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The postnatal development of blood vessels in the optic nerve of normotensive and hypertensive rats

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INTRODUCTION

It has been suggested that there may be a correlation between development in the nervous system and the level of vascularisation (Skoff, Toland & Nast, 1980; Strong, 1961), although controversy still exists (Sturrock, 1982). The axon cylinder diameter and glial cell number and size increase during development, with a consequent increase in metabolic requirement. Other features such as the onset of function and myelination may also be correlated with the development of adequate vascularisation (Foster, Connors & Waxman, 1982; Skoff *et al.* 1980).

It has been suggested that vascularisation in the central nervous system is dependent upon the efficiency of the circulatory system (Sturrock, 1982). It might then be expected that a higher level of vascularisation would develop in situations where the circulatory system becomes more 'efficient', as in hypertension.

In this study the postnatal vascularisation of the optic nerve is examined and correlated with structural development of the nerve in both normotensive and hypertensive rats.

MATERIALS AND METHODS

Two strains of rat were used in this study. The normotensive rat used was the Wistar Kyoto rat. The hypertensive rat used was the spontaneously hypertensive rat, a strain derived from the Wistar Kyoto rat. The blood pressure profiles of these two strains have been previously reported (Pang & Scott, 1981).

Optic nerves were examined in rats aged 2, 4, 8 and 12 weeks. At each time period, three normotensive and three genetically hypertensive rats were anaesthetised with pentobarbital and perfused through the heart with a fixative containing 2% paraformaldehyde and 2·5% glutaraldehyde in 0·1 M sodium cacodylate buffer. Each optic nerve was removed, cut into intracranial, canalicular and intraorbital segments, and immersed in fixative for a further hour. The tissues were then washed with 0·1 M sodium cacodylate containing 5·4% sucrose, post-fixed with 1% osmium tetroxide in 0·1 M sodium cacodylate buffer, stained *en bloc* with uranyl acetate, dehydrated in a graded series of ethanol, cleared in acetone and embedded in Araldite.

From each segment of optic nerve, transverse sections 1 μm thick were cut from three different parts. Thin sections for electron microscopy were also cut. The thick (1 μm) sections were stained with a modified methylene blue stain and examined by light microscopy. The number of blood vessels in each transverse section was

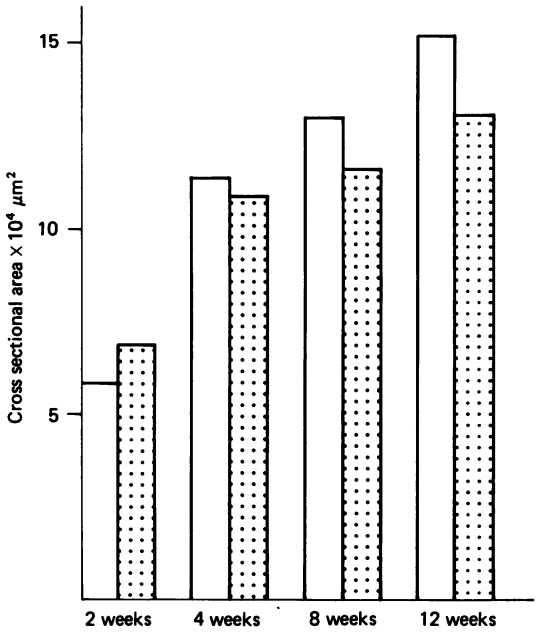


Fig. 1. Cross sectional area of optic nerves at 2, 4, 8 and 12 weeks in normotensive and hypertensive rats (stippled columns).

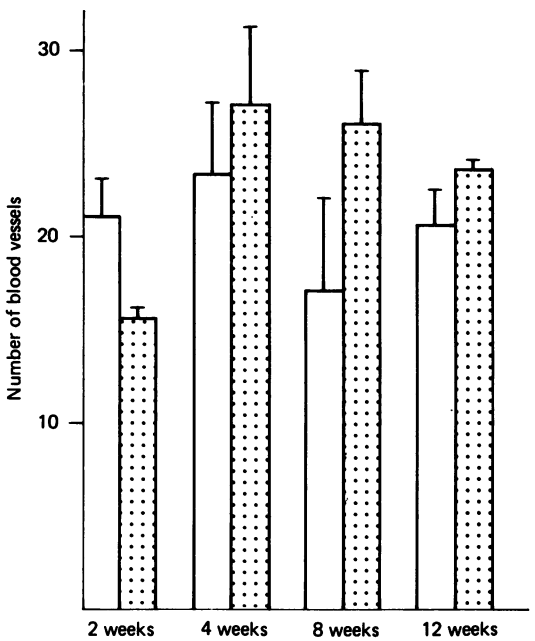


Fig. 2. Mean number of blood vessels in transverse sections of optic nerves at 2, 4, 8 and 12 weeks of age in normotensive and hypertensive rats (stippled columns). The error bar is the standard error of the mean. The number of blood vessels in the two strains is significantly different at each time period ($P < 0{\cdot}75$).

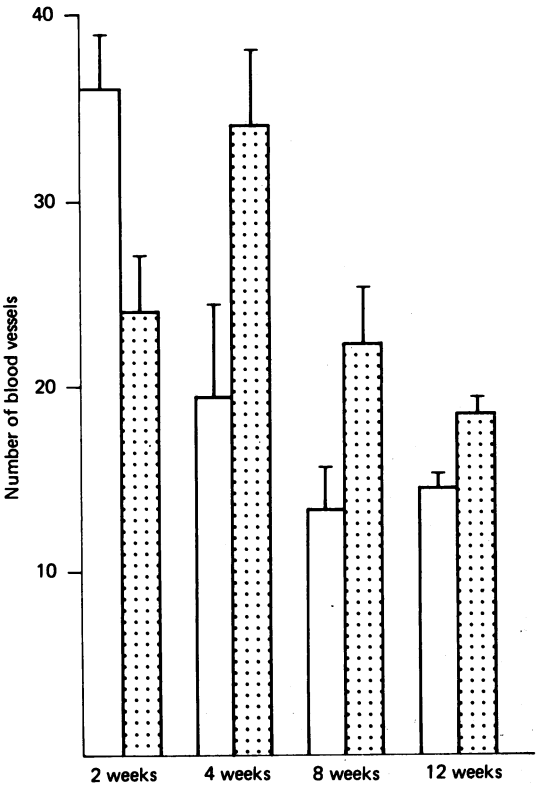


Fig. 3. Number of blood vessels per 1×10^5 μm^2 of cross sectional area of optic nerve at 2, 4, 8 and 12 weeks of age in normotensive and hypertensive rats (stippled columns). The error bar is the standard error of the mean. The density of blood vessels is significantly different between the two strains at each time period ($P < 0{\cdot}05$).

counted. Only those vessels not in direct contact with the surface were counted. Profiles which were obviously sections of the same vessel were counted as only one vessel. Each section from which a blood vessel count was made was photographed, and the area of the optic nerve measured from the negative. The density of blood vessels was thus determined.

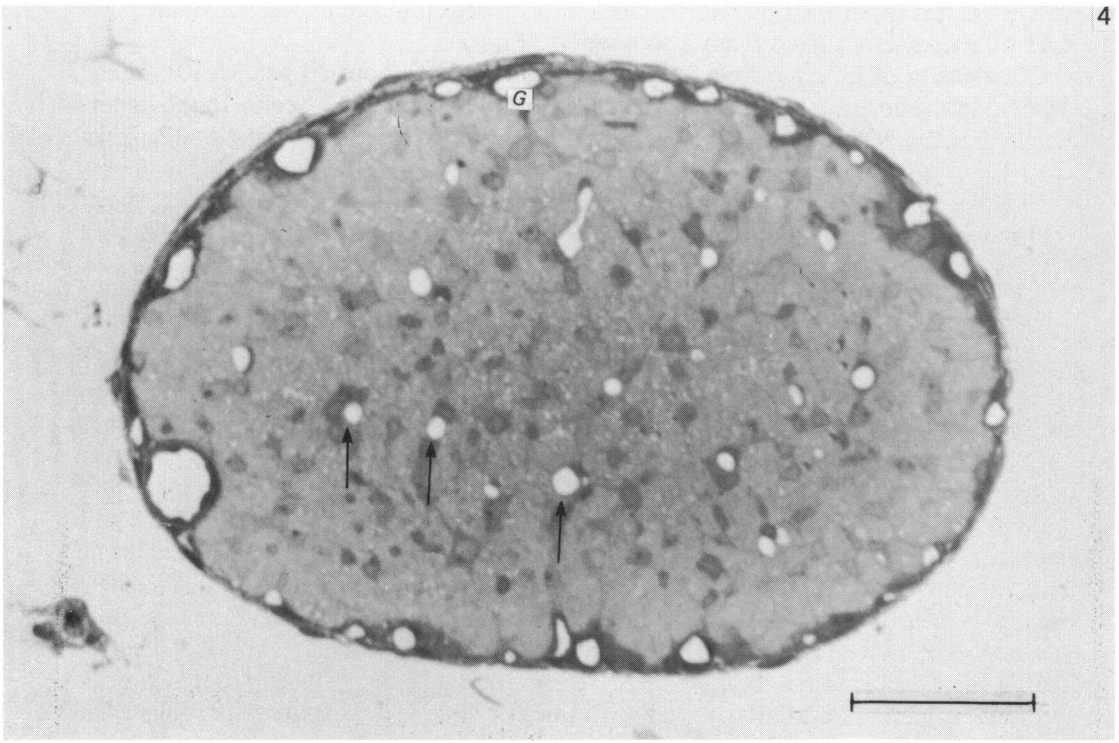
The thin sections were stained with lead citrate and examined in a Philips EM300 electron microscope. From the electron micrographs the percentage of fibres which were myelinated and the spectrum of axon diameters were determined at each age. Axon diameter was obtained by measuring the narrowest width of the axon cylinder. Micrographs used for axon diameter measurements were taken at random from all areas of the nerves.

RESULTS

Light microscopy

The cross sectional area of the optic nerve increased in both strains from 2 to 12 weeks of age as shown in Figure 1. At 2 weeks the cross sectional area was about 6×10^4 μm^2 and increased steadily to about 14×10^4 μm^2 at 12 weeks. No significant difference in cross sectional area was noted between the two strains at any age.

In the normotensive rat there was little change in the number of blood vessels seen



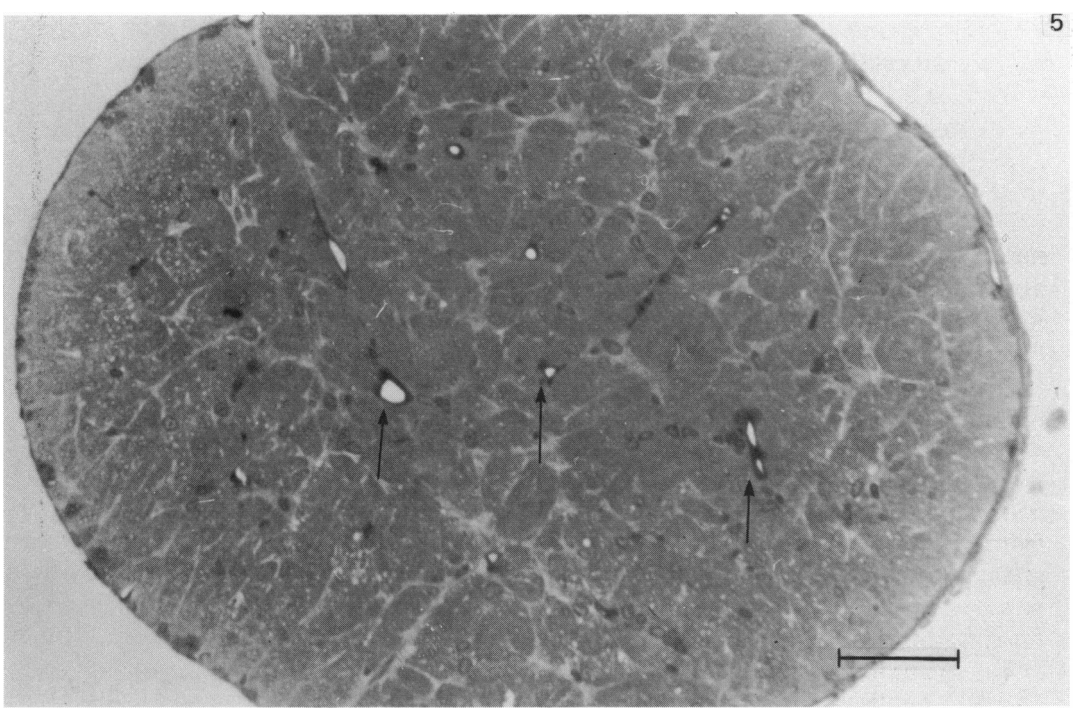


Fig. 4. A light micrograph of a transverse section of the optic nerve of a two weeks old hypertensive rat. Blood vessels are indicated by the arrows. Glial cell nuclei (G) are found throughout the section. Calibration mark, 50 μm.

Fig. 5. A light micrograph of a transverse section of the optic nerve of an eight weeks old normotensive rat. By this age the optic nerve cross sectional area has increased compared with that of the two weeks old in Fig. 4. Blood vessels are indicated by the arrows. Calibration mark, 50 μm.

in a cross section of the optic nerve, from 2 to 12 weeks. In the hypertensive rat however an increase occurred from 2 to 4 weeks (Fig. 2).

The density of blood vessels fell from a high level of about 36 per 1×10^5 μm^2 at 2 weeks in the normotensive rat, to about 15 per 1×10^5 μm^2 at 12 weeks. In the hypertensive rat the density first increased from 2 to 4 weeks, then fell from 4 to 12 weeks (Fig. 3).

The distribution of blood vessels appeared to be the same in the two strains in that the blood vessels were distributed uniformly throughout the nerve, each blood vessel lying equidistant from its neighbours (Figs. 4, 5). A lamina of optic nerve tissue at the surface of the nerve usually contained few blood vessels, the tissues presumably being supplied from vessels on the surface.

As seen by light microscopy, the vessels were of two types. One type of vessel had a thick wall surrounded by layers of collagen fibres, while the other possessed a thin wall and had the appearance of a true capillary.

Electron microscopy

At 2 weeks of age, the blood vessels in both strains consisted of thick endothelial cells (Fig. 6), with associated pericytes. These cells were separated from the axons by one or two layers of glial cells. Few collagen fibres were present around the blood vessel. In older animals both the collagen and glial layers increased in thickness (Fig. 7), and the endothelial cells became thinner.

The pericytes associated with optic nerve blood vessels were either single cells with few organelles and a kidney shaped nucleus (Fig. 8), or more active cells with an extensive Golgi complex and dilated granular endoplasmic reticulum.

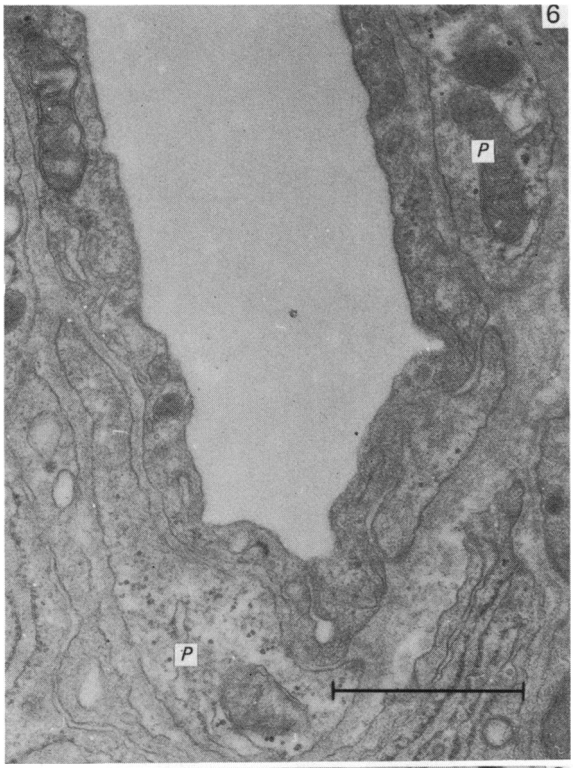
Mitotic figures were seen in both glial and endothelial cells. Most of the dividing endothelial cells were observed in specimens from 2 weeks old rats (Fig. 9).

At birth few fibres were myelinated. At 2 weeks of age, only about 1 % of the fibres were myelinated, at 4 weeks about 75 % were myelinated and by 8 weeks almost all fibres were myelinated although in the 12 weeks old spontaneously hypertensive rat, some unmyelinated fibres were still present. The spectrum of fibre diameters changed during development, as shown in Figure 10. At 2 weeks of age the mean fibre diameter was 0·5 μm, with a range of 0·5 to 1 μm. The fibres increased in diameter with age so that at 12 weeks the mean fibre diameter was 1 μm, with a range of 0·5 to 2 μm.

DISCUSSION

There are significant differences between the normotensive and hypertensive rats in the pattern of vascularisation of the optic nerve. The hypertensive rat lags behind the normotensive rat initially, but continues to develop so that at 12 weeks the density of blood vessels is significantly greater. The number of blood vessels is stable from 2 to 12 weeks in the normotensive rats, but increases from 2 to 4 weeks, thereafter remaining steady until 12 weeks in the hypertensive rats. The changes in blood vessel number and density do not appear to be correlated with either myelination of fibres or changes in the fibre diameter spectrum.

The increased vascularity of the hypertensive rat optic nerve from 4 weeks onwards, is consistent with the hypothesis that vascularisation is dependent upon the efficiency of the circulatory system (Sturrock, 1982). The circulatory system of the spontaneously hypertensive rat operates at an altered level of efficiency when compared with the normotensive rat. Bohlen, Gore & Hutchins (1977) have examined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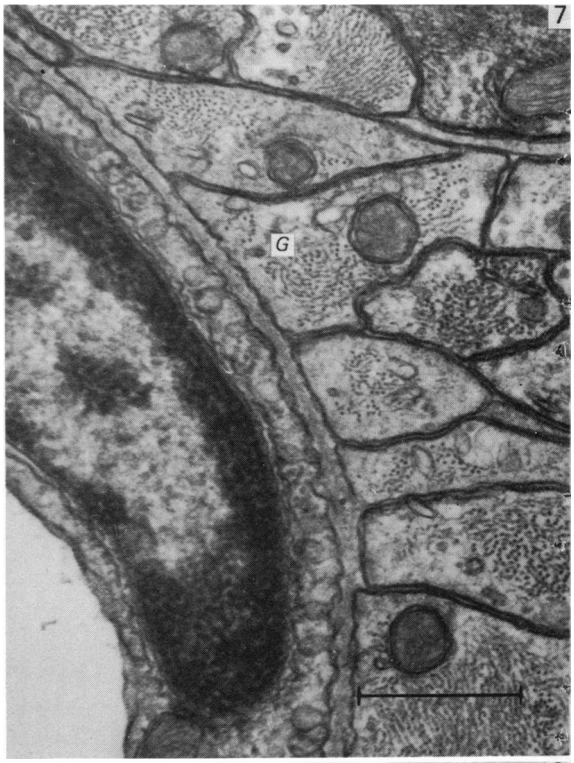
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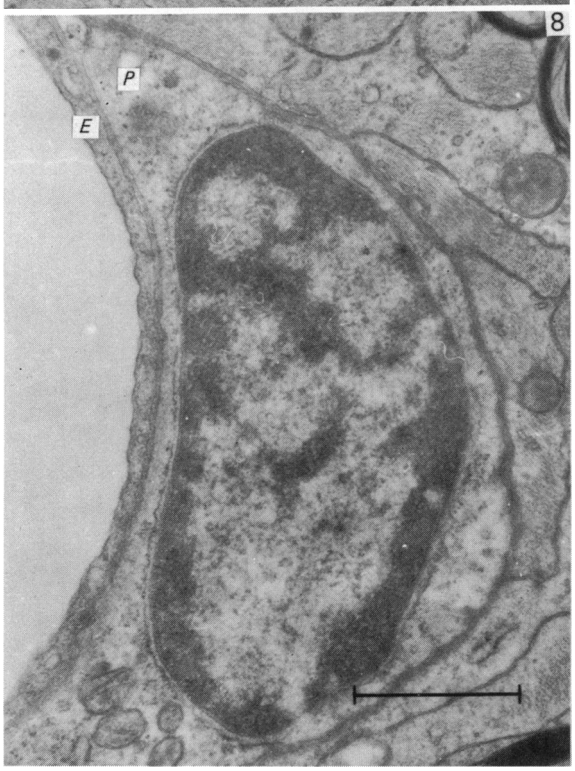
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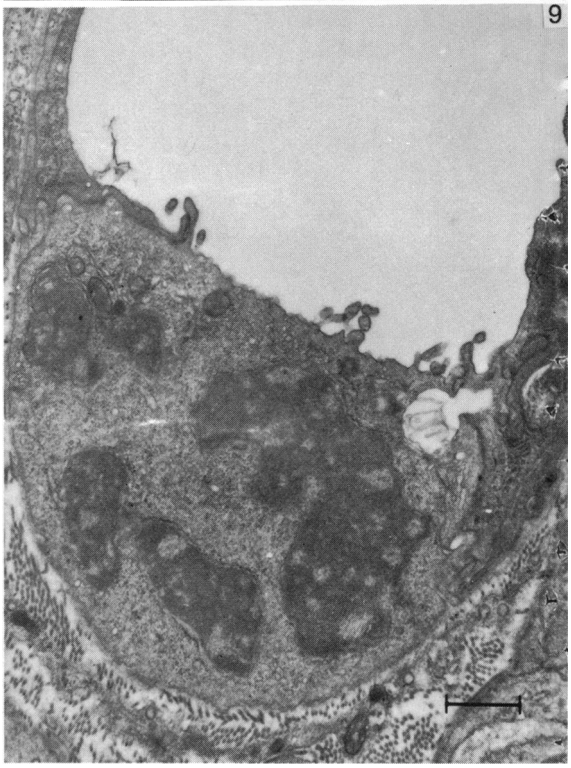
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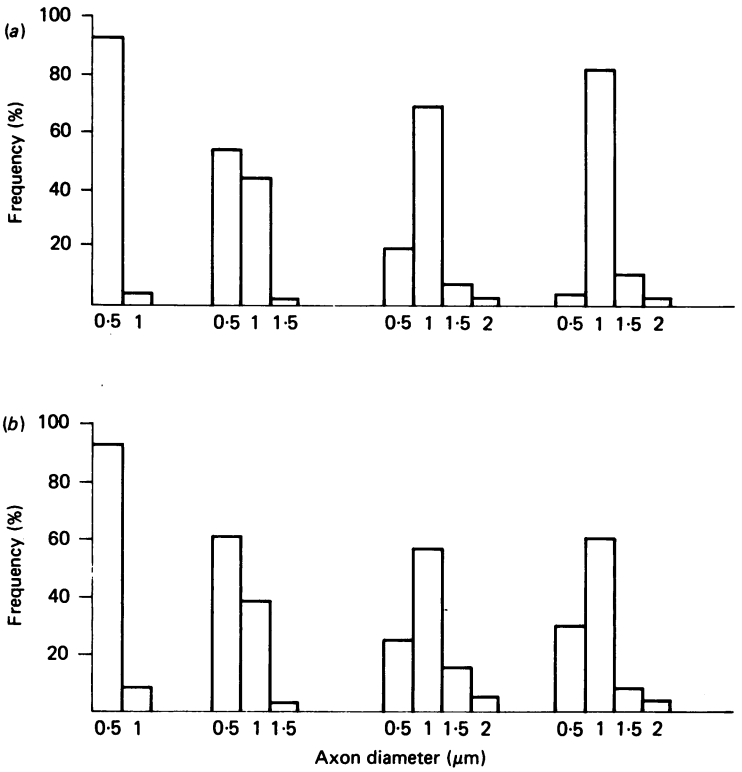

Fig. 10. Frequency distribution of optic nerve axons from normotensive (*a*) and hypertensive rats (*b*) of 2, 4, 8 and 12 weeks of age. One thousand fibres in each strain were measured.

the pressures in the microvasculature of normotensive and hypertensive rats. Both the systemic and microvascular pressures in spontaneously hypertensive rats are 30–35 % higher than those measured in normotensive rats.

A reduction in arteriole and capillary length and number has been reported (Chen, Prewitt & Dowell, 1981; Hutchins & Darnell, 1974). However, Hutchins & Darnell report an approximately twofold increase in the number of smallest venules. This suggests that the increase found in the present study may be due to an increase in small venules.

The cause of the increase in vascularity of the optic nerve in hypertensive rats is not known. It does, however, appear to be without effect on the structural development of the optic nerve.

Fig. 6. An electron micrograph of a blood vessel of a two weeks old hypertensive rat. The endothelial cell is relatively thick. Pericytes (P) are present. Calibration mark, 1 μm.

Fig. 7. An electron micrograph of a blood vessel of a 12 weeks old normotensive rat. The endothelial cell of the capillary has several layers of glial processes (G) applied to it. Calibration mark, 0·5 μm.

Fig. 8. An electron micrograph demonstrating a typical pericyte from the optic nerve of a 12 weeks old normotensive rat. The pericyte, (P), with a large nucleus, is applied to a thin endothelial cell (E). Calibration mark, 1 μm.

Fig. 9. An electron micrograph demonstrating cell division in an endothelial cell of a blood vessel from the optic nerve of a two weeks old hypertensive rat. Calibration mark, 1 μm.

SUMMARY

The blood vessels in the optic nerve of normotensive and hypertensive rats have been examined at 2, 4, 8 and 12 weeks of age. The pattern of development was found to be different in the two strains, with the number of blood vessels in the hypertensive rat optic nerve being lower at 2 weeks, but greater at 12 weeks than the normotensive rat. There appeared to be no correlation between vascularity and either myelination or changes in the fibre diameter spectrum at the ages studied. It is concluded that while the cause of the increased vascularity of the optic nerve in hypertensive rats is not known, it appears to be without effect in the structural development of the optic nerve.

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