coverslip, no apparent change occurred for some 30–60 sec; but then, over the succeeding 15–20 min, carbon labeling in the walls of leaking vessels developed progressively (Fig 5–8). The carbon deposits in the side and upper walls of the vessels could be seen distinctly; those on the lower walls were seen less clearly owing to the stream of flowing blood cells. At first the carbon deposits were small spots, lines, or smudges but these gradually increased in both size and density. Some areas of blackening remained discrete, but not uncommonly adjacent areas coalesced and in some instances this resulted in dense labeling of an entire vascular segment (Fig 9).

Labeling of this kind occurred predominantly in the walls of venules. Arterioles were never involved (Fig 5–9). Capillaries were always affected, but to a far lesser extent than venules. However, in both venules and capillaries, the intramural carbon deposits were extremely patchy (Fig 9 and 10). Thus, within affected vascular networks, it was common to find heavily labeled vascular segments adjacent to segments which were either sparsely labeled or unlabeled (Fig 9). These findings were the same whether or not the histamine solution came into contact with the major ileal vessels.

Serotonin

Effects on Vascular Caliber and Blood Flow

Application to Ileal Artery and Vein. When the serotonin solution (0.01 mg/ml) was applied to the vascular bundle containing the ileal artery and vein, there was no apparent change for 15–20 sec; but then the artery rapidly contracted to approximately 20% of its original diameter—eg, from 250 to 50 μ (Fig 11 and 12). In some instances the artery showed irregularities of caliber due to focal constrictions; in other instances it appeared uniformly narrowed. Unless the serotonin was irrigated away with Ringer's solution, the artery remained in spasm for 30 min or longer. Within 30–60 sec of the arterial contraction, the

accompanying vein showed a contraction which resulted in narrowing to approximately 80% of its original diameter—eg, from 750 to 600 μ (Fig 11 and 12).

As a result of the contractions of these larger vessels, there was complete or near complete cessation of blood flow through the small vessels forming the vascular networks in the mesenteric windows. However, this interruption of blood flow was not accompanied by dilatation of capillaries or venules or by any change in caliber of the small vessels.

Application Limited to the Minute Vessels. In most instances when the serotonin solution was applied to the edge of a coverslip lying within a mesenteric window so that the solution did not come into contact with the major ileal artery or vein (but only with the network of small blood vessels beneath the coverslip), there was no detectable alteration either in vascular caliber or in rate of blood flow in any of the network vessels (Fig 13 and 14). Occasionally, however, as with histamine, there followed a just detectable arteriolar dilatation with an accompanying increased rate of blood flow and minor capillary and venular dilatation. In no instance was there any slowing of blood flow.

Effect on Vascular Permeability

As just described, when the serotonin solution was applied to the ileal artery and vein (as well as to the network of minute vessels beneath the coverslip), flow within the vascular network became extremely sluggish or ceased altogether. Under these circumstances, although clumps of carbon were sometimes seen within the vessel lumen, carbon labeling of the vessel walls always failed to develop. However, when the serotonin solution was applied carefully to the mesentery at the edge of the coverslip so that the solution came into contact with only the small blood vessels beneath the coverslip, carbon labeling of vessels developed in the same way as occurred with histamine (Fig 15–18). Again, as with histamine, although the labeling was predominantly venular, it was not exclusively so, and, whether affecting venules or capillaries, it was characteristically patchy in its distribution.

These results have been illustrated in a cine film.[6]

Discussion

The present experiments show that the increased permeability of venules and capillaries caused by histamine and serotonin occurs in the absence of venous constriction. Whenever histamine was applied to the vessels of the rat mesentery, there was no discernible change in the caliber of either artery or vein or any slowing of blood flow;

nevertheless, widespread leakiness in venules and capillaries always followed. When serotonin was applied to the exposed mesenteric surface, there was prolonged spasm of the major ileal branches of the superior mesenteric artery and vein. This caused extreme slowing or cessation of blood flow in all mesenteric vessels, but, since the arterial contraction was much greater than the venous, no distention of venules or capillaries followed. In any event, under these conditions of sluggish flow, intramural carbon deposition failed to occur. But significantly, when serotonin was applied only to the minute vessels located beneath a coverslip within the mesenteric windows in such a way that it did not come into contact with the larger arteries and veins, widespread pathologic vascular leakiness occurred despite the absence of slowed blood flow or significant venular dilatation.

These findings, confirming those of Majno and La Gattuta [4] and Majno, Gilmore, and Leventhal,[5] run counter to Rowley's [3] proposal that the increased venular permeability caused by mediators such as histamine and serotonin is primarily due to a pronounced venous constriction which leads to damming up of blood in venules whose walls eventually leak by bursting. True, a rise in venous pressure increases the rate of fluid loss by transudation [7] and if, in a particular species or anatomic situation a mediator caused venous constriction, this could result in an increase in the rate of fluid leakage. However, the question we have attempted to answer is whether the abnormal separation which occurs between the endothelial cells of mediator-treated vessels is *necessarily* the result of a raised intravascular pressure stretching the vessel wall. The present results indicate that the mediators tested have a direct effect on the vessel walls which is quite independent of any effects they may have on the local hemodynamic situation. This was seen most clearly in those experiments where serotonin, coming into contact with only the minute vessels, caused widespread leakiness in venules which showed no measurable alteration in caliber. It is therefore quite clear that venous constriction and venular dilatation are not necessary steps in the development of abnormal leakiness in venules.

A further point in relation to Rowley's [3] hypothesis is that in the present experiments, although abnormally leaky vessels were predominantly venules, capillaries too were affected. Thus, notwithstanding the application of Laplace's law to this situation (for a given intraluminal pressure, the intramural tension, and hence tension on interendothelial cell junctions, will be greater in the larger radius venules than in the capillaries), it appears that, if significant, this factor is greatly overshadowed by the direct effects of chemical mediators on the endothelial

cells and their junctions. This conclusion, obtained by using blood vessels of the rat mesentery, is in complete accord with that of Majno and La Gattuta [4] and Majno, Gilmore, and Leventhal,[5] who made an in-vivo study of the vessels of the rat and rabbit cremaster. In spite of differences in the type of tissue studied and in the magnitude of the changes in vascular caliber and blood flow observed by the two experimental groups, neither group has found the venous constriction reported by Rowley.[3]

Since Rowley's [3] hypothesis appears no longer tenable, it seems reasonable to speculate a little on other possible mechanisms of the permeability-increasing action of histamine and similar drugs. Here, unfortunately, the evidence is sparse; but two observations in the relevant literature seem to be beyond dispute. First, Majno and Palade's [1] finding that the leakiness of affected vessels can be explained in terms of the gaps which they noted between vascular endothelial cells; and second, Majno, Palade, and Schoefl's [2] observation that these leaks have a curious and quite characteristic distribution, being located predominantly in the walls of venules. Clearly, any suggested mode of drug action must be compatible with these observations.

Regarding the characteristic distribution of the leaks, we would like to suggest that the pattern of leakiness may possibly be related to two observations from the physiologic literature. First, Rous, Gilding, and Smith's [8] observation that minute blood vessels of mammals exhibit a "gradient of permeability" such that permeability to large dye molecules increases from the arteriolar end of capillaries to the distal end of the venules. Second, Landis' [9] finding that high molecular weight albumin-bound dye (T1824) injected intra-arterially into the minute blood vessels of the frog mesentery, rapidly leaked from capillaries and venules at very discrete points which, though irregularly distributed over capillaries and venules, were both larger and more concentrated in the venules. Landis [9] and Landis and Pappenheimer [10] interpret these findings as providing additional evidence in favor of the concept of large pores, structures which earlier had been postulated by Grotte [11] and Mayerson *et al* [12] to account for their data on the passage of large molecules across the walls of capillary beds. Now, although the question of how large molecules pass across the walls of small blood vessels is still controversial, we are struck by the seemingly close parallel between the estimated frequency and distribution of the postulated physiologic large pores,[9-11] on the one hand, and the frequency and distribution of histamine-induced leaks, on the other. It has not been possible to make an accurate assessment of the overall concentration of hista-

mine-induced leaks, but examination of our photomicrographic records suggests that their frequency is of the same order of magnitude as the estimated frequency of large pores [10,11] (3/100 μ^2 of vessel surface). At the same time, being located predominantly in the venules, the distribution of the histamine-induced leaks appears generally similar to that of the dye-disclosed physiologic leaks which Landis [9] has ascribed to the large pore system. Therefore, although the evidence is no more than suggestive, these interesting similarities prompt us to propose that the pathologic interendothelial cell leaks occur preferentially at the sites of the postulated large pores.

Besides the problem of accounting for the location of the leaks, however, there is the question of how histamine and similar agents act to produce the endothelial cell separations which form the structural basis of pathologic leaks. It is well established that endothelial cells, along with other cell types, are actively motile in vitro [13–16] and, after certain pathologic stimuli at least, they appear to contract in vivo.[17] Accordingly, we agree with Majno and Leventhal [18] and Majno, Gilmore, and Leventhal [5] on the likelihood that histamine and similar agents cause endothelial cells to contract. However, we would like to propose that the permeability-increasing drugs have their greatest effect on those endothelial cells which border the postulated large pores. Here, we suggest, the drug could have two actions; first, loosening the attachments of the margins of the endothelial cells bordering the large pores so that they separate from one another and from the underlying basement membrane; and second, causing an active contraction (retraction) of the bordering endothelial cell margins. The two actions combined could then cause enlargement of the pore to the extent that a pathologic leak could develop.

Summary

To study the effects of histamine and serotonin on the blood vessels of the rat's mesentery, an in-vivo cinephotomicrographic technique was used to monitor changes in vascular caliber, blood flow, and increased permeability. Indicating abnormally increased permeability, histamine induced the deposition of intravenously injected colloidal carbon particles in the walls of venules and capillaries; concomittantly there was no change in the caliber of arteries or veins and no slowing of blood flow or any consistent alteration in the vascular caliber of the minute vessels—ie, arterioles, capillaries, and venules. Serotonin produced spasm of the large vessels, arteries more than veins, to such an extent that blood flow ceased in the minute vessels and no carbon labeling oc-

curred. However, when serotonin was applied only to the minute vessels, widespread venular and capillary leakage developed despite the absence of slowed blood flow and venular distention.

These findings indicate that increased venular permeability occurs in the absence of venous constriction and therefore support the view that the histamine-type mediators of increased permeability induce vascular leakage by exerting a direct effect upon endothelial cells or their junctions rather than by raising intravenular pressures. It is proposed that their prime mode of action is to retract the margins of endothelial cells which border large pores in the vessel wall.

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Legends for Figures

Fig 1. Method for making microscopic observations of exteriorized rat mesentery. Anesthetized rat lies on its left side on a laminated wood and cork board which is supported by the microscope stage. Mesentery of the distal ileum is laid out over a raised Perspex disc which fits in a recessed hole in the wood and cork board; bowel is held in position by pins, and mesentery is transilluminated by the microscope condenser. A thin plastic coverslip is located over a network of minute blood vessels within one of the mesenteric windows.

Fig 2. General arrangement of blood vessels supplying the distal ileum *(i)* and networks of minute vessels *(arrows)* which occupy mesenteric windows *(w)*. Extending out from root of the mesentery are paired arteries *(a)* and veins *(v)* which, within sheath of fatty tissue, divide to form vascular arcades which follow the mesenteric border of the ileum. From these major ileal vessels arise large branches supplying the distal ileum and minute branches supplying the mesenteric windows. These minute arterial and venous branches supplying the mesentery are linked to one another through networks of capillaries and venules; larger vessels of these networks can be seen in two mesenteric windows. A plastic coverslip overlies one such network.

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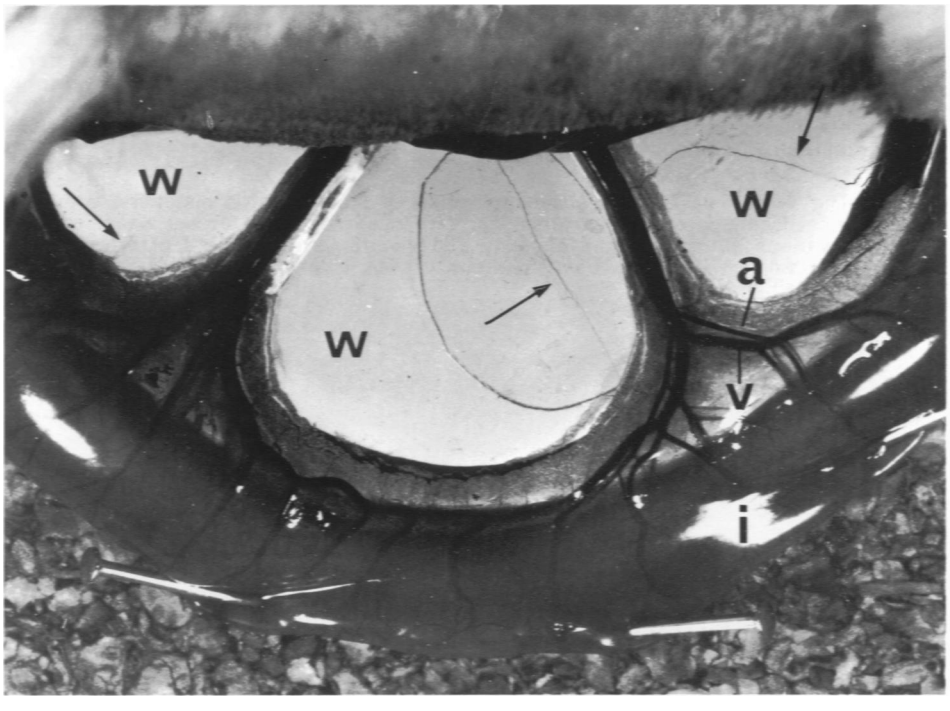

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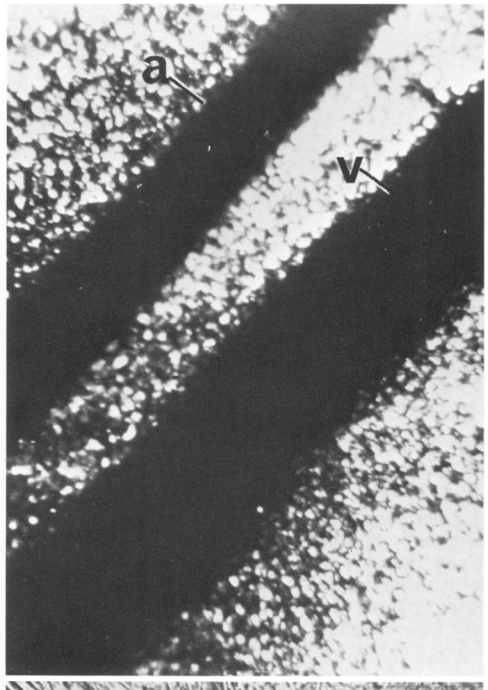
Fig 3. Very low magnification photomicrograph of fat sheathed ileal artery (*a*) and vein (*v*) before histamine solution was applied. × 25.

Fig 4. Same artery and vein 2 min after application of 0.04 ml of histamine solution, 2 mg/ml. No significant alteration in the caliber of either artery or vein occurred. × 25.

Fig 5. Low power photomicrograph of portion of network of minute blood vessels in a mesenteric window before application of histamine. Arteriole (*a*) and venule (*v*) are seen traversing thin space between mesothelial layers lining the mesentery. Elsewhere within this space are bundles of collagen fibers which give rise to a feltwork pattern. Since blood cells within the vessel lumen are moving rapidly, they produce a blurred image which gives the impression of a moving stream. Note absence of intramural carbon deposits. × 100.

Fig 6. The same field 21 min after application of 0.01 ml of a histamine solution, 2 mg/ml. Black deposits of colloidal carbon, localized within vessel walls, can be seen at various sites along the capillary and venule (*arrows*); walls of arteriole are free of carbon. Note that caliber of various vessels is unchanged. × 100.



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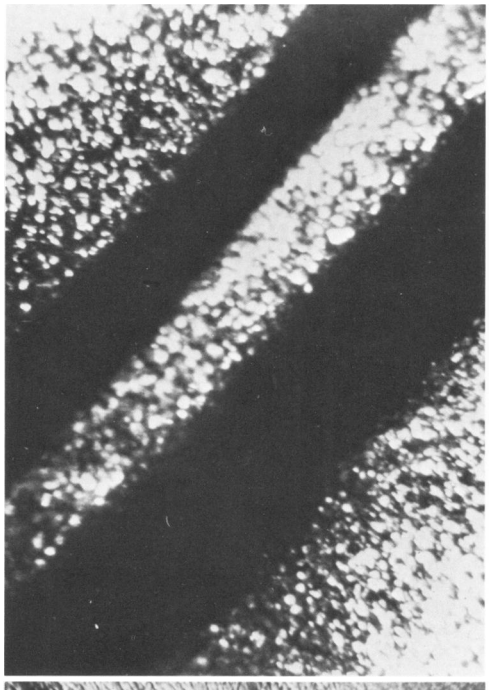

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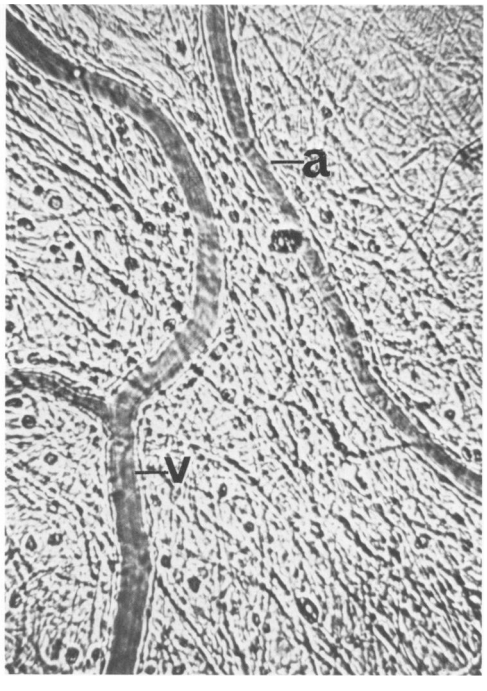

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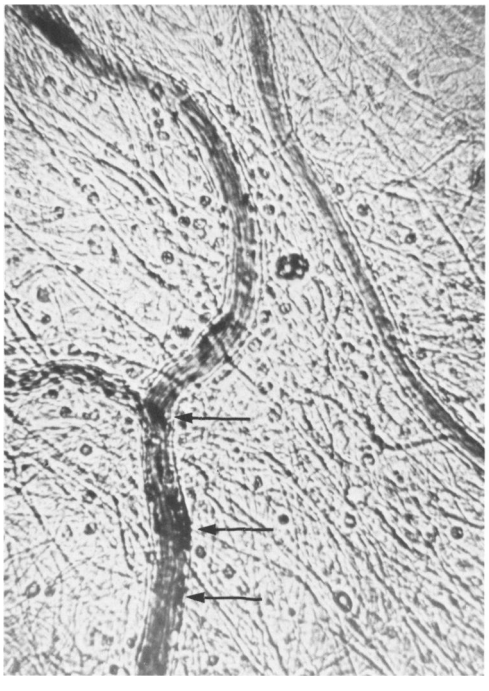

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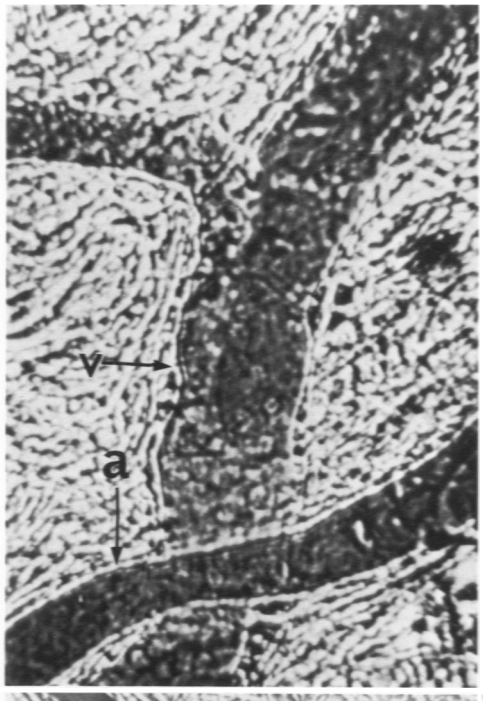
Fig 7. Higher magnification view of mesenteric venules (*v*) and arteriole (*a*) prior to the application of 0.04 ml of histamine solution, 2 mg/ml. × 270.

Fig 8. The same field 7½ min after applying histamine solution. Circulating colloidal carbon, leaking from vessel lumen at discrete loci, has formed dense black deposits which, in some instances, can be seen against background of moving blood stream. Deposits vary in size and shape and are distributed somewhat irregularly over blood vessel wall. In some places carbon deposits appear to have spread longitudinally outside vessel wall (*arrows*). Walls of arteriole have remained free of carbon deposits. × 270.

Fig 9. Low power microscopic view of portion of network of minute vessels 25 min after the application of histamine solution, 2 mg/ml. Carbon deposits are extremely patchy in distribution. In one vascular segment, deposits were so numerous or extensive that they coalesced to make the segment appear completely black (between *arrows*); in adjacent vascular segments the carbon labeling has been much lighter so that one sees only small black spots and streaks in side walls and in wall overlying the moving stream of blood. Again, note that the arteriole (*a*) remains unlabeled. × 125.

Fig 10. Detail of intramural carbon deposits as seen in a venule 25 min after histamine solution, 2 mg/ml, was applied to the mesentery. Deposits which have occurred at irregularly located but discrete loci can be seen against the moving stream of blood. Note that amount of carbon which has leaked out varies considerably from one place to another. × 250.



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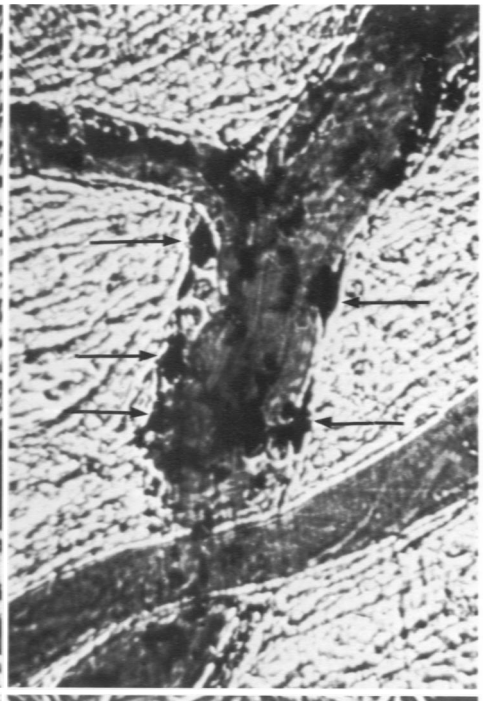

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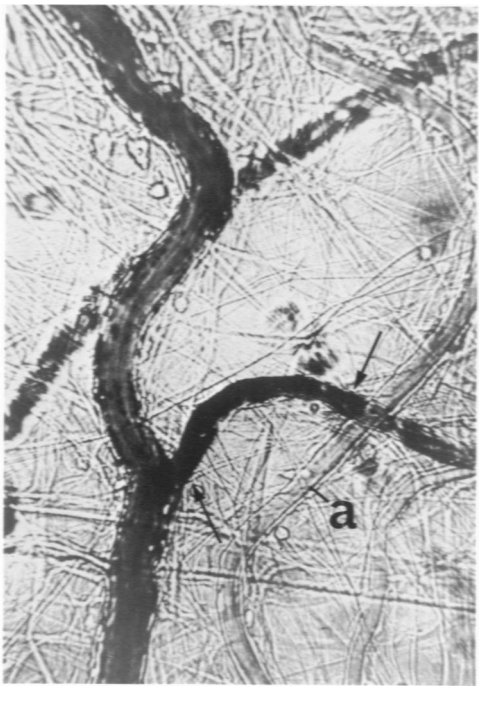

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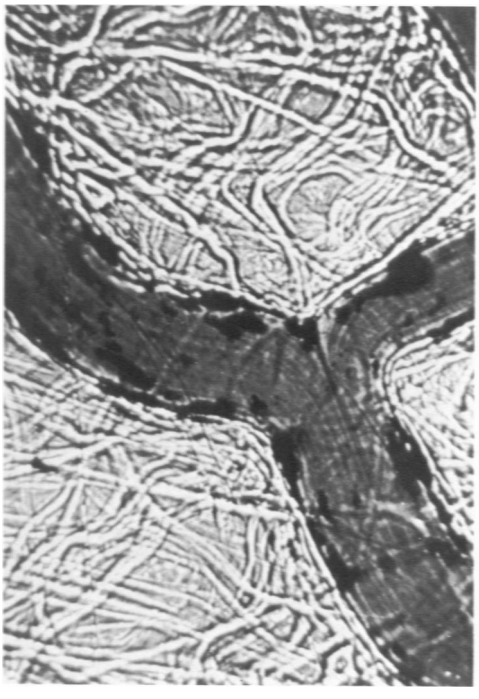

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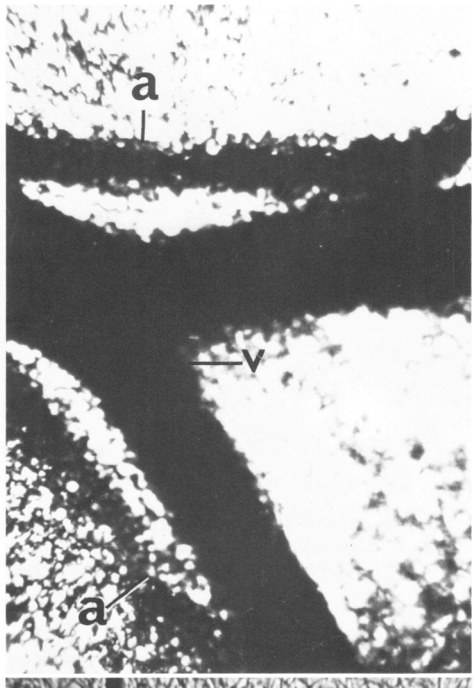
Fig 11. Fat-ensheathed artery (*a*) and vein (*v*) at point of bifurcation just proximal to mesenteric border of the ileum. This very low power photomicrograph was taken before application of serotonin, 0.01 mg/ml solution. × 25.

Fig 12. Same field 1.5 min after applying serotonin solution. Artery is greatly contracted and, due to light scattering caused by surrounding fatty tissue, it appears to have almost disappeared (*arrows*). By contrast, the vein is but slightly contracted. × 25.

Fig 13. Low power photomicrograph of portion of network of minute vessels in the rat mesentery before 0.02 ml of serotonin, 0.01 mg/ml solution, was added to the mesentery at margin of coverslip in such a way that serotonin did not contact any major ileal vessels. × 60.

Fig 14. Blood vessels of the same vascular network 2 min after serotonin solution was added. Caliber of venule appears unchanged. × 60.



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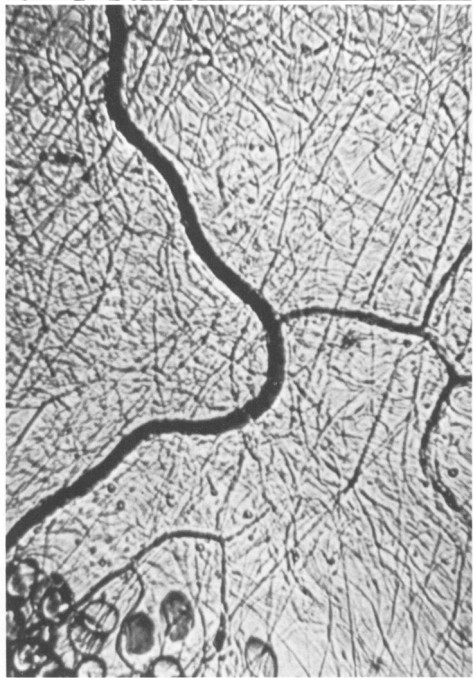

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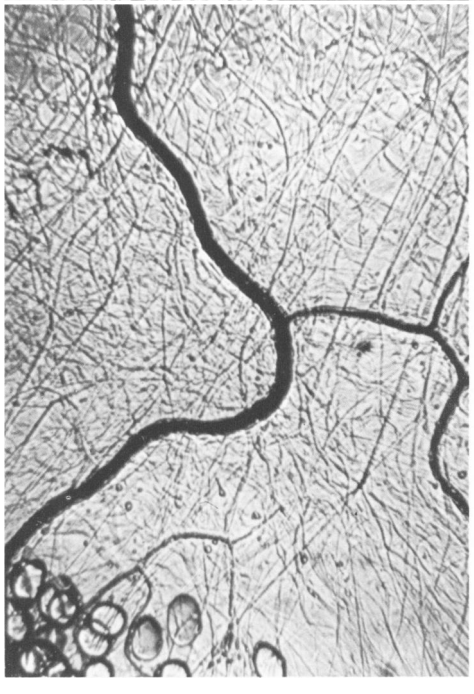

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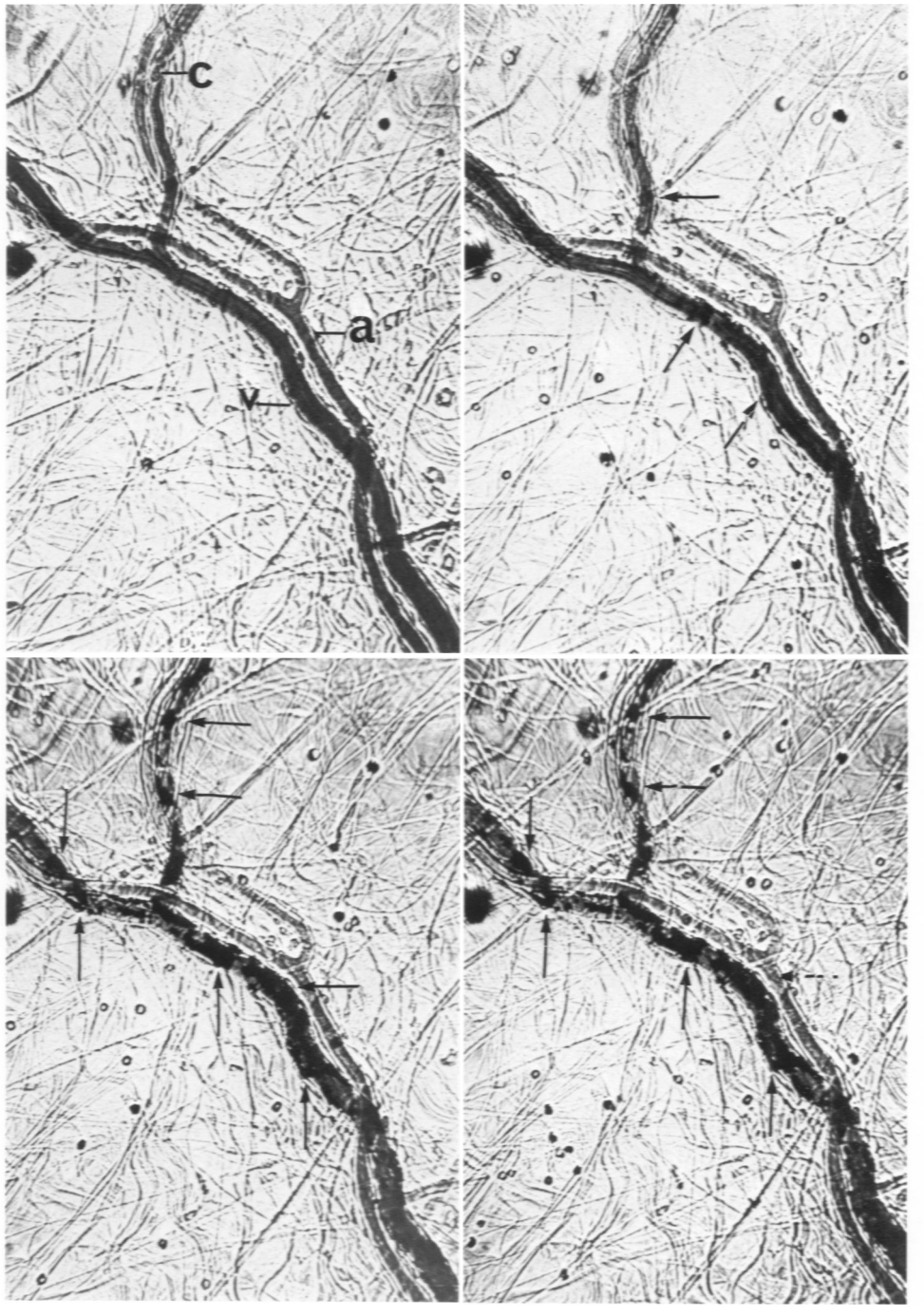
Fig 15–18. Series of photomicrographs showing progressive buildup of carbon deposits in walls of capillaries and venules of a mesenteric vascular network subjected to 0.02 ml of serotonin solution, 0.01 mg/ml, applied so that none reached the major ileal vessels. × 250.

Fig 15. Capillary (*c*), venule (*v*), and branched arteriole (*a*) prior to application of serotonin.

Fig 16. Same field 2½ min following application of serotonin. Intramural deposits of colloidal carbon have begun to appear in the capillary and the venule (*arrows*).

Fig 17. Earlier deposits have increased in size 15 min after applying serotonin, and there are additional deposits which have also developed to considerable size (*arrows*).

Fig. 18. Intramural deposits of carbon (*arrows*) do not appear to have further increased in size or density 18 min after adding serotonin. Note that arteriole wall has remained free of carbon deposition.



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