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Effects of experimental diabetes in the noradrenergic and cholinergic nerves of the rat small intestine

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Summary. An histochemical research on cholinergic and noradrenergic fibres of the adventitia layer and of the myenteric plexus of the terminal ileum from rats with streptozotocin-induced diabetes, after 20 weeks of evolution of the illness, was carried out to study changes in the innervation of the gut.

The cholinergic nerves, revealed through their acetylcholinesterase activity, did not present alterations, but an evident reduction in number of the noradrenergic nerves and swollen intensely fluorescent varicosities, were observed, both in the perivascular and myenteric plexus of terminal ileum from diabetic animals.

Key words: Diabetic neuropathy, Rats, Adrenergic, Cholinergic, Small intestine

Introduction

The occurrence of alterations in the intestinal motility during the course of diabetes mellitus appears to be related to changes in the autonomic innervation of the bowel. The fact that clinical studies have failed to clearly demonstrate this relation may justify an attentive study of both submucous and myenteric plexus.

The intestinal symptoms in experimental-induced diabetes in rats are very similar to those observed in human diabetes, thus the results of experimental models might be applied to the study of the illness in humans, in order to gain a better knowledge of the alterations caused on the nerves of the Enteric System by this illness.

Recent studies on experimental diabetes have shown alterations in the adrenergic, cholinergic and peptidergic innervation in the gastrointestinal tract of streptozotozin-treated diabetic rat (Schmidt et al., 1981, 1988; Lincol et al., 1984; Belai et al., 1985, 1988, suggesting autonomic neuropathy as the direct cause of the gastrointestinal disfunction displayed in diabetes (Diani et al., 1976, 1979).

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Materials and methods

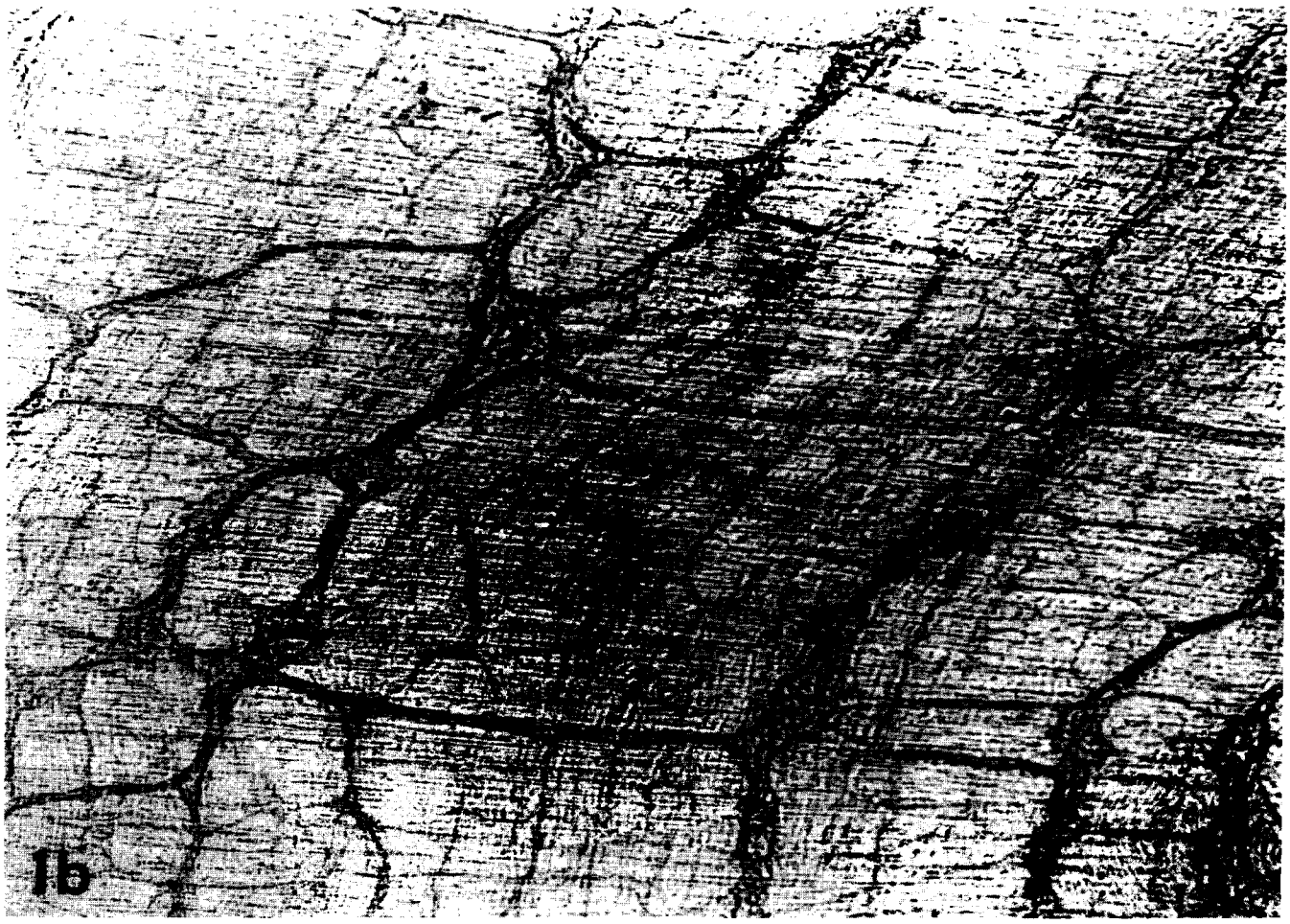
Diabetes was induced in 10 male Wistar rats with, initial weights of 250-300 gr, by intraperitoneal injection of streptozotocin (STZ, 65 mg/kg in citrate buffer 0.05M, pH 4.5). The same number of age/weight matched control animals received citrate buffer alone. All the animals were kept for 20 weeks under the same conditions and fed with food and water ad libitum. 48 h after the injection, plasma glucose was controlled weekly with VISIDEX strips (Ames).

Method for Cholinesterase «in toto»

The method used was a combination of those of El-Badawi and Schenck (1967) and Qayyum and Fatani (1985). Segments of terminal ileum were cut open lengthwise, stretched out and pinned on thin cork sheets, and immersed in 2% glyoxylic acid solution, ph 7.2, for 5-30 min at room temperature. After that, the segments were transferred to slides and under a stereoscopic microscope, with the aid of microsurgery instruments, the mucous layer was carefully removed and the two muscular layers were opened to expose the myenteric plexus. Both layers were post-fixed in 10% paraformaldehyde in PBS solution for 5 min, washed throughly and then incubated with the specific medium for cholinesterase. Cholinesterase activity was controlled every 15 min through the microscope, the best results being obtained between 1 and 2 h after the start. The samples were dehydrated in alcohols of increasing concentration, cleared in xylol and mounted in DPX.

Method for Adrenergic fibres (Furness and Costa, 1975)

Dissection, fixation and delamination proceeded as described above. The segments were exposed to parafor-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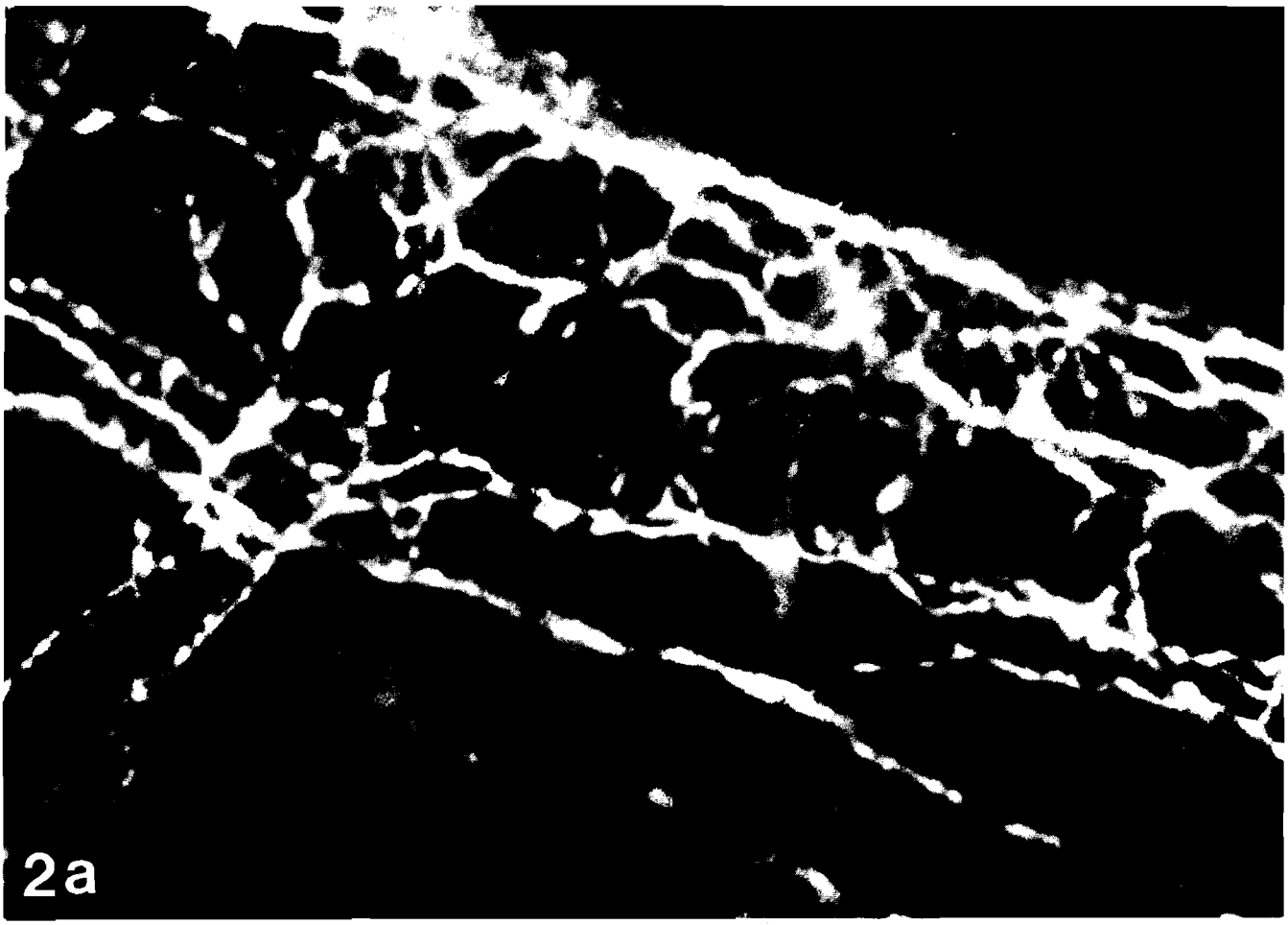

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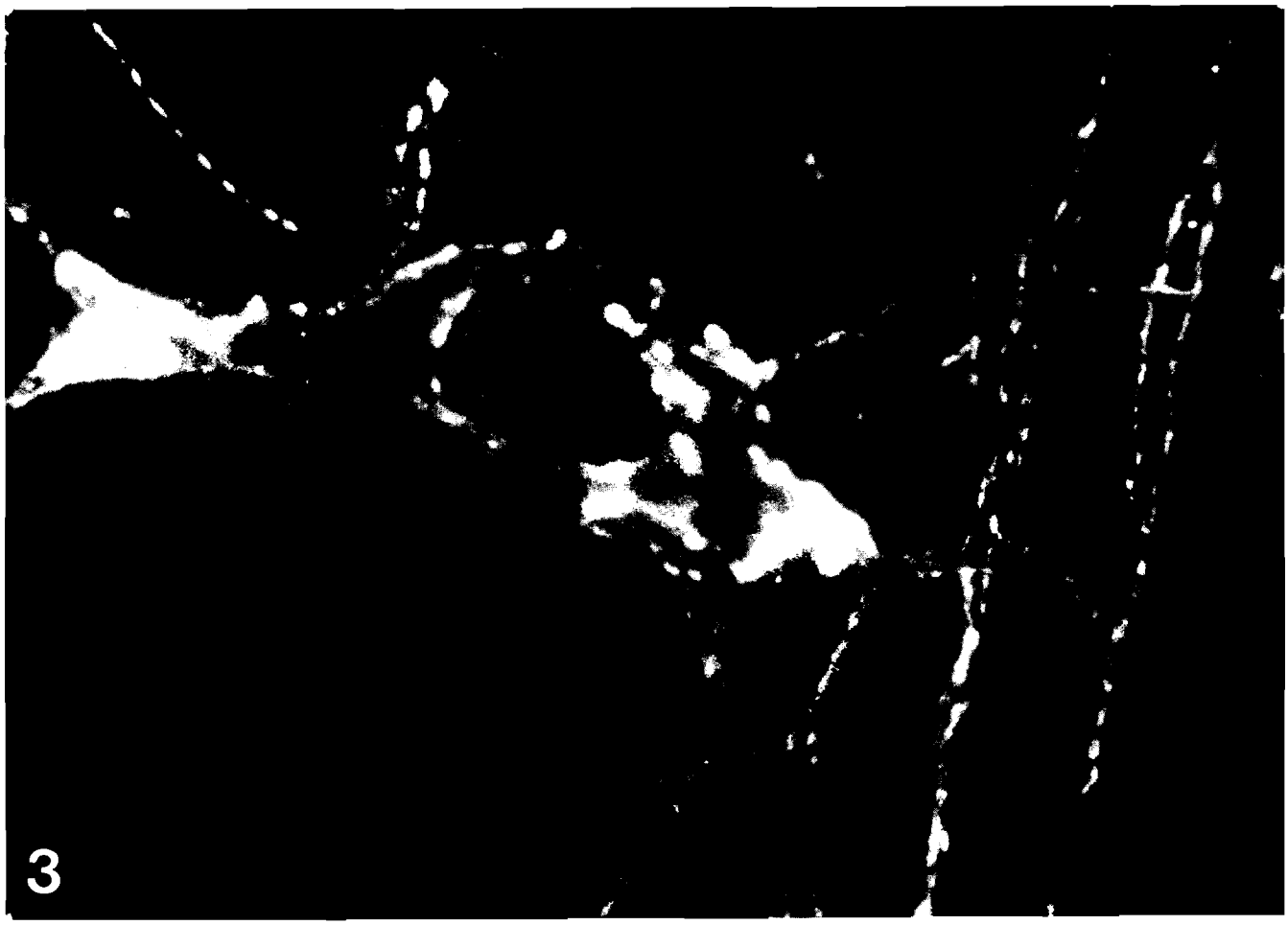
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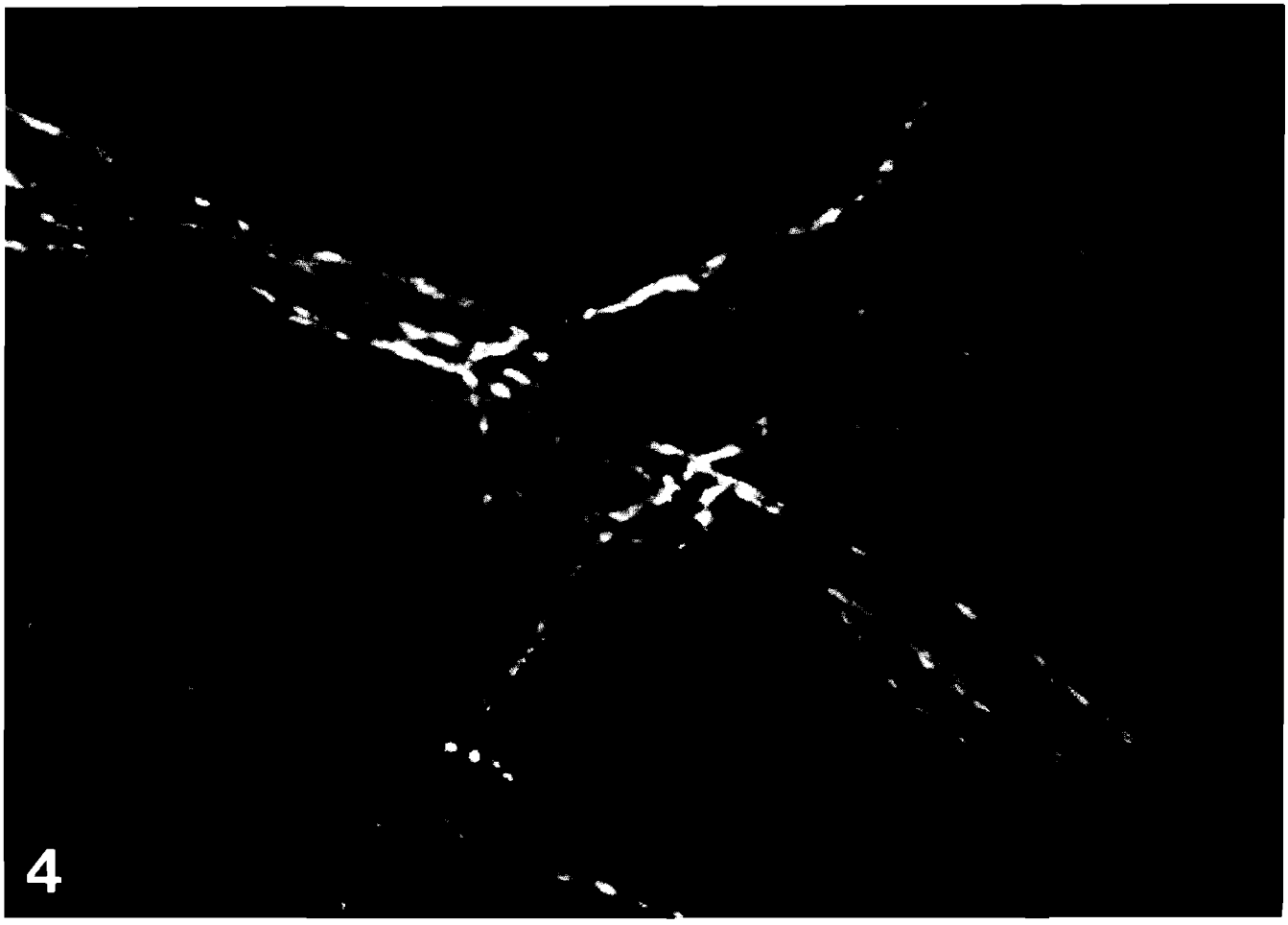
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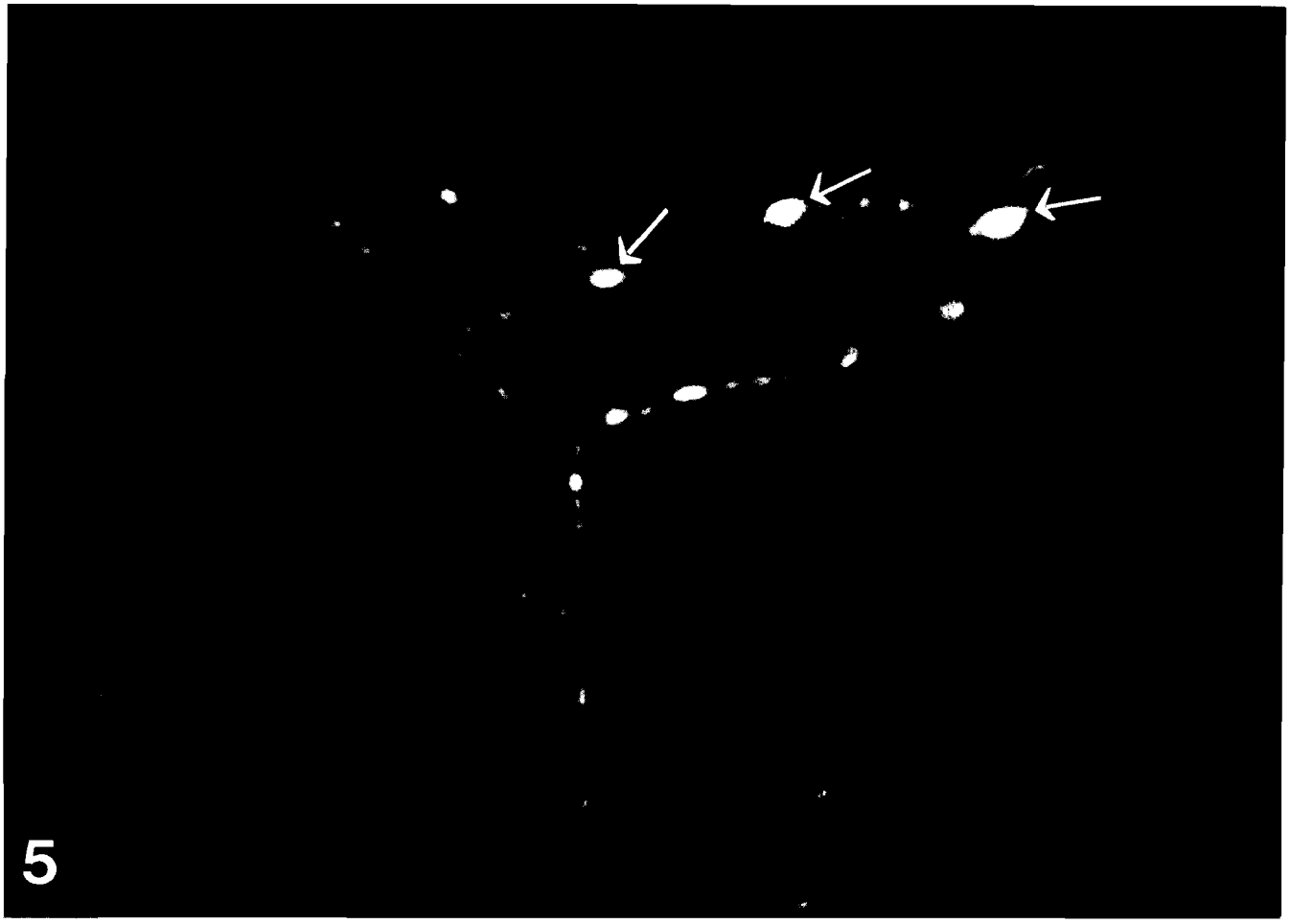

Fig. 1. Acetylcholinesterase staining of the myenteric plexus of the ileum from control **(a)** and diabetic **(b)** rats. × 20

Fig. 2. Catecholaminergic fluoresecence of the perivascular plexus of the adventitia layer of the ileum from control **(a)** and diabetic **(b)** rats. Note the reduction of the noradrenergic fibres in **(b)** in comparison with **(a)** × 20

Fig. 3. Myenteric plexus of the ileum from a control rat, with abundant varicose noradrenergic fibres surrounding the myenteric ganglion. × 20

Fig. 4. Myenteric plexus of the ileum from a control rat, with abundant interconnecting noradrenergic fibres. × 20

Fig. 5. Myenteric plexus of the ileum from a diabetic rat, showing marked reduction in the number of noradrenergic fibres and the presence of swollen intensely fluorescent varicosities (arrows). × 20

maldehyde vapours at 80° C for 1 h, mounted and examined through a fluorescence microscope (Leitz-Orthoplan).

Results

All diabetic animals showed loss of corporal weight and levels of plasma glucose superior to 400 mg/100 ml. On opening the abdomen, marked distention of both small and large bowel was observed, with occurrence of thick and amorphous faeces.

Acetylcholinesterase histochemistry of the ileum in both diabetic and control animals showed a similar tinctorial pattern in the cell bodies of the myenteric ganglia, and a dense network of AChE-positive nervous fibres in the myenteric plexus (Figs. 1a, 1b).

The catecholaminergic fluorescence induced by vapours of paraformaldehyde showed in the adventitia layer of the terminal ileum from control animals, perivascular plexus with abundant noradrenergic fibres forming dense and intensely fluorescent networks all along the route of the blood vessels (Fig. 2a). In the diabetic animals a marked reduction in number of noradrenergic fibres was observed, together with a diminished intensity of the catecholamine fluorescence around the vessels (Fig. 2b).

At the same time, the terminal ileum from control rats showed abundant and intensely fluorescent noradrenergic fibres around the myenteric ganglia, with abundant networks of interconnection. These networks were formed by delicate fibres presenting small varicosities (Figs. 3, 4). In the diabetic rats, the reduction of the intensity of fluorescence stood out, together with a decrease in the number of noradrenergic fibres. This

decrease was observed not only in the edges of the myenteric ganglia, but in the fibres of interconnection also. In the same way, numerous swollen and intensely fluorescent varicosities were seen (Fig. 5).

Discussion

Our observations in the present study suggest the existence of autonomic neuropathy in animals with streptozotocin-induced diabetes.

The histochemical studies performed on the myenteric plexus of the distal ileum showed no alterations in the cholinergic innervation, neither in the ganglionic bodies nor in their fibrilar prolongations, suggesting that the diabetic animals maintained undamaged the cholinergic nervous activity.

These observations agree with those of Lincoln et al. (1984), who carried out their experimental model on animals with induced diabetes and 8 weeks of evolution of the illness, which lead us to think that a significative increase on the time of evolution of the diabetes does not affect the cholinergic innervation of the myenteric plexus of terminal ileum.

On the other hand, the observations with catecholamine fluorescence suggest a significative decrease in the adrenergic innervation of perivascular fibres of the adventitia layer of the ileal wall. The described alterations of the perivascular noradrenergic fibres have not been reported up until now by any author, neither by ultrastructural nor histochemical methods.

In the same way, the adrenergic nerves of the ileal myenteric plexus showed a decrease in the number of terminal axons, together with a reduction in the intensity of fluorescence and occurrence of swollen varicosities. These alterations, similar to those described by Lincoln et al. (1984), are strongly suggestive of autonomic neuropathy in the ileum because of the diabetes. The degenerative changes of the amielinic fibres of the small bowel described ultrastructurally by Diani et al. (1979) in the myenteric plexus could correspond to the varicosities observed in the present study. The degeneration of noradrenergic nerves produced by treatment with 6-hydroxidopamine (Gordon-Weeks and Gabella, 1977), shows similar images of swollen varicosities, together with a decrease in the number of adrenergic nervous fibres.

The combined occurrence of similar modifications in the noradrenergic nerves of blood vessels of the ileum wall as well as in those innervating the intramural myenteric ganglia, together with the results obtained in our previous ultrastructural study of prevertebral ganglia in animals with induced diabetes (unpublished data), induces us to think of a common origin for these fibres, situated in the prevertebral ganglia (celiac and mesenteric superior). Probably, the origin of all observed alterations in the sympathetic postganglionic fibres is in these ganglia.

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