Virchows Arch (1995) 426:549–556

ORIGINAL ARTICLE

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Histopathological criteria for intestinal neuronal dysplasia of the submucosal plexus (type B)

Received: 23 August 1994 / Accepted: 30 March 1995

Abstract The aim of this study was to review critically the diagnostic features of intestinal neuronal dysplasia type B (IND B). Over a period of 5 years colonic mucosal biopsies of 773 children with symptoms of chronic constipation were examined. Four biopsies taken 2–10 cm above the pectinate line were cut in serial sections and histochemical lactate dehydrogenase, succinate dehydrogenase, (SDH) and acetylcholinesterase (AChE) reactions performed. Presence of giant ganglia of the submucosal plexus, being characterized by more than seven nerve cells, established the diagnosis of IND B. Giant ganglia were found to be age-independent changes, while hyperplasia of the submucosal plexus, increase of AChE activity in nerve fibres of the lamina propria and low SDH activity in nerve cells proved to be age-dependent findings which disappear during the maturation of the enteric nervous system. Using these criteria IND B was diagnosed in 209 children. In 64 of these patients a combination of IND B and aganglionosis (Hirschsprung's disease) was found. IND B seems to be related to premature expression of laminin A during embryogenesis, resulting in premature nerve cell differentiation in the myenteric and submucosal plexus, which in turn blocks neuroblast colonization of the rectum. IND B, hypoganglionosis and aganglionosis, which are often combined, may therefore be considered to be different manifestations of the same developmental abnormality.

Key words Intestinal neuronal dysplasia (type B) · Submucosal plexus · Colorectal innervation defects · Hypoganglionosis · Myenteric plexus

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Introduction

The introduction of enzyme histochemical acetylcholinesterase (AChE) and dehydrogenase reactions in the routine diagnosis of Hirschsprung's disease (aganglionosis) [30, 32, 33, 34, 35, 36] has resulted in the identification of abnormalities of the submucosal plexus [29]. Description and understanding of intestinal neuronal dysplasia (IND), accompanied by symptoms of pseudo-obstruction is incomplete, since 1983 when Fadda et al. [9] first distinguished two different types of abnormality of vegetative gut innervation: IND of the sympathetic innervation of the colon (IND type A) and IND of the submucosal plexus (IND type B). IND A is mainly caused by an immaturity or hypoplasia of sympathetic gut innervation. It is a rare disease comprising less than 2% of all dysganglionoses [32, 34, 55]. IND B, characterized by abnormalities in the development of the submucosal plexus, is almost as frequent as Hirschsprung's disease [34] and has been the subject of many studies [5, 10, 15, 40, 46, 47, 48, 49, 56, 57, 58, 59, 60].

The application of irrelevant diagnostic criteria, the examination of too few sections per biopsy and the use of nonspecific nerve cell staining resulted in confusion concerning the histological characteristics of IND B [56, 59]. To overcome this problem, a consensus conference on dysganglionosis was organized by German pathologists in 1991, which attempted to standardize the technical prerequisites for the diagnosis of and the histological findings in IND B [4]. The aim of this paper is to analyse critically the diagnostic criteria of IND B changes in 145 children with isolated IND B, and 64 with a combination of aganglionosis and IND B. In particular, the age-dependency of the lesions was studied.

The development and function of the innervation of the distal colon should be reviewed to place these observations in context. The distal gut receives, in addition to the vagus nerves, parasympathetic nerve fibres from the sacral roots S2–S4 (extramural parasympathetic nerves) which innervate the descending colon and the rectosigmoid. This innervation decreases exponentially in a

proximal direction [21, 35] and ends at the splenic flexure of the colon. It develops between embryonic weeks 5–7, at a time in which neuroblasts (intramural innervation) start migrating from the oesophagus to the anal ring [31, 44]. Subsequently, submucosal ganglia form from migrating myenteric neuroblasts which pass through the circular muscle into the submucosa along the routes of afferent nerve fibers [12]. The nerve cells from the submucosal neuroblasts spread in the submucosa and develop pressure receptors in the mucosa. The submucosal plexus depolarizes the myenteric plexus if its pressure receptors are stimulated by stool. Relaxation of the circular muscle is accompanied synchronously by contraction of the longitudinal muscle. As shown by Goerttler [14], the dilatation of the colon is not directly mediated by sympathic innervation (sympathic nerve fibres end at the myenteric plexus and only modulate the cholinergic activity of the myenteric plexus, influencing the tone of the circular muscle in this way [2, 16, 42, 43]). By an effect of the lattice-like connective tissue matrix of the circular muscle, which is passively stretched by the contraction of the longitudinal muscle, the gut is dilated [14].

Materials and methods

Colonic mucosal biopsies obtained from 773 children (mean age 15±9 months) with symptoms of constipation were examined. Four 3–4 mm^3 biopsies were taken 1–2 cm, 3–4 cm, 8–9 cm and 10–12 cm above the pectinate line; all tissue samples had a sufficient amount of submucosa. All biopsies in which IND B was diagnosed, were re-examined by five independent investigators.

The native tissue probes were mounted with cryogel on cryostat carriers exactly vertical to the surface of the mucosa. The biopsies were cut at −10° C to −15° C stepwise in 15 µm thick serial sections (120–160 sections per biopsy). The sections were distributed onto five slides and dried at room temperature. Mucosal biopsies of more than 5 mm^2 were cut parallel to the mucosal surface, which gives a much better overview of the submucosal plexus. Two slides were used for an AChE reaction ([24]; reaction time 90 min at 37° C), two slides were incubated for a lactate dehydrogenase (LDH) reaction ([20]; incubation time 10–13 min at 37° C) and one slide was stained for succinic dehydrogenase (SDH) containing structures ([41]; incubation time 90 min at 37° C). One of the two AChE-stained slides was dehydrated in a series of increasing alcohol concentrations and counterstained with haemalum. To avoid time-dependent fading of the haemalum counterstaining, the sections were covered with a water-compatible polyacryl resin (Crystal Mount, Biomeda Corp., Foster City, CA), polymerized at 60° C and mounted via xylol with Eukitt (O. Kindler, Freiburg, Germany) or Canada balsam.

All histochemical reactions were washed in tap water, fixed in 4% neutral formalin and mounted with a coverslip and glycerine gelatine or after air drying via xylol with Eukitt.

Cholinergic nerves and ganglia are selectively stained by the AChE reaction. This reaction, however, does not differentiate between nerve cells and glial cells. In the AChE reaction, non-specific esterase is blocked by iso-octamethylpyrophosphoramide or phenazine methosulphate. In the LDH reaction, no polyvinylpyrrolidone is used, which allows a more selective nerve cell staining. The SDH reaction selectively stains mature but not immature nerve cells.

Results

Over a 5-year period, colonic mucosal biopsies obtained from 773 children with symptoms of constipation were examined. In 209 children (mean age 18±12 months) the mucosal biopsies showed the characteristics of IND B; 64 of these children (mean age 12±6 month) were found to have Hirschsprung's diseases (aganglionosis) associated with IND B and another 82 patients had classical Hirschsprung's disease (mean age 4±2 months). The mucosal changes which led to the diagnosis of IND B could be divided into age-independent and age-dependent lesions.

Age-independent findings in IND B

The normal rectal mucosa contains only few ganglion cells within 2 cm of the pectinate line. Above this region, however, there is a progressive increase in the density of ganglia and submucosal nerve cells in a caudocranial direction. The ganglia consist of between two and five LDH-positive nerve cells. Nerve cells within nerve fibres are lacking.

In IND B, the most characteristic finding is the occurrence of giant ganglia, containing on average seven-to-ten nerve cells (Fig. 1), but occasionally showing up to 16 LDH-positive nerve cells (Fig. 2). These giant ganglia comprise only 3%–5% of all ganglia seen in a given case, and are usually not observed in the distal rectum (within 6–7 cm of the pectinate line). Moreover, bud-like nerve cell groups are found along or around thick afferent nerve fibres (Fig. 3), and nerve cells were also seen within nerve fibres forming pearlstring-like files (Fig. 4). Finally, in many cases the muscularis mucosae and sometimes also the lamina propria contains heterotopic nerve cells and ganglia of the submucosal plexus (Fig. 5).

Only 55%–65% of the sections with vertical cuts of the mucosa show nerve cells or ganglia. The yield of nerve cells and ganglia was much higher in biopsies larger than 5 mm^2, which were cut parallel to the mucosal surface.

Children older than 4 years with IND B and primary chronic constipation often had, in addition to giant ganglia, hypoganglionosis, hypogenesis or heterotopia of the myenteric plexus.

Age-dependent findings in IND B

There are two significant and some minor changes in IND B which are age dependent. First, rectal mucosal biopsies from newborns often reveal conspicuously dense submucosal plexus formations, which disappear at the age of 9–18 months. Second, increased AChE activity is found in nerve fibres of the lamina propria (Fig. 6), a change which also disappears after the age of 9–18 months.

Minor temporary changes in young IND B patients are the presence of small nerve cells, a more or less dis-

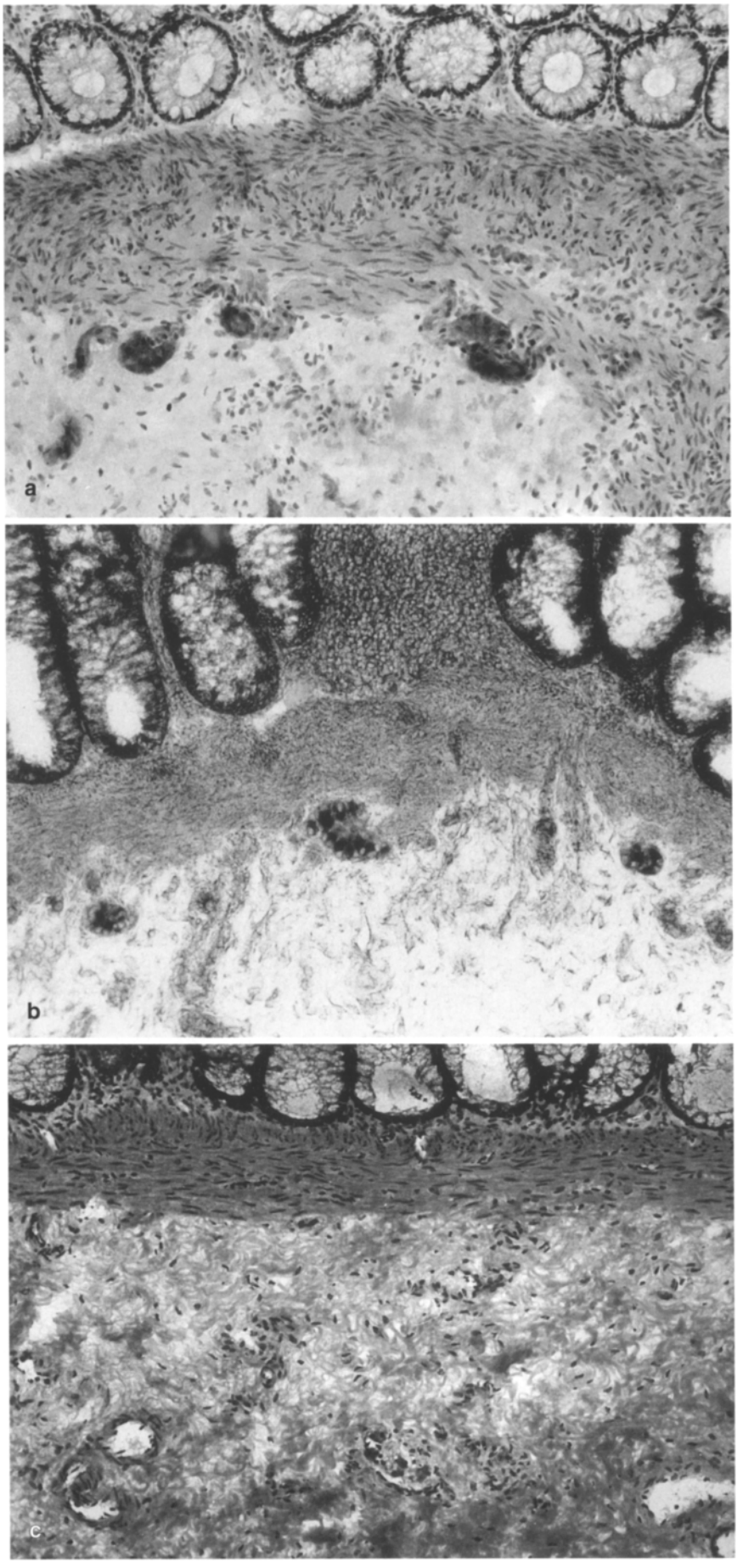

Fig. 1 **a** Haemalum counterstained acetylcholinesterase (AChE) reaction of a biopsy with intestinal neuronal dysplasia of the submucosal plexus (type B; IND B), ×90. Comparison with **b** shows that due to the lack of selective nerve cell staining no diagnosis is possible. **b** Lactate dehydrogenase (LDH) reaction of the same biopsy clearly demonstrates a IND B-specific giant ganglion with 11 nerve cells, ×90. **c** Haemalum and eosin staining, ×90

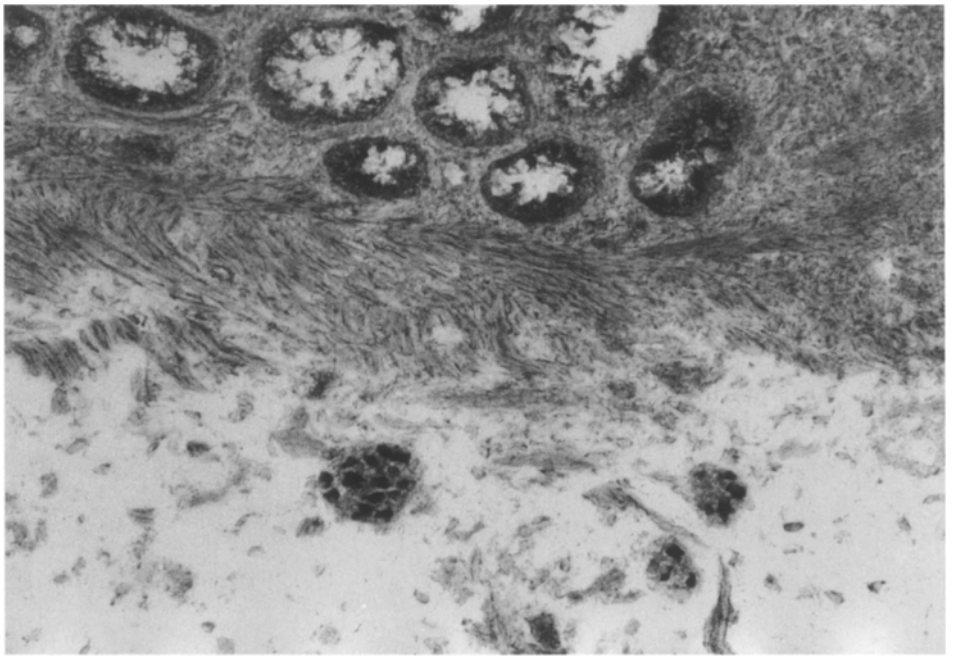

Fig. 2 Giant ganglion with 16 hypoplastic nerve cells. LDH reaction, ×90

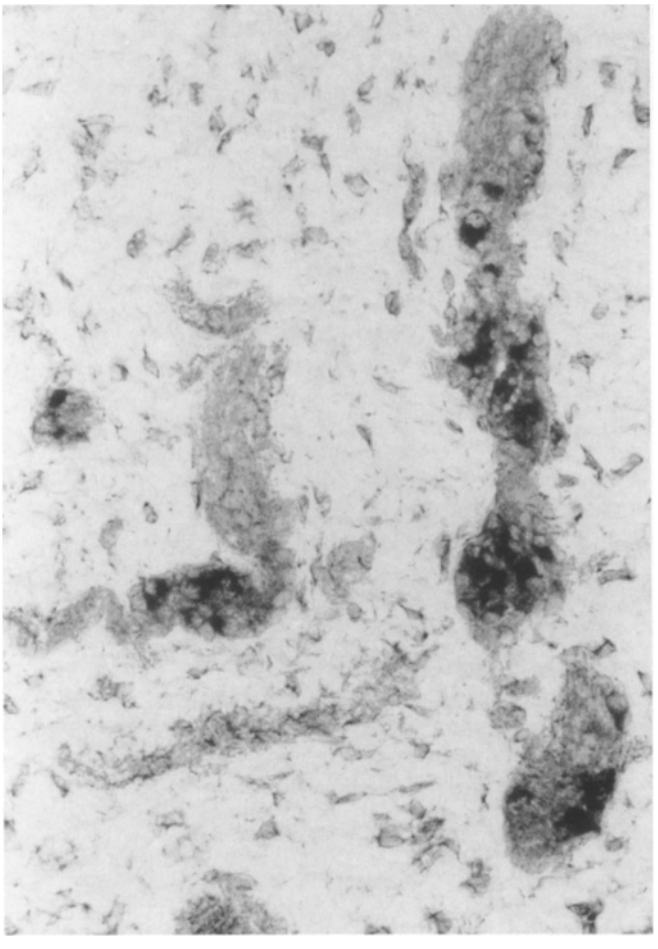

Fig. 3 Bud-like nerve cell groups along afferent nerve fibres with anisomorphous nerve cells. LDH reaction, ×150

tinct nerve cell anisocytosis and sometimes very variable LDH activity. In the first months of five SDH activity was very low in nerve cells.

Combination of aganglionosis and IND B

IND B associated with aganglionosis did not differ from the isolated from of IND B, but the diagnosis is often only established much later (median age 12±6 months) than in classical Hirschsprung's disease (median age 4±2 months). Aganglionosis in these cases showed a much looser network of intensely AChE positive nerve fibres in the lamina propria and muscularis mucosae of the rectum. The extension of the extramural parasympathetic nerve fibre net, arising from the sacral roots S2–S4, was also smaller than in cases of classical Hirschsprung's disease. Only in a few cases of IND B associated with Hirschsprung's was moderately increased AChE activity in parasympathetic nerve fibres of the lamina propria present in the region proximal to the aganglionic segment.

Discussion

The results of this investigation indicate that IND B patients have age-independent and age-dependent changes of the submucosal plexus. Only the age-independent changes establish the diagnosis of IND B, while the age-dependent changes which disappear with the maturation of the vegetative gut innervation are non-diagnostic.

The diagnostic finding of IND B is the presence of giant ganglia containing more than six nerve cells (normal ganglia have three to five nerve cells). This developmen-

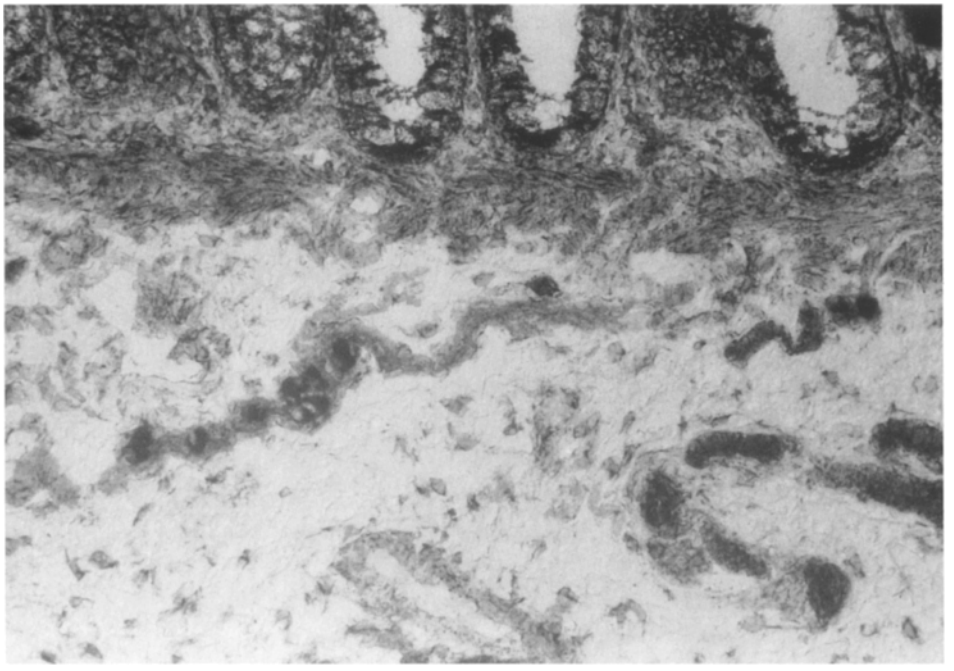

Fig. 4 Pearlstring-like localized nerve cells inside a thin nerve fibre observed in IND B. LDH reaction, ×90

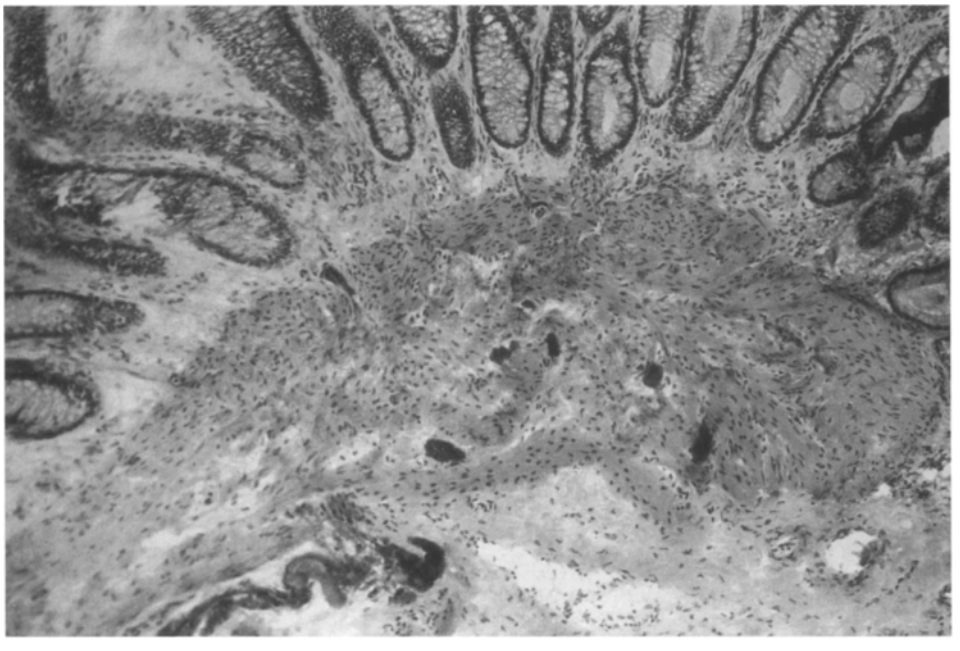

Fig. 5 Heterotopia of nerve cells of the submucosal plexus in the muscularis mucosae. LDH reaction, ×60

tal abnormality of the submucosal plexus is independent of maturation and can be observed from early childhood up to adult life [36, 61]. Additional findings are nerve cells lying in a line inside of thin parasympathetic nerve fibres (Fig. 4) and bud-like nerve cell groups along or inside thick nerves (Fig. 3).

In contrast to giant ganglia, hyperplasia of the submucosal plexus is an age-dependent phenomenon which is seen in the first months of life and may also be observed in biopsies without IND B. It is probably a sign of immaturity of the distal gut. With the growth of the rectum, this finding disappears [59]. Another consequence of maturation is the decrease in AChE activity in nerve fibres of the lamina propria, and the increase in SDH activity in nerve cells of the submucosal plexus. These two changes often coincide with a normalization of propulsive colon motility [50, 58]. SDH activity is always very low in nerve cells of the submucosal plexus in young children. Bertoni-Freddari et al. [3] have shown morphometrically that young nerve cells have few and small mitochondria (where SDH is localised) which increase in size and number up to adult life.

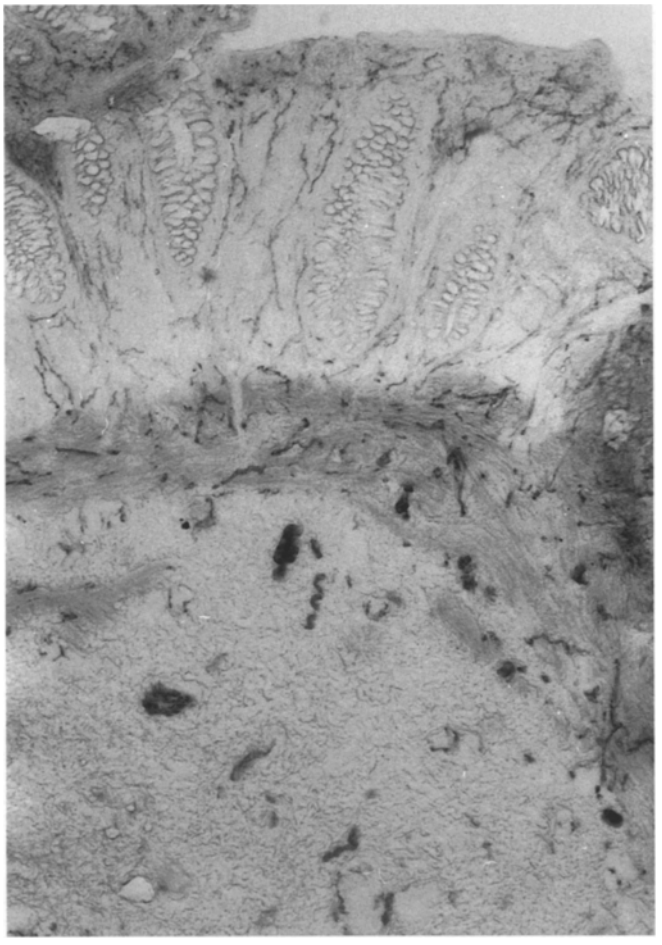

Fig. 6 IND B in a 2-month-old baby with a moderate increase in AChE activity in nerve fibres of the lamina propria. AChE reaction, ×60

Our study revealed that, for a proper diagnosis, four 3–4 mm^3 biopsies, taken between 2 and 12 cm above the pectinate line are necessary. These biopsies have to be cut stepwise in 15 µm thick serial sections leading to 120–160 sections per biopsy. We found it impossible to diagnose IND B in a small number of sections.We also did not rely on an AChE reaction only, because this reaction stains both Schwann cells and nerve cells in an identical manner.

Differences in technique and the use of different diagnostic criteria are possibly the main reasons for non-congruent results in this field. Schofield and Yunis [56] used only an AChE reaction with haemalum and eosin staining, which does not allow nerve cells to be distinguished from Schwann cells. The authors defined IND B as characterized by "... a slight to moderate increase in AChE stained fibres within the lamina propria ... greater than five submucosal ganglion clusters per high-power field or large (greater than ten cells) ... clusters of submucosal ganglion cells" [56]. The same authors defined IND B as follows: "Hyperganglionosis is at present time most often diagnosed as intestinal neuronal dysplasia" [57]. Most of these criteria – as increase in AChE activity in nerve fibres in the lamina propria and ganglion clusters in the submucosa (hyperganglionosis) – are not diagnostic for IND B. Only an increased number of nerve cells per ganglion is, according to our experience, specific for IND B.

Smith reported that he found only 7 cases with IND B in 2420 patients investigated between 1975 and 1991 [59]. This author also based his diagnosis only on AChE reactions and a maximum of six sections per case, a methodology which questions his results.

Pseudo-obstruction and constipation in IND B result from immaturity of the enteric nervous system, characterized by a lack of differentiation of the ganglia into Schwann cells and nerve cells, low SDH activity, increased AChE activity in nerve fibres of the lamina propria and ganglion clusters in the submucosa – which accompanies IND B in early childhood. As in Hirschsprung's disease [22], many children with IND B lack a recto-anal reflex [26, 27, 53]. All these symptoms can disappear, if the vegetative nervous system of the gut matures [46, 47, 50, 53, 54]. In almost 95% of young children with IND B gut motility normalizes in the first year of life [9, 53, 54]. But retarded maturation of the enteric nervous system for up to 4 years has also been reported by several authors [5, 6, 18, 56, 58].

IND B combined with aganglionosis [5, 10, 15, 17, 19, 25, 34] is not morphologically different from isolated IND B. In 64 patients with Hirschsprung's disease and IND B, the biopsy diagnosis was generally made at 12±6 months in comparison to 4±2 months in isolated Hirschsprung patients. This may be due to hypoplasia of the extramural parasympathetic innervation, arising from the sacral roots S2–S4, of the rectum and the immaturity of the enteric nervous system. Extended IND B has an apparently inhibitory effect on nerve fibre outgrowth from the extramural parasympathetic innervation in the distal colon. However, low or absent propulsive activity in the first months of life due to IND B in the proximal colon causes fairly late obstruction (12±6 months instead of 4±6 months in Hirschsprung's disease). If the IND B segment matures and normal propulsive activity develops, no major problems are to be expected after resection of the aganglionic segment [17, 52, 53–54].

It must be kept in mind that IND B can be combined not only with distal aganglionosis but also with developmental abnormalities of the myenteric plexus, for example hypoganglionosis, hypogenesis, heterotopia and hypoplasia. Such accompanying disorders are more frequently observed in older children and adults with primary chronic constipation. Surgical treatment depends on functional defects such as an absent recto-anal sphincter reflex [26, 27, 53] or a megacolon [10, 54]. In adults with primary chronic constipation, characteristics of IND B can often be found in the submucosa [61, 62]. The development of a megacolon in these patients ultimately requires surgical treatment [60, 61].

The demonstration of chromosomal abnormalities, mainly of chromosome 10, but also of chromosome 13 and 21 [1, 8, 28, 37, 38, 39, 51, 63], suggests that agan-

glionosis is a genetic disease. The observation of Kapur et al. concerning the effect of mesenchyme on the migration of neuroblasts [23] supports the idea of the involvement of trophic factors in the development of dysganglionosis. This observation was strengthened by the findings of Gershon et al. [11, 13] and Parikh et al. [45]. These authors have shown that abnormal early expression of laminin A during embryonic life promotes premature neuronal differentiation and inhibits neuroblast migration. Neuroblasts develop into neurons prematurely, which results in hypoganglionosis, or hypogenesis of the myenteric plexus, and ends in incomplete or absent neuroblast colonization of the distal colon (aganglionosis). Submucosal neuroblasts stop migration and start nerve cell differentiation (giant ganglia of IND B). Neuroblast migration sometimes ends gradually and variably long hypoganglionic segments develop proximal to the aganglionosis. These observations support the hypothesis that IND B, hypoganglionosis of the myenteric plexus and aganglionosis, which are often combined, are developmental abnormalities with an identical pathogenesis [7,13, 45]. This, however, contradicts the idea of Hanimann et al. that the combination of aganglionosis and IND B (which they called "HANID") is a separate disease-entity [17], different from isolated Hirschsprung's disease and isolated IND B.

Acknowledgements The authors thank Mrs. M. Kasper and Mrs. E. Meier for the careful preparation of the histochemical reactions, and Mrs. B. Villagrassa and Mrs. A. Meier-Ruge for patiently writing the many text versions. We thank the English linguist Mr. Paul Holt who often felt frustrated by the referees' comments and the clumsy English.

References

1. Angrist M, Kauffman E, Slaugenhaupt SA, Matise TC, Puffenberger EG, Washington SS, Lipson A, Cass DT, Reyna T, Weeks DE (1993) A gene for Hirschsprung's disease (megacolon) in the pericentromeric region of human chromosome 10. Nat Genet 4:325–326
2. Baumgarten HG (1967) Über die Verteilung von Catecholaminen im Darm des Menschen. Z Zellforsch 83:133–146
3. Bertoni-Freddari C, Fattoretti P, Casoli T, Spagna C, Meier-Ruge W, Ulrich J (1993) Morphological plasticity of synaptic mitochondria during aging. Brain Res 628:193–200
4. Borchard F, Meier-Ruge W, Wiebecke B, Briner J, Müntefering H, Födisch HJ, Holschneider AM, Schmidt A, Enck P, Stolte M (1991) Innervationsstörungen des Dickdarms – Klassifikation und Diagnostik. Pathologe 12:171–174
5. Briner J, Oswald HW, Hirsig J, Lehner M (1986) intestinal neuronal dysplasia – clinical and histochemical findings and its association with Hirschsprung's disease. Z Kinderchir 41: 282–286
6. Bussmann H, Roth H, Deimling O von, Nützenadel W (1990) Variabilität klinischer Symptome bei neuronaler intestinaler Dysplasie. Monatsschr Kinderheilkd 138:284–287
7. Cass DT, Zhang AL, Morthrope J (1992) Aganglionosis in rodents. J Pediatr Surg 27:351–356
8. Edery P, Lyonnet S, Mulligan LM, Pelet A, Dow E, Abel L, Holder S, Nihoul-Fekete C, Ponder BA, Munnich A (1994) Mutations of the RET proto-oncogene in Hirschsprung's disease. Nature 367:319–320
9. Fadda B, Maier WA, Meier-Ruge W, Schärli A, Daum R (1983) Neuronale intestinal Dysplasia. Eine kritische 10-Jahres-Analyse klinischer und bioptischer Diagnostik. Z Kinderchir 381:303–311
10. Fadda B, Pistor G, Meier-Ruge W, Hofmann-von Kap-herr S, Müntefering H, Espinosa R (1987) Symptoms, diagnosis and therapy of intestinal neuronal dysplasia masked by Hirschsprung's disease. Pediatr Surg Int 2:76–80
11. Gershon MD, Tennyson VM (1991) Microenvironmental factors in the normal and abnormal development of the enteric nervous system. Progr Clin Biol Res 373:257–276
12. Gershon MD, Epstein ML, Hegstrand L (1980) Colonisation of the chick gut by progenitors of enteric serotonergic neurons: distribution, differentiation and maturation within the gut. Dev Biol 77:41–51
13. Gershon MD, Chalazonitis A, Rothman TP (1993) From neural crest bo bowel: Development of the enteric nervous system. J Neurobiol 24:199–214
14. Goerttler K (1932) Der konstruktive Bau der menschlichen Darmwand. Gegenbaurs Morphol Jahrb 69:329–379
15. Gullotta F, Straaten G (1977) Hirschsprungsche Krankheit mit gleichzeitiger Aganglionose und sogenannter neuronaler Kolondysplasie (Dysganglionosis colica). Z Kinderchir 20:42–49
16. Hamberger B, Norberg KA (1965) Studies on some systems of adrenergic synaptic terminals in the abdominal ganglia of the cat. Acta Physiol Scand 65:235–242
17. Hanimann B, Inderbitzin D, Briner J, Sacher P (1992) Clinical relevance of Hirschsprung-associated intestinal neuronal dysplasia (HANID). Eur J Pediatr Surg 2:147–149
18. Harms KH, Bertele-Harms RM (1990) Variationen der neuronalen intestinalen Dysplasie Typ B. Der Kinderarzt 21:348–352
19. Heitz PU, Komminoth P (1990) Biopsy diagnosis of Hirschsprung's disease and related disorders. Curr Top Pathol 59: 257–275
20. Hess R, Scarpelli DG, Pearse AGE (1958) The cytochemical localization of oxidative enzymes. II. Pyridine nucleotide-linked dehydrogenase. J Biophys Biochem Cytol 4:753–760
21. Hinkel AS, Bender SW, Posselt HG, Meier-Ruge W, Störer B, Holschneider AM, Waag KL (1989) Biochemische Untersuchungen der Azetylcholinesterase (AChE) an Rektumbiopsien und Darmresektaten bei Morbus Hirschsprung (Abstract). Monatsschr Kinderheilkd 137:120
22. Holschneider AM (1982) Manometric diagnosis. In: Holschneider AM (ed) Hirschsprung's Disease. Hippokrates Verlag, Stuttgart, pp 72–86
23. Kapur RP, Yost C, Palmiter RD (1992) A transgenic model for studying development of the enteric nervous system in normal and aganglionic mice. Development 116:167–175
24. Karnovsky MJ, Roots L (1964) A "direct-coloring" thiocholine method for cholinesterase. J Histochem Cytochem 12: 219–221
25. Kessler S, Campbell JR (1985) Neuronal colonic dysplasia associated with short-segment Hirschsprung's disease. Arch Pathol Lab Med 109:532–533
26. Koletzko S, Ballauff A, Hadziselimovic F, Enck P (1992) Anorectal manometry in Hirschsprung's disease and intestinal neuronal dysplasia. In: Hadziselimovic F, Herzog B (eds) Pediatric Gastroenterology: Inflammatory Bowel Diseases and Morbus Hirschsprung. Kluwer Academic Publishers, Dordrecht, pp 263–281
27. Krebs C, Silva MC, Parra MA (1991) Anorectal electromanometry in the diagnosis of intestinal neuronal dysplasia in childhood. Eur J Pediatr Surg 1:40–44
28. Lyonnet S, Bolino A, Pelet A, Abel L, Nihoul-Fekete C, Briard ML, Mok-Siu V, Kaariainen H, Martucciello G, Lerone M (1993) A gene for Hirschsprung's disease maps to the proximal long arm of chromosom 10. Nat Genet 4:346–350
29. Meier-Ruge W (1971) Über ein Erkrankungsbild des Colon mit Hirschsprung-Symptomatik. Verh Dtsch Ges Pathol 55:506–509

30. Meier-Ruge W (1974) Hirschsprung's disease: Its aetology, pathogenesis and differential diagnosis. Curr Top Pathol 59:131–179
31. Meier-Ruge W (1983) Zur Pathogenese anorektaler Anomalien und Dysganglionose. In: Hofmann-von Kap-herr S, Schärli A, Sauer H, Enger E (eds) Anorektale Fehlbildungen. Gustav Fischer, Stuttgart, pp 19–22
32. Meier-Ruge W (1985) Angeborene Dysganglionosen des Colon. Der Kinderarzt 16:151–164
33. Meier-Ruge W (1990) Das morphologische Erscheinungsbild der neuronalen Dysplasie des Plexus submucosus. Der Kinderarzt 21:837–844
34. Meier-Ruge W (1992) Epidemiology of congenital innervation defects of the distal colon.Virchows Arch [A] 420:171–177
35. Meier-Ruge W, Hunziker O, Tobler HJ, Walliser C (1972) The pathophysiology of aganglionosis of the entire colon (Zuelzer-Wilson Syndrome). Morphometric investigations of the extent of sacral parasympathetic innervation of the circular muscle of the aganglionic colon. Beitr Pathol 147:228–236
36. Meier-Ruge W, Käufeler RE, Brönnimann P (1992) Classification of inborn malformation of distal gut innervation. In: Hadziselimovic F, Herzog B (eds) Pediatric Gastroenterology: Inflammatory Bowel Disease and Morbus Hirschsprung. Academic Publishers, Dordrecht, pp 177–201
37. Meijers JHC, Sanden MP van der, Tibboel D, Kamp AWM van der, Luider TM, Molenaar JC (1992) Colonization characteristics of enteric neural crest cells: embryological aspects of Hirschsprung's disease. J Pediatr Surg 27:811–814
38. Moore SW, Rode H, Millar AJW, Albertyn R, Cywes S (1991) Familial aspects of Hirschsprung's disease. Eur J Pediatr Surg 1:57–100
39. Moore SW, Kaschula ROC, Cywes S (1993) Familian and genetic aspects of intestinal neuronal dysplasia and Hirschsprung's disease. Pediatr Surg Int 8:406–409
40. Munakata K, Morita K, Okabe I, Seuoka H (1985) Clinical and histological studies of neuronal dysplasia. J Pediatr Surg 20:231–235
41. Nachlas MM, Tsou KC, Souza E, Cheng CS, Seligman AM (1957) Cytochemical demonstration of succinic dehydrogenase by the use of a new p-nitrophenyl substituted ditetrazole. J Histochem Cytochem 5:420–436
42. Norberg KA (1964) Adrenergic innervation of the intestinal wall studied by fluorescence microscopy. Int J Neuropharmacol 3:379–382
43. Norberg KA (1967) Transmitter histochemistry of the sympathetic adrenergic nervous system. Brain Res 5:125–170
44. Okamoto E, Ueda T (1967) Embryogenesis of intramural ganglia of the gut and its relation to Hirschsprung's disease. J Pediatr Surg 2:437–443
45. Parikh DH, Tam PKH, Lloyd DA, Velzen D van, Edgar DH (1992) Quantitative and qualitative analysis of the extracellular matrix protein, laminin, in Hirschsprung's disease. J Pediatr Surg 27:991–996
46. Pistor G (1989) Functional colonic ultrasonography: normal findings of colonic motility and follow-up in intestinal neuronal dysplasia. Prog Pediatr Surg 24:155-164
47. Pistor G (1990) Die Colonsonographie als bildgebender Verlaufsparameter bei neuronaler Dysplasie. Der Kinderarzt 21: 182–186
48. Pistor G, Hofmann-von Kap-herr S, Grüssner R, Munakata K, Müntefering H (1987) intestinal neuronal dysplasia – Modern diagnosis and therapy. Pediatr Surg Int 2:352–358
49. Puri P, Lake BD, Nixon HH, Mishalany H, Claireaux AE (1977) Neuronal colon dysplasia: an unusual association of Hirschsprung's disease. J Pediatr Surg 12:681–685
50. Rintala R, Rapola J, Louhimo I (1989) intestinal neuronal dysplasia. Prog Pediatr Surg 24:186–192
51. Romeo G, Ronchetto P, Luo Y, Barone V, Seri M, Ceccherini J, Pasini B, Bocciardi R, Lesone M, Kaariainen H (1994) Point mutations affecting the tyrosine kinase domain of the RET proto-oncogene in Hirschsprung's disease. Nature 367:377-378
52. Sacher P, Briner J, Stauffer UG (1991) Unusual cases of intestinal neuronal dysplasia. Pediatr Surg Int 6:225–226
53. Schärli AF (1992) intestinal neuronal dysplasia. Pediatr Surg Int 7:2–7
54. Schärli AF (1992) Surgery for Hirschsprung's disease and intestinal neuronal dysplasia. In: Hadziselimovic F, Herzog B (eds) Pediatric Gastroenterology: Inflammatory Bowel Disease and Morbus Hirschsprung. Kluwer Academic Publishers, Dordrecht, pp 287–296
55. Schärli AF, Meier-Ruge W (1981) Localized and disseminated forms of intestinal neuronal dysplasia mimicking Hirschsprung's disease. J Pediatr Surg 16:164–170
56. Schofield DE, Yunis EJ (1991) Intestinal neuronal dysplasia. J Pediatr Gastroenterol Nutr 12:182–189
57. Schofield DE, Yunis EJ (1992) What is intestinal neuronal dysplasia? Pathol Ann 27:249–262
58. Simpser E, Kahn E, Kenigsberg K, Duffy L, Markowitz J, Daum F (1991) intestinal neuronal dysplasia: quantitative diagnostic criteria and clinical management. J Pediatr Gastroenterol Nutr 12:61–64
59. Smith VV (1992) Isolated intestinal neuronal dysplasia: a descriptive histological pattern of a distinct clinicopathological entity? In: Hadziselimovic F, Herzog B (eds) Pediatric Gastroenterology: Inflammatory Bowel Diseases and Morbus Hirschsprung. Kluwer Academic Publishers, Dordrecht, pp 203–213
60. Stoss F (1990) Neuronal dysplasia. Consideration of the pathogenesis and treatment of primary chronic constipation in adults. Int J Colorectal Dis 5:106–112
61. Stoss F, Meier-Ruge W (1991) Diagnosis of neuronal colon dysplasia in primary chronic constipation and sigmoid diverticulosis – endoscopic biopsy and enzyme-histochemical examination. Surg Endosc 5:146–149
62. Wiebecke B, Müller-Lissner S (1990) Neuronale intestinale Dysplasie (IND) Typ B beim Erwachsenen. Verh Dtsch Ges Pathol 74:525
63. Wolgemuth DJ, Behringer RR, Mostoller MP, Brinster RL, Palmiter RD (1989) Transgenic mice overexpressing the mouse homeobox-containing gene Hox-1,4 exhibit abnormal gut development. Nature 337:466–467