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Vascular and neural changes in the rat optic nerve following induction of diabetes with streptozotocin

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INTRODUCTION

Structural changes in the vasculature have been reported to occur in genetically diabetic animals, or following induction of diabetes in experimental animals. The changes seen vary from one vascular bed to another but certain alterations are consistent. Most of the studies have concerned the vessel beds other than those in the central or peripheral nervous systems, with the exception of extensive studies on retinal vasculature (reviewed by McMillan, 1975). There is little information on changes which occur in the vasculature of the central nervous system, or how these changes effect structure and function within this system.

Recently it has been demonstrated that changes occur in the vessels of the optic nerve during development of hypertension in the rat (Scott & Foote, 1984). No similar study has been made following the induction of diabetes. It is widely believed that visual defects which develop in diabetic animals and patients are due to retinal vascular changes. However it has been suggested that, in some cases, changes occurring in the optic nerve are responsible for the visual system deficits (Kestenbaum, 1961; Walsh & Hoyt, 1969). Moore (1920), Skillern & Lockhard (1959) and Topilow & Bisland (1952) have attributed the optic nerve involvement to toxic metabolic changes affecting patients with longstanding or severe, uncontrolled diabetes. Others (Miller & Smith, 1966; Lubow & Makley, 1971) have suggested that optic nerve involvement in diabetes is due to a vascular phenomenon related to ischaemia.

In this study changes have been examined which occur in the morphology of the optic nerve vasculature following induction of diabetes, and the effects on neural components of the optic nerve are also studied.

MATERIALS AND METHODS

Four weeks old Sprague–Dawley rats were injected through the femoral vein with streptozotocin (50 mg/kg, donated by Upjohn). At daily intervals for one week after injection, urine was checked for glucose; thereafter, at weekly intervals, the rats were weighed and their urine checked for glucose and ketones (Ames labstix). The rats were included in the study if at the time when they were killed their blood glucose exceeded 400 mg/dl as measured by an Ames glucometer. All rats used in this study were diabetic by two days after injection. At 8 and 12 weeks after treatment, six diabetic and six age-matched control rats were anaesthetised with pentobarbital (35 mg/kg) and their blood pressure measured via a femoral cannula. Arterial pressures were measured in order to exclude effects of hypertension on vascular structure. All rats were normotensive. The diabetic and control rats were perfused

Table 1. *Morphometry of optic nerves from control and diabetic rats*

Rats	Volume of axons	Volume of glia	Number of vessels per cross section	Area of optic nerve ($\times 10^5$ μm^2)	Density of blood vessels (number/ 2×10^5 μm^2)
Control	86·4 ± 3	13·6 ± 3	20·6 ± 1·9	2·06 ± 0·2	19·8 ± 2·3
Diabetic 8 weeks	65·1 ± 8*	34·9 ± 8*	21·9 ± 1·4	1·94 ± 0·23	29·3 ± 3·4*
Diabetic 12 weeks	33 ± 7**	67 ± 7**	47·3 ± 8*	2·06 ± 0·23	45 ± 1·5**

By analysis of variance, significant differences were found ($P < 0.05$) between the values for the control rats and rats diabetic for 8 weeks (*), and between values for the control, 8 weeks diabetic and 12 weeks diabetic rats (**). $n = 6$.

through the heart with a fixative containing 2% glutaraldehyde and 2% formaldehyde in 0·1 M sodium cacodylate buffer. The optic nerves from each rat were processed for electron microscopy and embedded in Araldite resin.

Transverse sections, 1 μm thick, were cut from intraorbital, canalicular and intracranial sections of each optic nerve. The number of blood vessels in each cross section was counted from three sections of each of three regions of the optic nerve. All vessels were counted except those in contact with the pial surface. Each cross section counted was photographed and the area of the cross section determined. Thin sections were cut from three regions in each nerve for examination by electron microscopy. Electron micrographs were taken at various magnifications. From these electron micrographs a determination was made of axon calibre and of the % volume of glial and axonal elements using a 100 point grid, according to previously published methods (Scott & Foote, 1984).

RESULTS

All the rats used in the study were diabetic from four weeks of age until death.

The mean arterial pressure (mm Hg) of the diabetic rats at death was 96 ± 6 at eight weeks and 98 ± 4 at twelve weeks. The mean arterial pressure of the control rats was 91 ± 4 at eight weeks and 94 ± 9 at twelve weeks.

Blood vessels

The distribution of blood vessels was similar in both sets of rats. The vessels appeared to be distributed uniformly throughout the transverse section as shown in Figures 1 *a*, *b* and *c*. A band of optic nerve tissue lying just below the surface appeared to have no, or few, vessels. In only a few cases was a large central vessel seen. Most vessels ran in the long axis of the nerve, with only occasional vessels cut obliquely. As shown in Table 1, both the number and density of blood vessels was significantly increased in diabetic rats eight and twelve weeks following induction of diabetes. The mean cross sectional area of the diabetic and control rat optic nerve was not significantly different.

The structure of the blood vessels appeared to be similar in the control and diabetic rats. Most of the vessels were postcapillary venules, with pericytes. Examples of these vessels are shown in Figures 2 *a* and *b*. As can be seen from these examples,

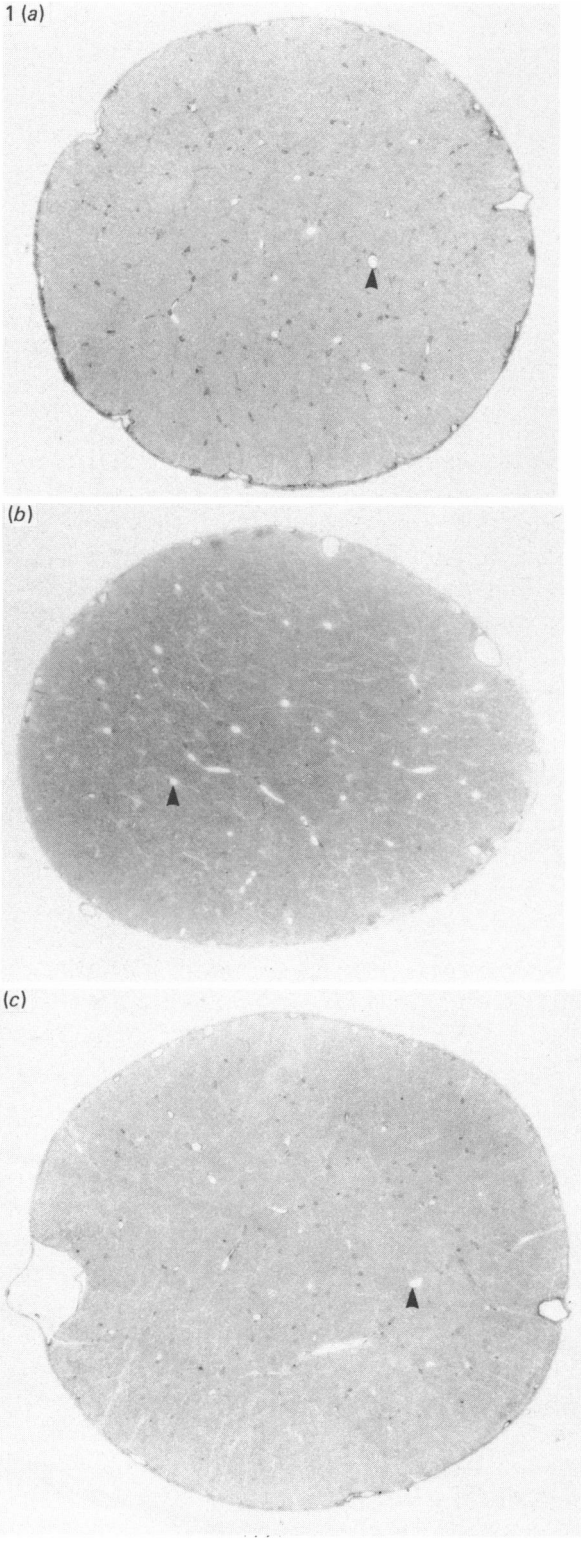

Fig. 1(*a–c*). Light micrographs of optic nerve transverse sections from a normal rat (*a*), and from rats with eight (*b*) or twelve weeks (*c*) duration of diabetes. The blood vessels are indicated by arrowheads. × 180.

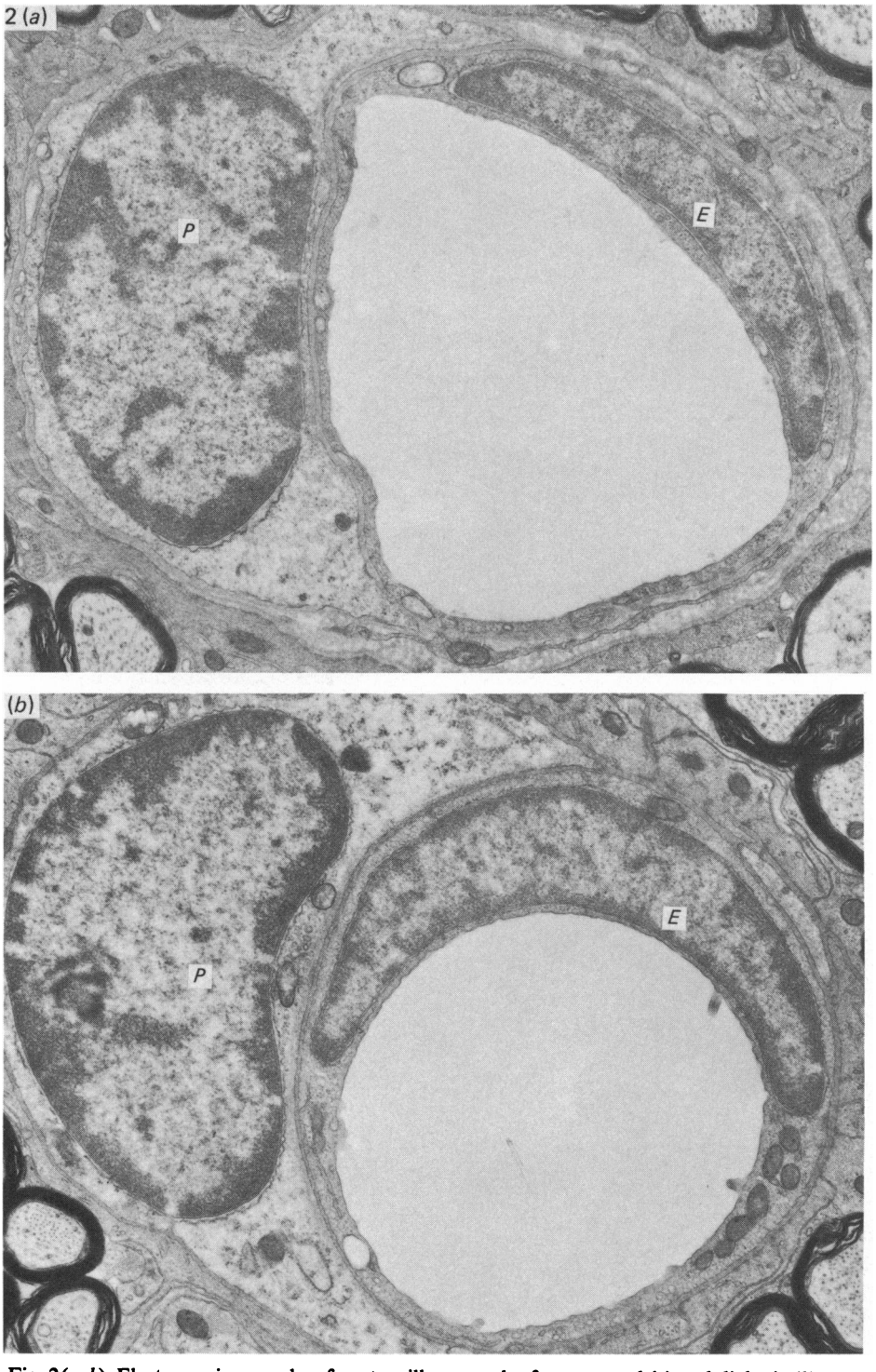

Fig. 2(*a–b*). Electron micrographs of postcapillary venules from normal (*a*) and diabetic (*b*) rat optic nerves. The endothelial cells (*E*) and pericytes (*P*) are shown, with a thin basement membrane in between. ×15500.

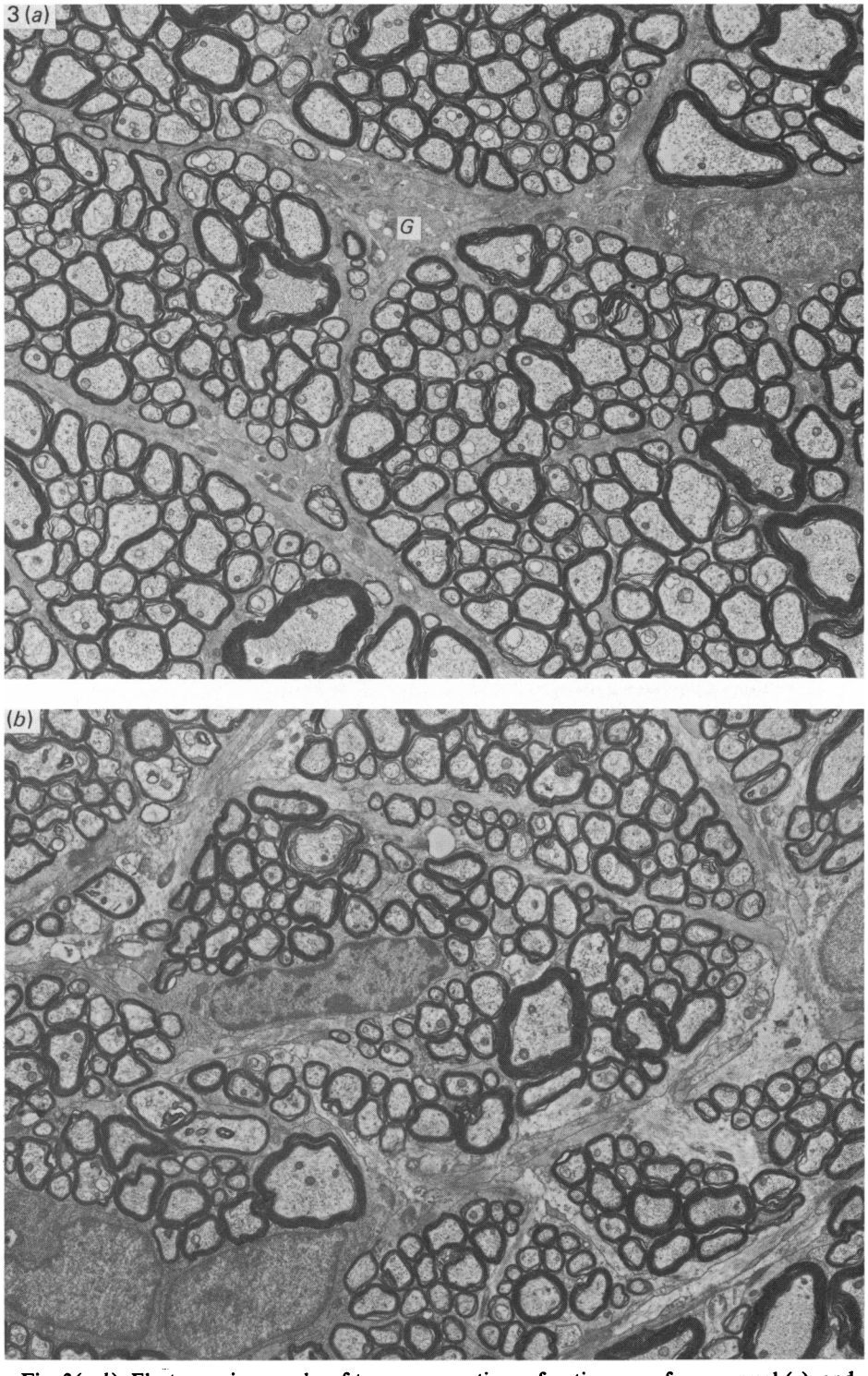

Fig. 3(*a–b*). Electron micrographs of transverse sections of optic nerves from normal (*a*), and diabetic (*b*) optic nerves. The glial processes (G) subdivide the fibres into bundles which are larger in the normal than in the diabetic. × 5000.

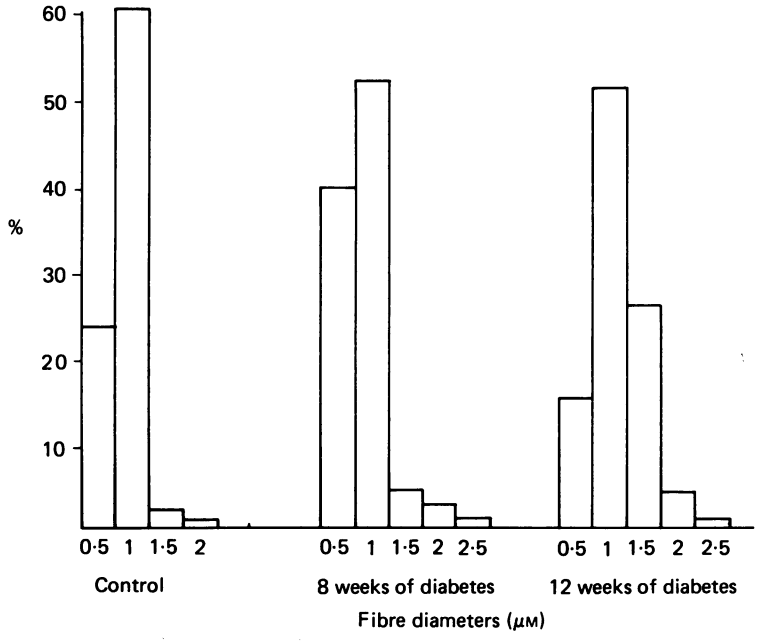

Fig. 4. Histograms of the distribution of fibre diameters in μm, in optic nerves from normal (control) rats, and in rats diabetic for eight or twelve weeks.

the endothelial cells had a normal appearance. No changes were noticed in endothelial junctional complexes, or in the basement membrane. The pericytes were, as shown, comparable in structure.

Glia and axons.

From point-counting, it was determined that the percentage volume of glial elements increased and the percentage volume of axons decreased in optic nerves from diabetic rats (Table 1). This change could be seen by light microscopy and by electron microscopy (Fig. 3). The glial processes appeared to subdivide the fibres into smaller bundles, in the diabetic rats. The fibre diameter spectrum was not very different, with most fibres being about 1 μm in diameter (Fig. 4). The axons in the diabetic optic nerve were normal in appearance including the myelin sheath. In a few cases, the axons in both diabetic and normal animals showed signs of degeneration.

DISCUSSION

It is shown that there are vascular and neural changes in the optic nerve following induction of diabetes with streptozotocin. The changes are progressive with the duration of the disease. It is possible that the alterations observed could contribute to visual impairment.

Ocular complications of diabetes have been described in detail (Konher, 1972). However, few reports deal with optic nerve involvement. It has been suggested that optic nerve involvement is a specific complication of diabetes (Kestenbaum, 1961; Walsh & Hoyt, 1969), but the nature of the changes and their pathogenesis has not been described. Previous reports have attributed the optic neuritis of diabetes either to biochemical alterations in glial cells similar to those that have been suggested to

occur at other sites (Thomas & Lascelles, 1965; Gabbay, Merola & Field, 1966; Stewart *et al.* 1967; Gabbay & Snider, 1970; Gabbay, 1971; Mattingly & Fischer, 1983), or to undetermined vascular phenomena (Miller & Smith, 1966, Lubow & Makley, 1971). Freund, Carmon & Cohen (1965) have assumed that diabetic optic involvement is due to diffuse vascular changes, while others suggest that the cause is capillary involvement in the area of the optic disc (Yanko, Ticho & Ivry, 1972).

The changes seen in this study support the suggestion of vascular changes in involvement of the optic nerve in diabetes. The increase in the number of small calibre vessels is consistent with that seen at other sites such as the retina (L'Esperance, 1978) and kidney (Osterby, 1975), and could contribute to structural and functional changes in the axons and glia.

Since the optic nerve is part of the central nervous system, any increase in blood vessels must be accompanied by an increase in the percentage volume of glial cells, since all optic nerve blood vessels have an astrocytic coat. However, since there is no difference in the cross sectional areas of the normal and diabetic optic nerves, a reduction in the percentage volume of axons must represent a real loss of axons in the optic nerve. The reduction in percentage volume of axons is significant. The loss of axons appears to be in all diameter classes since no significant change in fibre diameter spectrum occurs. Only a few axons containing pigment or with degenerating myelin sheaths have been seen. Unless the loss occurred at an early stage in the development of the disease, the signs of axon degeneration would be more obvious. Errors in the application of the point-counting technique or in the measurement of optic nerve area could have been responsible for the results obtained; however the same methods have been applied to all specimens, and there is no large variation between specimens. It may be necessary to examine the optic nerve within the first eight weeks after induction of diabetes to resolve the apparent enigma of axon loss.

A similar change in the density of blood vessels has been reported in the optic nerve following development of hypertension (Scott & Foote, 1984), but no changes in glial/axonal relationships were reported. Changes in axons have been reported in peripheral nerves in diabetic animals, particularly in smaller sized myelinated fibres (Mattingly & Fischer, 1983).

The cause of the change in axon/glial relationships in this study has not been determined, although it is most likely to be related to the accompanying vascular changes.

SUMMARY

This study was undertaken to determine whether or not changes occur in blood vessels, axons or glia of the optic nerve as the result of streptozotocin-induced diabetes.

Diabetes was induced in 4 weeks old Sprague–Dawley rats. At 12 and 16 weeks of age, the rats were killed and the optic nerves prepared for examination. The number and density of blood vessels was found to be significantly increased in the diabetic rats. No alteration in the structure of the blood vessels was noted. A decrease in the percentage volume of axons and an increase in the percentage volume of glial elements accompanied the increase in blood vessels in the diabetic rats. No difference was found in the spectrum of fibre diameters.

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