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Acetylcholinesterase-Activity in Rectal Mucosa of Children with Obstipation

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Summary. The results are presented of a clinical-enzyme histochemical evaluation of the activity of acetylcholinesterase in the rectal mucosa of 46 children with obstipation. In four cases abundant and coarse acetylcholinesterase positive nerve fibres were present in the lamina propria of the mucosa. Only in these 4 cases was the diagnosis of Hirschsprung's disease supported by the clinical course.

Local accumulations of fine acetylcholinesterase positive nerve fibres or accumulations of acetylcholinesterase positive foamy or structureless material were not correlated with Hirschsprung's disease.

Key words: Hirschsprung's disease – Megacolon congenitum – Enzyme histochemistry – Acetylcholinesterase – Rectal biopsy.

Introduction

Since Hirschsprung's major contribution in 1887 to the differential diagnosis of obstipation, this disease has continued to pose diagnostic problems. At present, the diagnosis is based on the symptomatology, the results of colon radiography, rectomanometry and histological and histochemical findings. Histologically, the disease is manifest as a congenital absence of the intrinsic innervation (i.e. ganglion cells in the intestinal wall) in association with proliferation of the extrinsic parasympathetic nerves (from S2 to S4). The leads to proliferation of nerve fibres containing acetylcholinesterase in the involved segment of the gut, including the lamina propria where normally very few or no nerve fibres are found. In the opinion of many authors the histochemical investigation (Meier Ruge, 1968, 1974) of this phenomenon in material obtained by suction biopsy (Dobbins, 1965) is to be preferred to histological investigation of a larger biopsy

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because the histochemical approach is easier, safer end gives equally good results. The absence of ganglion cells in a small suction biopsy from the rectum is not of diagnostic value because of the highly irregular distribution of these cells at that site.

Nerve fibres in the mucosa are more easily demonstrated histochemically than with the conventional histological stains. However, in the rectal mucosa of patients without Hirschsprung's disease we occasionally observed acetylcholinesterase-activity. We therefore attempted to evaluate the usefulness of the histochemical method for the differential diagnosis of obstipation on the basis of correlation between the clinical signs and symptoms and the histopathological picture.

Material and Methods

In 1975 and 1976 we performed rectal suction biopsies by the method of Dobbins in a total of 46 patients. Their ages varied from 2 months to 11 years, with an average of 4 years and 7 months.

The main indication for biopsy was obstipation. In a few cases the diagnosis of Hirschsprung's disease had been seriously considered on clinical grounds. Biopsy could be undertaken freely because the method causes little disturbance and involves no risk. Premedication and laxatives are not required, but in all cases simple coagulation tests were performed before the biopsy procedure, to exclude a bleeding tendency. The biopsies were taken at a distance of 1–2 cm from the anal sphincter and immediately frozen in isopentane cooled to about $-170°$ C with liquid nitrogen, after which they were sectioned 10 μ thick in a cryostat at $-20°$ C. These sections were stained with haematoxylin-eosin, and the acetylcholinesterase activity of the nerve fibres was visualized enzyme-histochemically according to the method of Karnovsky and Roots (1964). Most of the biopsies were found to include submucosal tissue. The initial histochemical results showed that in 4 cases the biopsy had been performed too close to the anus, and a new specimen was taken. Two other patients were re-biopsied early in the study, after inexplicable cholinesterase activity was found in the rectal mucosa. As a result, 48 rectal suction biopsies deriving from 46 patients were evaluated. The seriousness of the clinical picture was assessed on the basis of the following variables: The repeated occurrence of severe symptoms suggestive of chronic obstruction, the age at which the obstipation began, and the laxative requirement during the follow-up period.

Results

In our material four categories could be distinguished on the basis of the microscopical findings:

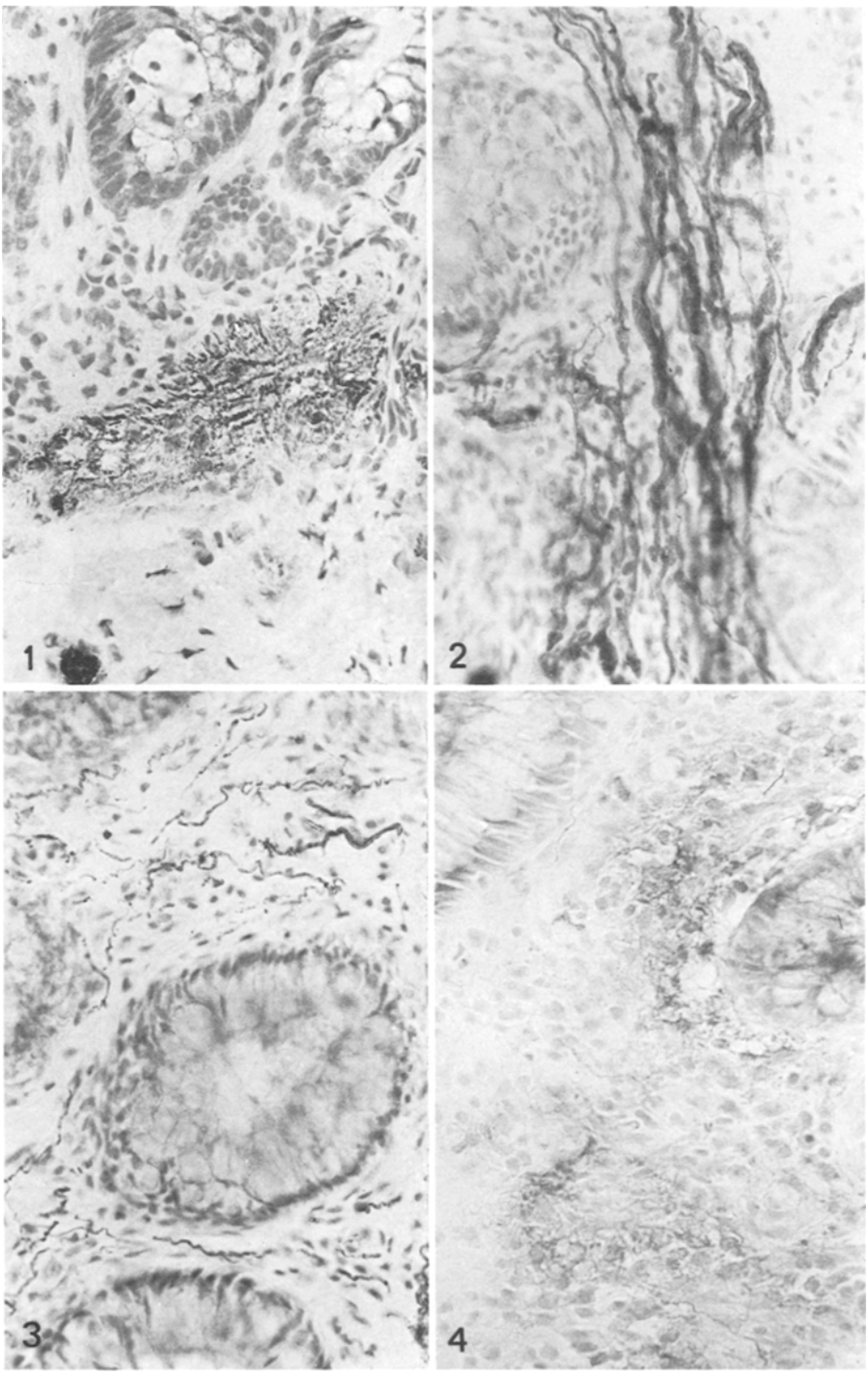
1. Normal intestinal tissue: In this group acetylcholinesterase-positive nerve fibres occurred only in the muscularis mucosa, submucosa and muscle tissue.

Fig. 1. Normal rectal mucosa. Acetylcholinesterase-positive nerve fibres are limited to the muscularis mucosa, and ganglion cells are present in the submucosa. × 300

Fig. 2. Marked proliferation of acetylcholinesterase-positive nerve fibres in the lamina propria, as seen in Hirschsprung's disease. × 300

Fig. 3. Local aggregations of acetylcholinesterase-positive nerve fibres in the lamina propria. × 300

Fig. 4. Fibrillar and foamy acetylcholinesterase-positive material in the lamina propria resembling nerve fibres. × 300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A few positive fibres were seen sporadically in the deep parts of the lamina propria of the mucosa. Ganglion cells were present as a rule in the submucosa (Fig. 1).

2. The picture described for Hirschsprung's disease (Meier-Ruge, 1968) i.e., not only acetylcholinesterase-positive nerve fibres in the muscularis mucosae, the submucosa, and the muscle tissue, but also numerous and often very coarse nerve fibres in the lamina propria of the mucosa. This group lacked ganglion cells in the submucosa (Fig. 2).

3. The picture seen in the normal controls but showing local aggregations of fine acetylcholinesterase-positive nerve fibres in the lamina propria. The submucosa often contained ganglion cells (Fig. 3).

4. In combination with any of the findings in the preceding three categories, accumulations of fibrillar, foamy, or amorphous acetylcholinesterase-positive material occurred in the lamina propria and on the surface of the biopsy (Fig. 4).

The results of the histochemical study were then compared with the clinical findings (see Table 1). In 32 patients the histochemical findings were consistent with the clinical findings (category I), and in 11 of these cases ganglion cells were present in the submucosa. Three of the patients in this group had developed the obstipation after the age of 1 year. In 12 other patients the obstipation was completely cured in the sense that a laxative or a laxative diet was no longer required. Of the remaining patients, 3 were not completely cured and 3 withdrew from the follow up. Only in the four cases with abundant and coarse acetylcholinesterase positive nerve fibres (category 2) was the diagnosis Hirschsprung's disease confirmed by clinical symptoms and follow up. In 10 cases varying amounts of acetylcholinesterase-positive fibres were found in the lamina propria of the mucosa (category 3), but the clinical picture was not that of Hirschsprung's disease. One patient showed both numerous fine acetylcholinesterase-positive nerve fibres and ganglion cells, and was completely cured. In the two re-biopsied patients ganglion cells were only found in the

Table 1. Acetylcholinesterase (ACE) activity in suction biopsies from the rectal mucosa of 46 patients with obstipation

	No ACE (+) nerve fibres (category 1)	Coarse ACE (+) nerve fibres (category 2)	Fine ACE (+) nerve fibres (category 3)
a) Ganglion cells present in biopsy	11	0	3[b]
b) Onset obstipation after age 1 yr	3	0	0
c) Spontaneous recovery	12[a]	0	4
d) Laxative and/or diet still required	3	4	2
e) Without follow up	3	0	1
Total	32	4	10

[a] Not including patients mentioned under a and b

[b] In 2 cases ganglion cells occurred only in second biopt

second biopsy. Four other patients, who were given laxatives and psychosocial guidance for some time are now completely cured. Two patients still need laxatives. One case cannot be evaluated because the follow-up period was too short.

Discussion

Initially, we encountered difficulties in the interpretation of some of the microscopical pictures, presented by the biopsies. All of these problems concerned the 3rd and 4th categories, i.e. cases in which local aggregations of nerve fibres or accumulations of fibrillar foamy or amorphous acetylcholinesterase-positive material were present in the lamina propria. At the beginning of the study we consistently interpreted aggregations of cholinesterase positive nerve fibres in the lamina propria as suggestive of Hirschsprung's disease. When doubt arose on this point because of the favorable clinical course in these patients and when furthermore, the findings in control material (including post mortem specimens) showed that local concentrations of fine acetylcholinesterase-positive nerve fibres occurred regularly in the lamina propria, we finally interpreted the 3rd category as non-pathological.

The fibrillar, foamy, or amorphous acetylcholinesterase-positive material of the 4th category, often showed, under low magnification, pictures suggesting proliferation of nerve fibres, but thorough investigation at higher magnification showed that this was not the case. Negative PAS and Sudan staining and the absence of acid phosphatase activity showed that these were not foam cells (i.e., macrophages). The local presence of erythrocyte ghosts in these regions and the knowledge that acetylcholinesterase activitiy occurs in erythrocytes (Brestkin and Ivanona, 1970) makes it likely that this picture is an artefact arising from lysis of erythrocytes. Furthermore, peroxidase staining demonstrated the presence of neutrophilic leucocytes at these sites. On this basis we evaluated these category 4 anomalies as artefacts. It thus became apparent that the diagnosis Hirschsprung's disease could only be made on the basis of the very pronounced anomalies of category 2. In the three other categories the structure of the wall was invariably normal, and therefore Hirschsprung's disease could be excluded in 42 of the 46 cases. The fact that the diagnosis was conformed histochemically in only 4 patients seems to us to be easily explained by our readyness to undertake biopsy and the fact that there were no very young children in the present series.

Only a radiological investigation of the colon seemed to be indicated in 10 cases. In 6 patients who were considered clinically not to have Hirschsprung's disease, radiography also failed to provide evidence of the disease. In 2 of the 4 patients diagnosed as having Hirschsprung's disease, the radiograms confirmed the diagnosis; in the third patient only megacolon was seen, and in the fourth the colon could not be investigated radiographically. Now we have experience with enzyme-histochemical methods of establishing the diagnosis of Hirschsprung's disease, we advocate them as an easy and secure procedure.

However, we are aware of the possibility of artefacts and the presence of fine acetylcholinesterase-positive fibres in normal rectal mucosa.

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