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Zonal aganglionosis

An enzyme and immunohistochemical study of two cases

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Summary. The existence of zonal aganglionosis, a rare variant of Hirschsprung's disease, is often questioned. An extensive enzyme and immunohistochemical study was performed on gut specimens of two patients presenting with bilious vomiting and abdominal distension to find evidence of the existence of double zonal aganglionosis and to characterize the abnormalities of the enteric nervous system.

The hypotheses concerning the pathogenesis of this neurogenic disorder are reviewed.

The results of our study confirm the existence of zonal aganglionosis. The clinical presentation may be similar to classical Hirschsprung's disease.

Key words: Hirschsprung's disease – Zonal aganglionosis – Immunohistochemistry – Pathogenesis

Introduction

Classical Hirschsprung's disease consists of a single area of aganglionosis extending proximally from the anal margin for varying distances (Meier-Ruge 1974; Taguchi et al. 1983). A rare variant of Hirschsprung's disease is zonal aganglionosis. Thirteen well-documented cases have been reported in the literature (Taguchi et al. 1983). Two types of zonal aganglionosis are distinguished i.e. single and double zonal aganglionosis (Kadair et al. 1977).

Single zonal aganglionosis (nine cases) is characterised by an aganglionic bowel segment preceded and followed by a bowel segment with a normally developed myenteric plexus (de Chadarévian et al. 1982). In double zonal aganglionosis (four cases) the aganglionic segments are separated by an area with normal ganglion cells, the so-called "skip-area". The distal aganglionic part may extend to the anal margin or fall short of it.

The existence of these rare variants of Hirschsprung's disease is often questioned because of poor documentation (Swenson 1959; Yunis 1983). In the present study we describe two well-documented cases of double zonal aganglionosis out of a series of 76 patients with Hirschsprung's disease.

To characterize the abnormalities of the enteric nervous tissue and perineural supporting tissue an extensive enzyme-histochemical study (acetylcholinesterase, acid phosphatase) and immunohistochemical study (neuron-specific enolase, neurofilament and S100 protein) was performed. The results are discussed against the background of existing hypotheses concerning the pathogenesis of zonal aganglionosis.

Case reports

Patient 1. A full-term, male baby (birth weight 4,000 gms), presented with bilious vomiting and abdominal distension on his first day of life. Only after 36 h he passed a small amount of meconium. A barium enema revealed a small left colon and a marked distension of the colon from the splenic flexure to the caecum. An end-colostomy was established in the transverse colon. A biopsy from the site of the colostomy revealed ganglion cells.

Rectal suction biopsy revealed an increase of acetylcholinesterase-positive nerve fibers in the submucosa, muscularis mucosae and lamina propria, in the absence of ganglion cells (Fig. 1); this immunohistochemical pattern is indicative of Hirschsprung's disease (Meier-Ruge 1974).

At the age of seven months the patient was readmitted to the hospital with recurrent intenstinal obstructions, supposedly caused by adhesions. The patient was discharged in good condition after surgical treatment of the adhesions. Within three weeks readmission and laparotomy were necessary because of a recurrent ileus. At laparotomy the ileum appeared

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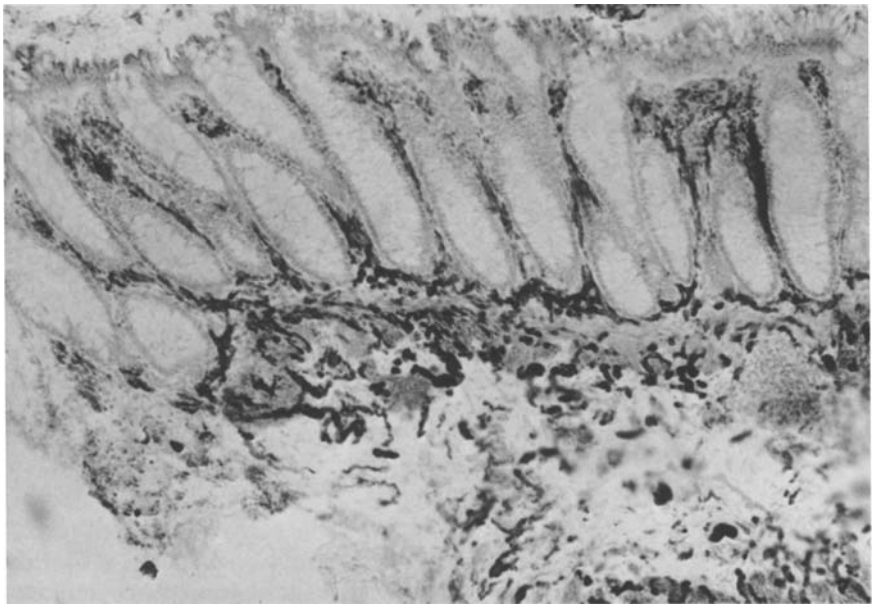


Fig. 1. Acetylcholinesterase staining of rectal biopsy: there is an increase of AcCh positive nerve fibers in the lamina propria, muscularis mucosae and submucosa in the absence of ganglion cells (×125)

to be distended except for the most distal 20 cm. In this dilated part an ileostomy was made and the bowel between ileostomy and colostomy was removed.

At the site of the ileostomy ganglion cells were present; in the resected ileum (20 cm) they were absent. The postoperative course was uneventful and the patient was discharged in good condition two weeks postoperatively.

At the age of 13 months a Duhamel-Lester Martin operation was performed. Thirty centimetres of the ileum was anastomosed side to side to the rectum, sigmoid and descending colon. The remaining part of the transverse colon (6 cm) and an ileum segment (3 cm) were resected for surgical reasons. The patient was discharged in good condition. There remained the problem of periodic constipation. A partial sphincterotomy was needed at the age of 2 years and 4 months. At the age of 4.5 years the patient is doing well.

Patient 2. A female newborn (birth weight 3,650 gms) presented with bilious vomiting and abdominal distension on her second day of life. She did not pass meconium for the first 24 h. Her brother was known to have Hirschsprung's disease.

A plain abdominal X-ray showed a marked distention of the small bowel. A barium enema revealed a colon normal in calibre with dull angulations. At emergency laparotomy the small bowel was distended, while the last 7 cm of the ileum and the total colon looked contracted. A Meckel's diverticulum was present 30 cm from the valvula Bauhini. A double barrel stoma was established in the distended ileum. Histologic examination of the biopsy at the site of the stoma failed to show ganglion cells. Rectal suction biopsy revealed an increase of acetylcholinesterase positive nerve fibres in the lamina propria and muscularis mucosae. No ganglion cells were found in the submucosa. These findings are consistent with Hirschsprung's disease (Meier-Ruge 1974).

The rather uneventful postoperative recovery led to the assumption that the ileostomy was on the verge of the aganglionic bowel segment. Reoperation was not performed and the patient was discharged after six weeks.

At the age of six months she was readmitted because in the long run the ileostomy was not functioning properly. A new stoma was established on the basis of frozen sections, 13.5 cm distant from the previous one. Subsequent histological examination did not fully justify this site because the last 5 cm did contain ganglion cells. Two weeks postoperatively the patient was discharged in good condition.

At the age of 9 months she was admitted for a Duhamel-Lester Martin operation. Thirty centimetres of the ileum was anastomosed side to side to the rectum, sigmoid and descending colon. The distal aganglionic ileum (7 cm) and 18 cm of the ascending colon and transverse colon were resected. Two weeks postoperatively she developed fever on the basis of an enterocolitis. Clostridium difficile was cultured and she was treated with Vancomycine and Metronidazol. Eventually she was discharged in good condition. After discontinuation of the antibiotic treatment she intermittently suffered from diarrhoea caused by bacterial overgrowth. Four months after the Lester Martin operation a partial sphincterotomy was performed. At the age of 2.5 years she is doing reasonably well.

Table 1. Characteristics of the antisera

Antiserum	Animal	Specificity	Source
Neurofilament	Mouse	Neurons (Klück et al. 1984)	Monosan
Neuron-specific enolase	Rabbit	Nerves enteric nervous systen (Ferri et al. 1982) Ganglion cells (Vinores and May 1985)	DAKO, Denmark
S100 (A and B)	Rabbit	Schwann cells, neural sheaths (Ferri et al. 1982; Taguchi et al. 1985)	DAKO, Denmark

Table 2. Normal staining patterns in colon specimens

Staining	Identification	Mucosa	Submucous plexus	Circular muscle layer	Myenteric plexus	Longitudinal muscle layer
Acetylcholinesterase	nerve fibres	+/−	++	+	++	+
Acid phosphatase	ganglion cells	−	+	−	+	−
Neuron-specific enolase	ganglion cells	−	++	−	++	−
	nerve fibres	+/−	++	+	++	+
Neurofilament	nerve fibres	−	+	−	+	−
S100	Schwann cells neural sheaths	+/−	++	+	++	+

Abbreviations: − = absent; +/− = scarce; + = moderate; ++ = intense

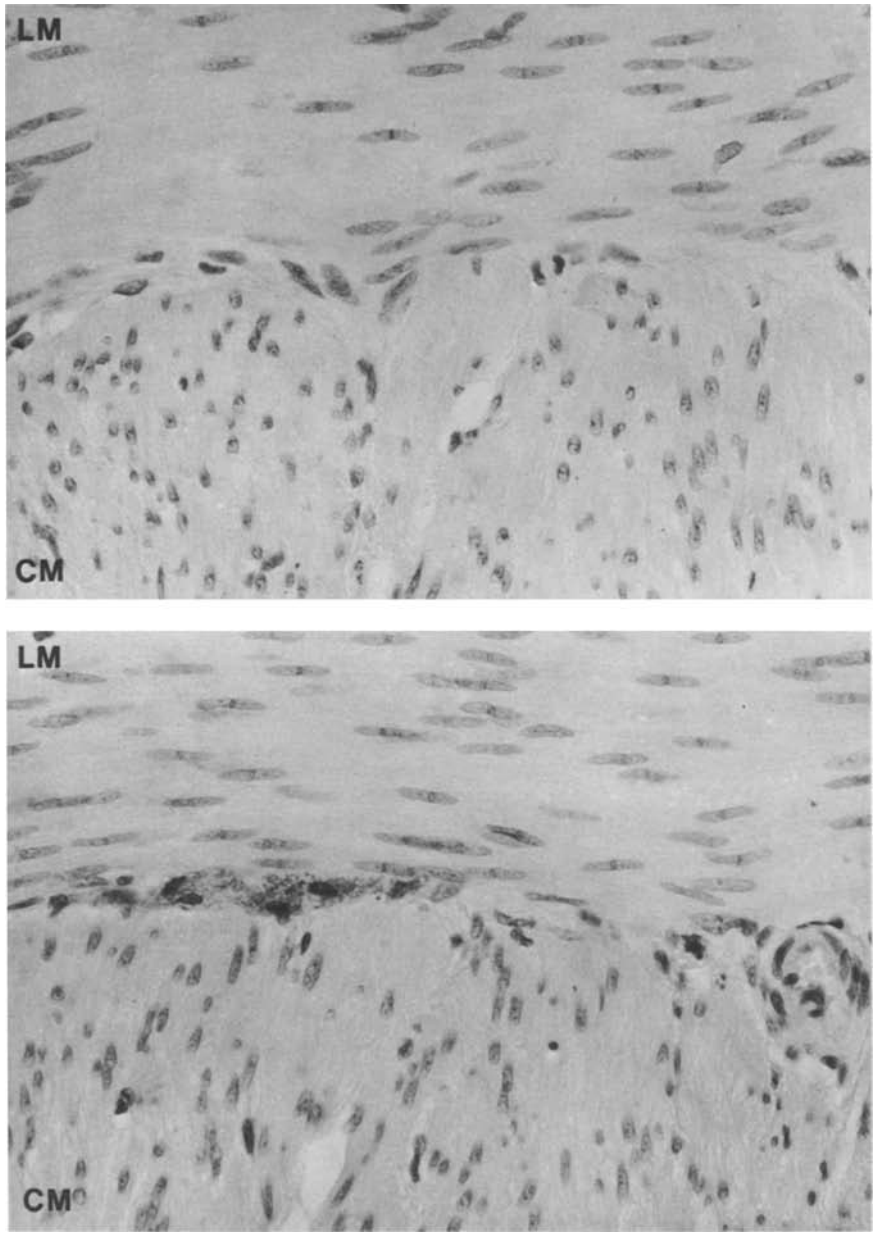


Fig. 2. NF (**a**) and S100 (**b**) immunostaining of the muscle layers (*CM*: circular muscle layer, *LM*: longitudinal muscle layer) of the aganglionic termial ileum (segment B, case 1). There is an agenesis of the myenteric plexus, no NF immunoreactivity and very scarce S100 immunoreactive nerve sheaths and Schwann cells between the muscle layers (×322)

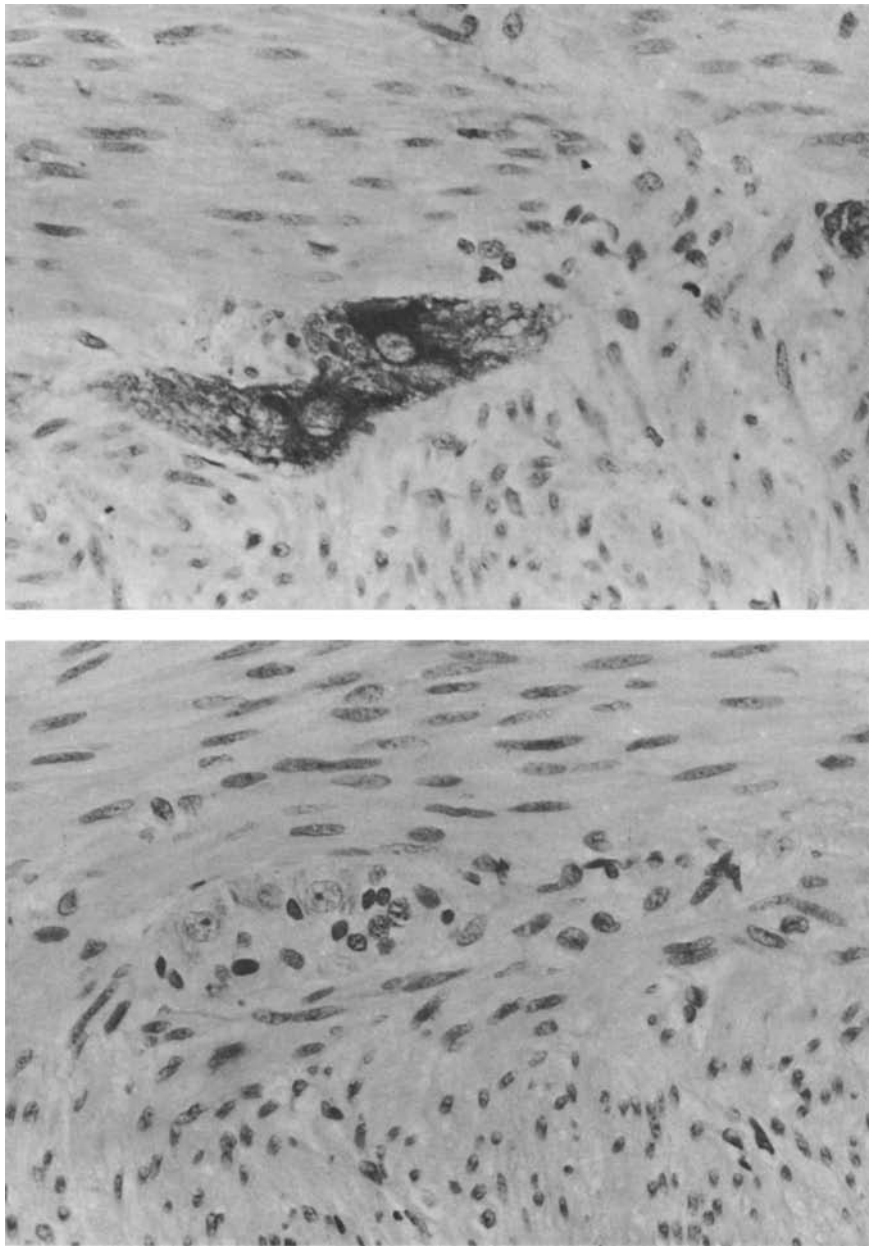


Fig. 3. (**a**) NSE immunostaining of ganglion cells in the well developed myenteric plexus (×322). (**b**) NF immunostaining: no NF immunoreactivity of the nerve fibres in the plexus (×322). (**c**) S100 immunostaining of nerve sheaths and satellite cells around the S100 negative ganglion cells (×322)

Materials and methods

Sequential (distances of 1–2 cm), full thickness sections of all the surgical resected gut specimens were made. Adjacent sections were immediately frozen in liquid nitrogen and formalin fixed, paraffine embedded for enzym-immunohistochemical study. Acetylcholinesterase (AcCh) activity was studied using an enzymhistochemical technique according to Goto et al. (1984). Ganglion cells were identified using an enzymhistochemical method for acid phosphatase (AP) according to Barka and Anderson (1962). Formalin fixed paraffine embedded tissue sections (5 um) were stained for neurofilament (NF), neuron specific enolase (NSE) and S100 protein (S100) using an indirect immuneperoxidase (NF) and peroxidase-antiperoxidase (PAP) technique (NSE, S100).

The characteristics of the antisera are shown in Table 1. Non-immune rabbit sera were used as negative controls. As positive controls cerebellum (NF) and vagus nerve (NSE, S100) were used.

Multiple sections were examined with elastic Van Gieson's staining to exclude vascular abnormalities.

Results

The staining pattern of AcCh, AP, NSE, NF and S100 in normal colon specimens are shown in Table 2. The AcCh staining is similar to that of NSE.

Nerve fibres of the submucous and myenteric plexus are strongly positive for NSE, whereas

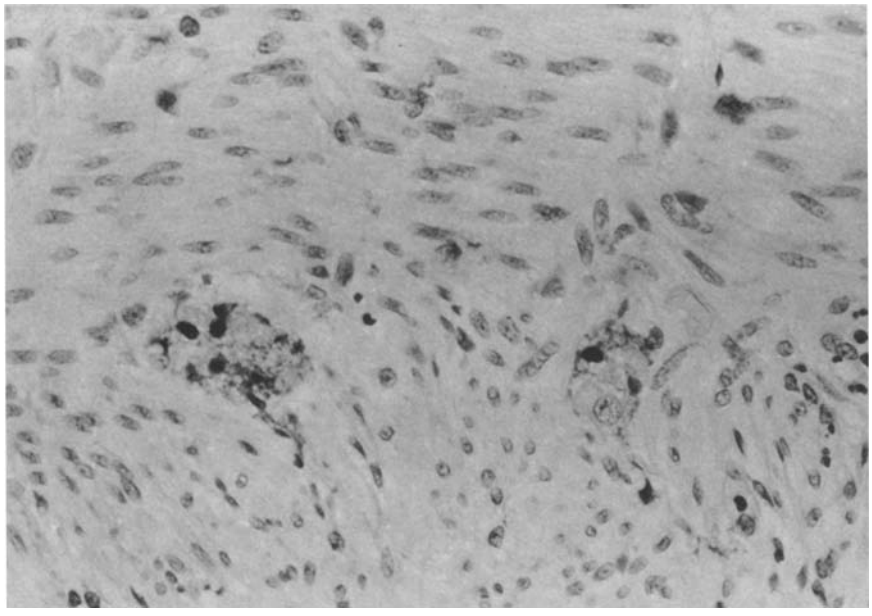


Fig. 3c

nerve processes of the basal layer of the mucosa are scarcely identified. Fine NSE positive fibers are present in the muscle layers. Ganglion cells have strong cytoplasmic immunoreactivity (Frykberg et al. 1985; Vinores and May 1985; Taguchi et al. 1985).

Some nerve fibres of the submucous and myenteric plexus have NF immune reactivity. There is a higher frequency of nerve fibre staining in the myenteric plexus than in the submucous plexus (Klück et al. 1984).

The S100 staining reveals positive nerve sheaths and Schwann cells (perineural tissue) in the submucous and myenteric plexus and to a lesser extent in the muscle layers (Taguchi et al. 1985). In the basal parts of the mucosa only very few nerve sheaths and Schwann cells are identified. The distribution is similar to that of NSE (Taguchi et al. 1985).

Patient 1. The resected ileum segment proximal to the final ileostomy (segment A = 3 cm) had a normal ganglionated plexus as shown in the AcCh, AP, NF and NSE stainings. In the mucosa, however, a moderate increase of AcCh positive nerve fibers was found. The perineural elements (Schwann cells and neural sheaths) of this segment revealed a normal staining pattern (S100).

The terminal ileum (segment B) distal to the ileostomy was aganglionic (NSE, AP) and did not show a myenteric or a submucous plexus at all (AcCh, NF, NSE) (Fig. 2). This segment was almost devoid of nerve fibres (AcCh, NSE) and perineural supporting tissue (S100).

The caecum, ascending colon and transverse colon including the 4 cm distal to the initial colostomy (segment C) had a well developed plexus as shown in the AcCh, NSE and S100 staining (Fig. 3). The plexus in the caecum and 10 cm of the ascending colon did not show any NF immunoreactivity.

The distal 6 cm of the colon specimen (segment D), however, was aganglionic (NSE, AP), while there were slightly hyperplastic nerve fibres in the submucous and myenteric plexus (AcCh, NSE). The nerve fibres had strong NF immunoreactivity. The perineural supporting tissue showed a small increase.

The results of the histochemical stainings are shown in Table 3.

Patient 2. Five centimetres of the ileum distal to the final ileostomy (segment A) contained ganglion cells (AP) and a normal AcCh, NSE pattern. The mucosa, however, showed a moderate increase of AcCh-positive nerve fibres. The perineural elements had a normal distribution (S100). The nerve fibres showed no immunoreactivity for NF.

The terminal ileum and caecum (segment B) did not have a submucous and myenteric plexus (NSE, and NF); this aganglionic segment was almost devoid of supporting tissue (S100).

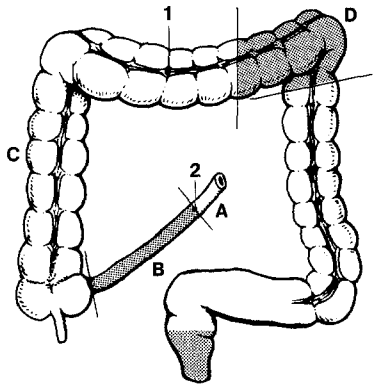
The ascending colon and transverse colon (segment C) had the same enzyme-histochemical characteristics as segment A.

The histochemical staining results of the second patient are shown in Table 3.

The submucosal, muscular and subserosal ar-

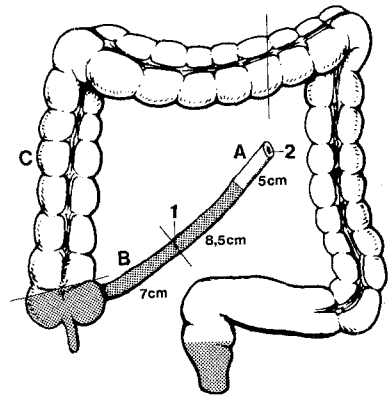
Table 3. Results of full-thickness examinations of sequential sections of surgical resected gut specimens of patient 1 and 2 using enzym- and immunohistochemical stainings

Patient 1



A: ileum (3 cm)
B: ileum (20 cm)
C: caecum, ascending colon, transverse colon (10 cm)
D: transverse colon (6 cm)
1: colostomy
2: ileostomy
■: aganglionic

Patient 2



A: ileum (5 cm)
B: ileum (7 cm), caecum
C: ascending colon, transverse colon (18 cm)
1: first ileostomy
2: second ileostomy
■: aganglionic

Staining	Identification	A	B	C	D	A	B	C
Acetyl-cholinesterase	nerve fibres:							
	lamina propria	i	a	n	n	i	x	i
	both plexus	n	a	n	i	n	x	n
Acid phosphatase	ganglion cells	n	a	n	a	n	x	n
Non-specific	ganglion cells	n	a	n	a	n	a	n
Enolase	nerve fibres	n	a/s	n	i	n	a/s	n
Neurofilament	nerve fibres	n	a	none	i	none	a	none
S100	Schwann cells, neural sheaths	n	a/s	n	i	n	a/s	n

Abbreviations: n=normal; i=increased; a=absent; none=no staining, nerve fibres present; x=no frozen tissue available; s=scarce.

teries and veins did not show any abnormalities. This finding excludes a neuronal loss because of vascular manipulation during the surgical procedures.

Discussion

In this study we demonstrate the existence of double zonal aganglionosis which is doubted by others (Swenson 1959, Yunis et al. 1983). In both patients rectal biopsies revealed Hirschsprung's disease on AcCh staining, while a segment of the ileum and in the second patient of the caecum as well showed an agenesis of the plexus (AcCh, AP). These findings indicate a long segment aganglionosis (Meier-Ruge 1974). However, the ascending colon and colon transversum and in patient 1 the caecum as well revealed a well-developed myenteric and submucous plexus. These findings are in agreement with the six cases of double zonal aganglionosis described in a recent literature review (Yunis et al. 1983). Remarkable is the great similarity between the location of the aganglionic segments in our cases and those described in the literature review (Yunis et al. 1983). The terminal ileum was always aganglionic and the intervening ganglionic segment consisted of the ascending colon and/or transverse colon. Our results with the enzyme- and immunohistochemical techniques undoubtedly demonstrated the existence of zonal aganglionosis.

It is important to know this rare variant of Hirschsprung's disease, since it might have a similar clinical presentation (McIver and Whitehead 1972). One should especially suspect this disorder in those patients who remain constipated after an ileo- or colostomy has been established.

Extension of increased AcCh activity in the proximal normal bowel segment has been described in the classical type of Hirschsprung's disease and the degree of this increase seems to be related to the age of the patient (Elema et al. 1973; Goto and Ikeda 1985). In those patients the agang-

lionosis of the distal segment was limited to the rectum.

In our two patients the mucosa of the proximal normal ileum segments (segments A) also revealed a moderate increase of AcCh positive nerve fibres.

There are a few hypotheses concerning the pathogenesis of aganglionosis. Embryologic studies show that the myenteric plexus is formed by neuroblasts which are distributed to the gastrointestinal tract by cranio-caudal migration during the fifth to the twelfth week of gestation (Okamoto and Ueda 1967). Most authors agree that neuroblasts forming the plexus of the proximal gastrointestinal tract are of vagal origin. There is still controversy concerning the origin of neuroblasts in the distal gastrointestinal tract. Some authors (Yntema and Hammond 1954) suggest that these neuroblasts are also of vagal origin, while other authors support the idea that they are derived from the prevertebral ganglia and/or sacral parasympathics (Kuntz 1910). In Hirschsprung's disease a cessation of this migration is suggested. In zonal-aganglionosis some authors postulate that there are "skip areas" in the neuroblast migration (Okamoto and Ueda 1967), while other authors suggest focal regression of ganglion cell precursors or ganglion cells themselves, possibly caused by anoxia (Touloukian and Duncan 1975) or chronic inflammation (Taguchi et al. 1983).

Our study shows that apart from abnormalities in the enteric nervous system in zonal aganglionosis there is also a defect in the perineural supporting tissue. The question whether zonal aganglionosis is a congenital or acquired disorder remains to be solved.

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