Cerebral Ischemia

III. Vascular Changes

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IN THE PREVIOUS PAPERS of this series [1,2] it was shown that if the blood flow to a rabbit brain is interrupted for 5 min. or longer and then restored, certain areas will continue to remain ischemic ("no-reflow phenomenon"). The obvious inference is that an obstacle to flow has developed during the ischemic period. In searching for possible vascular obstructions, it was shown that blood clotting and platelet thrombosis were probably not significant factors; a narrowing of the vascular lumen was more likely than a complete obstruction.[2] A preliminary account of the nature of the vascular obstruction has been published; [4,5] in this paper we will present a further study at the level of light and electron microscopy.

Materials and Methods

Light Microscopy

The brains studied by light microscopy were taken from the animals described in a previous paper.[2] Cerebral ischemia had been produced for 0, 2.5, 5, 7.5, 10, or 15 min., and the rabbits were killed either immediately or after a delay of 30 min. Just prior to death, the brains were perfused with carbon black; hence the areas of no reflow stood out clearly as white tissue against a gray-black background (they will be referred to hereafter as white areas).

The brains were fixed by immersion in 10% buffered formalin for 1 week. Twenty-four blocks of tissue from control and test brains were embedded in paraffin and sectioned. On the assumption that random sampling might miss a discrete, localized vascular obstruction, 6-μ serial sections were prepared of 2 white areas 4–5 mm. in diameter which had been excised with about 5 mm. of the surrounding tissue. The white areas were characterized by the presence of blood in the capillaries; because of this, the sections were stained routinely with Wright's blood stain, which produces a good contrast between the erythrocytes and the background.

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Electron Microscopy

The 14 rabbits studied by electron microscopy were divided into 3 groups. Four of the 6 control rabbits in Group I were submitted to the preparatory surgical procedures, including section of the basilar artery; the brain was then perfused with carbon black and glutaraldehyde as described below. The 2 other rabbits were simply anesthetized and perfused, without surgical procedures. The 5 rabbits in Group II were submitted to ischemia for 15 min. followed by immediate perfusion. The 3 rabbits in Group III underwent ischemia for 15 min. followed by delayed perfusion. After the cuff was released, these animals were allowed to survive for 30 min., with artificial respiration and maintenance of the blood pressure with Levophed (Winthrop Laboratories).[2] Perfusion was then accomplished as usual.

At the time of sacrifice we sought to fill all patent cerebral vessels with carbon black and at the same time to fix the brain for electron microscopy by intravascular perfusion with glutaraldehyde. It was not feasible to mix the glutaraldehyde with the carbon black beforehand, since the carbon failed to remain in suspension in such a mixture. Hence a double infusion was carried out as follows. Carbon black was allowed to flow into the carotid artery through a needle connected by tubing to a container 160 cm. above the animal (equivalent to a pressure of 120 mm. Hg). Concentrated glutaraldehyde (30% in H_2O) was injected into the stream of carbon as close as possible to the carotid artery, using a glass syringe and a fine needle inserted into the tubing. The fixative was injected at such a rate as to obtain an approximate concentration of 5% in the carbon black. The infusion was continued until the animal died, and 1–2 min. thereafter; usually 30 ml. was administered in this manner into each carotid artery. The head of the rabbit was rapidly cut off, and the calvarium was opened with a bone rongeur while fixative was poured onto the exposed brain. The fixative was 6.5% glutaraldehyde in cacodylate buffer with 3% sucrose.[3] The brain was then removed, immersed in the fixative, and cut into slices 2–3 mm. thick. After 30–40 min., these slices were firm enough to be cut into cubes about 1 mm. on edge. Samples were taken from the cortex only; the surface was examined to determine the degree of perfusion with carbon black, and the blocks were separated according to their origin from "black" (perfused), "white" (nonperfused), and "gray" (intermediate) areas. Total fixation time in glutaraldehyde was 2.5–3 hr. The tissues were postfixed in 2% OsO_4 in veronal buffer without sucrose, embedded in Epon 812, cut with a glass or diamond knife on an LKB Ultrotome, stained with uranyl acetate and lead citrate, and examined in a Philips 200 electron microscope.

Results

Light Microscopy

Relatively few changes could be detected with the light microscope following these brief periods of ischemia, even when the animals were allowed to survive for 30 min. The parenchyma in the test brains did not differ significantly from that of the controls. A consistent observation in the animals made ischemic for 5 min. or longer was the presence of red blood cells in the capillaries of those areas which appeared grossly as white, i.e., in the areas of no reflow. These vessels stood out in contrast to those of the neighboring tissue, which were filled with carbon black (Fig. 1–4). A special effort was made to detect platelet thrombi as possible causes of vascular obstruction. Only one convincing image was found, in

a venule emerging from a white area; another possible platelet thrombus is shown in Fig. 2, where it appears as a negative image outlined by the surrounding carbon black.

While searching for thrombi we found an entirely different phenomenon. A number of carbon-filled vessels contained white lacunae which appeared as punched-out holes (Fig. 1–10). These were at first dismissed as artifacts, but closer examination showed that they represented spherical structures contained in the lumen. They were most numerous in animals that had been allowed to survive 30 min. after the ischemia. Their distribution was fairly clear-cut: they were few in the vessels of the well-perfused areas, and most numerous at the edge of the perfusion defects. Within the white areas they were more difficult to see, because they were no longer outlined by carbon. A few were also present in control brains, usually in large, deep-seated venules. All were optically empty; thus it was easy to distinguish them from the occasional leukocytes, which contained obvious nuclei (and were usually smaller). Their contours were sometimes indented (Fig. 4), but most of them were rounded (Fig. 3 and 4) and measured 10–30 μ in diameter. They were present in small venules, arterioles, and capillaries. In the latter they were oval—i.e., stretched along the axis of the vessel—suggesting that they were deformed by compression. Often they appeared to occlude the lumen (Fig. 5–10). In larger venules they were either free in the lumen or adjacent to the wall (Fig. 4). In some arterioles they were so numerous as to suggest that the lumen was filled with "foamy" material (Fig. 5–8). These intraluminal bodies, seen as negative images by light microscopy, correspond almost certainly to the "blebs" observed in the same tissues by electron microscopy, and described in detail below.

Electron Microscopy

Controls. In the 2 animals which were simply anesthetized and perfused, the tissue appeared relatively well preserved. Some fixation artifacts were obvious (especially occasional dissociation of myelin sheaths); but the blood vessels showed no abnormalities. A representative area is shown in Fig. 11 and 12. In the 4 rabbits which had undergone the preliminary surgery (occlusion of the basilar artery) there was some glial swelling, though never to the same degree as in the rabbits subjected to ischemia (see below). In 2 of the animals with ligated basilar artery, a few small blebs (see below) were also found; none, however, to compare with those seen in the experimental animals.

Ischemia Followed by Immediate Perfusion. The blocks taken from

black, gray, and white areas showed changes of increasing severity in that order. Apart from the absence of intravascular carbon, typical of the white areas, the findings were qualitatively the same for all tissues. A consistent change was the swelling of the perivascular glial cells. At relatively low powers of magnification this change manifested itself as a clear space surrounding all or part of a vessel; higher powers showed that this was not an interstitial space, but part of one or more glial cells, clearly surrounded by the profiles of unit membranes, either continuous or broken Fig. 13–16). Contained within this membrane were mitochondria, surprisingly well preserved (Fig. 14–16), and occasional other cytoplasmic organelles, which seemed to float in a very clear and presumably watery background. Some degree of cellular swelling was occasionally observed in cells removed farther from the vessels, but not to the same extent as in the perivascular glia. The capillaries themselves often appeared deformed or flattened, as if compressed by the swollen glia. Some of the images seemed clearly to indicate obstruction of the microvasculature (Fig. 13–16), the lumen being reduced to a very fine slit a fraction of a micron in diameter, and visualized primarily by the carbon it contained (Fig. 15).

The lumen of the capillaries contained carbon black, together with a few red blood cells, and other structures which will be referred to henceforth as blebs. These were circular profiles, surrounded by a membrane (similar to the cellular membrane) and usually free in the lumen (Fig. 16). Most of them appeared optically empty; some contained one or a few small vesicles, and, rarely, one of these vesicles contained what appeared to be phagocytized carbon. Figure 16 shows a capillary which appears to be compressed by the perivascular glia; the lumen contains a bleb, which seems to resist the compression. Occasional blebs were very large—i.e., larger than red blood cells—and, molded by the lumen of the vessel, assumed an oblong or sausage-like shape with bulging ends. In some instances the outline of the bleb was so thin as to be almost evanescent, but was made more obvious by adherent particles of carbon black (Fig. 18). The origin of the blebs was difficult to observe (see below); however, there were several instances in which the endothelial cells gave rise to blister-like structures (Fig. 19 and 20) which could be interpreted as early stages of the free blebs.

Apart from the formation of blebs, the walls of the vessels, as a rule, were not significantly altered. Occasional endothelial cells, however, were diffusely swollen and contained swollen mitochondria (Fig. 21). No definite gaps were found in the vascular walls; in a few instances it seemed that particles of carbon black were being spilled out of the lumen through a break in the wall, but in each case the nature of the break rather sug-

gested an artifact, possibly due to the mechanical act of cutting the tissue into small blocks.

Ischemia Followed by Delayed Perfusion. The findings were practically identical with those for the previous group, except that the blebs were more conspicuous and more frequent. In several instances the endothelial cells showed clear-cut "blisters."

Discussion

The aim of these experiments was to determine the nature of the obstruction responsible for the no-reflow phenomenon. Electron microscopy yielded 2 negative findings of significance: (1) There was no evidence of fibrin in the vessels, and (2) platelet thrombi were not seen. Both facts agree with our previous study.[2] To our mind, the most important positive finding was the swelling of the perivascular glia.

Considered per se, of course, this is scarcely a novelty. Glial swelling was an established entity long before the advent of electron microscopy.[4-6] Since then, it has been confirmed many times with the electron microscope in a variety of conditions: cold injury in the rat and mouse,[7] incubation of brain slices,[8] alkyl tin poisoning in mice,[9-12] simple craniotomy or intracaval injection of distilled water in the rabbit,[13] compression by an inflated balloon in the cat,[14,15] injection of PPD from the tubercle bacillus in the rat,[16] injection of cryptococcal polysaccharide in the rat,[17,18] dental drill wounds in the mouse,[19] implanted tumors in the rabbit,[20] KCN poisoning and repeated asphyxia "in mammals,"[21] HCN poisoning in rats,[22] anoxic-ischemic encephalopathy in the rat,[23] asphyxia in newborn rabbits,[24] and around human tumors.[9,25,26]

Glial swelling also occurs when tissue is improperly fixed, and the significance here is probably the same: occurrence of cellular disease, in this instance, in response to fixation. In our material some perivascular glial swelling did occur in the control brains removed from animals which had been submitted only to ligature of the basilar artery; the degree, however, was much less than that observed in the ischemic animals. It is of interest that the effect of ischemia on the perivascular glia could not be evaluated in the specimens fixed in formalin for light microscopy, because perivascular swelling occurred in all specimens including the controls, the result being a clear space around all blood vessels (Fig. 1–10). This artifact is so ubiquitous that it was once considered a normal feature of brain tissue ("Space of Held"[5,27]).

In most of the reported cases the swelling has involved predominantly the perivascular astrocytes. It would appear, therefore, that these cells are either especially sensitive to injury or unusually susceptible to swelling

in response to injury.[6,28] The mechanism may vary with the causative agent[29] (e.g., breakdown of the sodium transport system in the cell membrane, increase in membrane permeability, or specific disturbances of cell metabolism), but the final result is an increase in volume of the cytoplasm by uptake of fluid.

While it is clear that our finding of perivascular glial swelling is not new, we are not aware that it has been previously shown to occur so promptly following the onset of ischemia. For example, Hills[23,30] reported astrocytic swelling beginning 2 hr. after the ischemic insult. Furthermore, little or no attention has been paid to the critical significance that this change may have for the subsequent perfusion of the tissue involved. In previous studies it has been noticed that the capillaries are "dwarfed" by comparison with the surrounding swollen glial processes,[13] or that these "surround closely" the capillary wall;[21] it has also been speculated that the transport function of the astrocyte may be impaired.[23] But the possibility of glial cells impinging upon the capillary lumen, and thus reducing flow, seems to have been overlooked.[28,31,32] It has been proposed by Lindenberg[33] that any condition leading to an increase of intracranial pressure may lead to secondary ischemia of the brain by arterial compression. The vessels implied here are the larger cerebral arteries. Spector[34] has suggested that this might be one of the pathogenetic factors in anoxic-ischemic encephalopathy of the rat.[35] This may well be the case, but this phenomenon is not relevant to our experiments, since our animals (at least those examined immediately after ischemia) could not have had cerebral swelling, in the absence of circulation; and, indeed no evidence of swelling was seen on gross examination in any of the brains, even those examined 30 min. after the ischemia.

It is rather odd that the compression of the capillaries by glia should have received so little consideration in view of the fact that encroachment by swollen glia has been described with regard to all other cerebral components: axons in alkyl tin intoxication of the rabbit,[10] neuronal bodies in incubated brain slices,[8] and extracellular spaces in asphyxiated mice.[36] The blood vessels are, after all, the only readily compressible structure in the brain and hence should be easy victims for compression whenever another cerebral structure undergoes a rapid increase in volume.

A second type of capillary obstruction, seen by electron microscopy, was due to the cellular blebs (Fig. 16–20). These structures, also, are familiar in electron microscopy as a result of poor fixation; hence, it is again essential to consider this possibility in our preparations. Small blebs were indeed found in some blocks of 2 out of the 5 controls. But they were found in all 5 brains perfused immediately after ischemia, and large blebs

were found in all 3 brains which were perfused after a delay of 30 min. It is therefore reasonable to conclude that we are dealing—in this case also —with a true result of ischemia and not with an artifact. This is also suggested by the occasional finding of an "occluding" bleb with carbon on one side only, as if the bleb, present before perfusion, had wholly or partially impeded the perfusion.

Most of the blebs appeared free in the vascular lumen, but a few were connected to the surface of an endothelial cell (Fig. 19 and 20). Since the connecting stalk is small in relation to the final size of the bleb, it is bound to be missed in most of the sections; thus it is likely that most blebs arose from endothelial cells, but we cannot exclude the possibility that they also originated from other cells in the vascular lumen (leukocytes and possibly platelets, but probably not erythrocytes).

Blebbing has been observed by phase microscopy in many types of cells, following exposure to a variety of adverse conditions such as anoxia, chemical toxins, antibody and complement, or various forms of radiations.[37–39] Though the mechanism of blebbing is not known, the final result is a ballooned cellular projection which may eventually become detached and float away. Bleb-like structures have been reported arising from the endothelium in anoxic-ischemic rat brains examined several hours after the insult.[30] Buckley produced localized injuries in rabbit ear chambers, and observed the formation of blebs from the surface of interstitial as well as intravascular cells.[40,41] Thus, there is no doubt that they can form in living tissues.

Since the largest blebs seen by electron microscopy were somewhat larger than red blood cells, they should have been visible by light microscopy. This was the case. They were generally larger in the histologic sections than on electron micrographs, presumably because further swelling had taken place during formalin fixation. It seems likely that they would present an obstacle to flow, especially when still attached to the surface of the endothelium. Sometimes they were so numerous within a small vessel as to appear as foamy material; this occurred especially in arterioles (Fig. 5–8). The "foamy contents" of glomerular loops, described in the ischemic kidneys by Sheehan and Davis,[42] most likely also correspond to a conglomeration of blebs.

It is possible that blebbing, as a form of abnormal cellular hydration, is pathogenetically related to the more diffuse type of endothelial swelling (Fig. 21) which has been seen under similar conditions by light[43] as well as by electron microscopy[30] and has been reported[30] to play a role as an obstruction to flow—but only after much longer periods of ischemia than those studied here. In our material, diffuse swelling of endothelial cells

was not very prominent, presumably because the lesions were examined at a very early stage. The endothelial cells are known to resist anoxia and other insults better than the perivascular glia.[6,9,14,30] At later stages of the postischemic damage, the endothelium of capillaries and venules becomes altered enough so that the changes, especially the reactive ones, can be seen by light microscopy.[43,44]

We reconstruct the sequence of events occurring during and after brain ischemia as follows. When blood flow stops, the cerebral capillaries contain their full complement of red cells and plasma. The progressive anoxia and disappearance of substrates causes failure of active sodium transport out of the brain cells and leads to a net movement of electrolytes and water from the extracellular to the intracellular phase. This causes cellular swelling. Since the plasma represents the largest localized collection of extracellular fluid in the brain, swelling is most marked in the cells bordering the capillaries—the endothelial cells and especially the perivascular glia. Movement of electrolytes and water from plasma into the perivascular cells leads (1) to a concentration of proteins and formed elements in the blood, and (2) to a narrowing of the capillary lumina. The resulting increase in blood viscosity and increase in vascular resistance should be expected to impair the return of blood flow. In the intact animal, the return of flow would be rendered even more precarious by the arterial hypotension which usually follows ischemia.[1,2] This interpretation is consistent with the findings of our previous study,[2] which led us to conclude that there was stenosis, rather than complete occlusion, of the fine cerebral vessels, and that fibrin clots and platelet thrombi did not contribute significantly to the phenomenon of no reflow.

If the pathogenesis of postischemic damage, as outlined above, is correct, the clinical implications are considerable, since the damage might be reduced by measures designed to lower blood viscosity, to raise blood pressure, and to prevent swelling of perivascular cells.

Summary

It was shown in a preceding study that rabbit brains, subjected to brief periods of total ischemia, develop areas which remain ischemic even after the circulation is restored (no-reflow phenomenon). The purpose of the present study was to establish the nature of the vascular obstruction. Electron microscopic examination of brains made ischemic for 15 min. revealed 2 relevant changes: (1) swelling of perivascular glial cells, which sometimes impinged upon the capillaries to such an extent that the capillary lumen was reduced to a fine slit, and (2) the presence, in the capillary lumen, of "blebs" which probably arose from endothelial cells and which

either projected into the lumen or became detached and acted as emboli. Blebs were also observed when the ischemic brains were examined by light microscopy. Neither platelet thrombi nor intravascular clotting appeared to be a significant factor in the etiology of the vascular obstruction.

It is proposed that the perivascular cells became swollen because of active sodium transport failure (on account of the ischemia), that the water responsible for this swelling came largely from the plasma, and that this led to an increase in blood viscosity, which also contributed to impairing the return of blood flow. These findings are discussed in relation to the irreversible damage caused by ischemia.

References

1. KOWADA, M., AMES, A. III, MAJNO, G., and WRIGHT, R. L. Cerebral ischemia. I. An improved experimental method for study; cardiovascular effects; and demonstration of an early vascular lesion in the rabbit. *J Neurosurg* In press.
2. AMES, A. III, WRIGHT, R. L., KOWADA, M., THURSTON, J. M., and MAJNO, G. Cerebral ischemia. II. The no-reflow phenomenon. *Amer J Path* 52:437–453, 1968.
3. REWCASTLE, N. B. Glutaric acid dialdehyde; a routine fixative for central nervous system electron microscopy. *Nature (London)* 205:207–208, 1965.
4. PENFIELD, W., and CONE, W. Acute swelling of oligodendroglia. A specific type of neuroglia change. *Arch Neurol Psych* 16:131–153, 1926.
5. GLEES, P. *Neuroglia, Morphology and Function.* Thomas, Springfield, Ill., 1955, 111 pp.
6. KLATZO, I., PIRAUX, A., and LASKOWSKI, E. J. The relationship between edema, blood-brain-barrier and tissue elements in a local brain injury. *J. Neuropath Exp Neurol* 17:548–564, 1958.
7. TORACK, R. M., TERRY, R. D., and ZIMMERMAN, H. M. The fine structure of cerebral fluid accumulation. I. Swelling secondary to cold injury. *Amer J Path* 35:1135–1147, 1959.
8. GERSCHENFELD, J. M., WARD, F., ZADUNAISKY, J. A., and DE ROBERTIS, E. D. P. Function of astroglia in the water-ion metabolism of the central nervous system. An electron microscope study. *Neurology (Minneap)* 9:412–425, 1959.
9. TORACK, R. M., TERRY, R. D., and ZIMMERMAN, H. M. The fine structure of cerebral fluid accumulation. II. Swelling produced by triethyl tin poisoning and its comparison with that in the human brain. *Amer J Path* 36:273–287, 1960.
10. ALEU, F. P., KATZMAN, R., and TERRY, R. D. Fine structure and electrolyte analyses of cerebral edema induced by alkyl tin intoxication. *J Neuropath Exp Neurol* 22:403–413, 1963.
11. BAKAY, L. Morphological and chemical studies in cerebral edema: triethyl tin-induced edema. *J Neurol Sci* 2:52–67, 1965.
12. BAKAY, L., and LEE, J. C. *Cerebral Edema.* Thomas, Springfield, Ill., 1965.
13. LUSE, S. A., and HARRIS, B. Electron microscopy of the brain in experimental edema. *J. Neurosurg* 17:439–446, 1960.
14. RAIMONDI, A. J., EVANS, J. P., and MULLAN, S. Studies of cerebral edema. III. Alterations in the white matter: an electron microscopic study using ferritin as a labeling compound. *Acta Neuropath (Berlin)* 2:177–197, 1963.
15. TANI, E., and EVANS, J. P. Electron microscope studies of cerebral swelling.

III. Alterations in the neuroglia and the blood vessels of the white matter. *Acta Neuropath (Berlin) 4*:624–639, 1965.

16. GONATAS, N. K., ZIMMERMAN, H. M., and LEVINE, S. Ultrastructure of inflammation with edema in the rat brain. *Amer J Path 42*:455–469, 1963.
17. HIRANO, A., ZIMMERMAN, H. M., and LEVINE, S. The fine structure of cerebral fluid accumulation. VII. Reactions of astrocytes to cryptococcal polysaccharide implantation. *J Neuropath Exp Neurol 24*:386–397, 1965.
18. HIRANO, A., ZIMMERMAN, H. M., and LEVINE, S. Fine structure of cerebral fluid accumulation. VI. Intracellular accumulation of fluid and cryptococcal polysaccharide in oligodendroglia. *Arch Neurol 12*:189–196, 1965.
19. SCHRÖDER, J. M., and WECHSLER, W. Ödem und Nekrose in der grauen und weissen Substanz beim experimentellen Hirntrauma (licht- und elektronenmikroskopische Untersuchungen). *Acta Neuropath (Berlin) 5*:82–111, 1965.
20. HERZOG, I., LEVY, W. A., and SCHEINBERG, L. C. Biochemical and morphologic studies of cerebral edema associated with intracerebral tumors in rabbits. *J Neuropath Exp Neurol 24*:244–255, 1965.
21. HEGER, H. Die frühen Alterationen des Nervegewebes nach Hypoxydose und die fortgeschrittene Nekrose im elektronenmikroskopischen Bild. International Congress Series No. 100, *Excerpta Medica Foundation, New York*, 1966. p. 64–78.
22. HIRANO, A., LEVINE, S., and ZIMMERMAN, H. M. Experimental cyanide encephalopathy: Electron microscopic observations of early lesions in white matter. *J. Neuropath Exp Neurol 26*:200–213, 1967.
23. HILLS, C. P. The ultrastructure of anoxic-ischaemic lesions in the cerebral cortex of the adult rat brain. *Guy Hosp Rep 113*:333–348, 1964.
24. CHEN, H.-C., LIN, C.-S., and LIEN, I-N. Vascular permeability in experimental kernicterus. An electron-microscopic study of the blood-brain barrier. *Amer J Path 51*:69–99, 1967.
25. NIESSING, K., and VOGELL, W. Elektronenmikroskopische Untersuchungen über Struktuveränderungen in der Hirnrinde beim Ödem und ihre Bedeutung für das Problem der Grundsubstanz. *Z Zellforsch 52*:216–237, 1960.
26. BEN-SHMUEL, A. Elektronenmikroskopische Untersuchungen über das im Marklager lokalisierte Hirnödem. *Z Zellforsch 64*:523–532, 1964.
27. WOOLAM, D. H. M., and MILLEN, J. W. Perivascular spaces of the mammalian central nervous system. *Biol Rev 29*:251–283, 1954.
28. KLATZO, I. Neuropathological aspects of brain edema (Presidential address). *J Neuropath Exp Neurol 26*:1–14, 1967.
29. KATZMAN, R., GONATAS, N., and LEVINE, S. Electrolytes and fluids in experimental focal leukoencephalopathy. *Arch Neurol (Chicago) 10*:58–65, 1964.
30. HILLS, C. P. Ultrastructural changes in the capillary bed of the rat cerebral cortex in anoxic-ischemic brain lesions. *Amer J Path 44*:531–551, 1964.
31. PALAY, S. L. "An Electron Microscopical Study of Neuroglia." In *Biology of Neuroglia*, Windle, W. F., Ed. Thomas, Springfield, Ill., 1958, p. 24.
32. KUFFLER, S. W., and NICHOLLS, J. G. The physiology of neuroglial cells. *Ergebn Physiol 57*:1–90, 1966.
33. LINDENBERG, R. Compression of brain arteries as pathogenetic factor for tissue necroses and their areas of predilection. *J Neuropath Exp Neurol 14*:223–243, 1955.
34. SPECTOR, R. G. Water content of the brain in anoxic ischaemic encephalopathy in adult rats. *Brit J Exp Path 42*:623–630, 1961.
35. LEVINE, S., and KLEIN, M. Ischemic infarction and swelling in the rat brain. *Arch Path (Chicago) 69*:544–553, 1960.
36. GOLDBERG, B., and GREEN, H. The cytotoxic action of immune gamma globulin

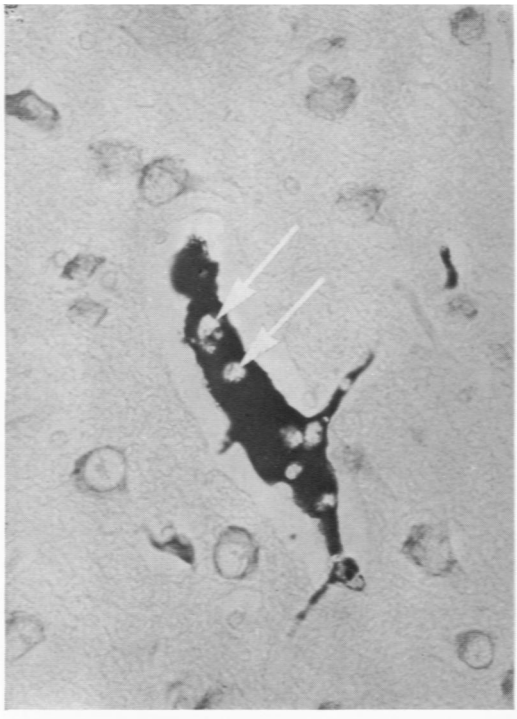
and complement on Krebs ascites tumor cells. I. Ultrastructural studies. *J Exp Med 109*:505–510, 1959.

37. ZOLLINGER, H. U. Cytologic studies with the phase microscope. I. The formation of "blisters" on cells in suspension (potocytosis), with observations on the nature of the cellular membrane. *Amer J Path 24*:545–568, 1948.
38. GOLDBERG, B., and GREEN, H. The cytotoxic action of immune gamma globulin and complement on Krebs ascites tumor cells. I. Ultrastructural studies. *J Exp Med 109*:505–510, 1959.
39. BESSIS, M. "Studies on Cell Agony and Death in an Attempt at Classification." In *Cellular Injury*, DE REUCK, A. V. S., and KNIGHT, J., Eds. Little, Boston, 1964, p. 287.
40. BUCKLEY, I. K. Tissue injury by high frequency electric current: observations with the Sandison-Clark ear chamber. I. Injury to small blood vessels. *Aust J Exp Biol Med Sci 38*:195–210, 1960.
41. BUCKLEY, I. K. Tissue injury with high frequency electric current. Observations with the Sandison-Clark ear chamber. II. Injury to small groups of cells. *Aust J Exp Biol Med Sci 38*:211–226, 1960.
42. SHEEHAN, H. L., and DAVIS, J. D. Renal ischaemia with failed reflow. *J Path Bact 78*:105–120, 1959.
43. LEVINE, S. Anoxic-ischemic encephalopathy in rats. *Amer J Path 36*:1–17, 1960.
44. SCHEINKER, I. M. Cerebral swelling. Histopathology, classification and clinical significance of brain edema. *J Neurosurg 4*:255–275, 1947.
45. MAJNO, G., AMES, A. III, CHIANG, J., WRIGHT, R. L. No reflow after cerebral ischaemia. (Letter) *Lancet 2*:569–570, 1967.

[*Illustrations follow*]

Legends for Figures

Fig. 1–4. Venules containing negative images of blebs (arrows) in brain submitted to 7.5 min. of ischemia and perfused with carbon 30 min. later. Blebs are so tenuous that they would easily escape notice if they were not outlined by carbon; some are round, others irregular. In Fig. 2 carbon outlines group of tiny bodies (P) which may correspond to one of rare platelet thrombi formed during 30 min. of reflow. Wright stain. × 380.



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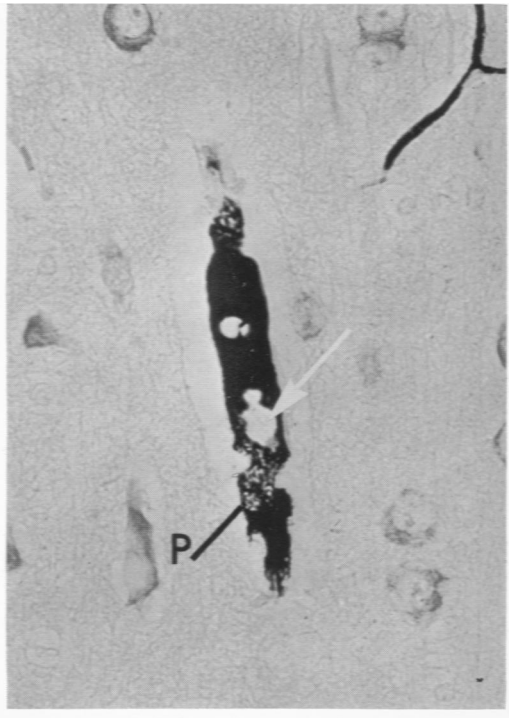

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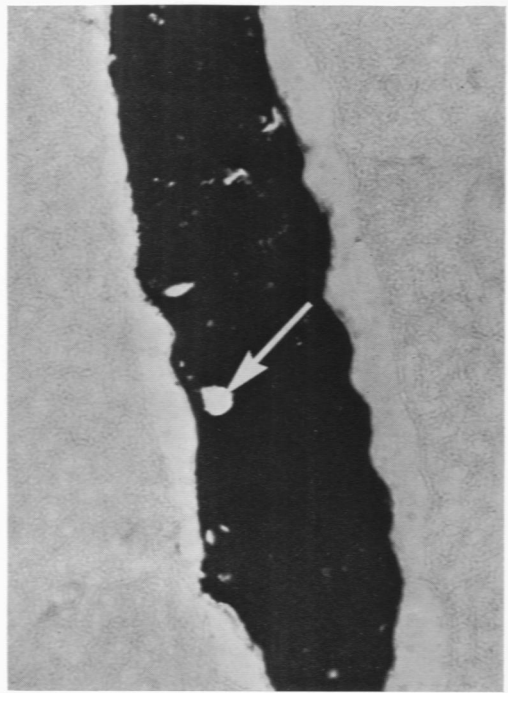

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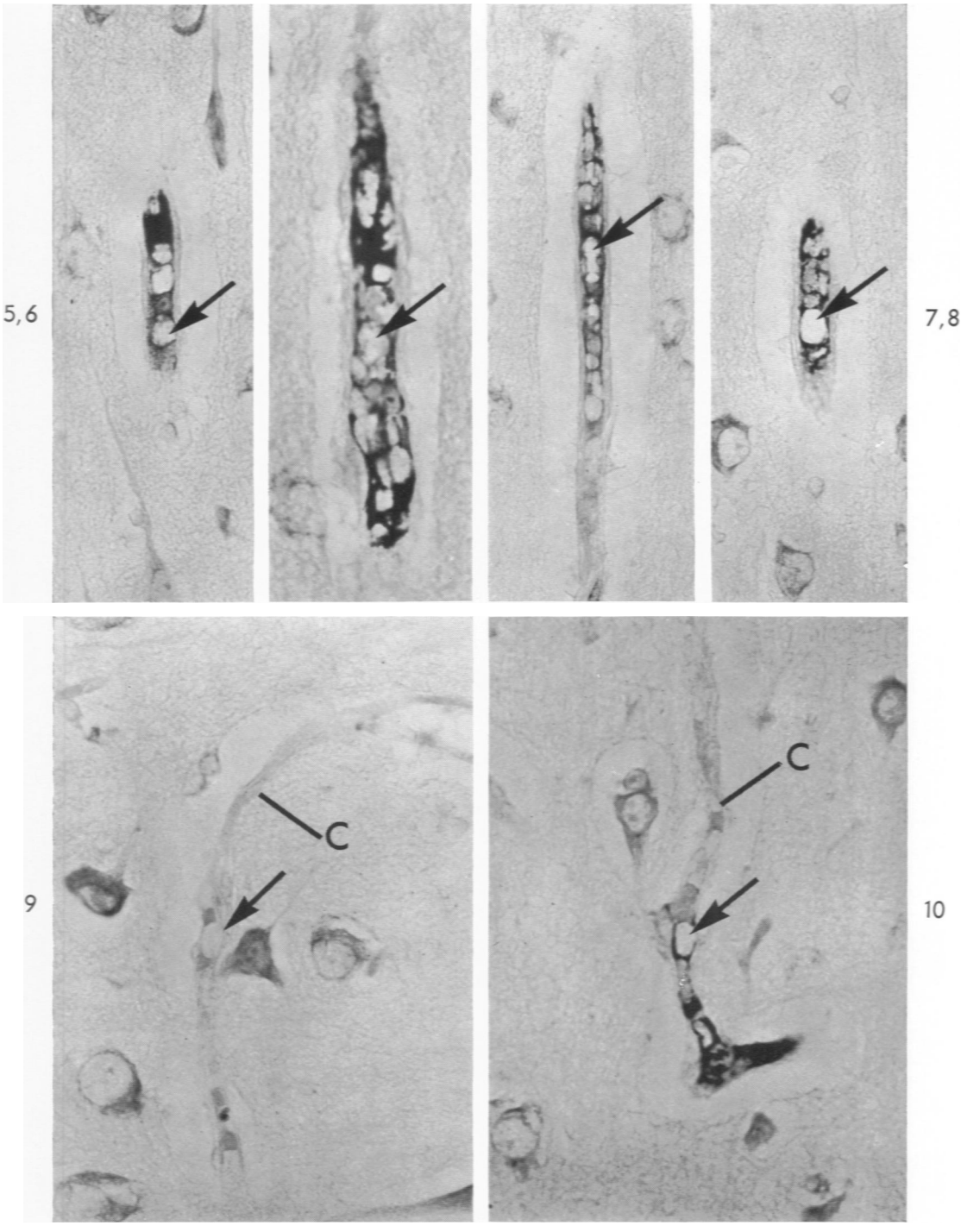

Fig. 5–10. Blebs in arterioles and capillaries. **Fig. 5–8.** Four arterioles; numerous blebs fill lumen. **Fig. 9 and 10.** Blebs (arrows) in 2 capillaries (C). In Fig. 9 bleb is elongated and deforms 2 adjacent erythrocytes. Fig. 5, 7, 8, × 350; Fig. 6, 9, 10, × 550.

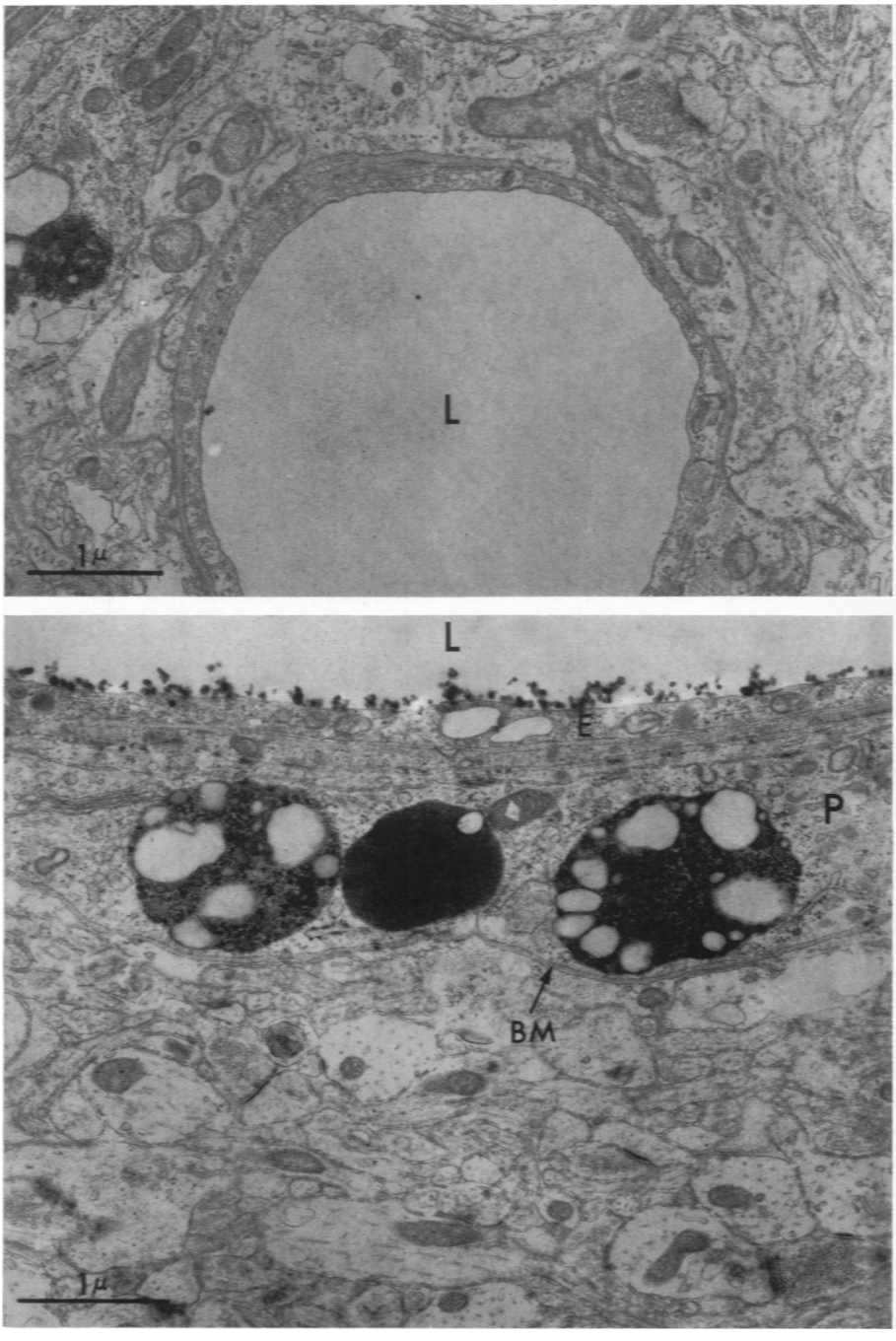

Fig. 11 and 12. Vessels from brain of sham-operated control rabbit. **Fig. 11.** Capillary or small venule. Endothelium appears normal; the perivascular astrocytic feet are not swollen. **Fig. 12.** Wall of venule, including large pericyte (P) surrounded by vascular basement membrane (BM) and containing 3 dense bodies. Lumen (L) contains some carbon particles.

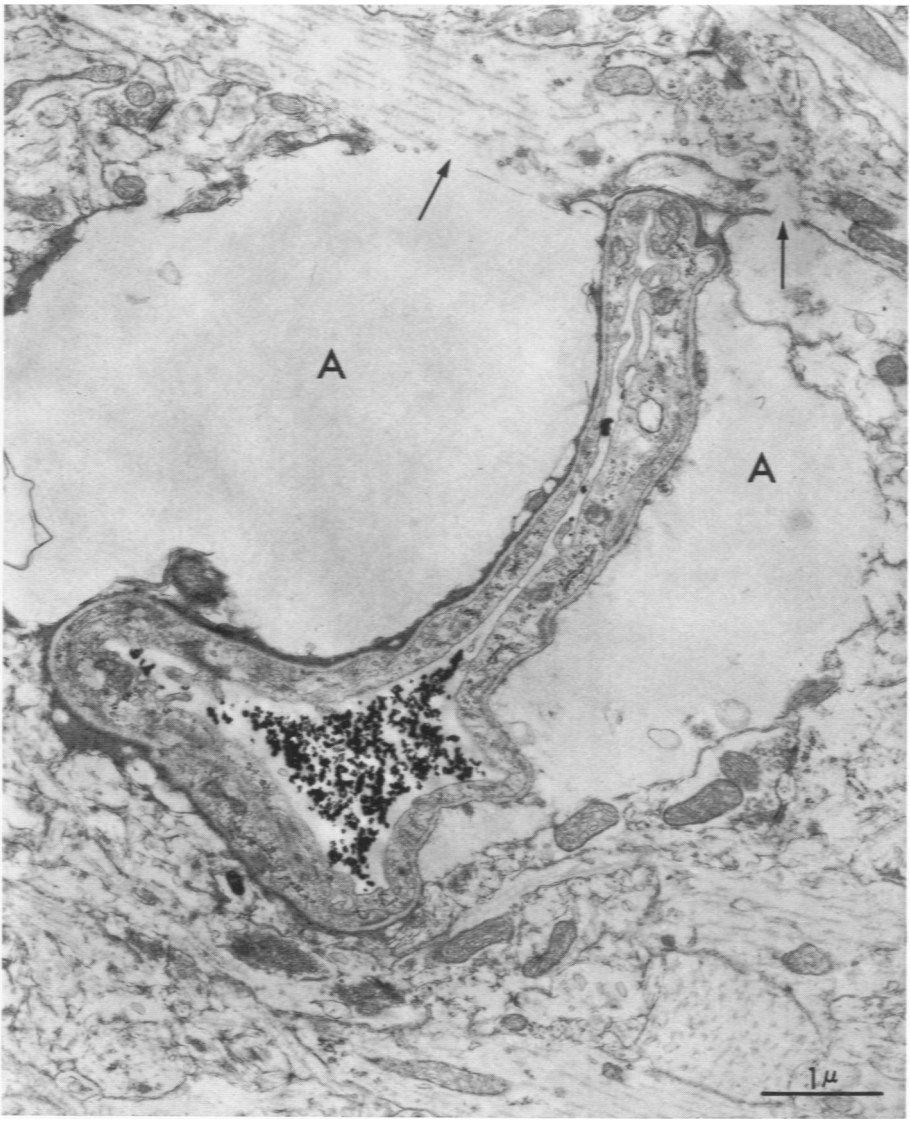

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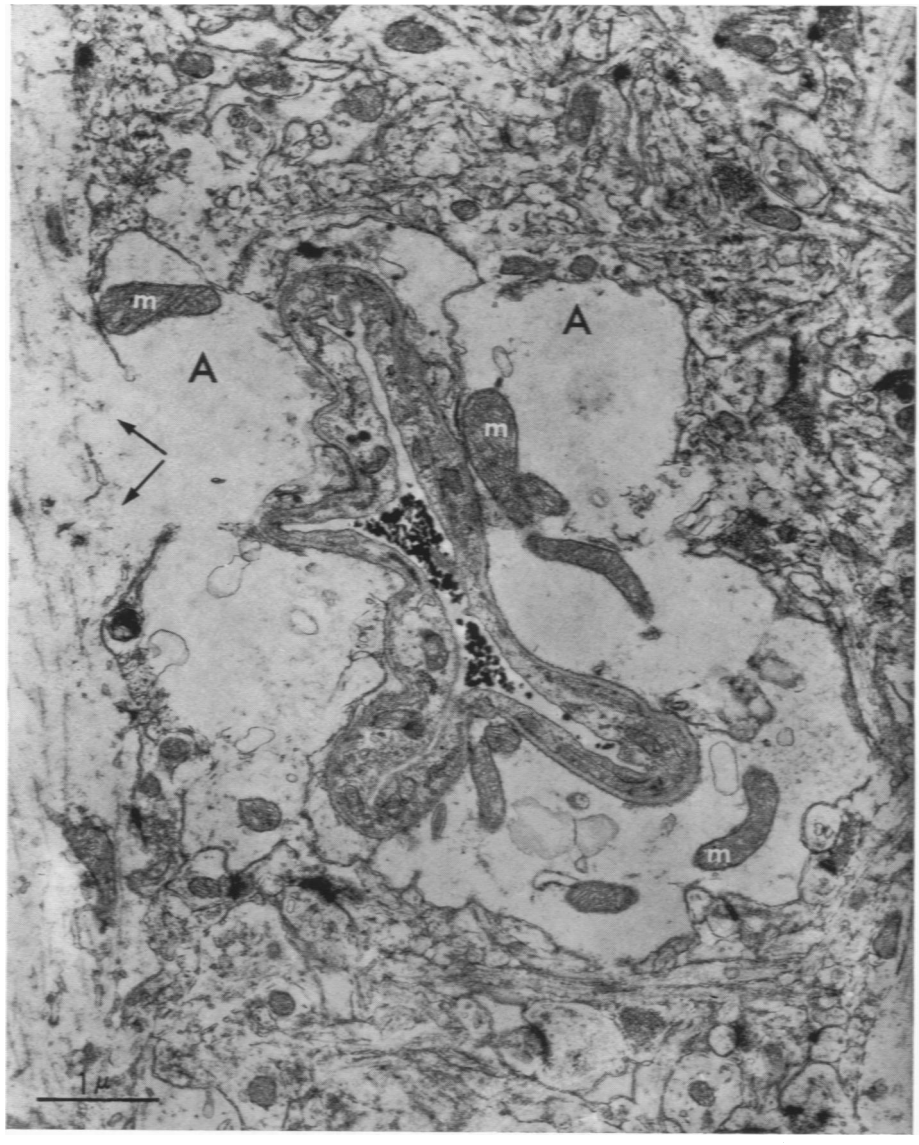
Fig. 13. Capillary from brain of rabbit killed after 15 min. of ischemia. Capillary appears to be squeezed between 2 greatly swollen astrocytic feet (A); since its lumen contains some carbon, it was reached by perfusion fluid despite apparent stenosis. Arrows point to disruptions of astrocytic cell membrane, possibly an artifact.



14

Fig. 14. Capillary from brain of rabbit killed after 15 min. of ischemia. Perivascular astrocytic feet (A) are greatly swollen and appear to compress capillary; its lumen, which contains some carbon black, is reduced to a slit. Disruptions of cell membranes (arrows) may be artifacts; note intact mitochondria (m).

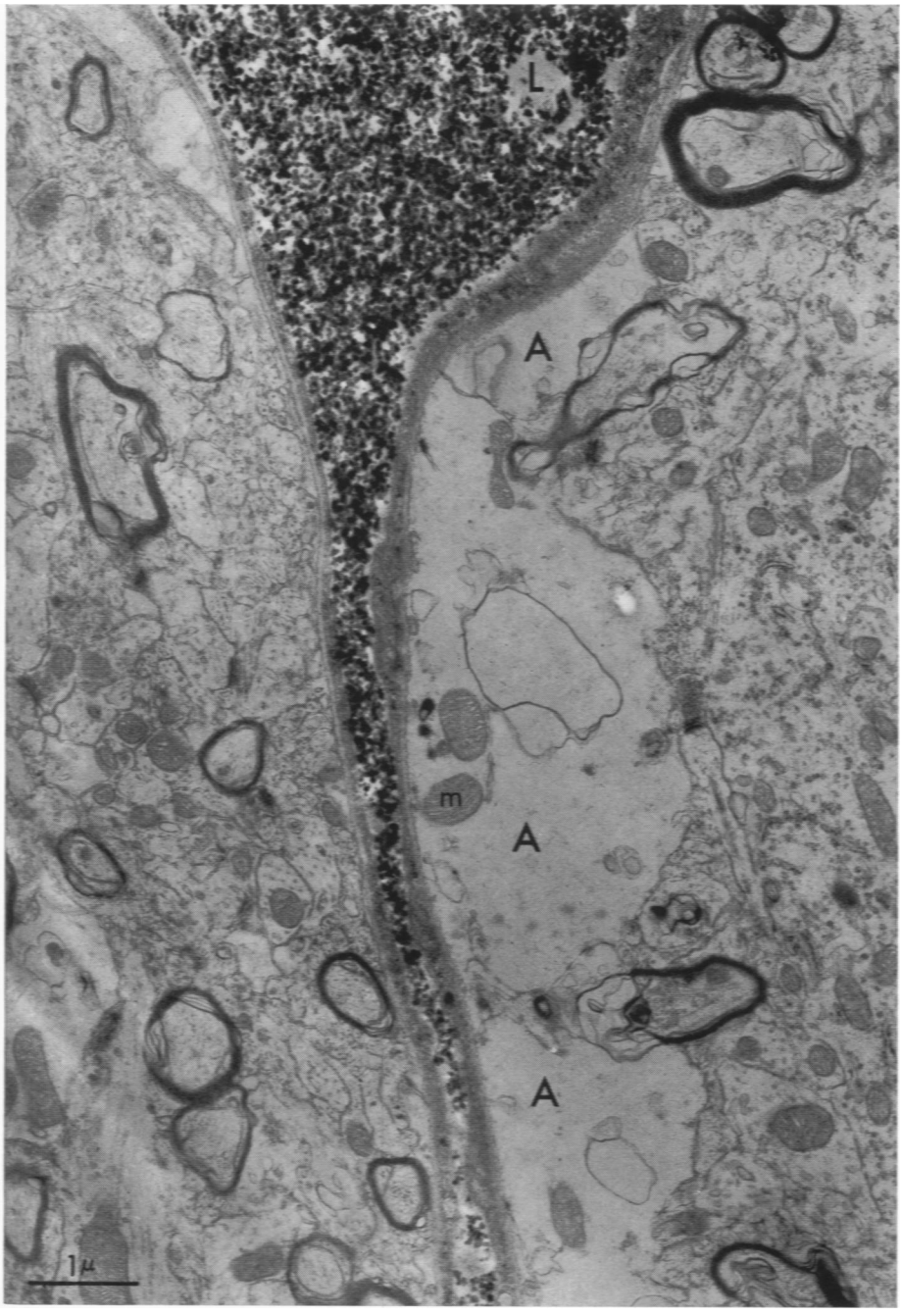


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Fig. 15. Cerebral capillary from brain of rabbit killed after 15 min. of ischemia. It appears to be compressed on right side only by swollen astrocytic feet (A). Lumen (L) contains densely packed carbon black; at bottom of picture it is so narrowed that it could be easily overlooked were it not emphasized by its carbon contents.

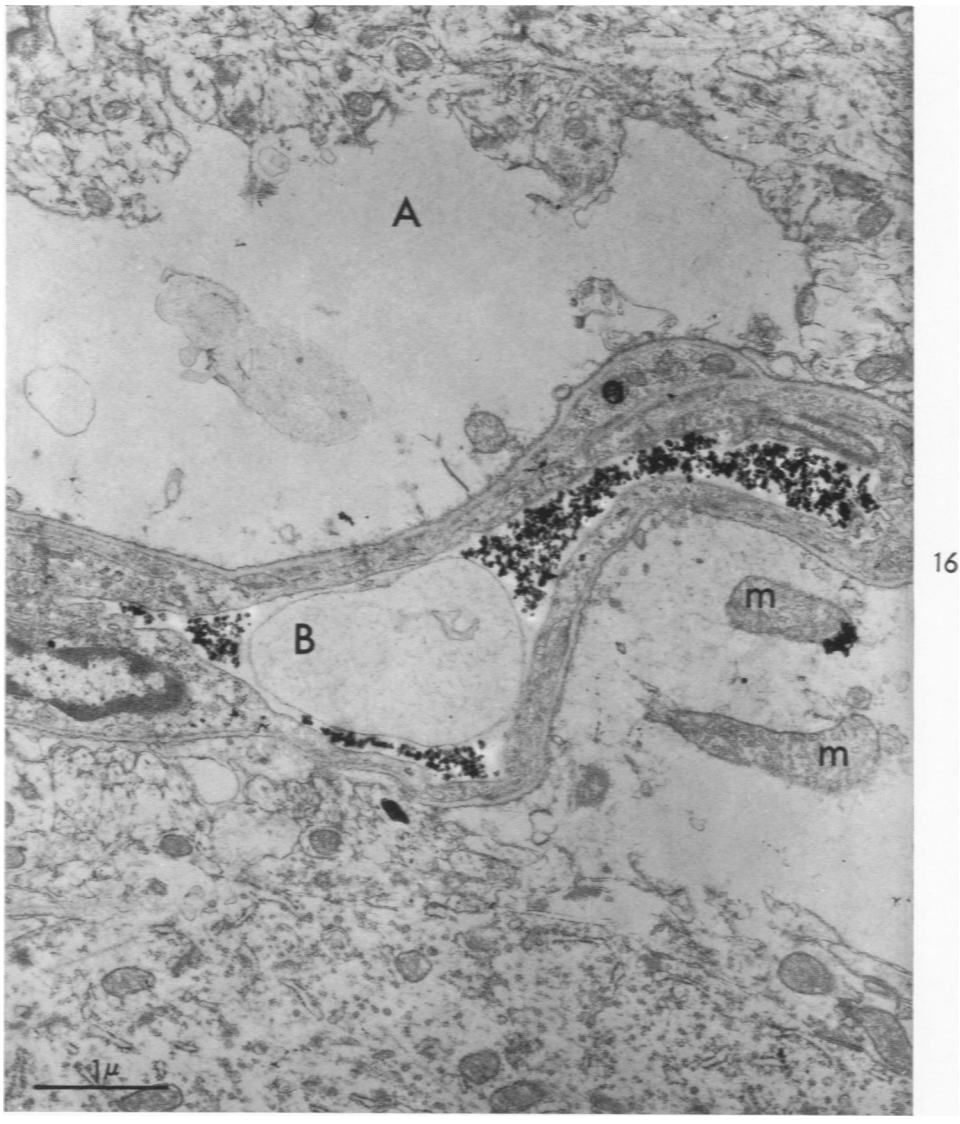
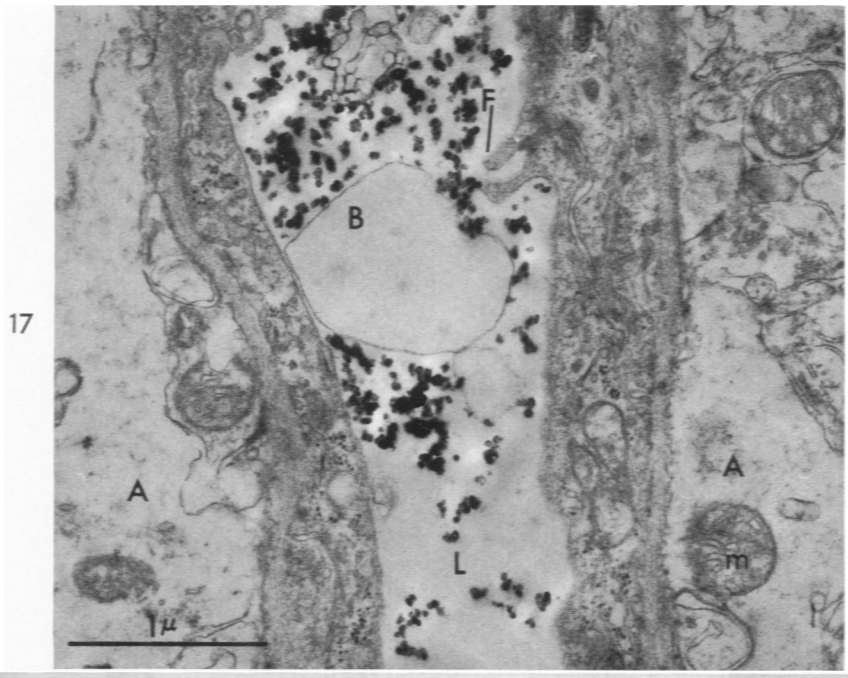


Fig. 16. Cerebral capillary from brain of rabbit killed after 15 min. of ischemia. It appears to be compressed from without by swollen astrocytic processes (A) and obstructed from within by bleb (B). Lumen now consists of fine slit, except where it is expanded by bleb (B).



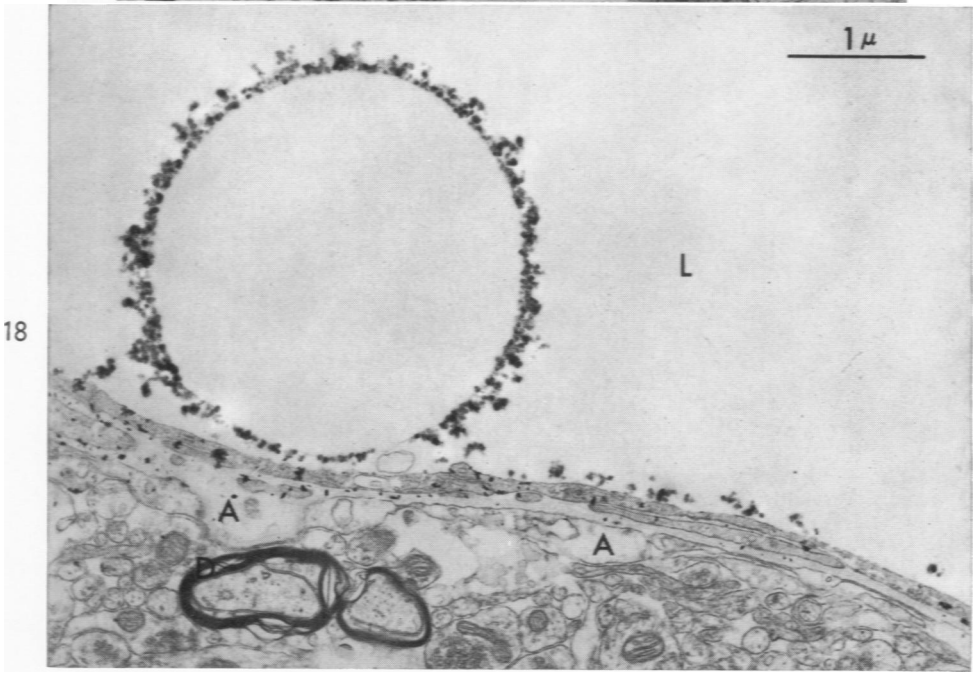
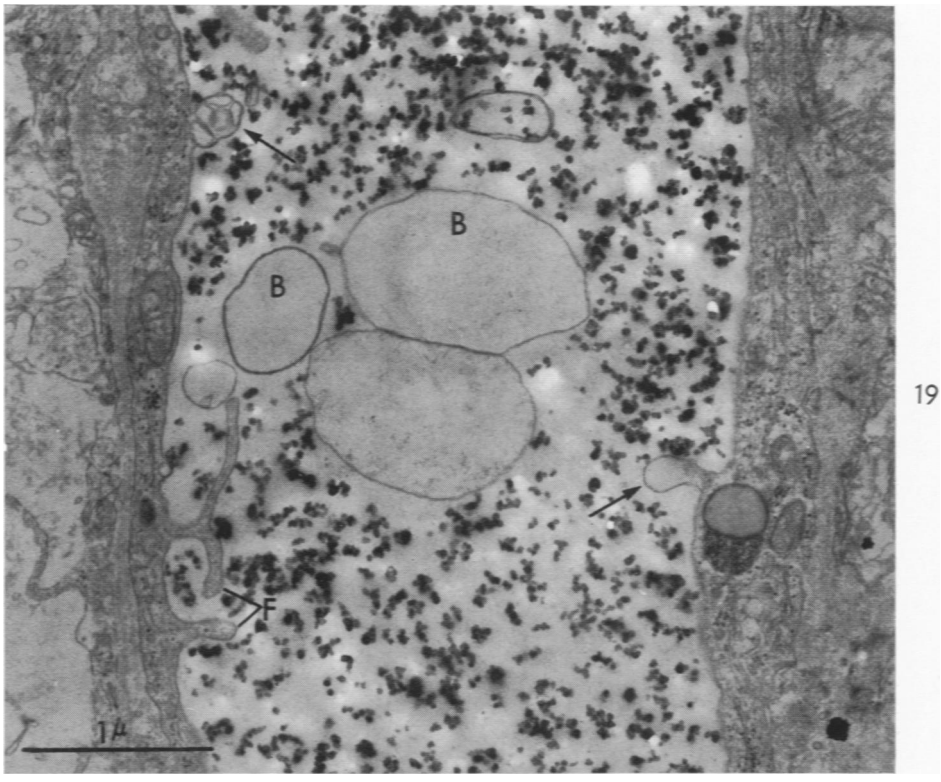


Fig. 17 and 18. Intravascular blebs in vessels of brains kept ischemic for 15 min. **Fig. 17.** Bleb (B) adjacent to 2 endothelial flaps (see also Fig. 19). There is more carbon in lumen above bleb than below; this was repeatedly observed, and suggests that bleb preexisted perfusion and constituted an obstacle to it. **Fig. 18.** One of many intravascular blebs outlined by carbon particles. Bleb of this size could be seen by light microscopy, especially if completely surrounded by carbon black. Note some astrocytic swelling (A) and some disruption of myelin sheaths.



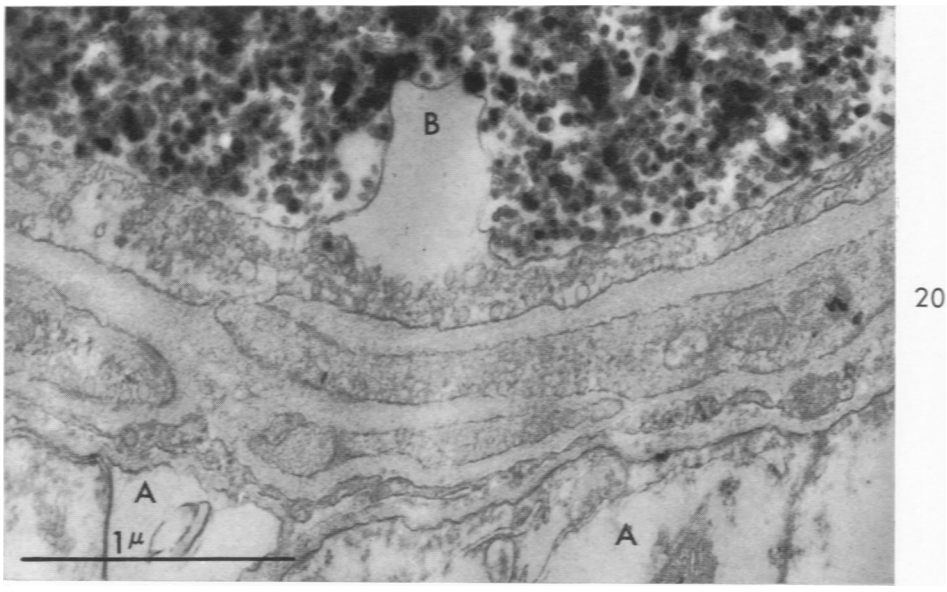


Fig. 19 and 20. Intravascular blebs and their origin (from brains fixed after 15 min. of ischemia). **Fig. 19.** Several blebs (B) in neighborhood of endothelial flaps (F). This image is often observed and suggests that former may arise from latter (see also Fig. 17). Arrows point to blebs arising from endothelial surface; upper bleb is honeycombed. **Fig. 20.** Bleb (B) arising from surface of endothelial cell in venule. Cell membrane is continuous all around bleb; endothelial vesicles and other organelles seem to be excluded from this structure. There is some astrocytic swelling (A). Lumen is full of carbon particles.

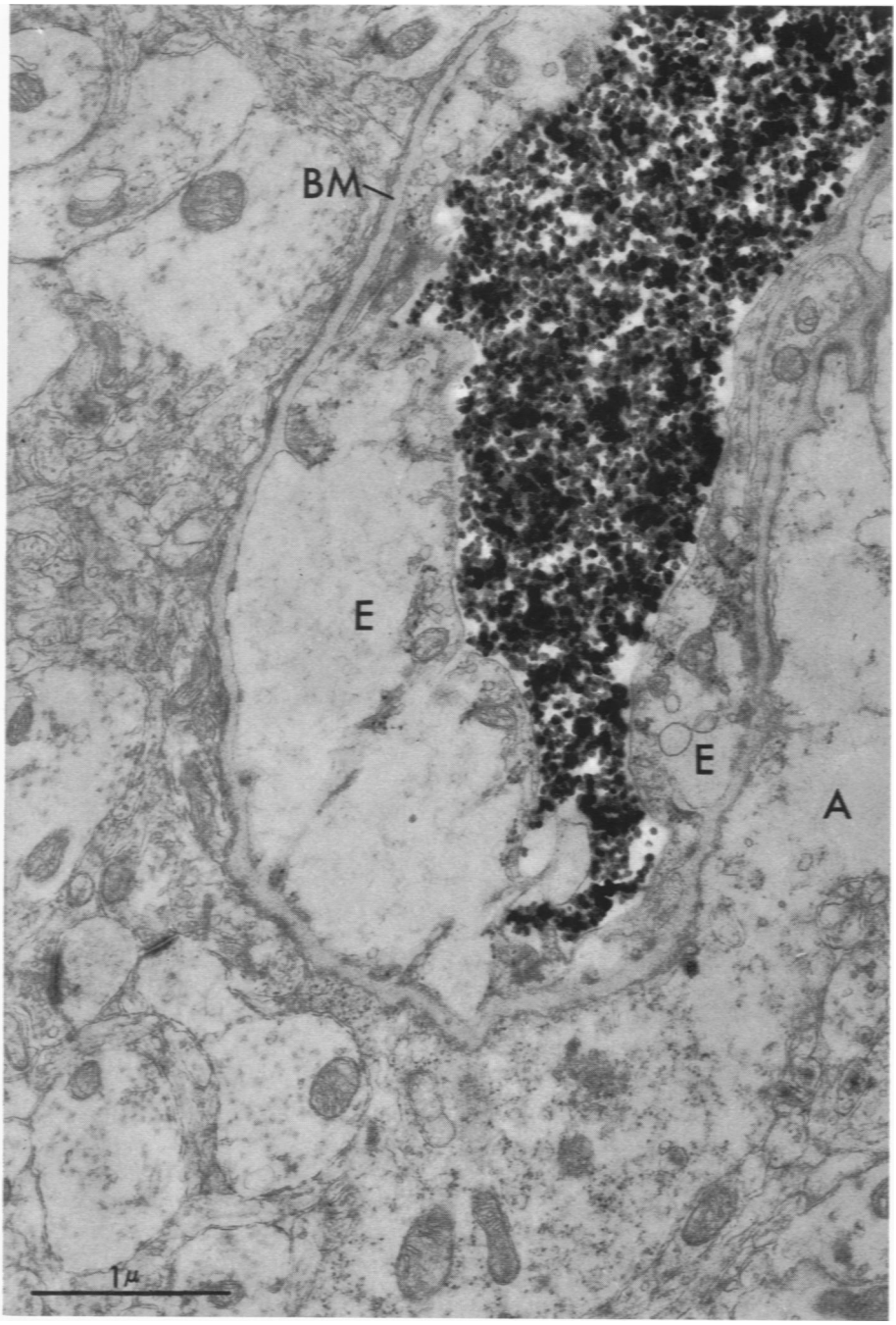


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Fig. 21. Endothelial swelling in cerebral capillary (15 min. of ischemia, fixation by perfusion 30 min. later). Overhydrated endothelial cells (E) bulge into lumen, which is filled with carbon black. There is no evidence that carbon is being spilled extravascularly, or that it has reached basement membrane (BM). Note also some astrocytic swelling (A).