Pathogenesis of hypertensive retinopathy
An experimental study in the monkey

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It has been known for many years that the retina is especially sensitive to sustained elevations of blood pressure, and that the extent of the accompanying injury, which in its severest form results in visual failure, is roughly proportional to the level and rate of rise of the diastolic pressure. In this introduction there is no need to review the various theories advanced through more than a century to explain this association; we need only state the modern view that it is fundamentally due to reactivity of the retinal arterioles to the raised intraluminal pressure. Whether this is a direct association or whether other unknown factors inherent in the hypertensive state are also involved is still uncertain.

In the so-called malignant or accelerated phase of hypertension the retinal precapillary arterioles in the posterior fundus become focally occluded and necrotic (histologically defined as "fibrinoid necrosis") resulting in haemorrhages and sero-sanguineous exudation, with ischaemic manifestations of neuro-retinal oedema and cotton-wool spots. Ophthalmoscopical evidence of such changes often provides the first clinical observation that the hypertension is entering a malignant stage and that other arterioles throughout the body, especially in the brain and kidney, are already or will soon also be involved. Thus the pathogenesis of the arteriolar changes in the retina is likely to be similar if not identical to that in the general circulation, and in experimental work the eye provides an exceptional opportunity for studying the whole problem of hypertensive vascular disease. The ready accessibility of the retinal vessels to observation *in vivo*, augmented by fluorescein angiography, together with the clarity and ease with which they may be examined *in vitro* in the excised retina, makes it possible to correlate progressive stages in the evolution of the vascular abnormalities with their structural and ultrastructural changes. Many workers have exploited this favourable situation and there are numerous reports in the literature

of experimental hypertensive retinopathy in various animals, including rats (Cramer, 1940; Abt and Brückner, 1950; Byrom, 1963; Engerman, Meyer, and Buesseler, 1964; Uyama, 1966, 1967; Giacomelli, Juechter, and Wiener, 1972), cats (Meyer, Waltz, and Gotoh, 1960), dogs (Keyes and Goldblatt, 1938; Fasciolo and Cramer, 1939; Cramer, 1940; Laughlin, Thomas, and Friedenwald, 1940), and monkeys (Keyes and Goldblatt, 1938).

These experiments, with the single exception of those of Giacomelli and others (1972), although clearly establishing the relationship between hypertension and retinopathy, are otherwise of rather limited value since the techniques of fluorescein angiography, retinal digestion, and electron microscopy were not available, so that virtually no progress in our understanding of the exact nature and evolution of the all-important arteriolar lesions could be made.

In the present report we describe in detail our own combined clinical and pathological studies of experimental hypertension in monkeys, including the experimental work already published in preliminary communications (Ashton, Peltier, and Garner, 1968a; Garner and Ashton, 1970; Ashton, 1972), and on the basis of our findings together with those in the literature we suggest a possible explanation of the pathogenesis of the retinopathy.

MATERIAL AND METHODS

We chose the monkey because its ocular anatomy and, by inference, its retinal haemodynamics are a replica of the human situation.

Surgical procedures

Young growing Cynomolgus monkeys were subjected to bilateral procedures aimed at reducing, but not preventing, renal blood flow. Under nembutal anaesthesia (35 mg. per kg. body weight) given by intraperitoneal injection, the renal artery on one side, usually the left, was exposed and a stainless steel clip of the Goldblatt type with a 1 mm. gap was slid over the artery and anchored in place

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by passing a fine silk ligature through holes drilled in the open ends of the clip.

After an interval of 2 or more weeks a second operation was performed, again under nembutal anaesthesia, in which the contralateral kidney was freed from its attachments as far as the vascular pedicle and placed within a rubber balloon after the manner described by Lörincz and Gorácz (1954). The size of the balloon in its unstretched state was always a little greater than that of the enclosed kidney and was not such as to compress the cortex directly.

Although this was the usual order of events, in some instances bilateral clips were applied or bilateral balloons were used, and in some animals, in which the initial procedures were ineffective, several operations were required. In all seventeen monkeys were used.

Blood pressure measurement

The blood pressure was measured preoperatively and at weekly intervals thereafter using a tail plethysmograph. Unfortunately diastolic pressures could not be recorded by this method and, since to do so would have required intra-arterial cannulation—a procedure which does not lend itself to frequent repetition—measurement was limited to readings of the systolic levels.

Biochemical and haematological investigations

In all but the earliest experiments, plasma urea levels were estimated at weekly intervals as an index of renal damage, while haemoglobin and, in some cases, reticulocyte numbers were estimated as an indication of intravascular haemolysis and bone marrow depression related to developing renal failure.

Retinal photography and analysis

Ten monkeys (HM 1, 3, 5, 6, 8, 10, 11, 12, 13, and 16) had retinal photographs and fluorescein angiograms, four (HM 5, 10, 11, and 13) on more than one occasion.

For the photography the animals were anaesthetized as described previously (Kohner, Dollery, Shakib, Henkind, Paterson, de Oliveira, and Bulpitt, 1970). Anaesthesia was induced with 2·5 per cent. thiopentone injected into a leg vein. The trachea was intubated with a 3 mm. cuffed endotracheal tube and anaesthesia maintained with halothane 0·5–2·5 per cent. according to requirement and nitrous oxide and oxygen in a mixture of 2:1.

For the angiograms 0·05–0·1 ml. of 5 per cent. fluorescein was injected into the common carotid artery through a gauge 25 needle fitted to a oo nylon catheter.

The pupil was dilated using 1 per cent. mydrilate and 10 per cent. phenylephrine. The lids were kept open with a standard non-guarded lid speculum. The cornea was irrigated by a continuous saline drip. To bring the required retinal arteries into focus, the monkey's head was rotated into the correct position. Spontaneous movement of the eye usually indicated that the anaesthetic was too light.

Photographs were taken with a vertically mounted Zeiss (Oberkochen) fundus camera. Kodachrome II film developed commercially was used for colour photographs and Kodak Tri X film for fluorescein angiography. These were developed in D 76 undiluted developer for 14½ min. at room temperature.

Arterial diameters were measured from the fluorescein angiograms (Bulpitt, Dollery, and Kohner, 1970), using a Vanguard motion-picture analyser, adapted to take still photographs, whereby the photograph is projected on to an illuminated screen. Readings taken with an X-Y plotter were printed directly on to a teletype for analysis.

The vessels measured were the superior temporal artery as near to the disc as possible and further out after giving off first and second branches. The two first second-order arteries on the macular side were also measured. The diameter of third-order vessels was studied where possible.

The mean of three readings was taken at each point on those frames which showed maximal filling of the arteries. Since the exact magnification was not known, the results are expressed in arbitrary units.

Pathological examination

Post mortem examination

A general *post mortem* examination, including the brain, was performed immediately after death in all cases. Specimens of various tissues were removed and paraffin sections were prepared in the conventional manner.

Injections

In almost all cases one eye was injected with either colloidal carbon or Berlin blue by infusion through a wide-bore needle placed in the left ventricle of the heart. After fixation in formol saline the eye was opened through the ora serrata and the retina removed for mounting as a flat preparation.

Digests

In general a portion of retina was subsequently partially digested in a proteolytic enzyme solution so as to provide a leash of retinal blood vessels free from surrounding neuroglial tissue.

The technique employed was a modification described by Ashton (1963) of the method introduced by Kuwabara and Cogan (1960). Initially the vessels were stained with oil red O and later with haematoxylin and eosin.

Light and electron microscopy

Provided fluorescein angiography showed evidence of focal vascular damage, the animals were killed within the next 24 hours and the eyes removed. Enucleation of the eye studied angiographically was performed in the anaesthetized state after the animal had received a lethal intraperitoneal dose of nembutal.

The globe was then immediately removed, opened in the coronal plane through the ora serrata, and placed in chilled 1 per cent. isotonic veronal buffered osmium tetroxide, and the vitreous was gently removed. (In some of the earlier experiments fixation was by means of 2·5 per cent. glutaraldehyde in Tyrode buffer at pH 7·4.) By comparison with fluorescein angiographic photographs selected areas of retina and underlying choroid were excised during the initial stages of fixation before the specimen was so blackened by the osmium as to obscure the vascular landmarks. After fixation the specimens were dehydrated in ascending concentrations of alcohol and embedded in Araldite.

Thick sections, cut on a Huxley microtome, were stained with 1 per cent. toluidine blue and studied by light microscopy to enable the preparation of thin sections from the

relevant area. Thin sections were stained with uranyl acetate and lead citrate and viewing and imaging was carried out using an AEI EM6 electron microscope.

CLINICAL FINDINGS

Blood pressure

The initial systolic blood pressure, recorded either preoperatively or at the time of the first operation, varied from 75 to 130 mm.Hg (Table I). After the induction of bilateral renal ischaemia, the eventual rise in the ten monkeys subsequently found to have evidence of retinal vasculopathy averaged 43.5 mm.Hg, whereas of five monkeys which survived the initial operation without developing a retinopathy only one (HM 9) showed any appreciable rise in final systolic pressure. With a single exception (HM 8), in which animal the retinopathy was limited to a few early cotton-wool spots and minimal leakage of fluorescein, retinal lesions were not seen in monkeys which failed to develop a systolic blood pressure of at least 150 mm.Hg. In general, any alteration in pressure attributable to unilateral renal ischaemia was minimal and occasionally represented a decrease. Comparison of the highest reading in each animal with the maximum recorded before the ultimate operation, *i.e.* before bilateral renal ischaemia was produced, showed an average rise of 56.7 mm.Hg in those with retinal lesions and 41.0 mm.Hg in those without.

Table I *Systolic blood pressure before and after induction of renal ischaemia*

Monkey	Systolic blood pressure					
	At or immediately before renal surgical operations			Maximum recorded	Final	Evidence of retinopathy
	1st	2nd	3rd			
HM 1	110	110		170	140	+
HM 2	120	130	140	160	140	−
HM 3	90	130	90	180	180	+
HM 4	110	105	105	150	100	−
HM 5		110	110	170	120	+
HM 6	118	90		155	140	+
HM 8	105			145	140	+
HM 9	90	75		150	150	−
HM 10	110			155	135	+
HM 11	130	135		200	200	+
HM 12		100		170	150	+
HM 13	80	140		210	180	+
HM 15	125	130	155	170	145	−
HM 16	110		115	205	190	+
HM 17	105	135	125	203	110	−

Renal function

Alterations in plasma urea levels are summarized in Table II. Five animals had plasma urea levels of under 40 mg./100 ml. immediately before death, but most animals developed a measure of renal dysfunction. Nevertheless, only one of the ten monkeys that presented with retinopathy had a serious degree of impairment as reflected by values of 100 mg./100

ml. or more, whereas of five animals which did not become sufficiently hypertensive to produce retinal lesions, three had terminal urea levels considerably in excess of this figure.

Table II *Plasma urea (mg./100 ml.) before and after induction of renal ischaemia*

Monkey	Plasma urea level					
	At or immediately before renal surgical operations			Maximum recorded	Final	Evidence of retinopathy
	1st	2nd	3rd			
HM 1			38	24	24	+
HM 2			38	60	60	−
HM 3			44	68	56	+
HM 4			72	120	120	−
HM 5			60	70	30	+
HM 6	56	27		83	83	+
HM 8		31		108	108	+
HM 9		35		204	204	−
HM 10	27	40		164	50	+
HM 11		33		60	34	+
HM 12		59		100	100	+
HM 13		27		100	25	+
HM 15		22	25	380	380	−
HM 16			36	56	56	+
HM 17	33	46	36	90	64	−

The principal effect of both renal artery clipping and encapsulation of the kidney in a rubber balloon was to reduce the amount of functional parenchyma in the renal cortex. Usually the ischaemia resulted in atrophy, particularly when hypertensive retinopathy was present, but in some there was overt necrosis associated with elevated blood urea and, where it was pronounced, death occurred from renal failure. Such vascular changes as were present were limited to a mild degree of intimal hyperplasia in the arcuate and interlobular arteries and occasional arterioles. Insudative changes in the arterioles, whether of fibrinoid or hyaline type, were not observed in any of the kidneys.

These findings emphasize the importance of achieving a critical level of renal ischaemia if hypertension is to be produced. Too little ischaemia failed to influence the blood pressure, possibly because it was insufficient to stimulate the homeostatic function of the kidney, while too much ischaemia resulted in a lethal degree of renal failure.

Haematology

In those monkeys examined for evidence of anaemia (HM 11, 12, 13, 15, 16, 17), an appreciable drop in the haemoglobin level was found only after operative procedures which incurred a degree of haemorrhage. Furthermore, this posthaemorrhagic anaemia was invariably followed by a reciprocal reticulocyte response and the haemoglobin levels showed a steady return to preoperative levels. The reticulocyte response in the four monkeys in which it was measured (HM 13, 15–17) rarely rose above 4.0 per cent. and

was usually much lower. Examination of blood films failed to show any red cell fragmentation.

There was therefore no evidence of significant bone marrow depression or of a microangiopathic haemolytic process, such as has been invoked in the pathogenesis of some cases of malignant hypertension in man by Linton, Gavras, Gleadle, Hutchison, Lawson, Lever, MacAdam, McNicol, and Robertson (1969).

Retina

(1) Development of retinopathy

On the basis of the fundus photographs the monkeys can be divided into two groups.

Group 1 (HM 3, 8, 11, 12, 13) showed only minimal lesions or none on fundoscopy or colour photography, but had fluorescein angiographic evidence of retinal vascular disease.

Group 2 (HM 1, 5, 6, 10, 16) showed marked hypertensive retinopathy with many features similar to those seen in accelerated hypertension in man (Fig. 1, opposite). The photographic findings are summarized in Table III (overleaf).

The interval between the final operation and the development of retinopathy averaged just under 4 weeks in monkeys with marked changes, whereas the corresponding interval in those with minor disturbance was almost 10 weeks.

(2) Optic disc lesions

Papilloedema was observed in only one monkey (HM 1) and even in this instance it was only of a mild degree. This monkey was hypertensive for longer than the others.

Hyperaemia of the disc, on colour photography, with leakage of fluorescein from the vessels on angiography, was seen in monkeys 3 (R. disc), 5, 10, and 16. In most instances discrete leaking spots were seen on one or more arteries at the disc (Fig. 2). New vessels arising from the disc with a preretinal haemorrhage were observed in one instance (HM 6). The optic disc was normal on colour photography and fluorescein angiography in five monkeys (HM 3, L. disc, 8, 11, 12, 13).

(3) Vascular lesions

(a) Colour photographs in vivo

Animals with mild retinopathy or none had apparently straighter and narrower major retinal vessels (Fig. 3) than those with more severe lesions in which the vessels were tortuous and dilated (Fig. 4). Focal or general narrowing of retinal vessels was not observed. Increased light reflex was noted in those with severe retinopathy.
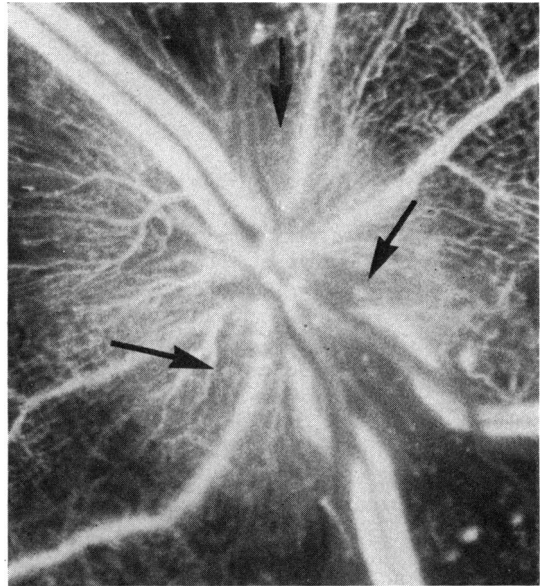

FIG. 2 *Fluorescence angiogram of R. disc of HM 10 in capillary phase. Arrows point to leaking spots*

(b) Fluorescein angiograms

(i) ARTERIAL CHANGES   The most characteristic abnormality seen was that of leaking areas on small arteries and terminal arterioles. This was the earliest lesion observed, even in the absence of funduscopic changes (Fig. 5), and was noted in every animal except HM 11 and the first photographs of HM 13. Such leaking arteries were always observed in areas of cotton-wool spots. These spots frequently appeared in clusters, and were most often seen within one disc diameter from the optic disc; their number gradually decreased as the distance from the optic disc increased. Only in the most severe retinopathies were they observed lateral to the macula (HM 5, 10, 16).

Increased tortuosity of the largest retinal vessels was noted in those with severe retinopathy. Arterial diameter measurements were possible in nine eyes (six with mild and three with severe retinopathy). The results indicate that the main superior temporal artery is dilated in severe as compared with mild retinopathy by between 25 and 44 per cent. depending on the distance from the disc (P = <0·06–0·01) (Table IV). No such difference was observed in the macular branches, but measurement of these smaller vessels is less accurate (Table IV).

In only one monkey (HM 10) were serial angiograms of sufficient quality to measure vessel diameter at the different times (Table III). In this animal, in which the retinopathy was severe on the first occasion,
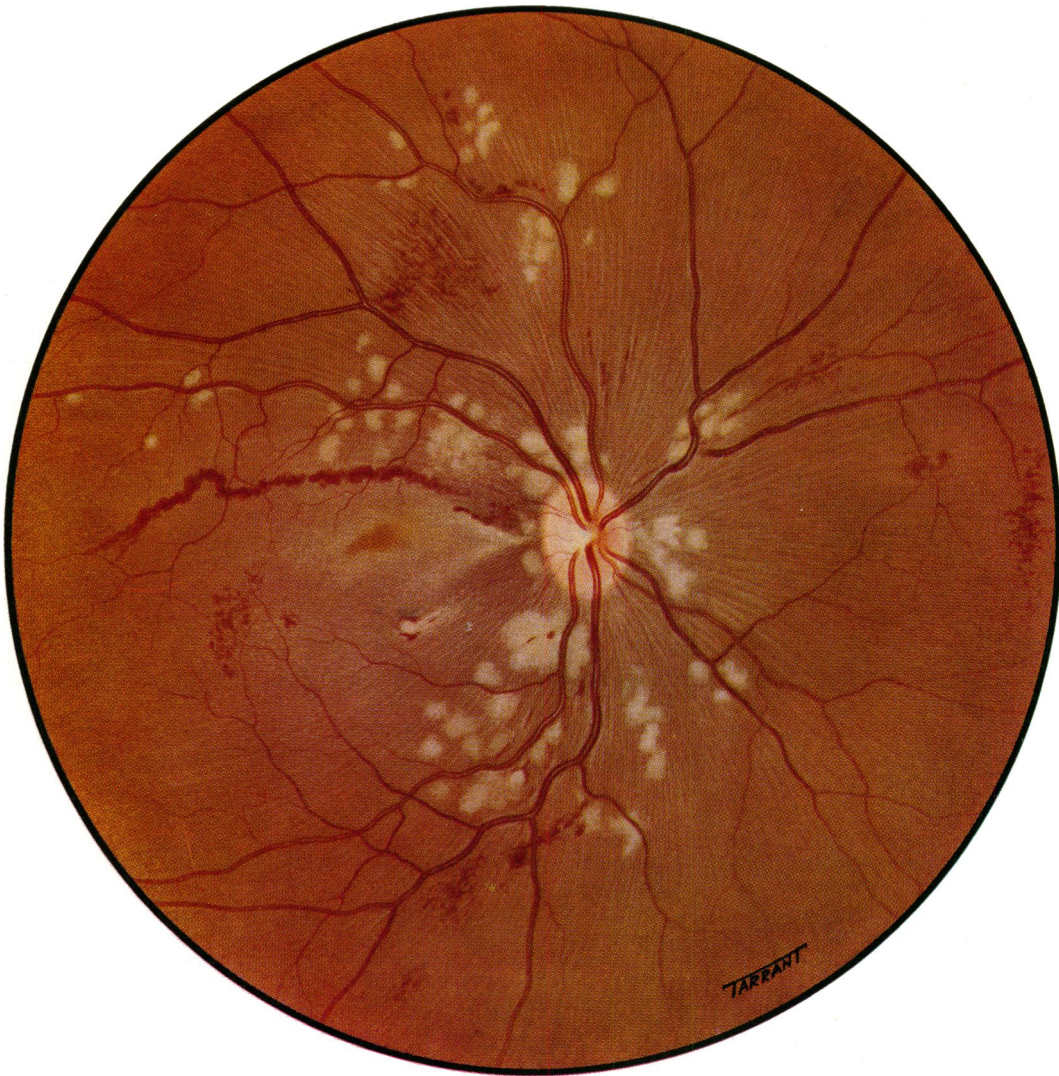
F I G . I   *HM I. Fundus painting of right eye 5 weeks after bilateral reduction of renal blood flow, showing neuro-retinal oedema, numerous cotton-wool spots, and haemorrhages*

Table III   *Hypertensive monkeys*

Retinopathy	Monkey no.	Duration of possible hypertension	Date of photographs	Photographic features		
				Colour		Fluorescein
Mild or none	HM 3	2·5 mths	2.5.1968	Disc:	R. Hyperaemic L. Normal	Disc:	R. Leaking spots, late leakage L. Normal
				Vessels: Extra- vascular:	Normal R. Pale areas L. One early cotton-wool spot (CWS)	Vessels:	Multiple leaking spots Delayed perfusion and possible non-perfusion of small areas Capillary abnormalities Arteriolar occlusion (L)
	HM 8	1 mth	25.3.1969	Disc: Vessels: Extra- vascular:	Normal Increased tortuosity Pale areas, ? early CWS	Disc: Vessels:	Normal Few well-defined leaking spots Small areas of delayed or non- perfusion
	HM 11	2·5 mths	23.10.1969 30.10.1969	Disc: Vessels: Extra- vascular:	Normal Normal Normal	Disc: Vessels:	Normal 23.10.69: Normal 30.10.69: Small area of non-perfusion
	HM 12	1 week	17.7.1969	Disc: Vessels: Extra- vascular:	Normal Normal Normal	Disc: Vessels:	Normal Small areas of delayed capillary perfusion Few leaking areas
	HM 13	1 mth	18.5.1970	Disc: Vessels: Extra- vascular:	Normal Normal Normal	Disc: Vessels:	Normal Normal
		5 mths	20.7.1970	Disc: Vessels: Extra- vascular:	Normal Normal ? Pale areas	Disc: Vessels:	Normal Multiple leaking spots
	HM 18	10 days	18.2.1971	Disc: Vessels: Extra- vascular:	Normal Normal No lesions	Disc: Vessels:	Normal Normal
Severe	HM 1			Disc: Vessels:	Papilloedema	Disc: Vessels:	Dilated capillaries, leakage + + Leaking spots
	HM 5	3 mths	23.5.1968	Disc: Vessels: Extra- vascular:	Hyperaemic Tortuous, dilated Increased light reflex CWS + + + Hard exudates Few haemorrhages Retinal oedema	Disc: Vessels:	Leaking spots, late leakage Leaking spots + + + Arterial occlusion Areas of delayed or non-perfusion Retention of dye in capillaries
	HM 6	1 mth	19.11.1968	Disc: Vessels: Extra- vascular:	New vessels Preretinal haemorrhages Not remarkable Preretinal haemorrhage CWS + + + Haemorrhages	Disc: Vessels:	Leaking spots, abnormal vessels Multiple leaking spots Areas of late or non-perfusion associated with leaking spots Late retention of dye in capillaries
	HM 10	2 mths	10.4.1969	Disc: Vessels: Extra- vascular:	Normal Slight increase in diameter and tortuosity CWS + + + Haemorrhages + + Retinal oedema	Disc: Vessels:	Leaking spots, late leakage Multiple leaking spots, many associa- ted with delayed or non-perfusion Late retention of dye in capillaries
		2 mths	17.4.1969	Disc: Vessels: Extra- vascular:	Hyperaemic Same as before Same as before + Early macular star	Disc: Vessels:	Similar to 10.4.1969 Similar to 10.4.1969
		3 mths	8.5.1969	Disc: Vessels: Extra- vascular:	Normal Same as before Change in CWS Macular star	Disc: Vessels:	Leaking spots Similar to 17.4.1969, but filling of capillary bed more uneven More areas of delayed perfusion Late retention of dye
	HM 16	4 mths	8.2.1971	Disc: Vessels: Extra- vascular:	Hyperaemic Dilated increased tortuosity Increased light reflex CWS + + + Few haemorrhages Retinal oedema	Disc: Vessels:	Leaking spots Late hyperfluorescence Leaking spots Arteriolar occlusion Delayed and non-perfusion of small areas Marked capillary abnormalities Late retention of dye

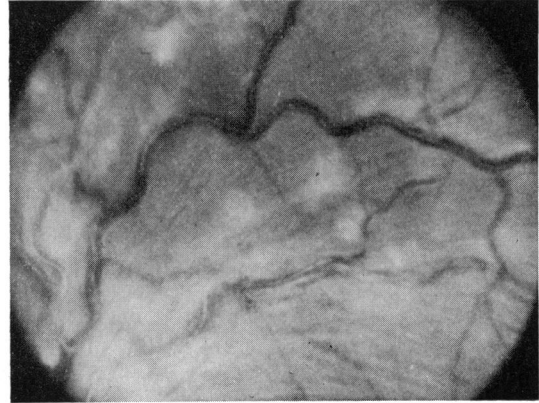HM 17 was found dead before fluorescein angiography could be carried out

FIG. 3 *From colour photograph of R. superior temporal quadrant of HM 3, showing normal major retinal vessels*
FIG. 4 *From colour photograph of L. superior temporal quadrant of HM 5 with severe retinopathy. Note increased tortuosity and dilatation of major retinal vessels*

FIG. 5 *L. superior temporal quadrant of HM5 with severe retinopathy. Fluorescence angiogram in capillary phase. Note discrete leaking spots (arrows)*

the vessels were significantly wider at the first study than on subsequent examinations.

Focal or generalized narrowing of arteries was not observed. Arteriolar occlusion and retrograde filling of the distal part of the vessel was observed in HM 3, 5, and 16. Arteriolar occlusion was also observed in some areas of cotton-wool spots. Obstruction of the vascular inflow in these animals could readily be detected by the failure of capillary perfusion at a time when surrounding capillaries were filled with fluorescein. In most instances the ischaemic area filled later from surrounding capillaries. Microaneurysms and circumferential leakage around cotton-wool spots, such as are seen in man, were not observed in any of the monkeys.

(*ii*) CAPILLARY CHANGES   Capillary perfusion was absent only in areas of cotton-wool spots, although these areas filled later in most instances. The capillary bed, however, appeared coarse and irregular and there was leakage of dye in later pictures, indicating endothelial cell damage (Fig. 6*a,b*). Surrounding the leakage spots were small areas in which capillary

Table IV *Vessel diameters (arbitrary units)*

Vessel		Vessel diameter (Mean ± S.E.)		
		'Mild' retinopathy	*'Severe' retinopathy*	*Significance* (P)
Main superior temporal artery	Off disc	128·1 ± 6·5	160·0 ± 16·5	0·06
	After first side branch	114·7 ± 8·3	158·0 ± 15·8	0·03
	After second side branch	99·1 ± 6·7	143·3 ± 16·0	0·01
Branch arteries	First macular side branch	72·7 ± 6·5	82·9 ± 13·4	N.S.
	Second macular side branch	94·1 ± 11·9	90·5 ± 17·8	N.S.

FIG. 6(a)   *From fluorescence angiogram of R. inferior temporal region of HM 16 during 1st passage of fluorescein. Arrow points at area of non-perfusion*
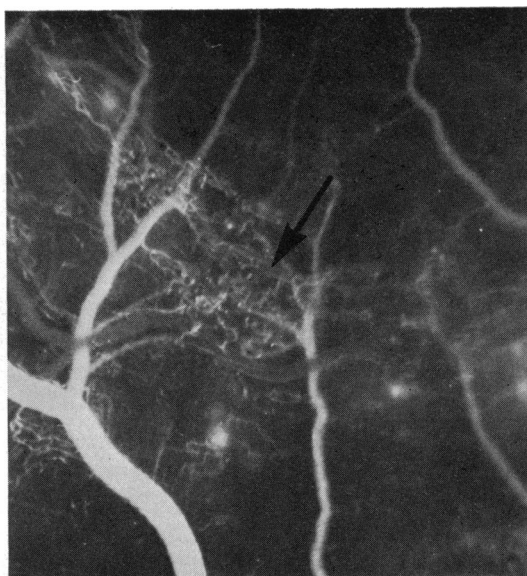
FIG. 6(b)   *Same as Fig. 6(a) but in late venous phase. Area now perfused (arrow) when normal capillaries empty. Note irregular hyperfluorescence of some capillaries*

FIG. 7   *From fluorescence angiogram of superior area of HM 10. Note areas of capillary non-perfusion associated with leaking vessels (arrows 1). Also coarsening of capillary bed and probable non-perfusion of areas not associated with leakage (arrows 2)*

perfusion was delayed and at times probably absent (Fig. 7). Because of extensive leakage on late pictures it was often impossible to be sure of non-perfusion.

The background mesh-work of capillaries appeared coarser than normal in some retinal areas even in the absence of leaking spots or obstructed arterioles (Fig. 7). This may mean closure of some capillaries, but the significance of this finding cannot be assessed because there were insufficient serial studies of animals before and during the development of hypertensive retinopathy.

In five animals (HM 5, 6, 8, 10, 16), there was prolonged retention of dye in some capillaries, with probable leakage into but not through the capillary wall (Fig. 8).

(*iii*) EXTRAVASCULAR LESIONS   These animals commonly showed extravascular lesions characteristic of hypertensive retinopathy.

Superficial linear or flame-shaped haemorrhages were present in five (HM 1, 3, 5, 6, 10) (Fig. 9). Preretinal haemorrhage was observed only in HM 6 (Fig. 10).

Cotton-wool spots, the commonest extravascular lesions seen, occurred in seven animals (HM 1, 3, 5, 6, 8, 10, 16). Many of these were just paler faint greyish areas in the retina, difficult to ascertain as cotton-wool spots until compared with the fluorescein

angiogram, where they were always associated with leaking arterioles. Their distribution was similar to that seen in accelerated hypertension in humans, *i.e.* they were more frequent in the immediate vicinity of the disc (Fig. 9) and were only rarely present lateral to the macula.

Hard waxy deep retinal exudates were seen in only three monkeys (HM 1, 3, 10). In two of these (3 and 10) a macular star developed (Fig. 11*a*, *b*, *c*) during the period of observation.

PATHOLOGICAL FINDINGS

Extraocular tissue pathology

Of the gross *post mortem* findings we need mention only the ischaemic kidney surrounded by the rubber balloon, and a marked left ventricular hypertrophy of the heart.

Hypertensive vascular disease in the extraocular tissues examined was rarely observed; evidence of fibrinous arteriolonecrosis was seen in only four animals, and was confined to the small intestine (Fig. 12) in all but one monkey (HM 6) which showed additional involvement of the adrenal capsule (Fig. 13). Rather more common was the presence of a pericapillary fibrinous exudate; this occurred in isolated foci in the cerebral cortex of HM 4–6, and 13 (Fig. 14), as well as in the myocardium of HM 3,
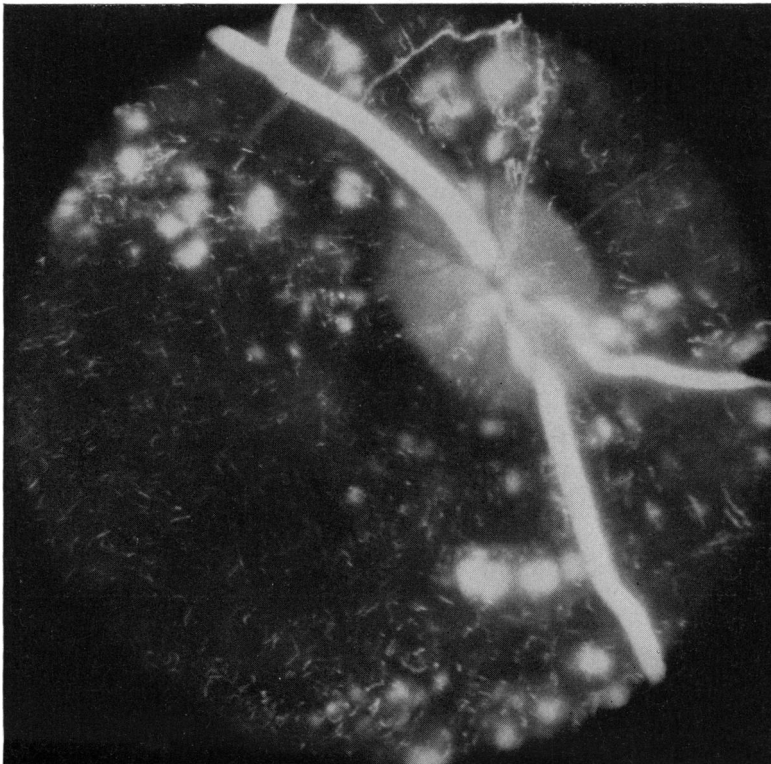
FIG. 8   *From fluorescence angiogram of HM 10 in late venous phase, showing retention of dye in capillaries and profuse leaking spots*
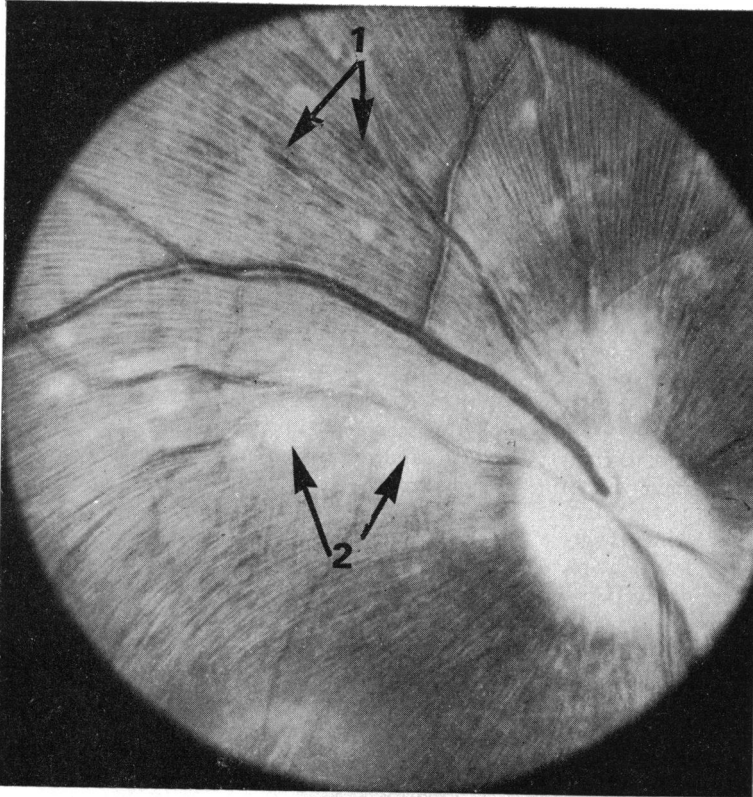
FIG. 9   *From colour photo-graph of R. superior temporal area of HM 10, showing flame-shaped haemorrhages (arrows 1) and cotton-wool spots (arrows 2)*
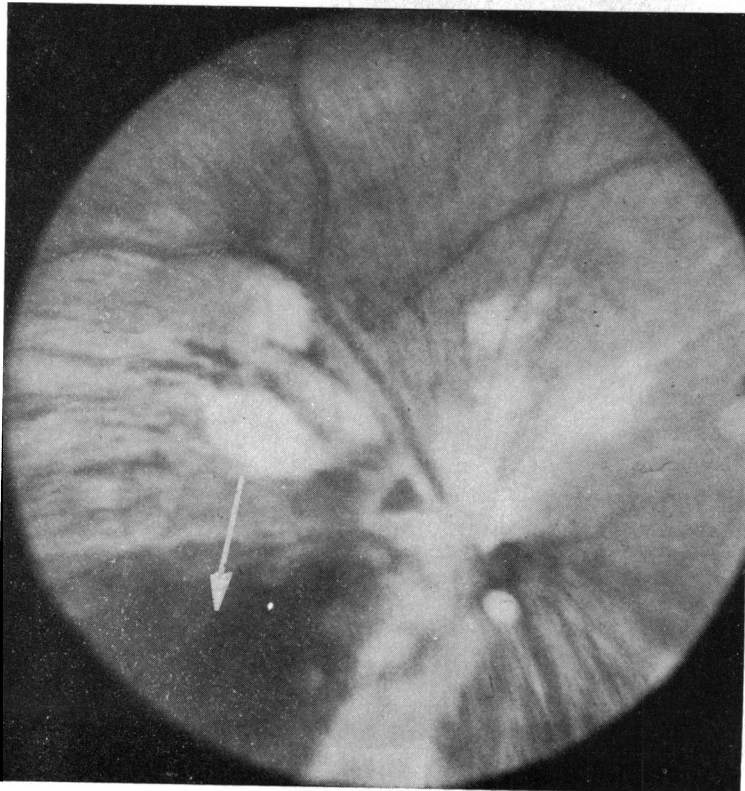

FIG. 10   *Preretinal haemor-rhage (arrow) arising from new vessels on disc (HM 6)*
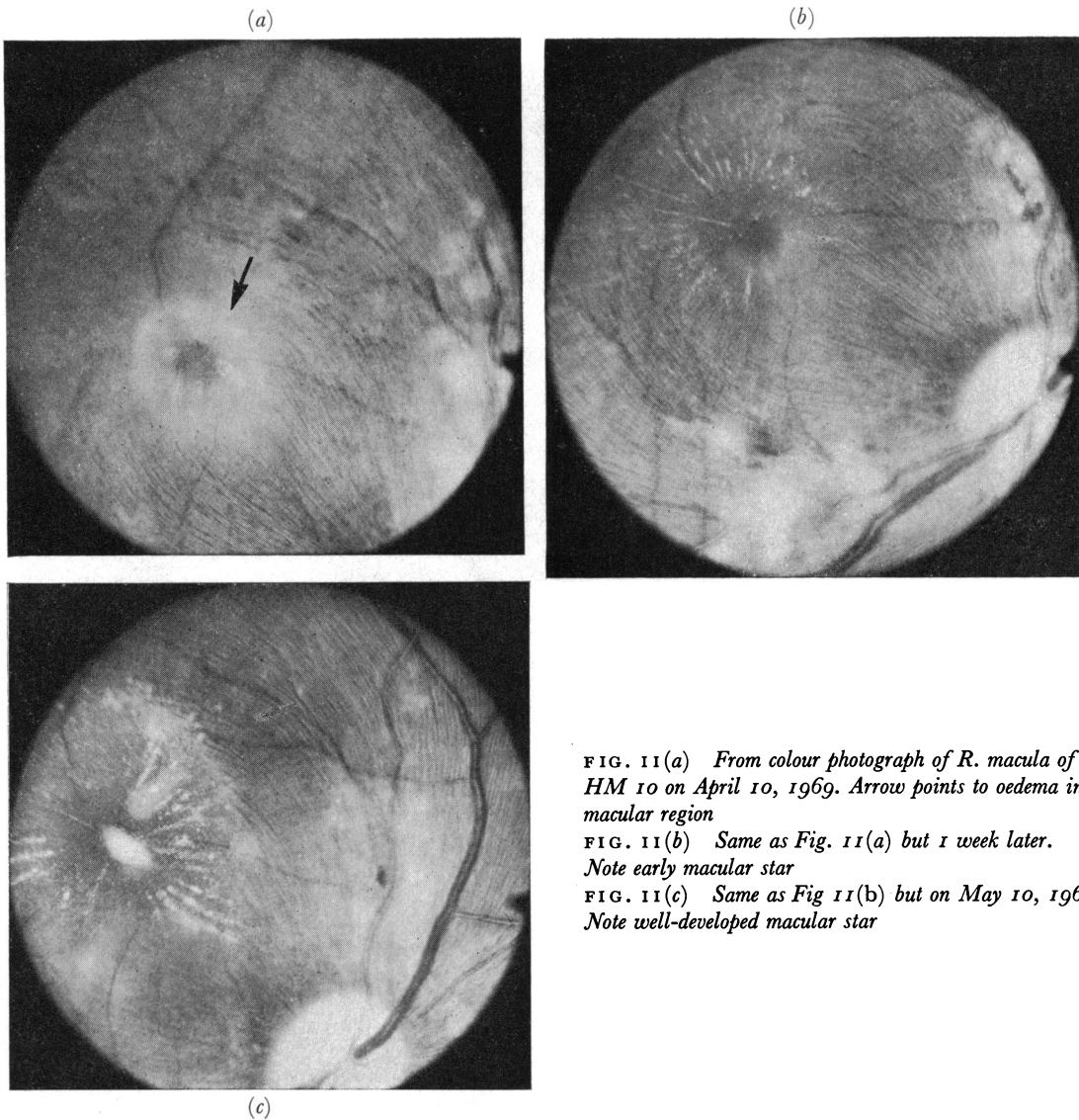
FIG. 11(a)   *From colour photograph of R. macula of HM 10 on April 10, 1969. Arrow points to oedema in macular region*

FIG. 11(b)   *Same as Fig. 11(a) but 1 week later. Note early macular star*

FIG. 11(c)   *Same as Fig 11(b) but on May 10, 1969. Note well-developed macular star*

6, 8, and 13. No evidence of hypertensive disturbance was found in the liver, spleen, or pancreas of any animal.

Light microscopy: Retina

Since the primary object of the project was to analyse the early vascular changes in hypertensive retinopathy, most of the animals were killed before florid retinal disease had time to develop, and consequently histological signs were usually mild. In the one animal in which mild papilloedema was observed (HM 1) there was protrusion of the optic nerve head forwards and laterally to form neuritic swellings in the border tissue of Kuhnt (Fig. 15).

Serous exudates, some containing recognizable fibrin, were observed in the posterior retina in six animals (HM 1, 3, 5, 6, 10, 13) (Fig. 16). They were located principally in the outer plexiform layer but smaller amounts of exudate were also to be found around the blood vessels in the inner retinal layers (Fig. 17). In the latter situation they were often accompanied by frank haemorrhage.

Cotton-wool spots, noted clinically in all but three monkeys (HM 11, 12, 13), were identical to those observed in man, presenting as collections of swollen axons—many containing densely-staining pseudonuclei ("cytoid bodies")—in the nerve fibre layer (Fig. 18).

Where vascular changes were observed, these were usually best demonstrated in flat digest and injected specimens. Insudation of lipo-hyaline mater-
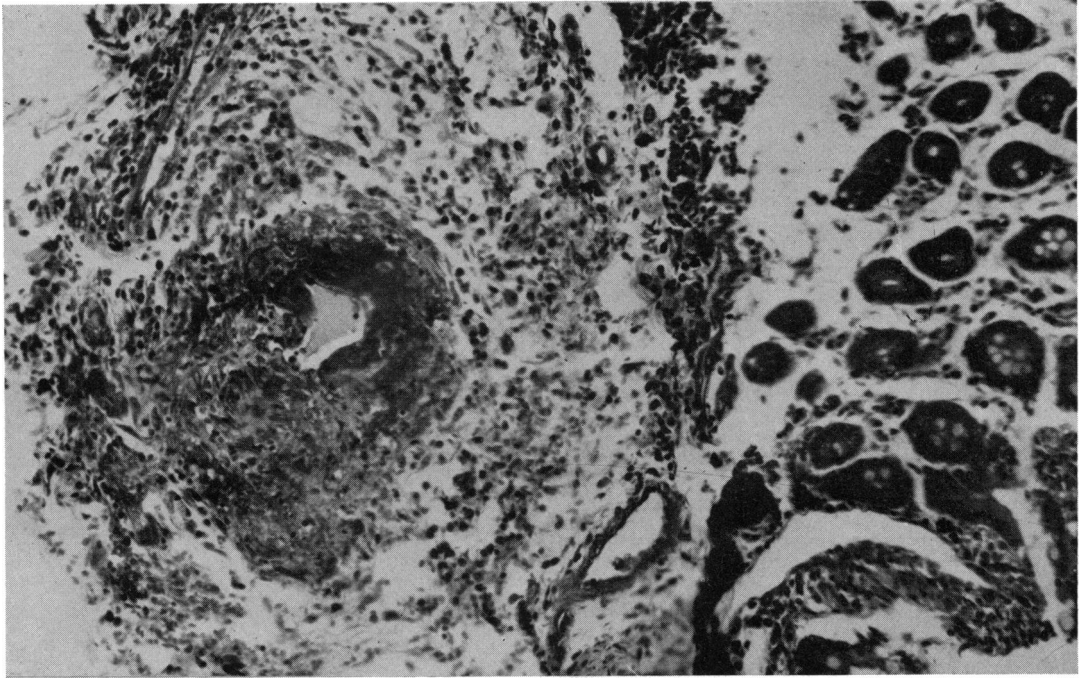
FIG. 12   *HM 6. Section of wall of small intestine, showing fibrinoid necrosis in wall of small arteriole. Picro-Mallory. × 175*
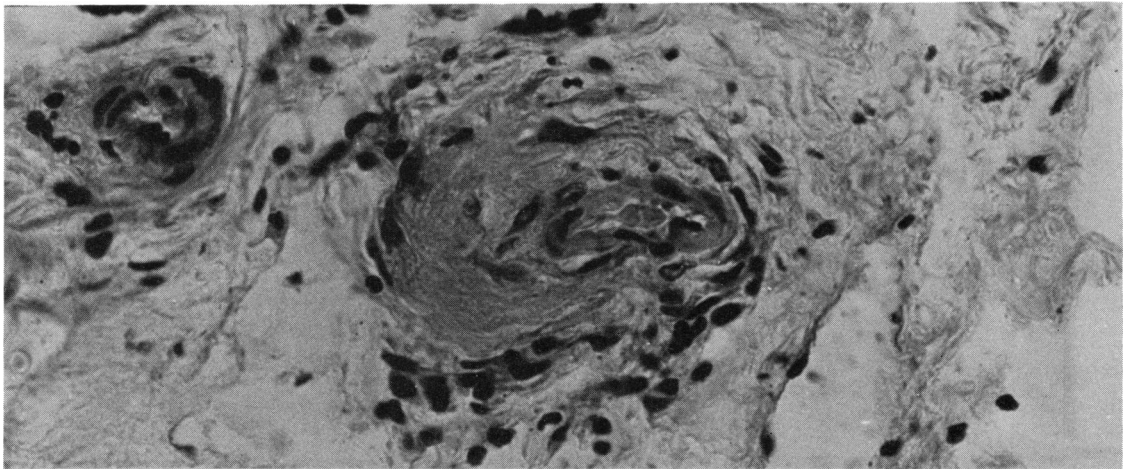
FIG. 13   *HM 6. Wall of necrotic arteriole in adrenal capsule thickened by accumulation of amorphous eosinophilic material; as a result there is gross narrowing of the vessel lumen. Haematoxylin and eosin. × 440*
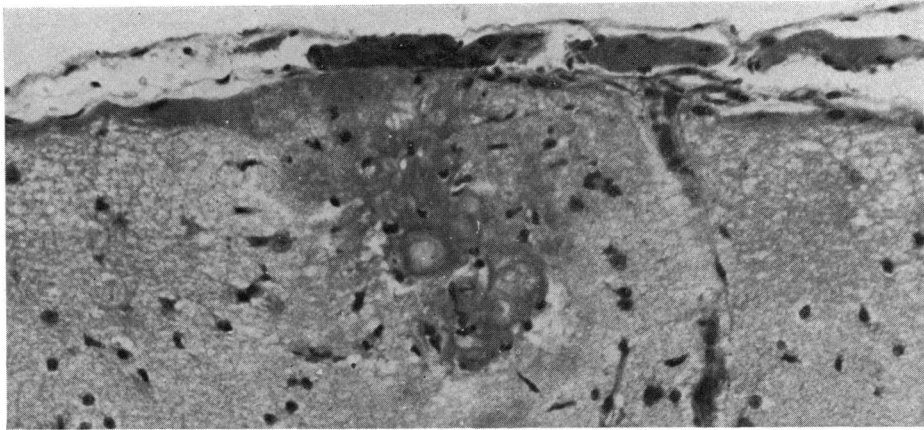
FIG. 14 *HM 13. One of several foci in cerebral cortex, showing exudation of fibrinous material from degenerate capillaries. Haematoxylin and eosin.* ×225
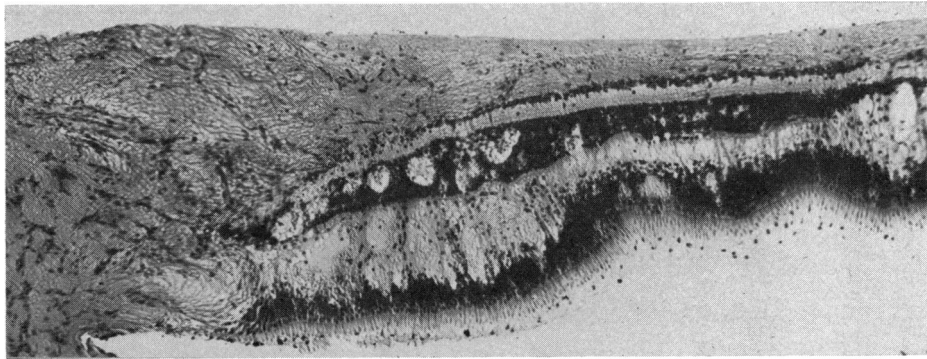
FIG. 15 *HM 1. Section of optic disc margin and peripapillary retina. Oedema and serous exudates in outer plexiform layer of retina associated with forward and lateral protrusion of oedematous nerve head. Haematoxylin and eosin.* ×58
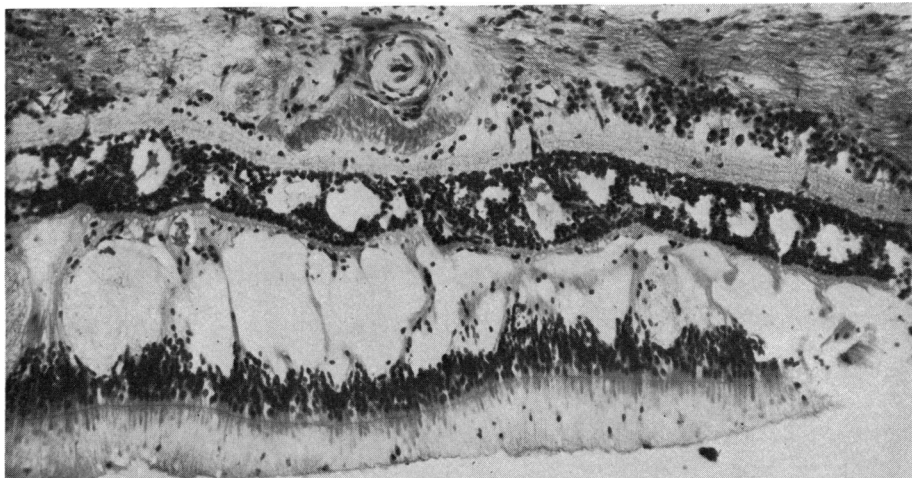
FIG. 16 *HM 1. Oedema and plasma exudates in outer plexiform layer of retina associated with arteriole in inner retina, showing marked fibrinous insudation and calibre reduction. Haematoxylin and eosin* ×110
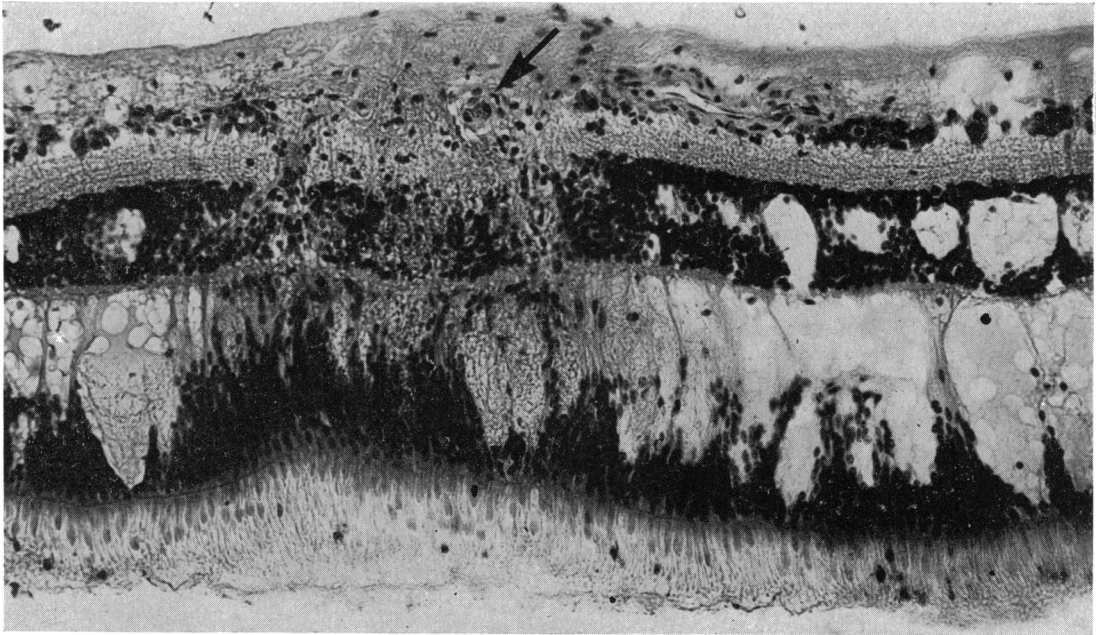
FIG. 17   *HM 1. Arteriole in inner layers of retina (arrowed) associated with extensive exudation of plasma into surrounding tissue and disruption of ganglion and inner nuclear layers. Haematoxylin and eosin.   × 180*
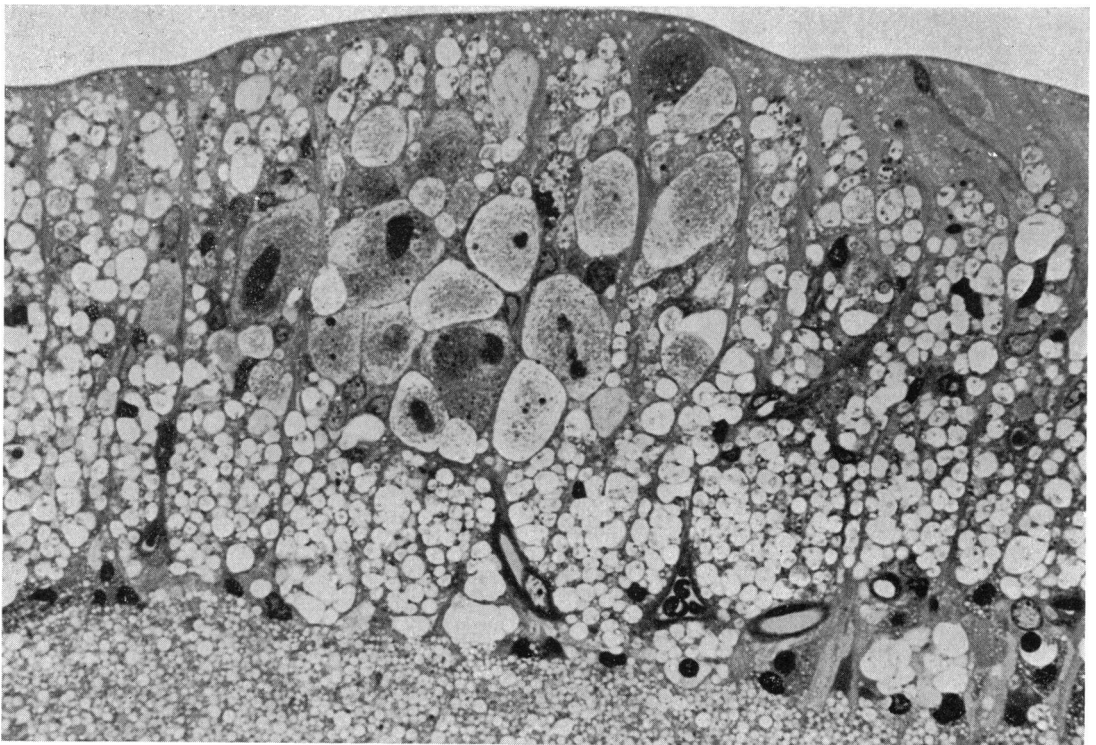
FIG. 18   *HM 5. Section through inner retina, showing part of cotton-wool spot composed of greatly swollen axons, many of which contain pseudonuclei (cytoid bodies). Toluidine blue/Araldite-embedded tissue.   × 475*

ial into the walls of precapillary arterioles was observed in four monkeys (HM 1, 5, 6, 16), while small areas of unperfused capillaries were seen in seven (HM 1, 3, 5, 10, 12, 13, 16). In no instance were capillary microaneurysms observed. Histologically the retinal pigment epithelium appeared normal throughout. In one animal with hypertensive retinopathy (HM 5), which had been injected with Berlin blue, a flat preparation of the bleached choroid stained with oil red O, showed numerous arterioles, especially in the peripapillary region, with focal lipid and protein insudation, leakage into the choroidal stroma, and grossly attenuated lumina.

Electron microscopy: Retina

(1) Arterioles
Findings varied from apparent normality to extensive necrosis but a striking feature of several retinae (HM 3, 6, 10, 13) was the presence of markedly constricted precapillary arterioles. In some instances the constriction amounted to virtual closure of the lumen. The smooth muscle coat of such vessels was in some cases normal, with a full complement of prominent myofibrils (Fig. 19), but in others there were varying degrees of degenerative change characterized by depletion of myofibrils and consequent increase in electron lucency (Figs 20–22). There was, however, no evidence of significant endothelial cell damage or degeneration in the constricted vessels, although the nuclei of both endothelium and smooth muscle frequently presented a corrugated appearance which was due to multiple invaginations of the nuclear membrane.

On occasion (HM 5, 6, 8, 10) cytoplasmic vacuolization and loss of myofibrils were seen in the smooth muscle of the larger first- and second-order arterioles, but this was usually of a fairly minor degree and never involved the entire media. Calibre reduction was not apparent in these vessels.

Rarely (HM 6) dilated patent arterioles of precapillary size showing extensive smooth muscle necrosis in the absence of detectable endothelial damage were observed. Some sections showed plasma leakage into the vessel wall which had seeped into the muscular coat, displacing and eventually replacing the degenerating muscle cells (Fig. 23). Advanced arteriolar necrosis represented by complete loss of cellular components resulting in a corrugated residue of basement membrane and inevitable obliteration of the lumen was seen in two animals (HM 5, 16) (Fig. 24).

Three animals (HM 3, 6, 16) showed precapillary arteriole closure or calibre reduction, associated not so much with signs suggestive of smooth muscle activity as with mural infiltration by an amorphous finely granular material of moderate electron density (Fig. 25), and dense banded fibrillar structures,

having a periodicity of approximately 22 nm and thus indicative of fibrin (Fig. 26). In these vessels, it seemed quite probable that the narrowed lumen was due principally to induration of the wall by the accumulated granular and fibrinous deposits. In other instances similar mural infiltration was seen in arterioles with open lumina (Fig. 27). Except for arterioles in which there was extensive necrosis involving the entire thickness of the wall, there was, as a rule, no clear indication how the plasma-derived insudate had gained access to the vascular media. The endothelial lining was intact, with no appreciable evidence of degenerative change and no breakdown of the intercellular junctions, in all but two (HM 10, 16). The most striking of these concerned a minute break just over 0.6 μ in width in the endothelial lining of a terminal arteriole infiltrated and surrounded by granular and fibrinous material: the break was apparently within the endothelial cytoplasm and did not seem to involve the intercellular junctions (Fig. 28). Elsewhere, even in the immediate neighbourhood of this isolated defect, the endothelium, although attenuated, was intact. Subsequent sections of the same vessel showed virtual obliteration of the lumen by large masses of intramural granular material and fibrin. In the other case the endothelium was found to be focally necrotic (Fig. 29).

Obliteration of the arteriole lumen by intravascular thrombosis was not observed.

Evidence of probable regenerative activity involving both endothelium and smooth muscle was seen in two animals (HM 3, 10).

(2) Capillaries

Capillary changes closely mimicked those of the arterioles and consisted most often (HM 3, 6, 8, 10, 12, 13) of closure of otherwise intact channels (Fig. 30). Sometimes this was associated with corrugation of the endothelial cell nuclei and an apparent increase in endothelial bulk. Only rarely was there any indication of increased pericyte volume (Fig. 31). In other capillaries the closure appeared to be the result of collapse (HM 6, 13) without any attendant signs suggestive of active contraction.

Both open and completely closed capillaries with degenerative changes involving pericytes alone (HM 3, 5, 6, 10) (Fig. 32) or pericytes and endothelium, often in association with deposits of amorphous material and fibrin in the wall (HM 1, 6, 13, 16) (Figs. 33, 34) were also observed. Some degenerate capillaries were patent and the cellular defect could be extremely focal and restricted to a single endothelial cell within an otherwise intact lining (Fig. 35). In some capillaries the endothelium was degenerate (Fig. 36) and in others it was both necrosed and disintegrated (Fig. 37). Occasional vessels in an even more advanced stage of necrosis were reduced to a
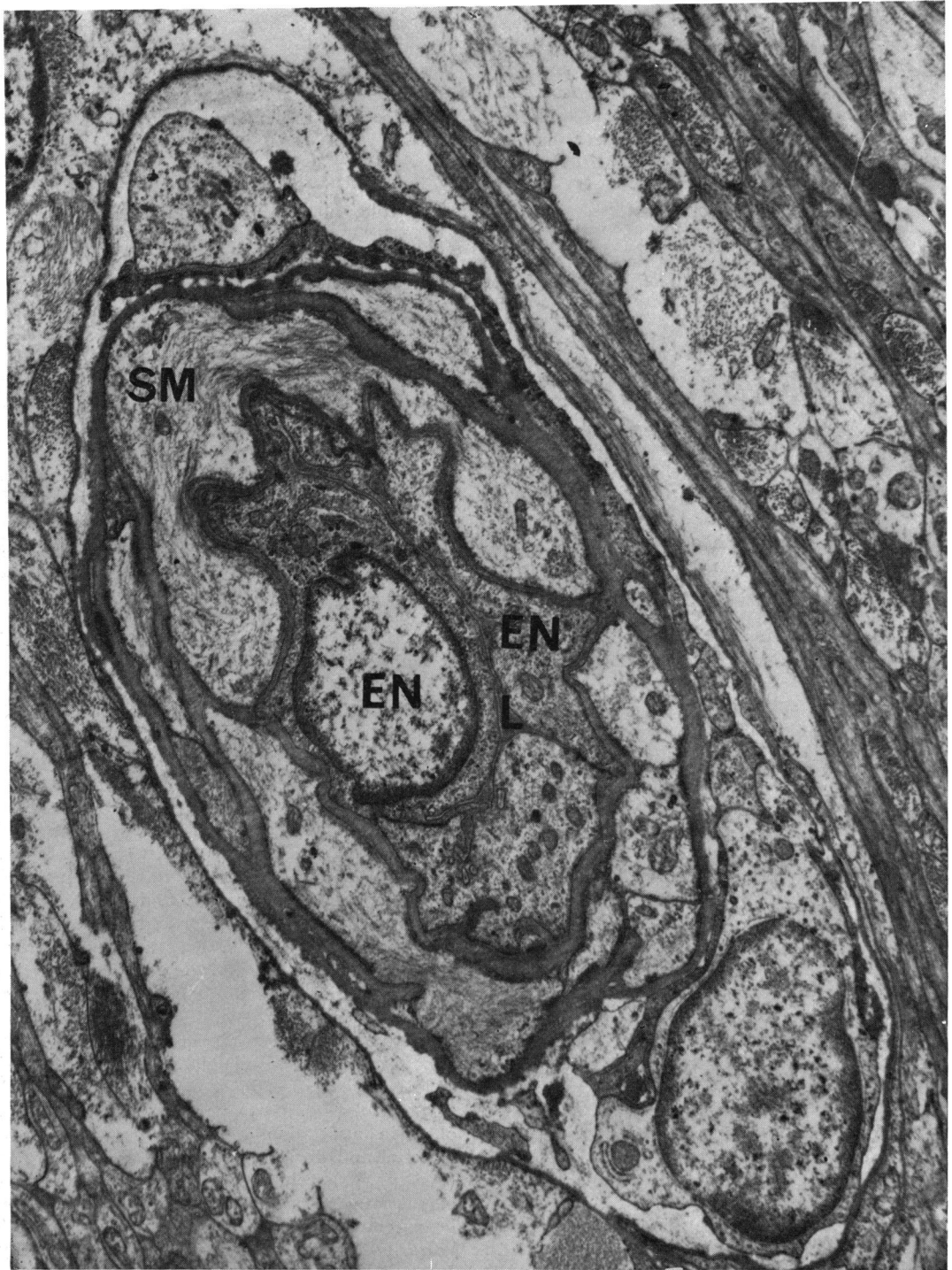
FIG. 19 *Electron micrograph of contracted arteriole with obliterated lumen (L.). Endothelial lining (EN) is intact and smooth muscle cells (SM), showing prominent myofilaments, also appear healthy.* × 13,000

FIG. 20 *Electron micrograph of contracted arteriole with obliterated lumen (L). Endothelial lining (EN) is intact but smooth muscle cells (SM) show swelling, loss of cytoplasmic density, and depletion of myofilaments.*   × 10,000
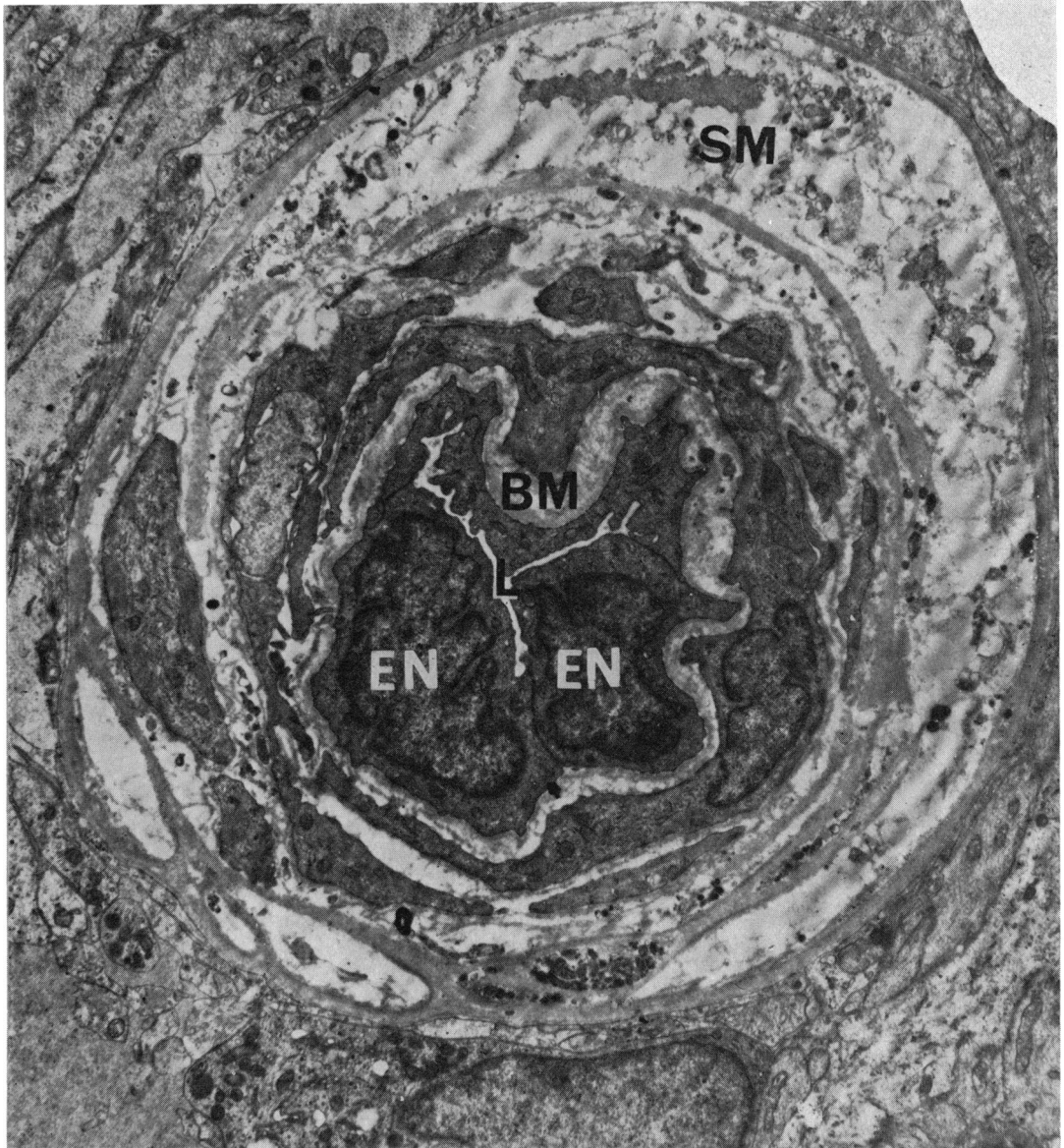
FIG. 21   *Contracted arteriole with obliterated lumen (L) and corrugated basement membrane (BM). Endothelium (EN) is intact but smooth muscle cells (SM) show oedema, loss of myofilaments, and necrotic disintegration. Electron micrograph.* ×8,000

FIG. 22 *Electron micrograph of wall of arteriole, showing varying degrees of degenerative changes in muscle cells. Note advanced necrosis in one muscle. Arrows denote cellular debris.* × 25,000

FIG. 23   *Arteriole with open lumen (L) and intact endothelial lining (EN). The endothelial cells on the left may actually be muscle cells repairing an endothelial breach. Smooth muscle cells are degenerate and in many places replaced by granular debris and plasma. Note also intra- and extramural polymerized deposits of fibrin (F). Electron micrograph.   × 20,000*
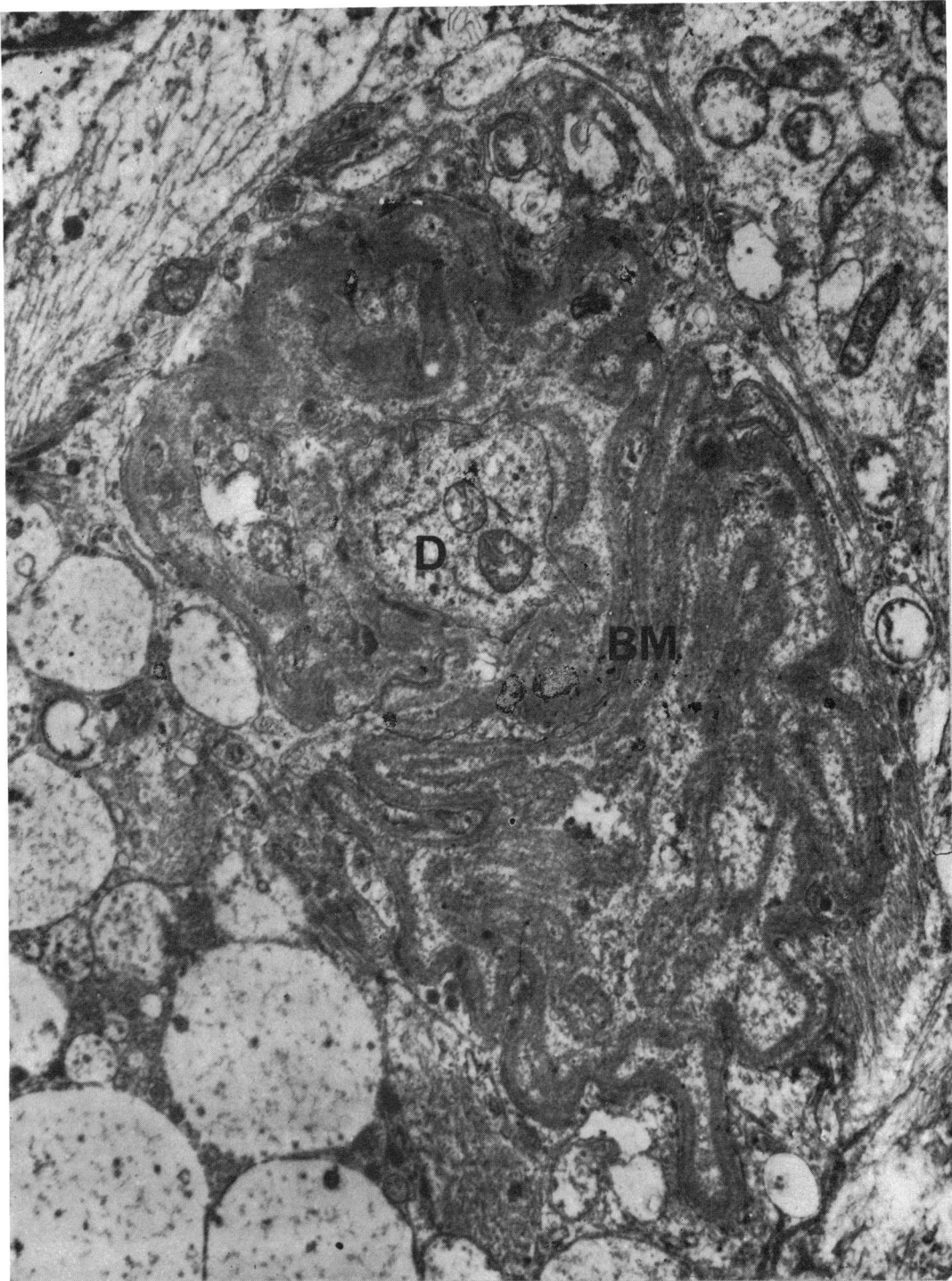
FIG. 24 *Corrugated basement membrane remnants (BM) and some cellular debris (D) representing terminal stage of obliterated and non-functioning arteriole. Electron micrograph.* × 14,000
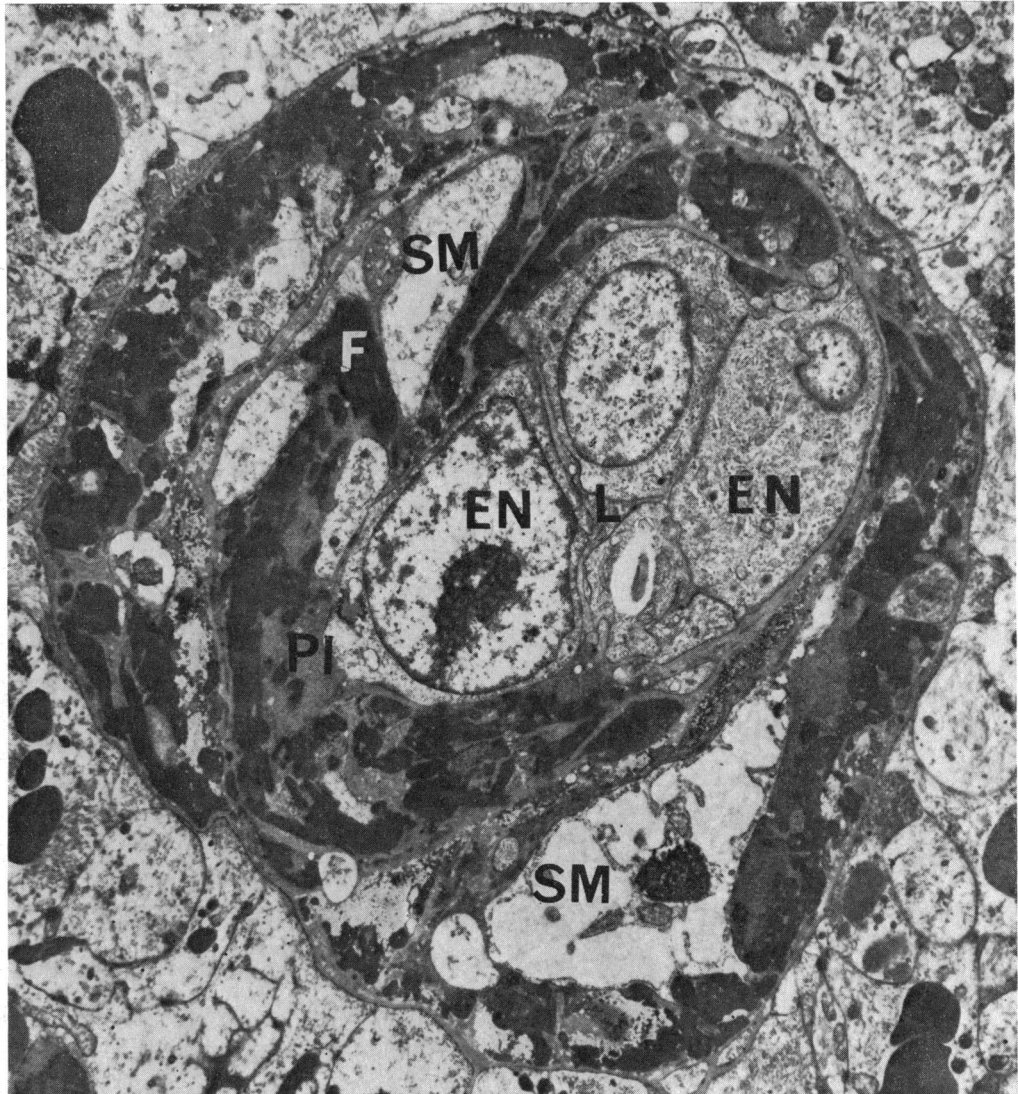
FIG. 25 *Electron micrograph of closed arteriole with obliterated lumen (L); endothelium (EN) is intact and appears healthy. Wall contains plasma (PL), fibrin (F), and vacuolated and degenerate muscle cells (SM).* ×8,000
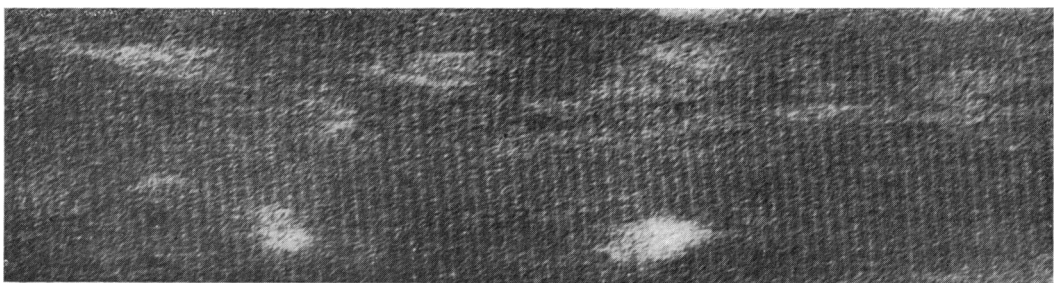
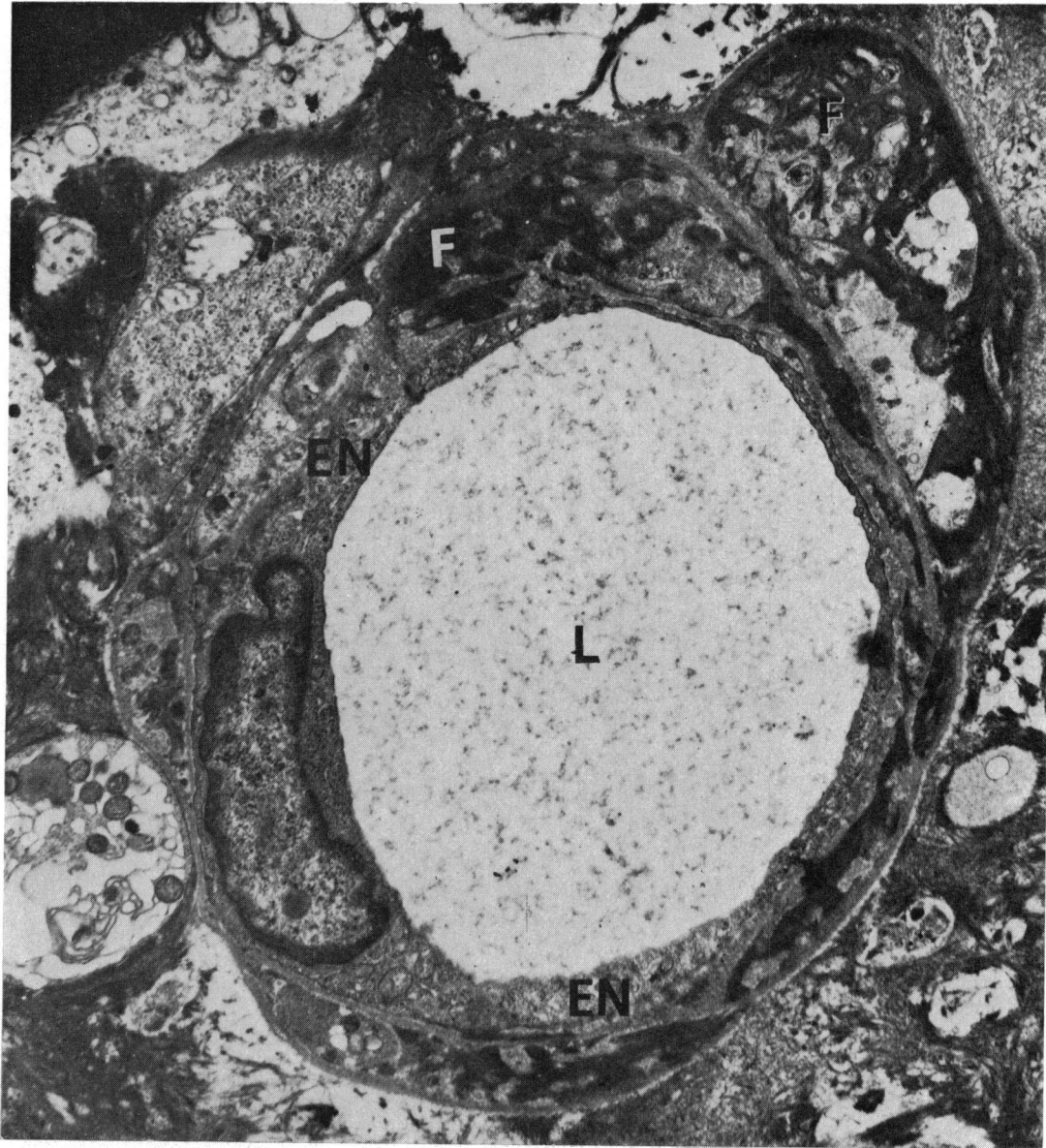FIG. 26 *Electron micrograph of polymerized fibrin, showing main periodicity of approximately 22 nm.* ×96,000

FIG. 27   *Electron micrograph of precapillary arteriole with dilated luman (L). Endothelial lining (EN) is intact but muscle cells are degenerate and infiltrated with finely granular material and polymerized electron dense fibrin (F), also seen in surrounding retina.*   × 10,000
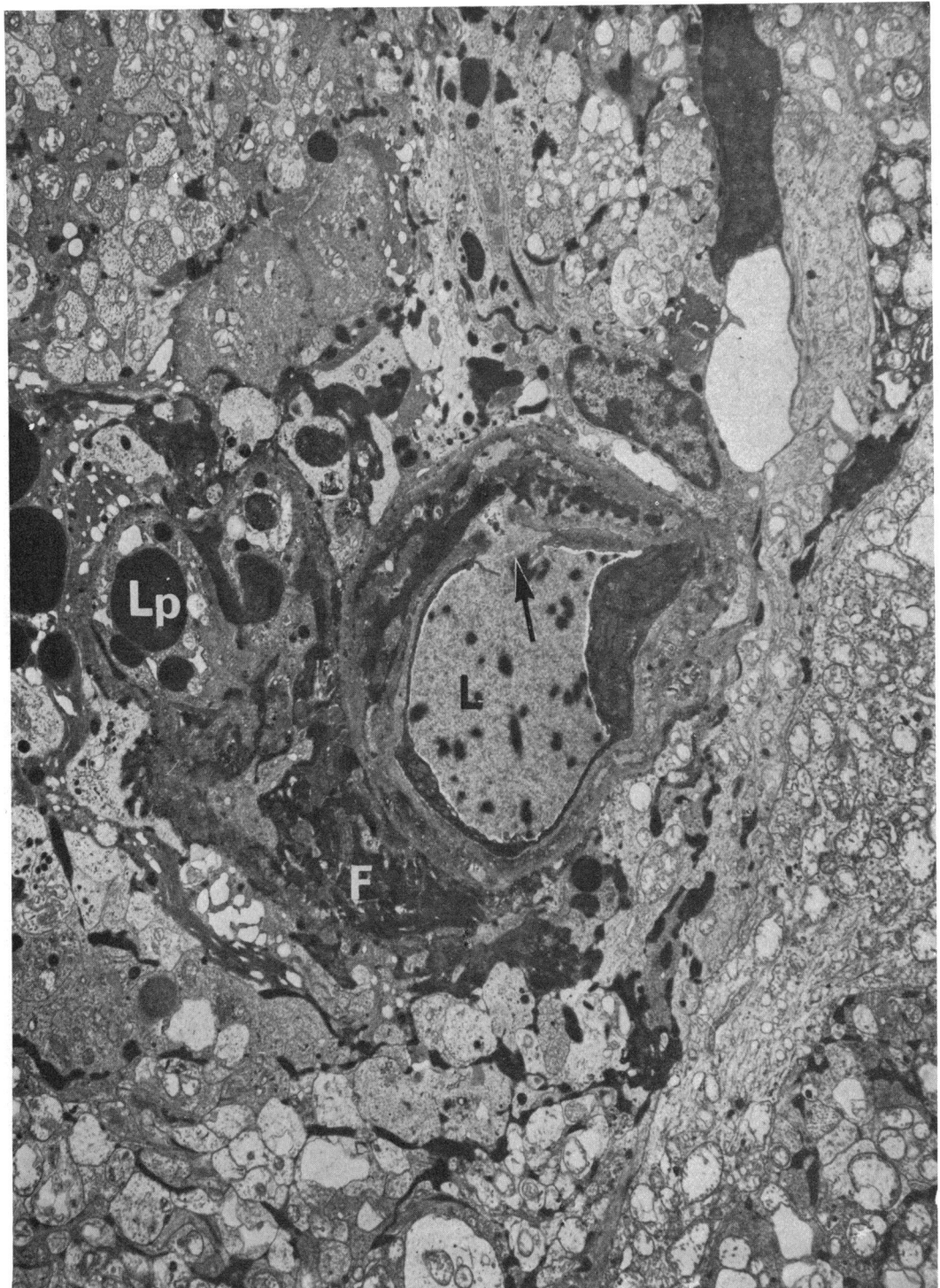
FIG. 28   *Small precapillary arteriole surrounded by fibrinous (F) and lipoidal (Lp) exudates in retina. Lumen (L) is dilated. Focal break (arrow) in endothelial lining.*   ×6,625
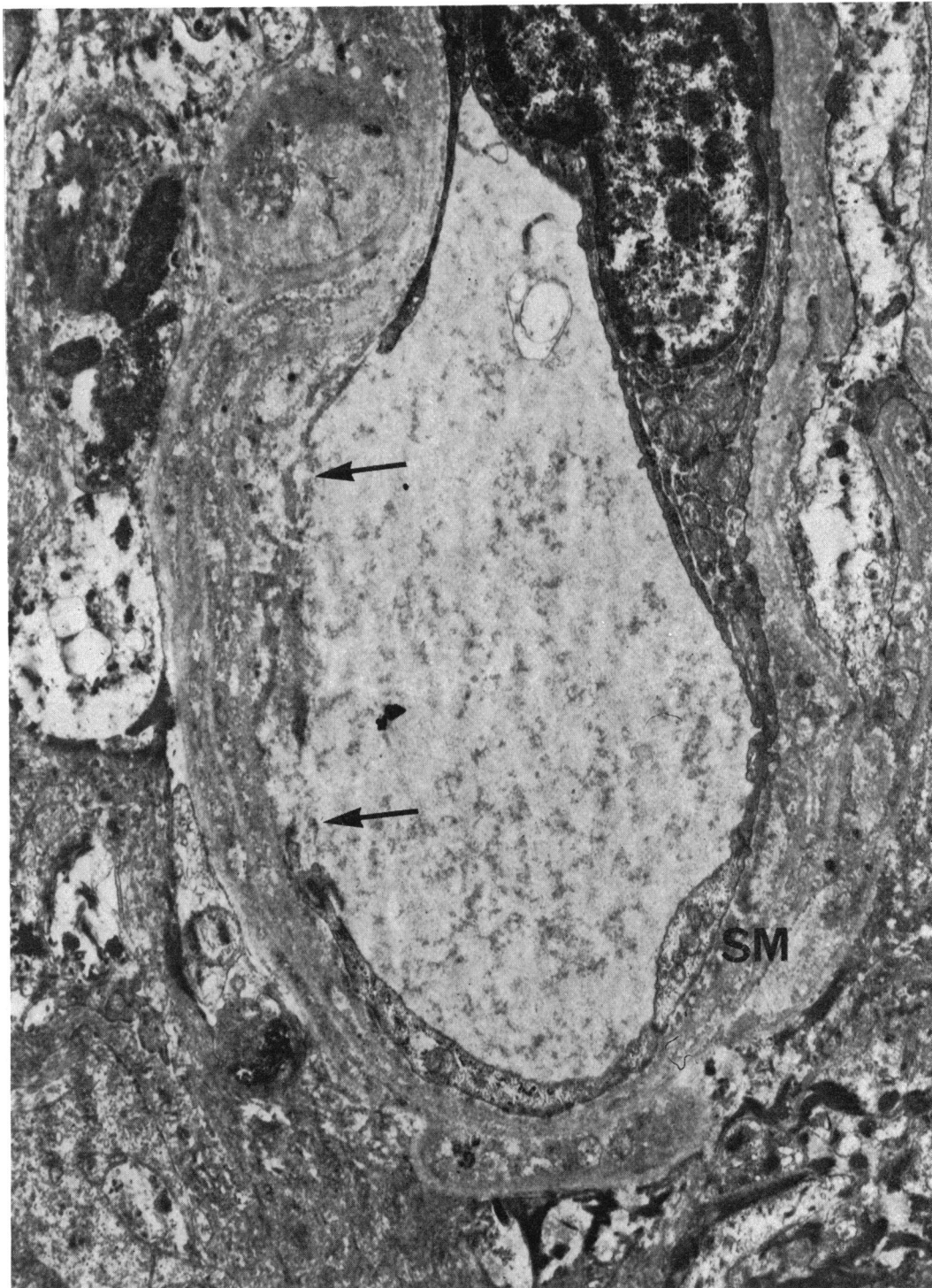
FIG. 29 *Electron micrograph of precapillary arteriole, surrounded by plasma and fibrinous exudate, showing endothelial necrosis producing a large gap (arrow). Muscle cells (SM) are degenerate.* × 15,000

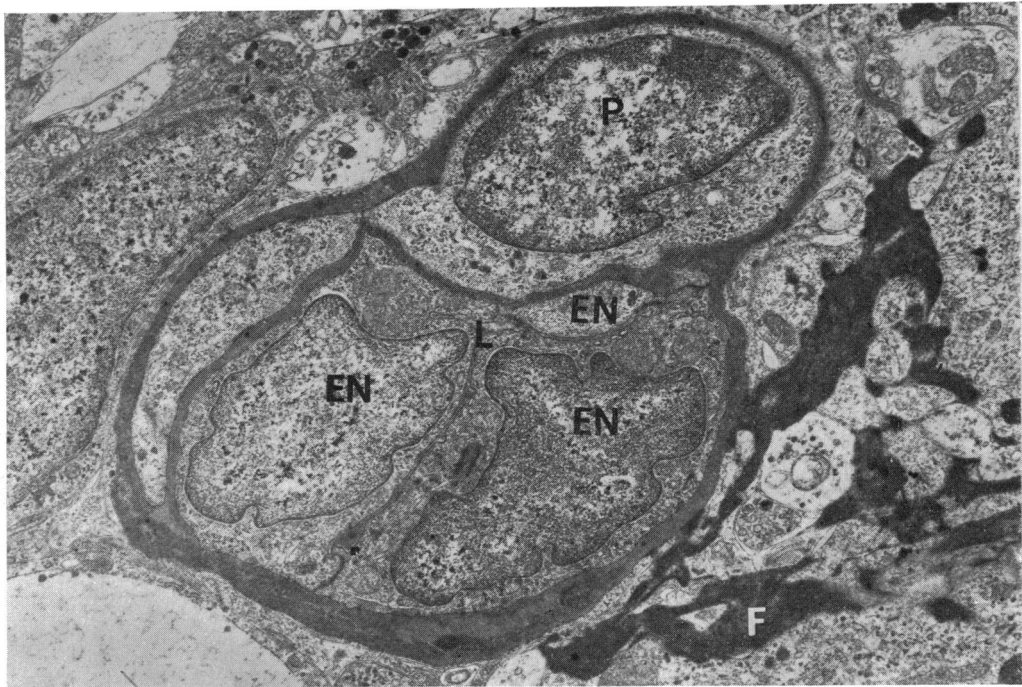FIG. 30   *Electron micrograph of tightly closed capillary with obliterated lumen (L). Endothelial cells (EN) and pericyte (P) appear normal. Fibrinous (F) and plasmoid exudate in adjacent retina.   × 11,250*
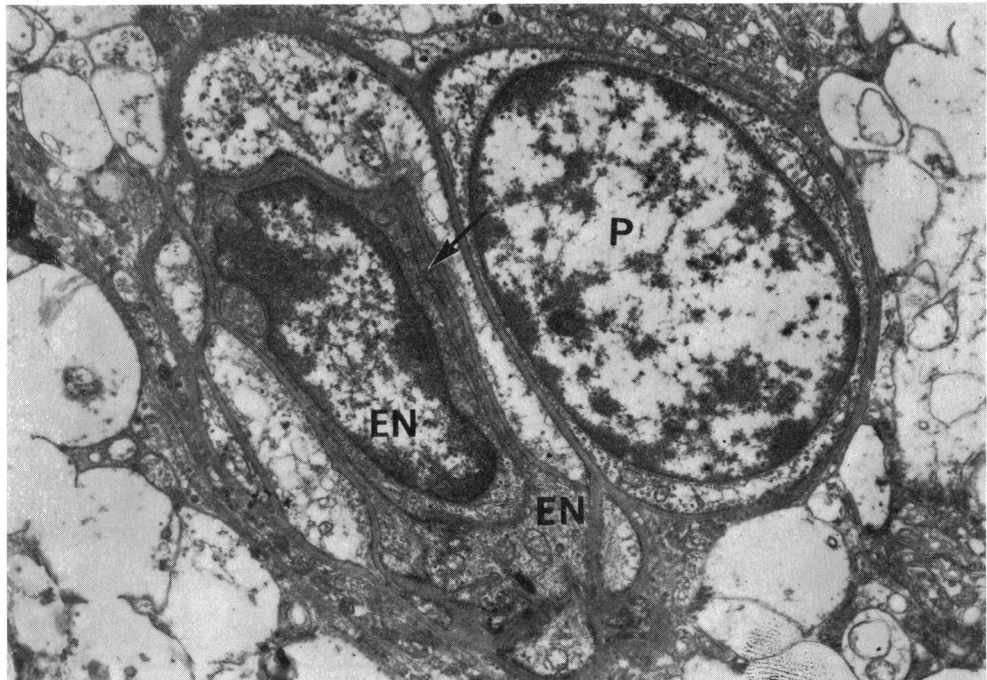

FIG. 31   *Electron micrograph of tightly closed large capillary with obliterated lumen (arrow). Endothelial cells (EN) are intact and appear healthy; pericytes (P) show some oedematous swelling.   × 10,000*
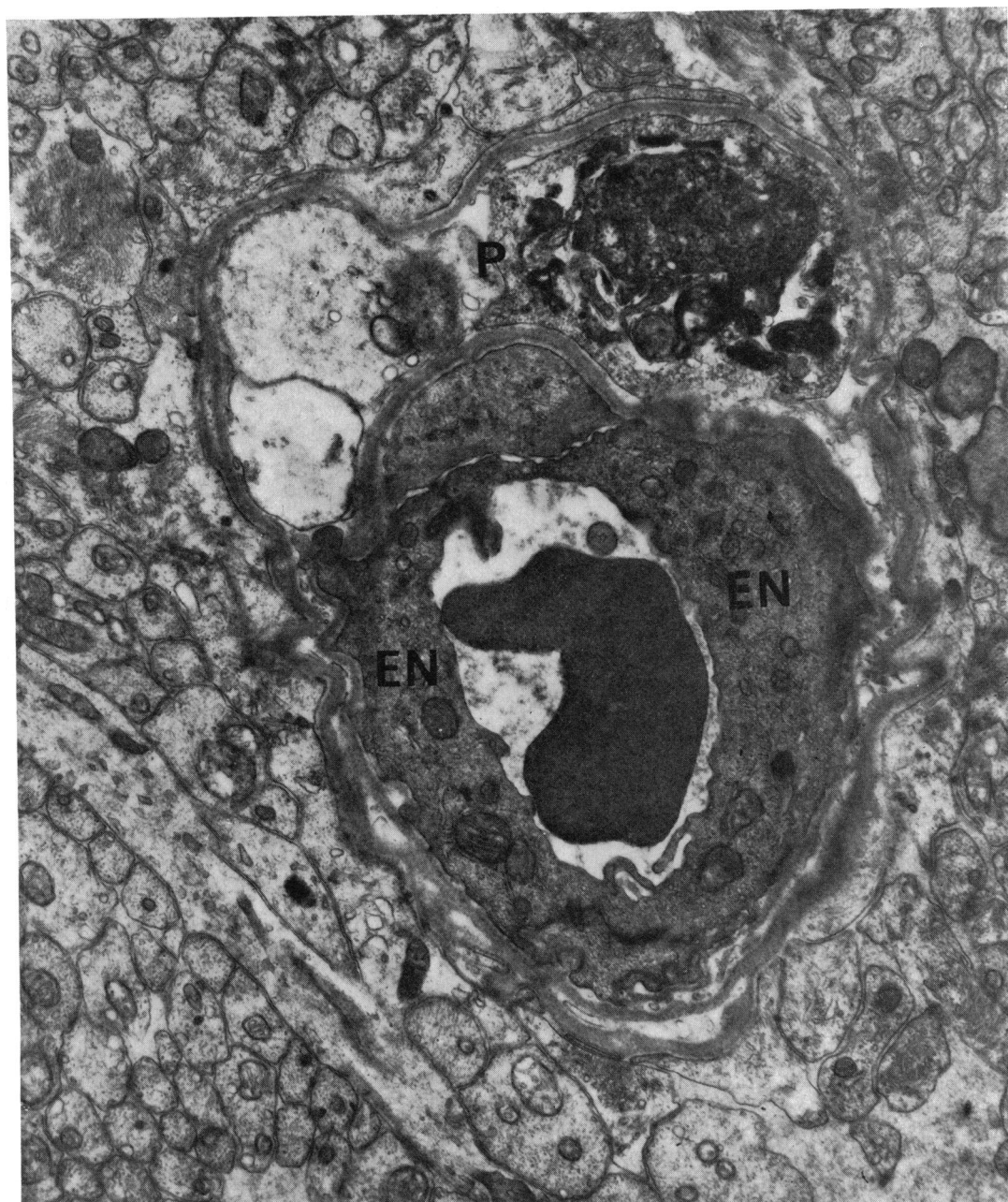
FIG. 32   *Electron micrograph of patent capillary, showing intact endothelial lining (EN). There is selective degeneration of the pericyte (P), showing cellular fragmentation and electron dense debris.*   × *14,000*
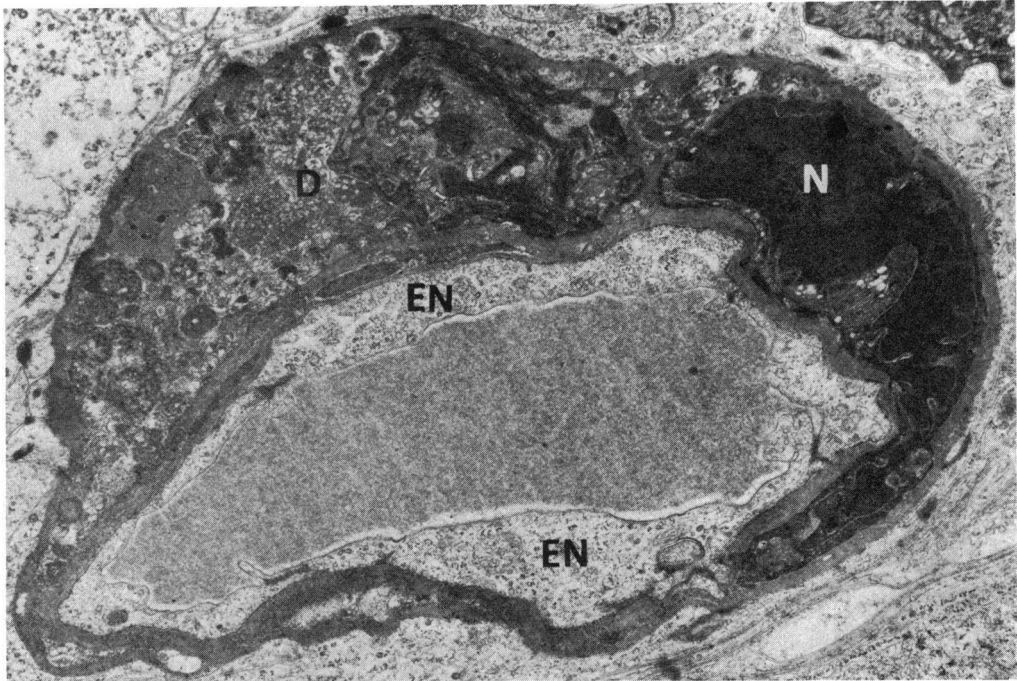
FIG. 33   *Electron micrograph of a capillary, showing an intact endothelial lining (EN). There is selective degeneration of the pericyte, showing cellular debris (D) and fragmentation, intermixed with plasma and fibrin and nuclear pyknosis (N).* × 14,600

FIG. 34   *Electron micrograph of a partially-closed capillary with a vacuolated cell in the lumen (L). There are sub-endothelial deposits of fibrin and plasma (arrow), and fibrinous exudate (F) in the surrounding retina.*   × 7,000
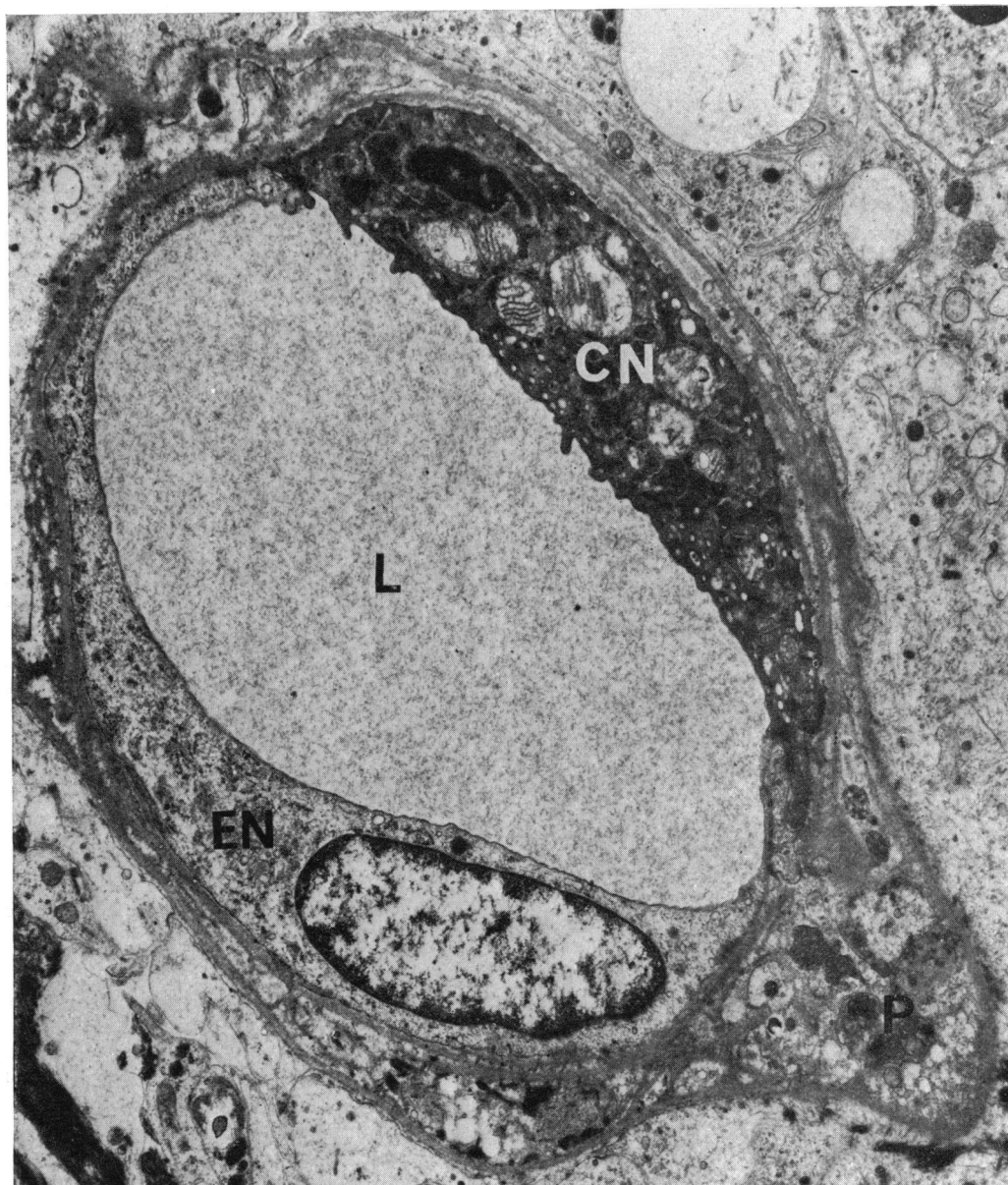
FIG. 35   *Electron micrograph of a large capillary with wide lumen (L). There is selective endothelial necrosis (CN), but the adjacent endothelial cell (EN) is normal; the pericyte (P) is degenerate.   × 13,000*

residue of convoluted basement membrane and scattered cell organelles (Fig. 38). Infiltration of the capillary wall by platelets was an exceptional and bizarre finding in one instance (Fig. 39). Capillary thrombosis was only rarely noted (Fig. 40).

(3) *Extravascular tissues*

Electron microscopy of the extravascular tissue showed that amorphous granular material, some of it including banded fibrils with the typical configuration of fibrin and indistinguishable from that found within the walls of many blood vessels, was often present in the interstitial spaces of the neural and glial tissue (HM 1, 3, 6, 10, 13, 16). These exudates had a predominantly perivascular distribution and sometimes included erythrocytes, platelets, and lipid-laden macrophages (Fig. 41).

Axonal swelling and disruption giving rise to "cytoid bodies" was also seen in the majority of monkeys (HM 1, 5, 6, 8, 10, 13). Such lesions were characterized by oedematous swelling of the disrupted axon and the proliferation of mitochondria, many of which were themselves distended. In other "cytoid bodies" there were also numerous microcysts, dense bodies, and neurofilaments, while some showed a central aggregation of dense bodies, granular material, and degenerate organelles to form a "pseudonucleus" (Fig. 42).

Electron microscopy of the *papilloedema* seen in animal HM 6 showed exactly similar changes: grossly swollen axons, with and without proliferating organelles, and disrupted extracellular spaces containing plasma and fibrin (Fig. 43).

DISCUSSION

The characteristic retinal features of malignant hypertension in man are cotton-wool spots, linear haemorrhages, and papilloedema. The presence of these changes in the eye correlates fairly well with a high blood pressure and the presence of fibrinoid necrosis in arterioles and other organs such as the kidney (Heptinstall, 1954). Without antihypertensive therapy 90 per cent. of patients with malignant hypertension are dead within one year of diagnosis (Keith, Wagener, and Barker, 1939); the outlook is considerably improved if the blood pressure is controlled (Breckenridge, Dollery, and Parry, 1970).

The arteriolar pathology of malignant hypertension was originally referred to as fibrinoid necrosis (Neumann, 1880), but modern work with the electron microscope (Fisher, Perez-Stable, and Pardo, 1966) and fluorescein-labelled antibodies has confirmed the presence of fibrin (Craig and Gitlin, 1957; Fennell, Reddy, and Vazquez, 1961) and some prefer the term "fibrinous vasculosis" (Lendrum, 1955, 1969). This is not to dispute that necrosis of arteriolar

smooth muscle contributes to the histological picture (Byrom and Dodson, 1948) although few would now maintain that the eosinophilic residues are purely or largely derived from necrotic muscle as previously claimed (Muirhead, Booth, and Montgomery, 1957; Hatt, Berjal, and Bonvalet, 1966).

If the nature of the vascular lesions is largely resolved the same cannot be said of their pathogenesis. Little is known about how and why plasma proteins gain entry to the vessel wall, particularly in the retinal circulation where the intercellular tight junctions are impervious to many of the stimuli that increase permeability in the other parts of the systemic vasculature (Ashton, 1965; Ashton and Cunha-Vaz, 1965; Cunha-Vaz and Shakib, 1967).

As *post mortem* material is unsuitable for studying the earliest stages of the development of hypertensive retinopathy we turned to an animal model. The lesions produced in the monkey retina as a result of inducing renal hypertension closely resembled in number and distribution those seen in man in respect of their ophthalmological and fluorescein angiographic features and their pathology. The only important difference was the relative absence of overt papilloedema in the animals, but this discrepancy may merely reflect the duration of the process, because the retinopathy was allowed to develop fully in only one animal, and in that experiment there was a measure of optic disc swelling.

Evidence of arteriolar disease in other organs, though present, was not prominent and this again is likely to be a reflection of the generally early state at which the experiments were terminated. It recalls the clinical impression, recorded by Volhard and Fahr (1914) and confirmed by Pickering (1968), that retinopathy is usually the first indication of an impending malignant phase.

Morphogenesis of experimental hypertensive retinopathy

(1) *Arteriolar changes*

The sequence of events leading to the incorporation of fibrin and other plasma components into the arteriolar wall, especially in the precapillary arterioles, is not entirely clear. Electron microscopy provides some evidence of preceding vasoconstriction in these vessels amounting almost to closure, but fluorescein angiographic findings *in vivo* are, perhaps, more readily interpreted in terms of failed autoregulation. Neither view alone finds unequivocal support and the merits of each will now be discussed, although it should be emphasized that the two concepts are by no means mutually exclusive. Indeed, it would seem that focal constriction in the precapillary arteriole, if it proves to be a genuine reaction, is most likely to be explicable within the framework of the concept of autoregulation.
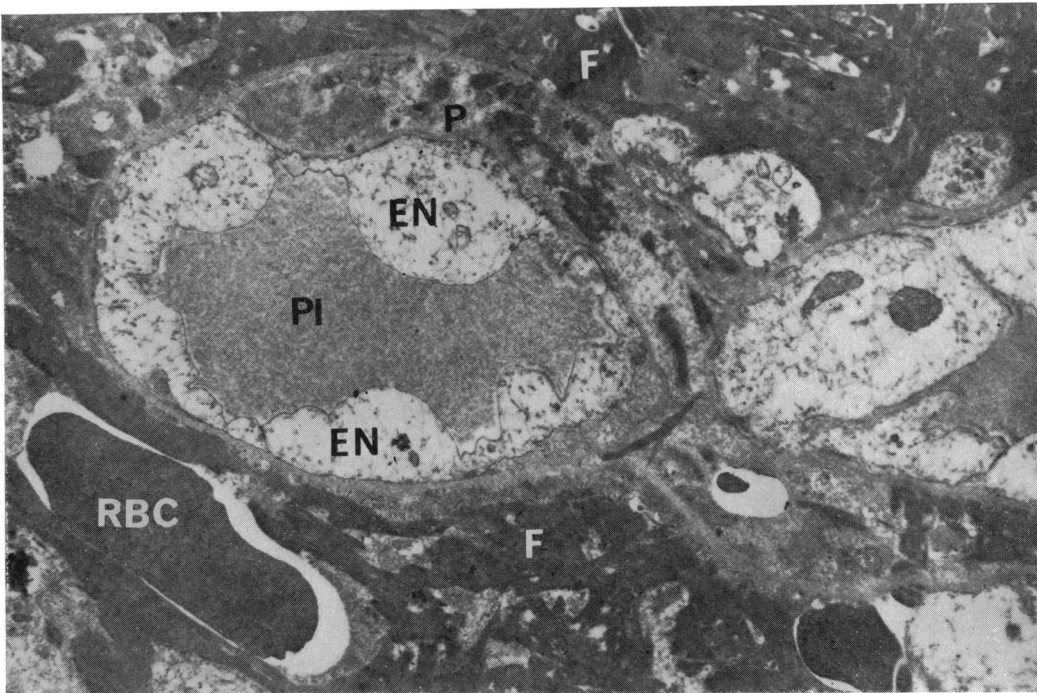
F I G. 36   *Electron micrograph of a patent capillary containing plasma (PL) in the lumen and showing a degenerate but intact endothelium (EN) and necrotic pericyte (P) infiltrated with fibrin and amorphous granular material. The pericapillary tissues are heavily infiltrated with fibrin (F), plasma, and red blood corpuscles (RBC).*   × 7,500
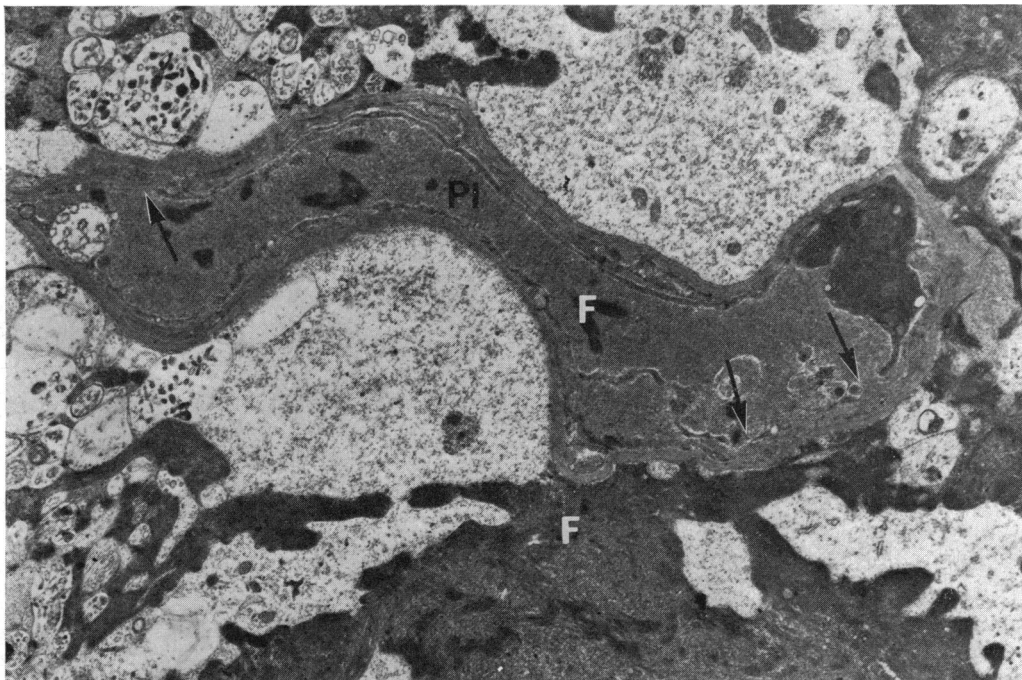

F I G. 37   *Electron micrograph of a capillary, cut obliquely, showing widespread necrotic disintegration of its endothelial lining. The lumen contains plasma (PL) and electron dense fibrin (F). Arrows denote breaks in endothelial lining. Note also plasmoid and fibrinous exudate (F) in surrounding retina.*   × 11,250
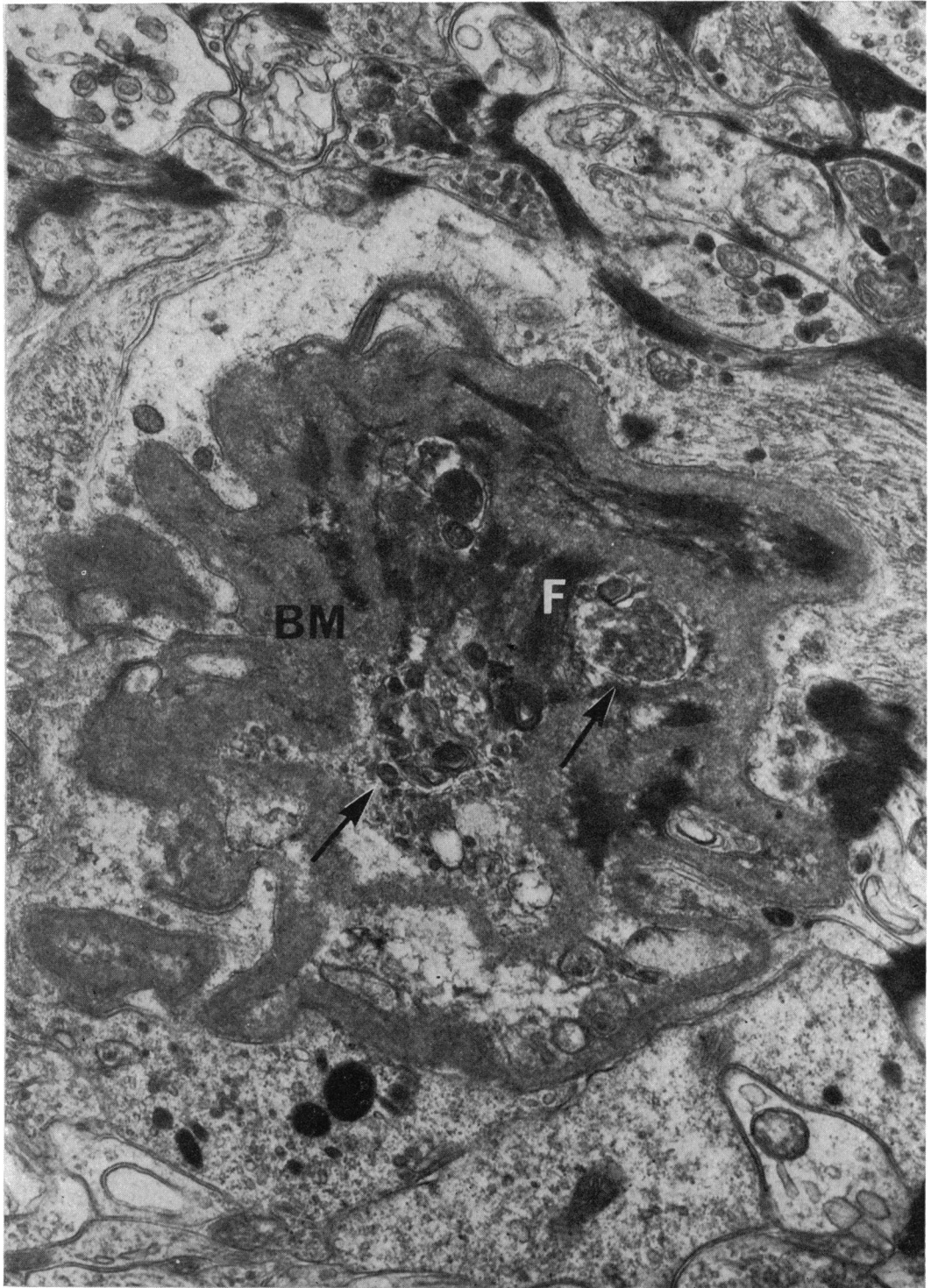
F I G. 38 *Convoluted basement membrane (BM) containing fibrinous exudate (F) and cellular debris (arrowed) representing the remnants of a capillary. Electron micrograph.* × 28,000
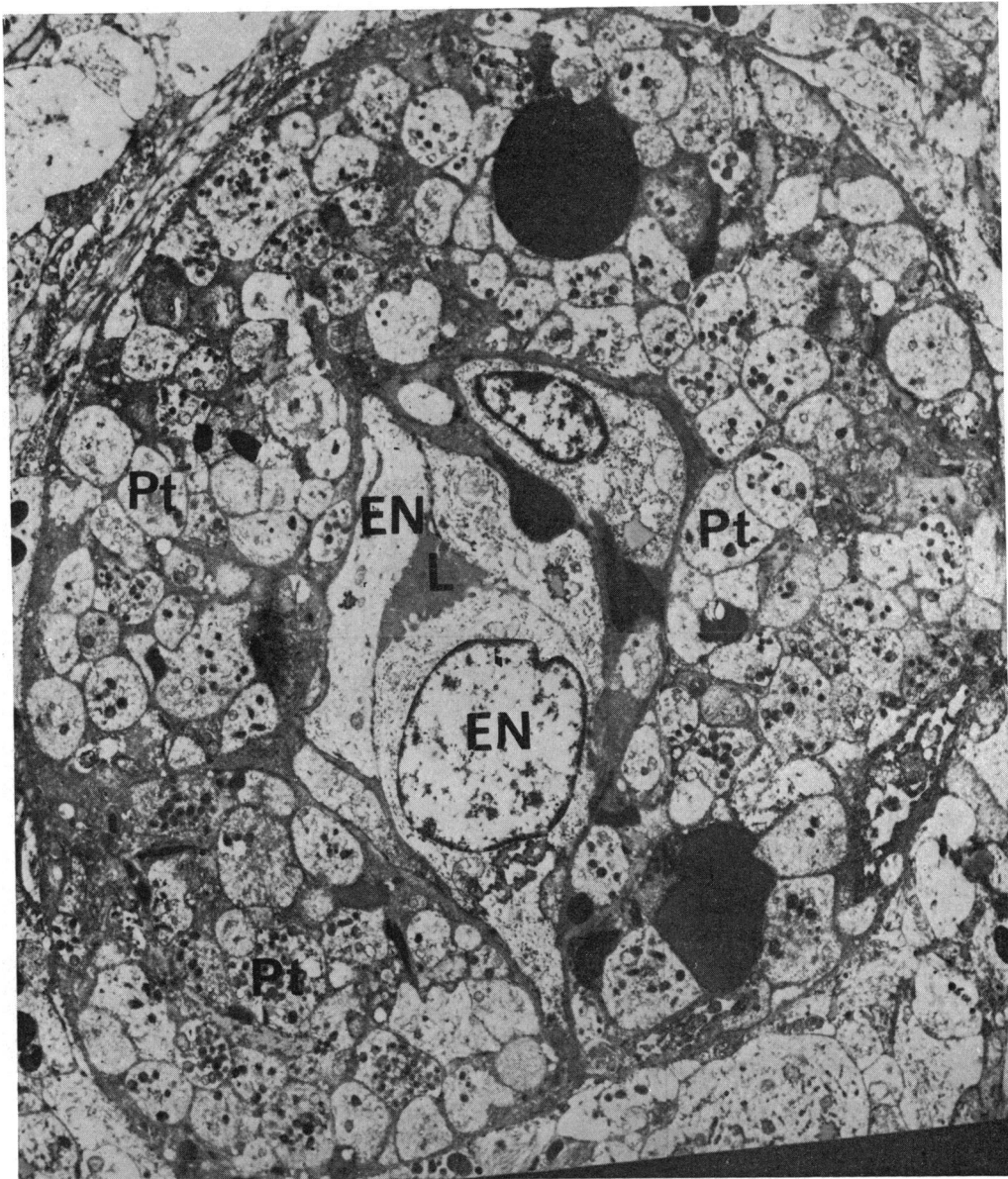
FIG. 39 *Closed arteriole, with a partially obliterated lumen (L), lined by intact but oedematous endothelial cells (EN). Note greatly distended wall due to infiltration by a mass of platelets (Pt), red blood corpuscles, fibrin, and plasma. Electron micrograph.   × 5,500*
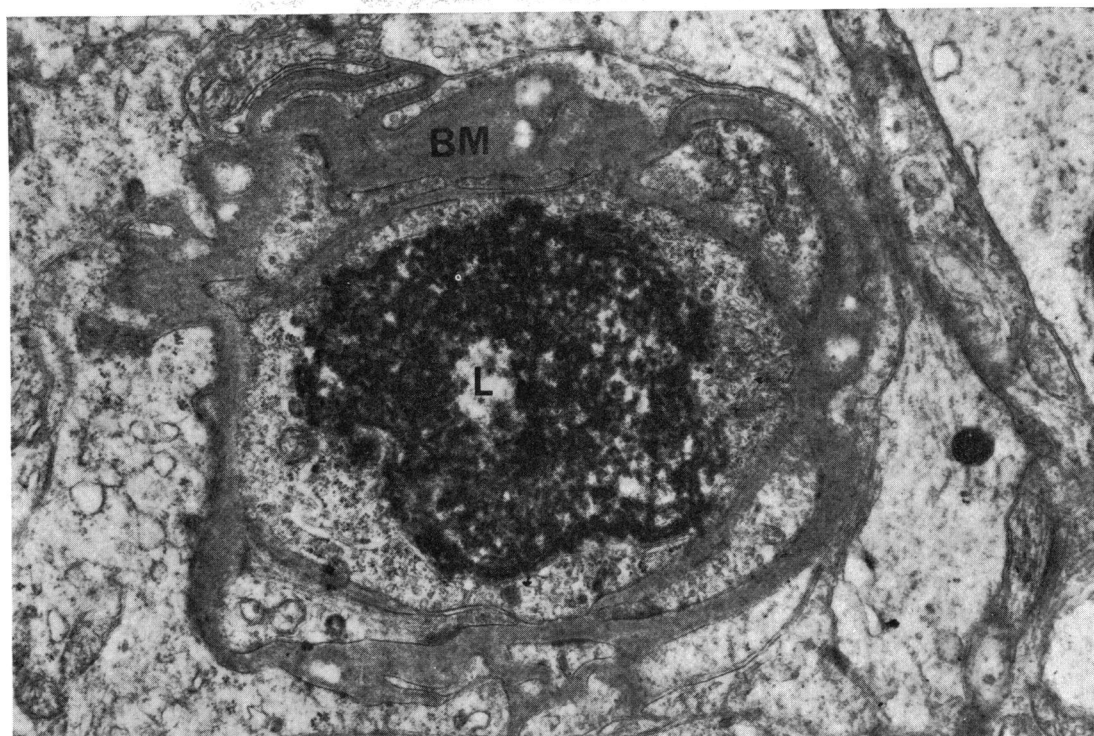
FIG. 40   *Electron micrograph of a thrombosed capillary with the lumen (L) completely occluded by a fibrinous thrombus. There are some proliferative changes in the basement membrane (BM).*   × 24,375
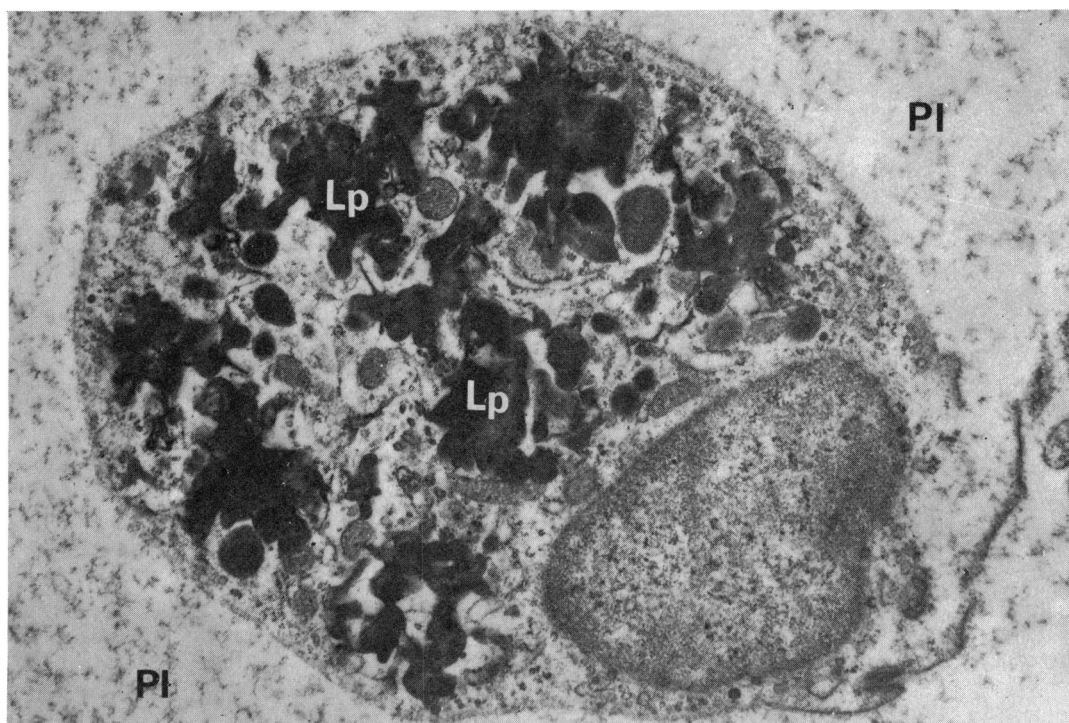
FIG. 41   *Electron micrograph of a macrophage in a cystoid space of the retina filled with plasmoid exudate (Pl). Note abundance of electron dense lipoidal inclusions (Lp).*   × 16,500
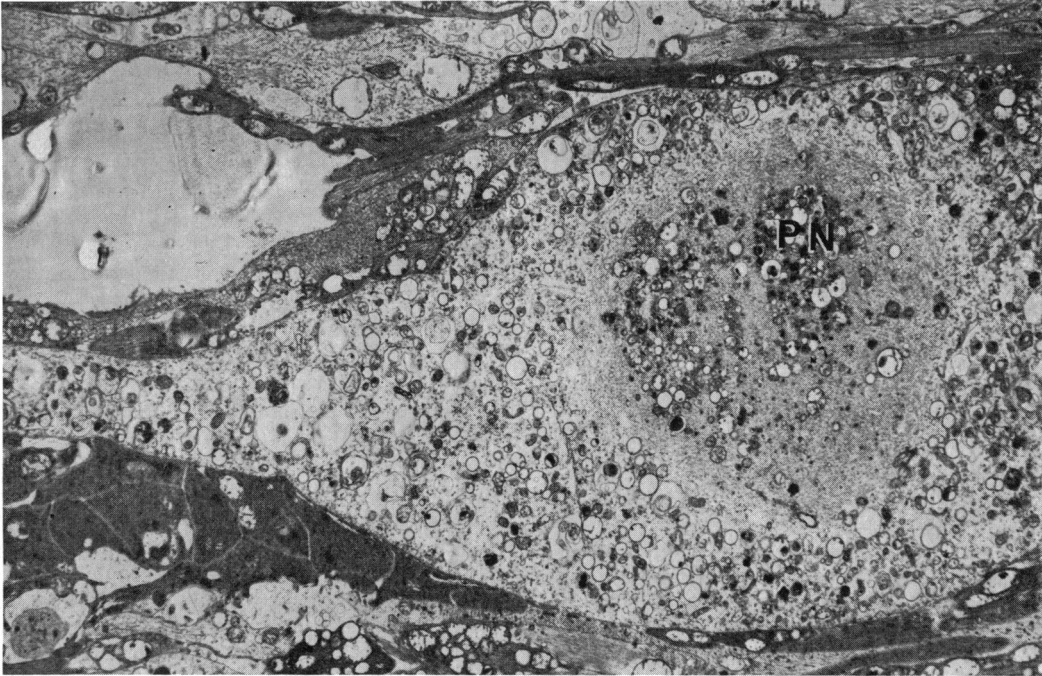
FIG. 42 *Electron micrograph of a cytoid body consisting of a swollen end of a disrupted axon filled with numerous cystic mitochondria, vesicular structures, dense bodies, and central aggregation of electron dense material and degenerate organelles constituting a "pseudonucleus" (PN). × 4,650*
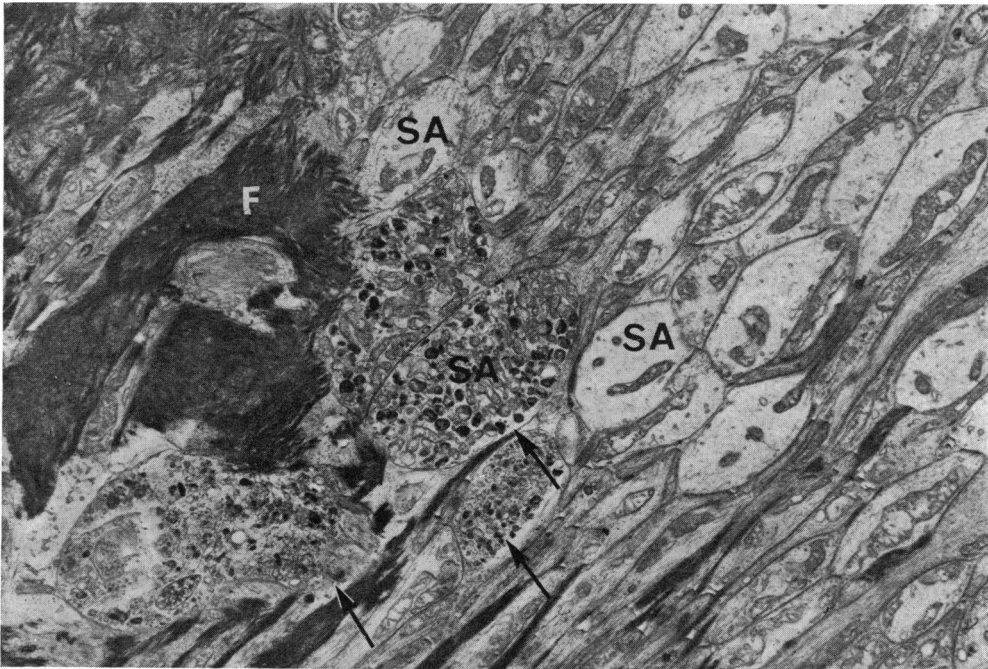
FIG. 43 *Electron micrograph of the rim of an oedematous disc in a hypertensive monkey. Many axons are swollen (SA), some contain proliferating organelles (arrows), and there is fibrin (F) in the extracellular space. × 6,750*

(*a*) FOCAL CONSTRICTION OF TERMINAL ARTERIOLES

One of the most striking features of the pathological material is the closure of precapillary arterioles, which show either no structural change or only varying degrees of smooth muscle degeneration. It is possible that this may have been a postenucleation artefact because the vessels were fixed at a time when the intravascular pressure was zero and the vessel walls sometimes swollen with oedema. Moreover, hypertensive smooth muscle is hyperexcitable (Gordon and Nogueira, 1962) and fixation can induce arterial constriction (Pease and Molinari, 1960; Matthews and Gardner, 1966).

It is, however, difficult to conceive that contraction would occur mainly in those vessels showing degenerative muscle unless they were abnormal before death. Artefactual contraction would also leave unexplained the absence of similar changes in adjacent vessels with healthy muscle in the same retina. Consequently, although the evidence is not incontrovertible, there would appear to be good reasons for interpreting the electron microscopical findings as representing changes *in vivo* in vessel calibre as an initial stage, and this view has been favoured as a working hypothesis in earlier publications (Garner and Ashton, 1970; Ashton, 1972).

Reversible focal arteriolar constriction has been demonstrated in a number of animals, including the monkey, in response to a raised arterial pressure. It has been observed in mesenteric arteries (Byrom, 1954; Giese, 1964), in intracranial vessels (Byrom, 1954, 1968, 1969; Meyer and others, 1960), and in retinal arterioles of rats (Abt and Brückner, 1950; Byrom, 1963). Cerebral vasospasm progressing to fibrinous vaculosis has also been recorded in monkeys (Rodda and Denny-Brown, 1966a, b).

Such reversible focal constriction in small arteries was not, however, observed with the ophthalmoscope or in retinal photographs of the monkeys in the present study; indeed, large vessels were dilated (Table IV). But constriction in small arteries is not excluded since their diameter is not measurable. The most striking early feature of the fluorescence angiograms was focal leakage of fluorescein and this occurred in some animals before there was extensive formation of cotton-wool spots. Obstruction of small arterioles with substantial areas of impaired capillary perfusion was a later feature. These discrepancies between the findings *in vivo* and the ultrastructural findings need not, however, imply a basic contradiction, since it is not implicit in the focal constriction concept that there would inevitably be complete cessation of blood flow and certainly not of plasma flow.

Should pronounced focal vasoconstriction be genuine, it would of necessity have preceded the advanced muscle necrosis and, consequently, since

degenerative changes in the arteriolar wall were frequently observed in the absence of fibrinous insudation, the sequence of events might have been as follows:

(1) An initial stage of extreme and unrelieved vasoconstriction at the level of the precapillary arterioles.

(2) Eventual relaxation of the constriction due, possibly, to ischaemic damage to the smooth muscle caused by prolonged reduction of blood flow.

(3) Focal rupture of a now poorly supported endothelium allowing plasma to leak into the damaged wall. This insudation could interfere even further with the integrity of the muscle coat and result in structural narrowing of the lumen.

These events are summarized diagrammatically in Fig. 44 (opposite).

This concept of focal vascular constriction as applied to hypertensive retinopathy in man has had a very chequered career and is currently in disfavour, but as Ashton (1972) points out precapillary arterioles are not readily observed by routine ophthalmoscopy, so that much of the existing controversy, which refers exclusively to larger arterioles, is not in fact relevant to the reactivity of these minute vessels.

(*b*) AUTOREGULATORY FAILURE

The second mechanism we must consider relates to the physiological process of autoregulation. A rise or fall in blood pressure in an intact animal or man does not usually bring about a corresponding change in blood flow through the tissues unless the alteration in pressure is large. At organ level the most important factor stabilizing blood flow appears to be self regulation or "autoregulation" to maintain an appropriate metabolic environment. It has been defined as "a continuous local adjustment of blood flow in proportion to the needs of the tissue for nutrients" (Guyton, Ross, Carrier, and Walker, 1964). This is achieved by constriction of the vessels as the pressure rises and by dilatation as the pressure falls.

Autoregulation is effective over a wide range of systemic arterial pressures, but at the extremes it fails. At the lower limit this is presumably because maximum vessel dilatation is inadequate to sustain the desired blood flow. At the upper limit the reasons for failure are less clear and possibly the muscle necrosis we found in the precapillary arterioles gives a clue that it occurs when the tension in the vessel wall becomes too great for the smooth muscle to sustain. It is of incidental interest that pathophysiological changes can alter the pressure level at which autoregulation fails. Lassen and Agnoli (1972), who studied the human cerebral circulation, observed autoregulatory failure at mean pressures ranging from 130 to 160 mm.Hg. They also noted that inhalation of carbon dioxide, which dilates the cerebral
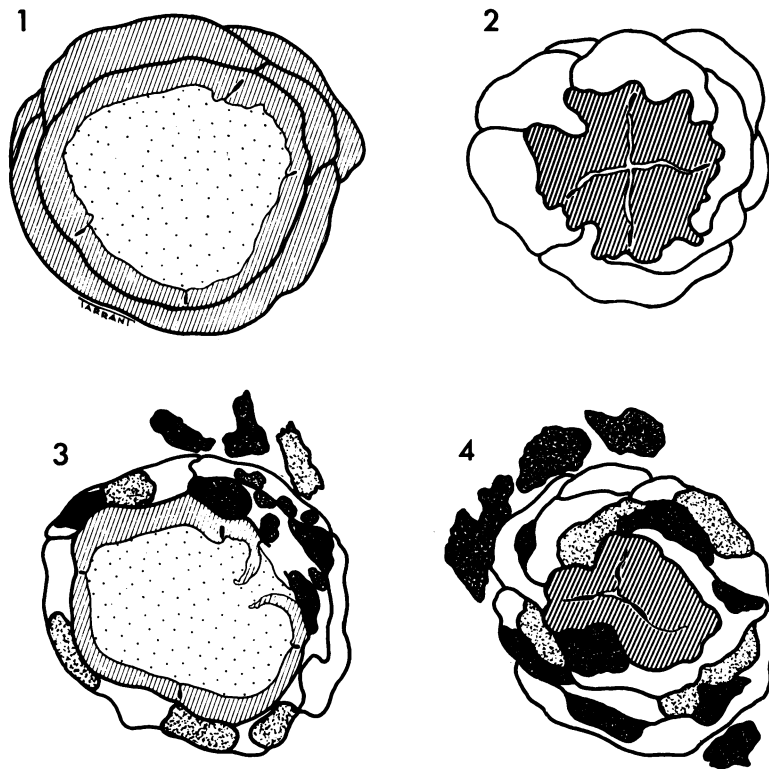
FIG. 44 *Diagrammatic representation of proposed sequence of events in development of vascular changes leading to fibrinoid necrosis in retinal precapillary arterioles.*
1. Normal vessel.
2. A sustained contraction leading to muscle degeneration.
3. Dilated lumen after passive reopening of vessel, rupture of endothelial barrier, leakage of blood components.
4. Secondary closure of vessel due to gross mural infiltration by plasma and fibrin—fibrinoid necrosis.

vessels, lowered the level at which the mechanism failed.

The pathological and ophthalmological observations in our own experiments suggest that the first event as the pressure rises is a general constriction of retinal arterioles. With further rises focal damage to the walls of minute arterioles becomes manifest. On the above analysis this could be due to a "breakthrough of autoregulation" (Lassen and Agnoli, 1972) with necrosis of muscle cells and resulting dilatation, allowing the insudation of plasma into the vessel wall and the deposition of fibrin. These could be the points that leak fluorescein. Some of these arterioles become obstructed by the swelling and disruption of their walls, and infarction of the retina downstream leads to the formation of cotton-wool spots.

The special involvement of the precapillary arterioles may be explained in the following way. As autoregulation fails some arterioles give way. The moment this begins an unstable situation is created.

The vessel dilates because some muscle weakness has occurred; as it dilates the tension in the wall increases in accordance with the law of Laplace:

Tension = Vessel Radius × Transmural Pressure

As the radius increases so will the transmural pressure in distal arterioles, because the increase in diameter will allow the pressure to be transmitted to the most distal segments of the arteriolar bed.

This concept, however, as set out above, means that excessive dilatation of the arterioles as seen in the photographs (Table III) is responsible for the pathological changes and does not explain the severe and purely focal constriction of the terminal arterioles we found by electron microscopy: initially without structural injury but later with developing necrosis. It may well be, however, that this focal constriction is none other than the upper limit of autoregulatory constriction, which in vessels of such minute calibre may alone be responsible for their closure; or possibly these small vessels, being further down the arterial

tree, retain their activity (Wise, Dollery, and Henkind, 1971) until a late stage and react severely when eventually exposed to the full force of the hypertension. In short, the whole process of autoregulation at its upper limit and the consequences of its breakdown may be traced in these minute terminal vessels, and in this way the two concepts we have been discussing could come together.

(2) *Fibrinous insudation*

In those instances in which recognizable fibrin was present within the walls of arterioles and capillaries it seems reasonable to suppose that the associated granular material was also derived from the plasma in the form of unpolymerized fibrinogen and other plasma proteins, muscle necrosis sometimes acting as a contributory factor.

The real problem is not so much the nature of the deposits as their mode of transit across the lining endothelium of the vessels. In previous reports (Ashton and others 1968a; Garner and Ashton, 1970), no statement was possible regarding this problem and only latterly, as described in this paper, have we found an unequivocal pathway.

Increased endothelial permeability due to unspecified mechanisms has been postulated in hypertension on a number of occasions and has variously been ascribed to the direct effect of raised intravascular pressure (Duncan, Cornfield, and Buck, 1962; Giese, 1964; Goldby and Beilin, 1972; Häggendal and Johansson, 1972), to associated hypercoaguability of the circulating blood in abnormal arterioles (Linton and others, 1969; Gavras, Brown, Brown, Lever, Linton, Macadam, McNicol, Robertson, and Wardrop, 1971) or even to increased fibrinolysis (Ooneda, Yoshida, Takatama, Sekiguchi and Kato, 1962), to electrolyte disturbance (Hatt and Dontcheff, 1959) or to some factor of renal origin (Giese, 1962; Asscher and Anson, 1963). More recently Pessina and Peart (1972) have produced evidence suggesting that renin and angiotensin may, in addition to their pressor effect, also exert a direct influence on vascular permeability. But, as Giese (1966) and Ashton (1972) have made clear, the focal distribution of fibrinous insudation is opposed to a generalized circulatory explanation and suggests that the hypertension exploits localized points of increased vulnerability in the vascular system, such as the foci of precapillary smooth muscle necrosis seen in the majority of monkeys.

Electron microscopy has provided evidence for at least three mechanisms whereby insudated plasma might cross the endothelial barrier:

(i) From a study of experimental hypertension in the rat, Ooneda, Ooyama, Matsuyama, Takatama, Yoshida, Sekiguchi, and Arai (1965) postulated an increase in pinocytosis. A similar mechanism has been described by others (Geer, Skelton, and McGill, 1958; Hatt, Rouiller, and Grosgogeat, 1959; Hatt, Dvojakovic and Cornet, 1962; Hüttner, Jellinek, and Kerényi, 1968; Hüttner, More, and Rona, 1970). This process would represent not only increased activity but also an abnormal function since the endothelium is normally impervious to fibrinogen (Mancini, Vilar, Dellacha, Davidson, Gomez, and Alvarez, 1962). In any case there was little or no evidence of increased pinocytotic activity in the present study.

(ii) Another route for insudation proposed by Ooneda and others (1965) is through disrupted intercellular junctions in the endothelial lining. This has since been described by others (Jellinek, Nagy, Hüttner, Balint, Kockze, and Kerényi, 1969; Wiener, Lattes, Meltzer, and Spiro, 1969; Giacomelli, Wiener, and Spiro, 1970; Hüttner and others, 1970) in a variety of tissues, including brain and retina, and has been attributed both to endothelial cell stretching (Aikawa and Koletsky, 1970) and to retraction (Giacomelli and others, 1970; Northover and Northover, 1970). Although both attenuated and apparently contracted endothelium were seen—evidence for the latter being nuclear corrugation as described by Majno, Shea, and Leventhal (1969)—a striking feature of the present study was the quite remarkable resistance of the tight encircling junctions between the endothelial cells in the retinal vessels to separation even where there was advanced necrosis. This makes the reports of Giacomelli and others (1970, 1972) all the more interesting, since their studies were related to the retinal and structurally comparable cerebral vessels. Using horseradish peroxidase as a marker, they demonstrated some widening of the intercellular junctions in the hypertensive state in rats but, as the peroxidase is a smaller molecule than most plasma proteins, it remains to be shown that this pathway would contribute significantly to the insudative process, especially as these same junctions were impervious to colloidal carbon.

(iii) A third possibility, and the only one our own findings support, is structural damage to the endothelium. This would accord with the view expressed by Ashton (1965) and Cunha-Vaz and Shakib (1967) that breakdown of the blood–retinal barrier is contingent on intimal necrosis. That we found endothelial injury so rarely in the early stages, before the development of complete vascular necrosis, is possibly a reflection of the very small size of such focal lesions and the propensity of vascular endothelium to regenerate. Evidence of regeneration was observed on a number of occasions in the form of mitochondrial and ribosomal proliferation and prominence of the endoplasmic reticulum. Goldby and Beilin (1972) have recently described a similar finding of plasma leakage

through gaps in degenerate endothelial cells lining the mesenteric arteries of rats made acutely hypertensive by intravenous infusion of angiotensin.

Since endothelial cell damage was not seen apart from necrosis of the underlying smooth muscle, it is highly probable that loss of support due to muscle necrosis facilitated mechanical damage by the elevated intravascular pressure.

(3) Capillary changes

Closed capillaries were a common finding in the present study and since they occurred in relation to defective arterioles can reasonably be ascribed, at least in part, to failure of perfusion. Ashton (1959) suggested that this capillary closure might be due to compression by the swollen ischaemic retina, and recent work on the effects of ischaemia on blood flow through the cerebral vessels of the rabbit by Kowada, Ames, Majno, and Wright (1968) and Ames, Wright, Kowada, Thurston, and Majno (1968) adds support to this concept. It was found that arterial occlusion is followed by anoxic oedema in the dependent brain tissue and this in turn compresses the capillary bed, so that on restoring the arterial circulation there is incomplete or uneven perfusion (Kowada and others, 1968; Ames and others, 1968). Further work from the same group indicates that the cerebral oedema so provoked is located chiefly in the perivascular glia and to a lesser extent in the capillary endothelium (Chiang, Kowada, Ames, Wright, and Majno, 1968). Since capillary closure is often linked with cotton-wool spot formation, it is quite possible that an equivalent "no-reflow" phenomenon operates in the retina, with the extravascular swelling residing principally in the nerve fibre layer. It is also possible that endothelial cell swelling contributes to the narrowing in some instances. Capillary closure in the brain secondary to tissue oedema was described over a century ago by Traube (1871), but was then attributed to the direct effect of elevated blood pressure in promoting excessive diffusion of fluid across the vessel wall; Hodge and Dollery (1964) suggested that retinal capillary closure was due to compression by exudate from defective precapillary arterioles. In some instances it was apparent that closure was the result of sero-fibrinous insudation, facilitated possibly by anoxia imposed by damage to the feeding arterioles.

A further possibility prompted by the results of the present study is that capillary closure may be brought about by endothelial cell contraction. That vascular endothelium may be able to contract is suggested by fluorescein-labelled antibody studies in the rat demonstrating the presence of actomyosin (Becker and Murphy, 1969), and the occasional finding of banded fibrillar structures similar to those occurring in smooth muscle (Röhlich and Olah, 1967).

The signs of endothelial regeneration seen in some capillaries were comparable to the findings in occasional patent arterioles and could be ascribed to postanoxic replication of surviving cells when there was an adequate restoration of perfusion.

(4) Extravascular changes

The location of cotton-wool spots distal to occluded precapillary arterioles agrees with the experience of others both in the experimental situation (Gay, Goldor, and Smith, 1964; Ashton and Henkind, 1965; Dollery, Henkind, Paterson, Ramalho, and Hill, 1966) and in naturally occurring vascular disturbances in man (Ashton and Harry, 1963; Ashton, Coomes, Garner, and Oliver, 1968b). That they were invariably associated with fluorescein leakage recalls the similar experience of Hodge and Dollery (1964) with regard to human hypertensive retinopathy, and suggests that exudation of plasma may contribute to the swelling, although it cannot be considered to play a necessary part in the formation of cotton-wool spots, since embolization with glass microspheres in the experimental animal can produce cotton-wool spots in the absence of fluorescein leakage (Dollery and others, 1966).

The sero-fibrinous exudates were clearly an extension of the insudative process within the walls of arterioles and capillaries. Their rarity on ophthalmoscopical and gross pathological examination in contrast to their frequent occurrence at a microscopical level is probably a measure of their size. The generalized retinal oedema can be attributed to autoregulatory failure, for the resulting rise in transmural pressure in distal arterioles and proximal capillaries will disturb the Starling equilibrium across the capillaries and permit increased transudation of fluid into the tissue. This mechanism has also been postulated to explain the oedema of acute hypertensive encephalopathy (Lassen and Agnoli, 1972; Strandgaard, Olesen, Skinhøj, and Lassen, 1973). The ultrastructural changes indicate that intracellular oedema of ischaemic origin is also of importance.

The problem of the pathogenesis of papilloedema in hypertension is too complex to be entirely explained by the evidence provided by our experiments, but it is of interest that the swollen disc shows exactly similar ultrastructural changes to the retina suggesting that papilloedema is also explicable on the same basis as oedema in the retina.

SUMMARY

Retinal changes in accelerated hypertension were studied in seventeen monkeys with experimental hypertension by means of ophthalmoscopy and colour and fluorescence photography during life, and by

injection and digest preparations and light and electron microscopy after the animals had been killed.

Cotton-wool spots developed in all but three monkeys. The arteries became tortuous and dilated and the light reflex decreased in those animals that became hypertensive. The earliest abnormality was a development of many points of fluorescein leakage on terminal arterioles or small arteries. Such leaking points were always present in relation to cotton-wool spots but were not confined to such areas. Focal narrowing of arteries was not observed but arteriolar occlusion and retrograde filling of the distal segment was present in three animals. Superficial linear haemorrhages were noted in five animals.

Light microscopy revealed cotton-wool spots which were identical to those observed in man with a collection of swollen axons containing densely staining pseudonuclei. Study of the arterioles by electron microscopy showed findings ranging from normality to extensive necrosis. Many precapillary arteries were constricted and some were virtually occluded. Degenerative changes were present in smooth muscle cells in the wall of many of the constricted arterioles. Many arteries also showed insudation into their wall of plasma which had seeped into the muscular coat displacing and sometimes entirely replacing the smooth muscle cells. Except for arterioles with advanced necrosis, there was no indication of how plasma insudation occurred. Two arterioles with extensive necrosis showed a break within the endothelial cell cytoplasm through which penetration of plasma proteins had probably occurred. The extravascular tissues showed collections of amorphous material, some of it with the typical banded configuration of fibrin.

The sequence of events proposed to explain these features is as follows:

(1) The arterioles constrict as the pressure rises, most likely as a result of vascular autoregulation. This may lead to occlusion of the precapillary arterioles and is associated with necrosis of vascular smooth muscle.

(2) Dilatation then occurs with insudation of plasma into the unsupported wall through a damaged endothelium. This stage probably corresponds to the autoregulatory break-point and is evidenced clinically by focal leakage of fluorescein.

(3) Progressive plasma insudation into the vessel wall with further muscle necrosis results in secondary occlusion and the typical picture of advanced fibrinoid necrosis.

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