THE CEREBRAL ARTERIOLES IN EXPERIMENTAL HYPERTENSION

II. The Development of Arteriolonecrosis

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Byrom[1] has shown that segmental arteriolar spasm precedes the development of focal arteriolonecrosis in the cerebral cortex of the rat with severe renal hypertension. In a previous paper[2] we have described segmental constrictions of the cerebral arterioles in monkeys rendered hypertensive by bilateral clipping of the renal arteries. These arteriolar constrictions were shown to be due to a focal contraction of the smooth muscle of the vessel wall which, although reversible by muscle relaxants, was remarkably persistent and slow to relax on reduction of the intraluminal pressure.

In this paper we present evidence of functional and structural changes which appear to precede the development of frank arteriolonecrosis in the wall of cerebral arterioles in monkeys with persistent severe hypertensive spasm.

Material and Methods

Young male and female cynomolgus monkeys weighing 2,200 to 3,300 gm were made hypertensive by a two-stage bilateral renal artery clipping. The arteries of the cerebral cortex were subsequently observed and photographed using techniques already described in detail.[2]

Records of the indirect arm and leg blood pressure measurements over periods of up to 19 weeks after the production of renal ischemia were obtained in 10 hypertensive and 2 normotensive monkeys before the animals were again anesthetized and the cerebral cortex exposed.

During observations of the cortex direct femoral artery blood pressure was recorded and a tracheal tube with flap valve permitted the breathing of oxygen or nitrogen. To lower blood pressure the body and lower limbs were allowed to hang vertically by removing their separate supporting surface. To demonstrate vascular damage in the cerebral cortex following general or local circulatory impairment, 5 ml of saturated freshly filtered and warmed trypan blue solution were injected into the femoral artery. In two experiments this infusion resulted in a dramatic and prolonged

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fall of femoral blood pressure from 140 to 160 mm systolic to circa 40 mm. The resulting cortical changes, resulting from stasis, presented features of special interest.

After sacrifice of the animal the brain was removed and fixed in formalin. On hardening, blocks of cerebral cortex were excised corresponding to the areas studied during the cortical observations. From small portions of these, paraffin sections were stained with hematoxylin and eosin, periodic acid-Schiff (PAS) reagent, phosphotungstic acid hematoxylin (PTAH) and azocarmine.

From the major part of each block of cortex, meningeal spreads were prepared and stained with hematoxylin and eosin. These spreads showed the same vessel pattern as that seen during the observations of the cortex *in vivo*, and a comparison with the photographs of the cortex enabled the microscopic appearances of any desired parts of the fixed and stained cerebral arterioles to be examined. A number of carefully identified small segments of these arterioles were selected and excised from the spreads with a sharp scalpel under a dissecting microscope. These tiny pieces were embedded in paraffin and serial cross sections cut and restained, if necessary, before mounting.

Defective Arteriole Function

In the normotensive monkeys after intravenous injection of trypan blue, a critical reduction in cortical blood flow produced by a lowering of the blood pressure and the occlusion of the middle cerebral artery resulted in widespread endothelial damage in the central part of the territory of that artery. As in previous experiments[3] this ischemic anoxia caused a patchy blue haze to be seen next to each emerging venule on the brain surface; this resulted from diffusion of dye from the venous end of the capillaries in the underlying superficial cortex. After a time this was followed by dilatation of a few small surface venules with blood stasis, segmentation of clumped red cell masses and subsequent blue staining of the actual walls of the venules. Flow in arterioles continued. With continuation of reduced blood flow, adjacent venules became similarly involved with more and more vessels showing stasis until quite large veins became engorged by segmental masses of clumped red cells. Only at a final stage of cerebral circulatory impairment was segmentation of the blood column observed in the anastomotic arteriolar network.

In the hypertensive monkeys after intravenous injection of trypan blue, a critical reduction in cortical blood flow due to fall of blood pressure induced by tilting, was again first manifested by a patchy blue haze on the brain surface as the dye diffused from the damaged capillary endothelium in the underlying cortex. With continued impairment of cortical circulation, the first vessel changes were seen in a few scattered arterioles and these became well developed before engorgement with stasis and segmentation of blood commenced, even in the smaller venules. The arteriolar changes comprised a clumping and stasis of the contained blood with segregation into the dilated segments of the smaller anastomotic arterioles measuring 50 μ in diameter or less lying near the centers of the gyri. The constricted arteriolar segments appeared empty

of blood but their walls showed obvious blue staining. Staining was also seen in the dilated segments although the blue color in the wall tended to be obscured by the dark red color of the clumped blood masses in the lumen.

Isolated small arteriolar lesions of this type were observed at systolic blood pressure of 140 mm in an animal with systolic pressure of 260 mm at the beginning of the experiment. In another animal some were found after reduction to 120 mm. With greater falls of blood pressure (90 mm and below) large numbers appeared.

Although these events were more dramatically recorded in a series of color photographs they are satisfactorily demonstrated in monochrome. Blood stasis and clumping could be seen as a prominent dark granular mass in an arteriole (left center, Fig. 1). This contrasted with other arterioles (right, Fig. 1) which showed typical hypertensive segmental spasm. Blood stasis had not yet occurred in them. The meningeal spread (Fig. 2) in comparison allowed certain identification of arterial and venous channels. Segmental arteriolar constrictions could still be seen in the meningeal spread (Fig. 2). Details of the blood stasis in the arteriole are shown at a higher magnification in a photograph of this cortex (Fig. 4) taken 2 minutes after Figure 1. The masses of clumped red cells were darker than the lighter trypan blue stained arteriole wall. Some stasis and clumping of red cells commenced by now in a network of small venules in the lower part of the photograph (Fig. 4). The same area in the meningeal spread at a similar magnification clarified the pattern of veins and arteries in the cortex (Fig. 5).

In a more advanced phase of the process there were scattered arterioles and now venules also with stasis and blood segmentation (Fig. 8). Stasis in the arteriole and venule in the middle of the occipital cortex (Fig. 9) and the same area in the meningeal spread (Fig. 10) clearly differentiated the arterioles and venules. There was no staining of the wall of the curved venule above (Fig. 9) but the greyness in the wall of the Z-shaped arteriole below showed obvious arteriole staining by the trypan blue.

As has been shown previously [3] an anoxic anoxia in the normotensive animals had the same effect as ischemic anoxia. In the hypertensive monkeys, nitrogen breathing resulted in a rapid progression of this same sequence of events. In the animals with prolonged hypertension and segmental arteriolar spasm, the production of either an anoxic or an ischemic anoxia resulted in early blood stasis and segmentation in the arteriole lumen, together with trypan blue staining of the arteriole wall. This indicated an impairment of the circulation and damage to the wall in these small anastomotic arterioles.

Damage to the Arteriole Wall

In the fixed and stained meningeal spread preparations, the constricted segments of the larger arterioles and of the smaller arterioles, in which blood stasis with trypan blue staining had not been seen during the observations of the cortex, showed a nuclear condensation and pattern believed to indicate contraction of the smooth muscle.[2] In transverse sections cut from these vessels (Figs. 3A and 3B) no degenerative changes were seen in the muscle cytoplasm or nuclei. There was no cellular hyperplasia and no evidence of arteriolonecrosis. In fixed and stained meningeal spread preparations the arterioles in which stasis and segmentation of blood with trypan blue staining had been observed in the cortex very obviously contained fewer nuclei and many of the remaining nuclei were elongated, thin and twisted with an apparent irregular condensation of chromatin (Figs. 6 and 10). There was a loss of normal muscle cytoplasmic fibrillation but no fibrinoid change had as yet occurred. These changes involved all parts of the arteriole which had shown evidence of damage by trypan blue staining *in vivo*. In transverse sections cut from these vessels, the nuclear loss and cytoplasmic degeneration were clearly demonstrated (Figs. 7A and 7B) and contrasted with the sections from the contracted (Fig. 3B) or dilated (Fig. 3A) segments of the arterioles with simple hypertensive spasm. In the damaged arterioles there were strikingly few nuclei in the arteriolar muscle and the cytoplasm showed some smudging but fibrinoid eosinophilia was not evident. The wall was markedly thinned when stretched round a blood mass in its lumen (Fig. 11).

During the observations the cortex had been exposed for up to 8 hours and, indeed, an inevitably inflammatory reaction resulted. This is clearly seen in the meninges in the spread preparation illustrated in Figure 6. This period was, however, far too short for any microscopic changes to have developed in the arteriolar wall as a result of trauma to the exposed vessels. These arteriolar changes must be ascribed to the hypertension and the lesions in the arteriole wall were interpreted as the initial or early stages of a hypertensive arteriolonecrosis.

Arteriolonecrosis

In none of the experiments were we able to observe the full development of arteriolonecrosis in the living cerebral cortex. In one monkey which died with hypertensive encephalopathy and uremia we were, however, able to study the arteriolonecrosis in meningeal spread preparations and in histologic sections of the brain. This animal had shown a femoral artery blood pressure of 272/214 mm Hg on the 25th day after

the left renal artery clipping. The right renal artery was clipped on the 30th day and on the 38th day the femoral systolic blood pressure was something over 300 mm Hg with a diastolic pressure of 267 mm. On the 45th day he was noted to be listless and apathetic and during the next two days became very weak with myoclonic muscle contractions, nystagmus, coma, bloody diarrhea and death with a blood urea nitrogen of 142 mg per 100 ml. At necropsy there were hemorrhagic lesions in the colon with histologic vascular changes typical of malignant hypertension. The left kidney presented the histologic appearance of renal ischemia and the right kidney contained several large infarcts. The brain was swollen and in the boundary zones between the territories supplied by the middle and posterior cerebral arteries in the cortex of the occipital lobes there were a number of petechial hemorrhages.

In the meningeal spread preparations there were lesions indicative of malignant hypertension involving some of the smaller distributing arterioles and many of the small penetrating arterioles. The distributing arterioles affected were those with a 50 μ outside diameter in the fine anastomotic ramifications over the centers of the gyri. In these vessels segments measuring from 100 to 500 μ in length showed arteriolonecrosis (Fig. 12). The arteriolonecrotic involvement seemed haphazard. Short affected segments were seen in the middle of a length of an unbranched vessel or near to a branch origin or, occasionally, much longer involved segments appeared to extend along the arteriole from one branch to the next. The arteriolonecrotic segments exhibited swelling of the wall of the vessel with an intense fibrinoid eosinophilia and loss of cytoplasmic and nuclear detail with pyknosis. Numbers of neutrophils were collected as a bulbous or fusiform cuff in and around each necrotic length of vessel.

In the meningeal spreads, the proximal parts of the small penetrating cortical arterioles remained attached to the pial plexus vessels when the cortex was scraped away from the pia and were easily recognized as they lay floating in the mountant of the preparation. Large numbers of these arterioles, generally rather less than 50 μ in diameter, showed similar arteriolonecrotic segments which extended from the origin (Fig. 13) or from just distal to the origin (Fig. 14) down into the underlying cortex.

In the histologic preparations of the brain, the larger arterioles of the meninges were uninvolved. Arteriolonecrosis in some of the smaller pial vessels, however, was clearly due to the involvement of the small anastomotic network arterioles in the pial plexus (Fig. 15). There was arteriolonecrosis in the small penetrating arterioles with involvement from, or just beyond, their origin. On the other hand lesions of these vessels deep in the cortex (Fig. 16) indicated a more extensive involvement than was evident in the meningeal spreads.

Discussion

Trypan blue has been used as a sensitive indicator of vascular damage.[4] Staining by the dye of the walls of the small blood vessels in the brain in general or local circulatory failure has been shown[3] to occur with a characteristic pattern and sequence. This has been associated with the advent of stasis and segmentation of the blood column in the venules and later in the arterioles. In these circumstances, it seems likely that the endothelial damage is due to an ischemia resulting from the blood stasis. In the present series of hypertensive animals with prolonged segmental spasm of cerebral arterioles, stasis and staining were observed to occur in some of these arterioles before venular stasis became established. The development of stasis and staining in the anastomotic arterioles at this stage of circulatory failure provided further evidence of functional impairment of the meningeal collaterals in the hypertensive animal.

In the rat, Byrom and Dodson[5] described a simple coagulative muscle necrosis in renal arterioles after repeated artificial overdistention of the arterial tree. The histologic appearances of these arterioles with disappearance of nuclei and loss of muscle structure resemble those in the trypan blue stained cerebral arterioles in the present monkey experiments. The present arteriolar lesions were related to the long continued spasm resulting from the hypertension, and showed similar segmental intensification. Trypan blue staining of these arterioles could be partly due to initial damage of the muscle by the prolonged hypertension and, with the wall of the arteriole thus damaged, slowing and stasis of blood could be expected to occur[6] sooner than in a normal arteriole. Subsequent to blood stasis, further trypan blue staining would be the inevitable result of endothelial ischemia.

Byrom's beautiful demonstration of the vascular lesions in the brain of the severely hypertensive rat[1] did not show the development of arteriolonecrosis in arterioles with hypertensive segmental spasm. In the hypertensive monkey Meyer, Waltz and Gotoh[7] described segmental arteriolar spasm but they were unsuccessful in producing arteriolonecrosis. In the present experiments, although many arterioles with segmental spasm were studied, only a few showed cytoplasmic and nuclear degenerative changes. The distribution of these lesions coincided with the distribution of fibrinoid arteriolonecrosis. Cytologic and histologic appearances suggested these changes were those of a commencing arteriolonecrosis.

Summary

Studies of the cerebral cortex were carried out in anesthetized monkeys with experimental renal hypertension. The resulting severe persistent segmental arteriolar spasm showed stasis and segmentation of the blood. There was also evidence of vascular damage manifested by trypan blue staining in some of these arterioles in spasm. This occurred at a much earlier stage in circulatory failure than in the normotensive monkey. Critical levels of systolic blood pressure as high as 140 mm and 120 mm Hg were observed.

Examination of the arterioles in meningeal spread preparations and in histologic sections showed changes in the arteriole wall which appeared to represent the commencement of arteriolonecrosis. Cerebral fibrinoid arteriolonecrosis was demonstrated in the hypertensive monkey in histologic sections and in meningeal spreads. The distribution of these necrotic lesions in the cerebral arterioles was similar to the earlier changes described.

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[*Illustrations follow*]

Legends for Figures

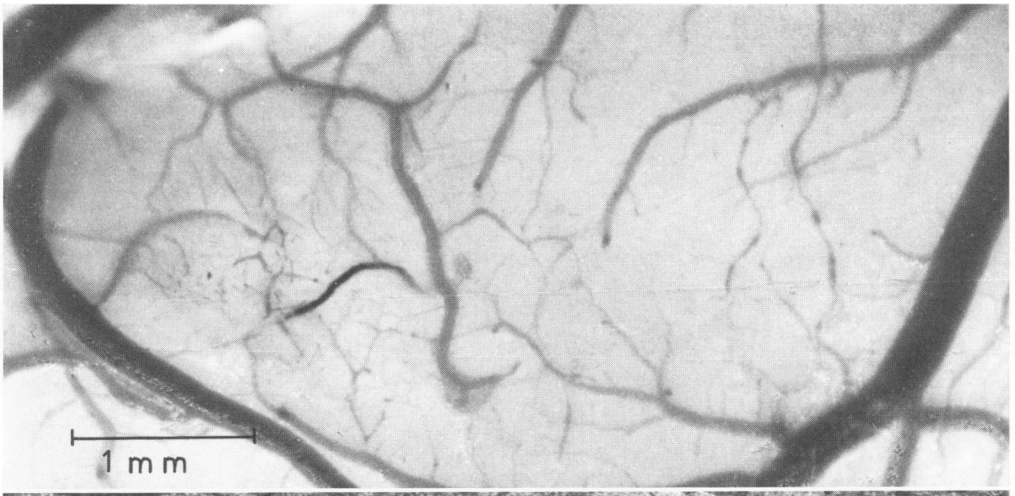
Photographs were prepared from sections and spreads stained with hematoxylin and eosin.

Fig. 1. Posterolateral part of the angular gyrus in a monkey with renal hypertension after impairment of the cortical blood flow by lowering of the blood pressure. Blood stasis and segmentation of clumped red cells appear in an anastomotic arteriole in the left center. On the right, there is typical hypertensive segmental spasm in 2 arterioles in which blood stasis has not yet occurred. × 24.

Fig. 2. Meningeal spread from the same area of the cerebral cortex shown in Figure 1. The general layout of arterial and venous channels is demonstrated. The wall of the arteriole seen with blood stasis in Figure 1 exhibits degenerative changes with loss of nuclei. This contrasts with the arterioles on the right in which some segmental constrictions persist. × 22.

Fig. 3A. A cross section of an arteriole shown at A in Figure 2. Uncontracted smooth muscle of the dilated arteriole segment contains elongated nuclei. No degenerative changes are present. × 810.

Fig. 3B. A cross section of the arteriole shown at B in Figure 2. Contracted smooth muscle appears in the constricted arteriole segment. Two shortened and twisted muscle nuclei lie partly superimposed. × 810.



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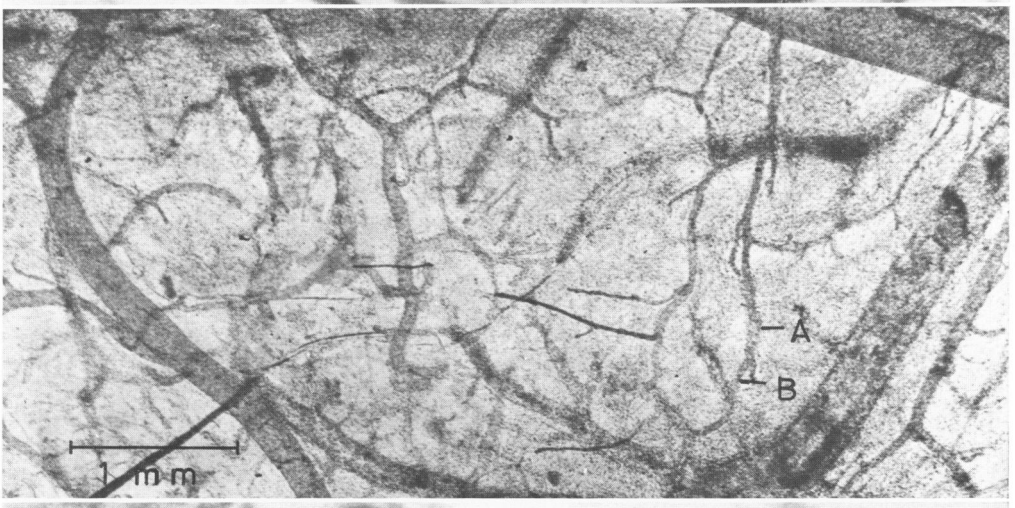

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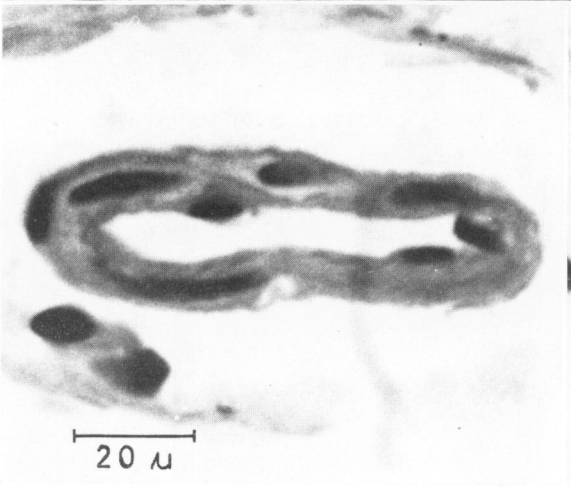

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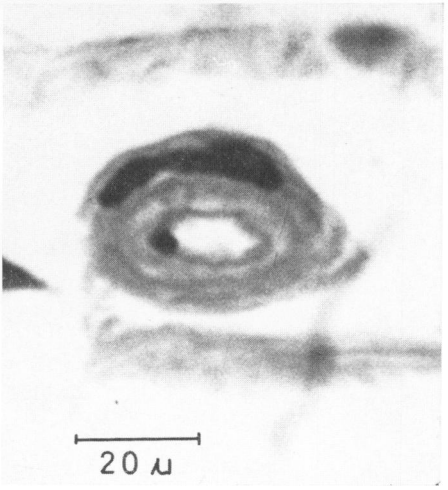


3B

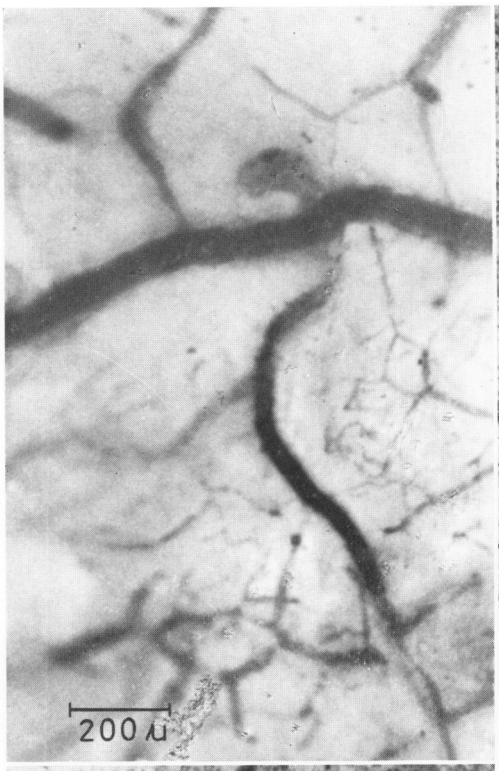
FIG. 4. A higher magnification of the left central part of the cortex shown in Figure 1, taken two minutes later. The arteriolar stasis is well established and clumped red blood cells appear black. The trypan blue stained arteriole wall appears grey. Stasis and clumping of red cells is commencing in a network of small venules in the lower part of the photograph. × 62.

FIG. 5. A meningeal spread from the same area shown in Figure 4. The pattern of arterioles and venules is demonstrated. × 66.

FIG. 6. The central part of the meningeal spread shown in Figure 5. Loss of cytoplasmic structure and few nuclei appear in the muscle of the arteriole wall. × 136.

FIG. 7A. A cross section of the arteriole at A in Figure 5. There is loss of nuclei in the degenerated muscle of the arteriole wall. × 830.

FIG. 7B. A cross section of the arteriole shown at B in Figure 5. Similar degenerative changes are apparent. The flattening of the arteriole seen in this and the preceding figure is the result of pressure applied during preparation of the meningeal spread. There are remnants of the red cell masses in the lumen. × 830.



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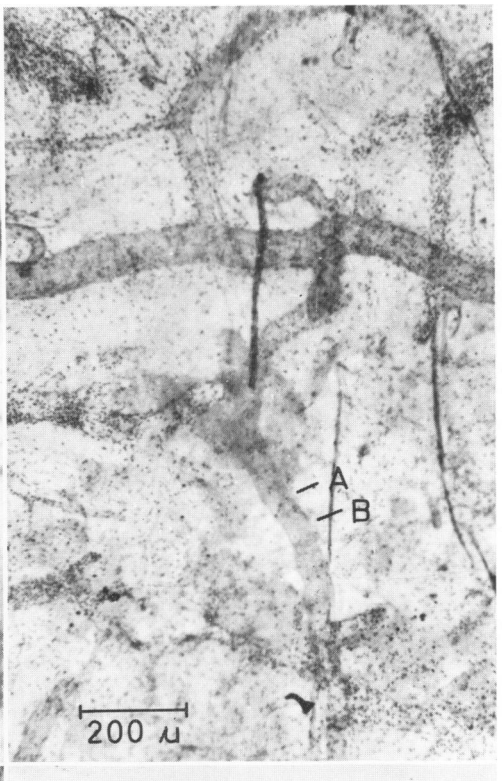

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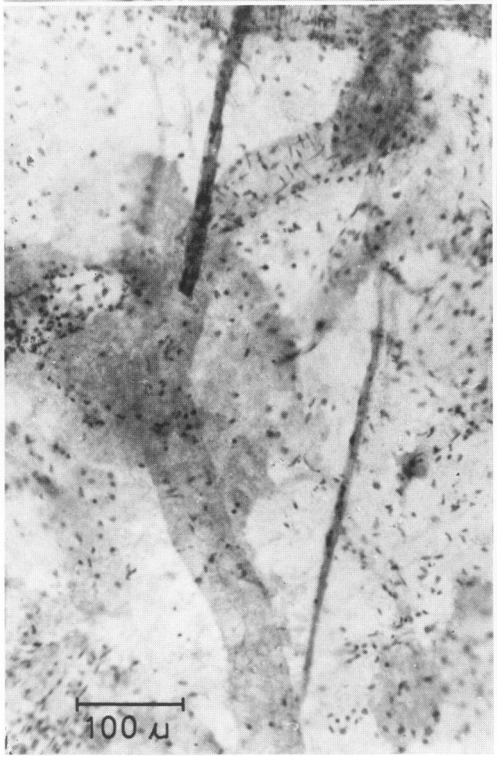

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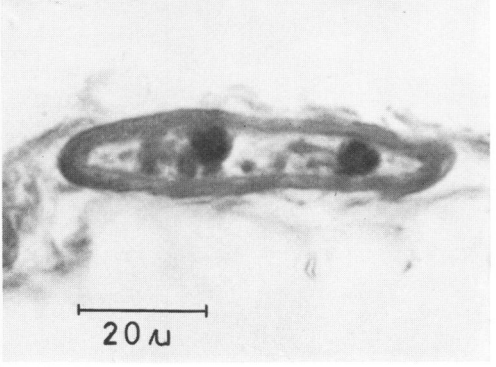

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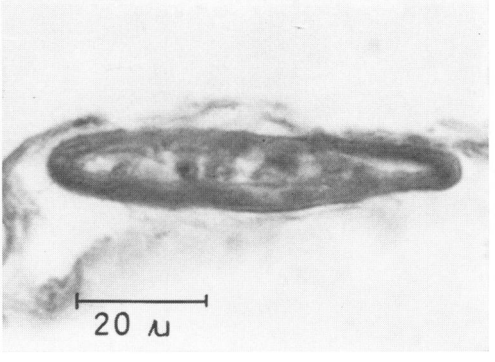


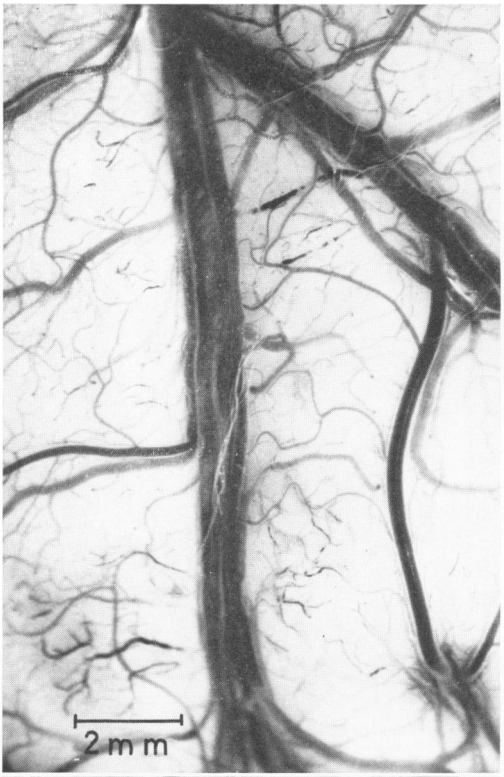
7B

FIG. 8. The occipital cortex of another monkey with renal hypertension. After impairment of cortical blood flow there is more advanced blood stasis and segmentation in arterioles and veins. The trypan blue haze from capillary diffusion appears as a greying of the cortex in the lower left of the photograph. × 7.

FIG. 9. Details of blood stasis are shown in arterioles and venules in the left central part of Figure 8. The greyness of the Z-shaped arteriole is due to trypan blue staining of its wall. The venule wall is as yet unstained by the dye. × 52.

FIG. 10. The meningeal spread from same area of cortex shown in Figure 9. The pattern of arterioles and venules is demonstrated. The Z-shaped vessel with stasis in Figure 9 is shown to be an arteriole and the vessel above it is a venule. There is degenerative change in the arteriole wall. × 55.

FIG. 11. A cross section of the arteriole shown at A in Figure 10. The lumen is distended by a platelet conglutination and the wall exhibits degenerative changes. × 1070.



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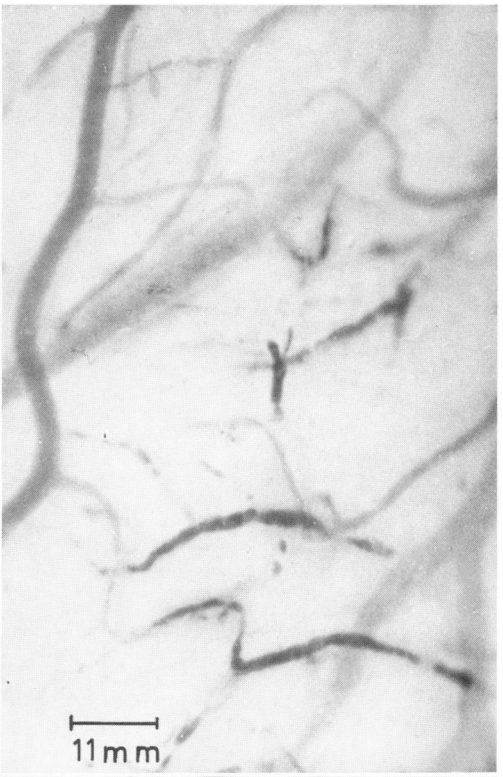

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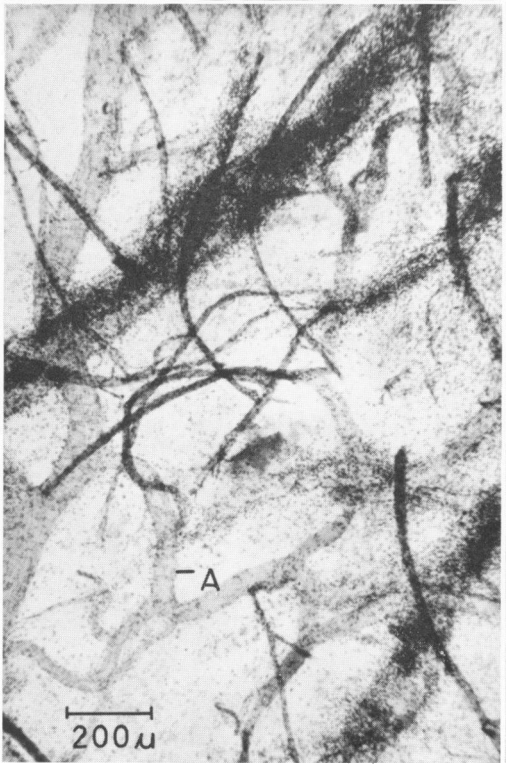

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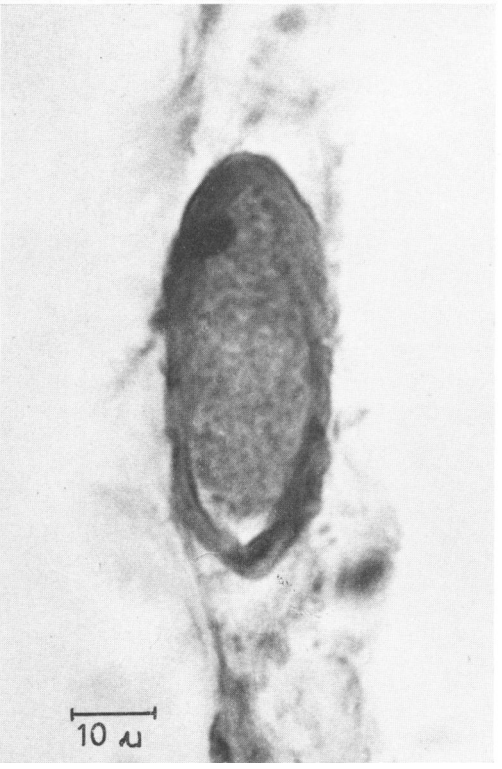

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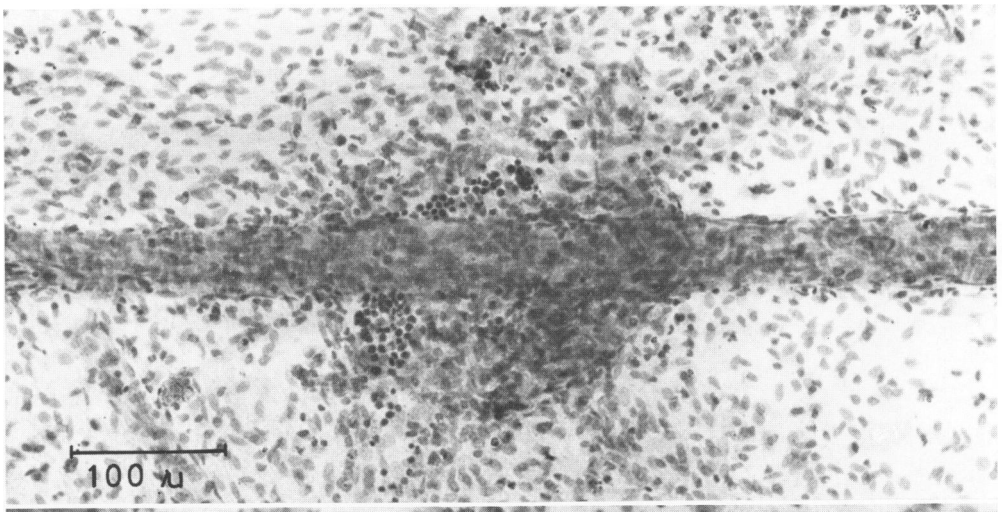
FIG. 12. An anastomotic arteriole measuring 40 μ in diameter in a meningeal spread from a monkey with malignant hypertension. A segment of arteriolonecrosis measures 300 μ in length and is unrelated to any branching. There is fibrinoid change and a bulbous cuff of neutrophil exudate. The cortex in this animal had not been exposed during life. × 200.

FIG. 13. The meningeal spread from the same monkey shown in Figure 12. The origin of a penetrating cortical arteriole measuring 20 μ in diameter appears to be from a pial plexus measuring 40 μ in diameter. Arteriolonecrosis involves both vessels. × 370.

FIG. 14. The meningeal spread from the same monkey shown in Figure 13. A penetrating cortical arteriole measuring 25 μ in diameter arises from the pial plexus. There is arteriolonecrosis in the penetrating arteriole distal to its origin. × 380.



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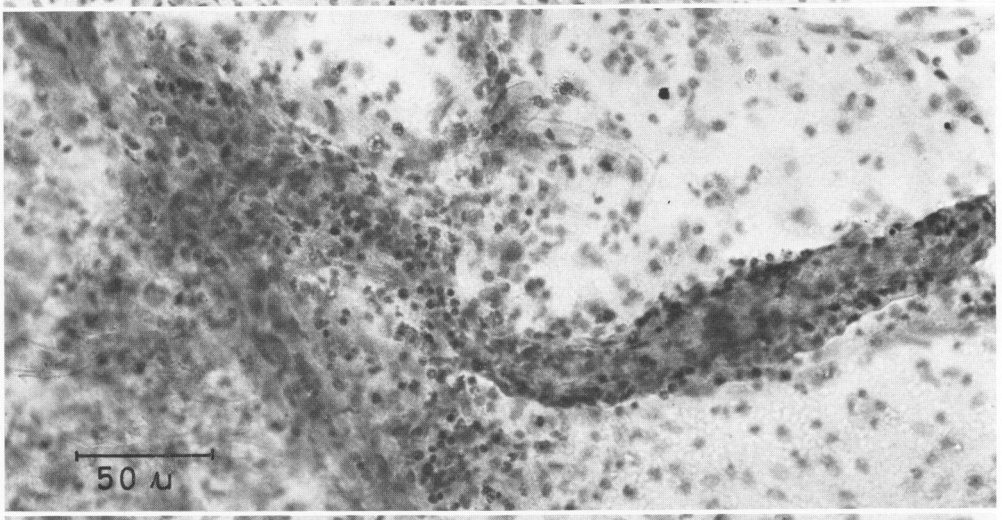

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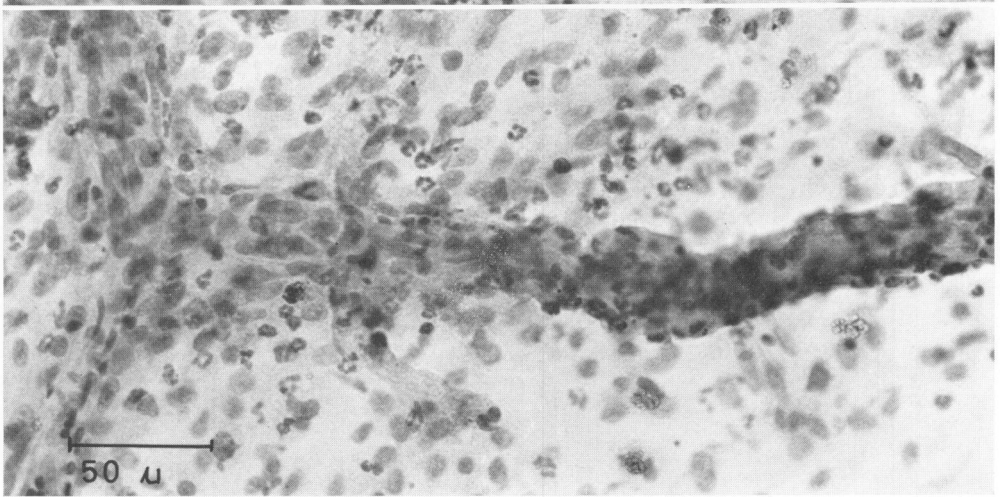

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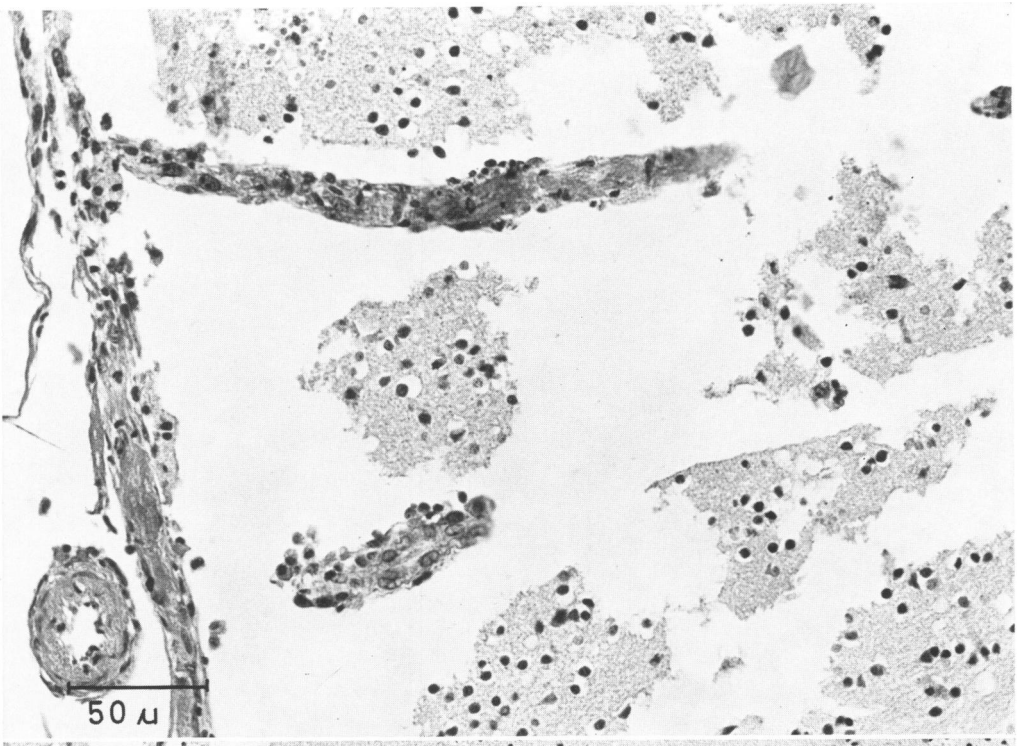
FIG. 15. Occipital cortex in the same monkey shown in Figures 12 to 14. There is muscle contraction in a meningeal arteriole measuring 40 μ in diameter and a pial plexus arteriole measuring 20 μ. Arteriolonecrosis appears at the lower left. A penetrating arteriole measuring 20 μ in diameter (upper) exhibits arteriolonecrosis in the cortex but the origin is unaffected. $\times$ 360.

FIG. 16. The distribution of fibrinoid necrosis in the penetrating arterioles is shown in the deeper cerebral cortex of the same monkey referred to in Figure 15. $\times$ 160.



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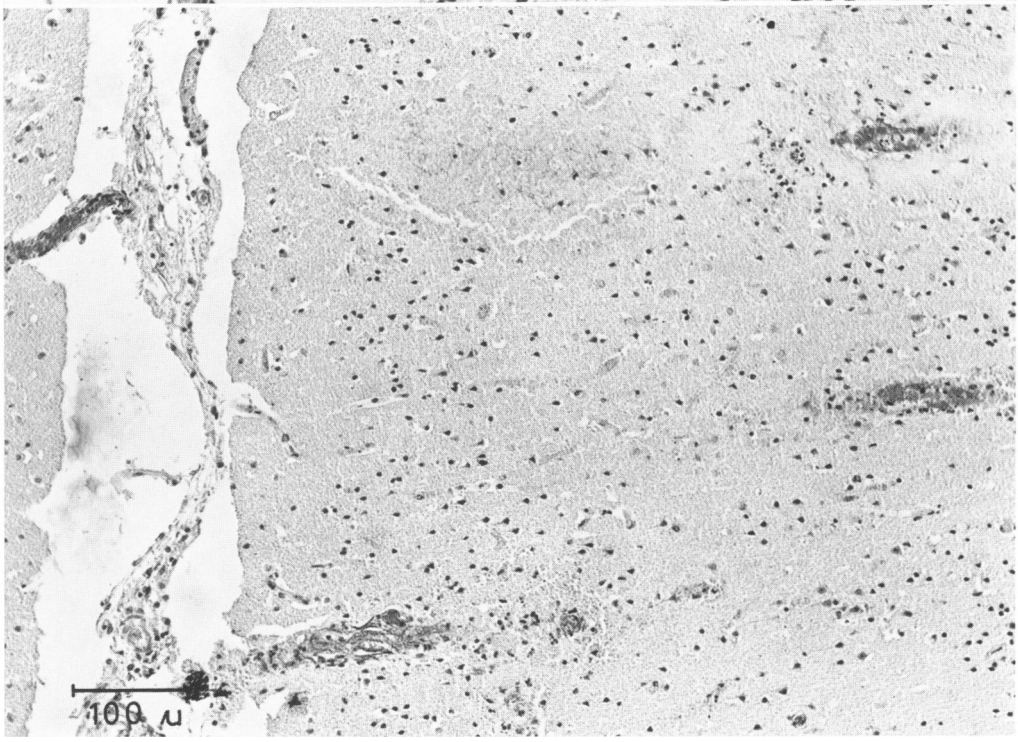

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