On the pathogenesis of intestinal aganglionosis. A consideration based on electron microscopic observations of experimental and clinical aganglionoses

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Summary. A close resemblance in fine structure was observed between the aganglionic colon, produced in rats by serosal application of 0.1% benzalkonium chloride solution, and the aganglionic descending colon, or more proximal segments, of Hirschsprung's disease. The pathogenesis of the disease is discussed.

The importance of genetic factors in the pathogenesis of Hirschsprung's disease has been pointed out by some workers[1,2], whereas other authors[3,4] emphasise the participation of environmental factors. Previously the authors[5,6] reported that serosal application of 0.1% benzalkonium chloride (BC) solution for 30 min to the descending colon of the rat produced aganglionosis. In the present investigation, the authors performed electron microscopic observations on experimental and clinical aganglionoses, and attempted to make suggestions about the pathogenesis of the disease.

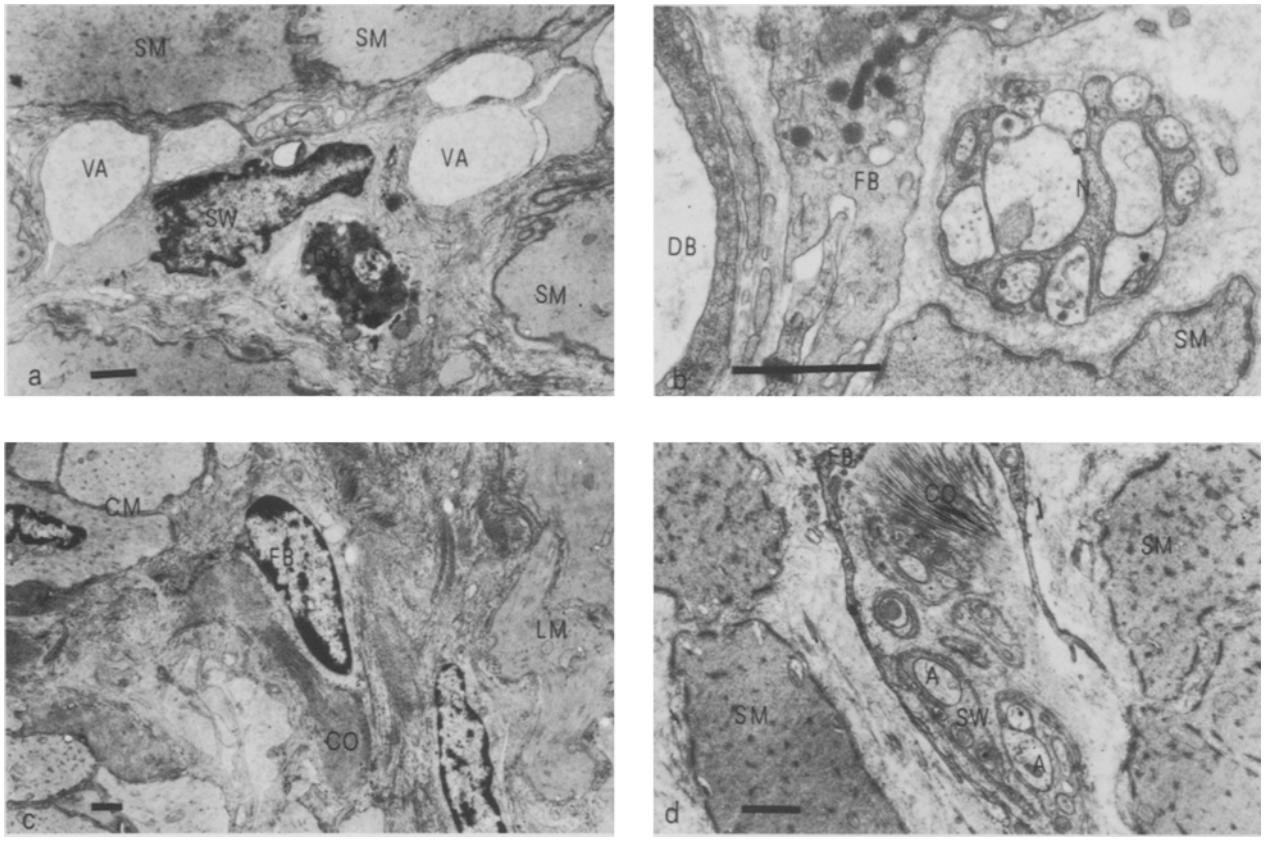
Materials and methods. Experimental aganglionosis of the descending colon was produced in 22 adult rats by serosal application of BC solution[5,6], and observed for 14 months at the longest. Operatively-removed aganglionic intestinal specimens were obtained from 15 cases, from 4 months to 6 years old, 10 male and 5 female, and consisting of 13 cases of the short segment type, 1 case of the long segment type and 1 case of the extensive type. Colonic specimens from several normal rats and humans were also observed. Specimens were fixed with 1% glutaraldehyde-paraformaldehyde and postfixed with 1% osmium tetraoxide, or, in some cases, fixed with potassium permanganate. After embedding in Epon 812, ultra-thin sectioning and double staining with uranyl acetate and lead acetate, the specimens were observed with a JEM-100U electron microscope.

Results. The descending colon, which had been treated with BC solution 7-12 weeks before examination, showed at places a widening of the space between the muscle layers up to 40 μm or more; this was filled up with collagen fibrils and some interspersed vessels, fibroblasts and residual Schwann cells; no Auerbach plexus was observed. In the circular muscle layer, smooth muscle cells invariably showed a saw-toothed contour, indicating contraction. The intercellular space was widened and, at places, Schwann cells containing vacuoles, probably originating from swollen axons, were observed, while normal axons had disappeared (figure, a); these findings continued up to 14 months of the observation period. More than 12 weeks after the treatment with BC solution, collagenous proliferation in the space between the muscle layers was still marked, and there was no regeneration of nerve plexi. In the circular muscle layer, muscle cells, with apparently normal cytoplasm, showed a saw-toothed contour. More than 7 months after the BC treatment, a few axons, probably of extraneous origin, which contained large cored vesicles and probably had regenerated after degeneration, were found by scrutiny of the circular muscle layer. There were also cases in which a few small regenerated nerve bundles were observed around vessels by scrutiny (figure, b). Increased nerve bundles, such as those seen in the aganglionic recto-sigmoid colon of Hirschsprung's disease, were never observed.

In the aganglionic intestinal wall of Hirschsprung's disease, the space between the muscle layers tended to be wider at relatively proximal aganglionic segments and there was marked proliferation of collagen fibrils (figure, c). The collagenous proliferation was found to contain fibroblasts, vessels and probably extraneous unmyelinated nerve bundles. Such nerve bundles were thick and abundant in the distal segment of the aganglionic intestine, and were reduced and disappeared as the more and more proximal aganglionic segment was examined. In the circular muscle layer, there were fine nerve bundles, consisting of 1, several or quite a large number of axons (figure, d). Such bundles were increased in the distal aganglionic segment as compared with the normal intestine. They were reduced as the proximal segment was reached, and in the descending colon or more proximal intestinal segment of a long segment, or extensive aganglionosis, they were only rarely observed. Smooth muscle cells generally showed a contracting contour. Collagenous proliferation in the intercellular space tended to increase as a more proximal intestinal segment was reached and was especially marked in the ascending colon and the ileum of an extensive aganglionosis. The findings obtained in this investigation, including the results described above, are summarized in the table.

Summary of electron microscopic findings

	a) Aganglionic rat's colon, treated with BC solution 7 weeks or more priorly	b) Aganglionic descending colon or proximal intestine of Hirschsprung's disease	c) Aganglionic sigmoid colon or distal intestine of Hirschsprung's disease
Space between the muscle layers			
Ganglion cells	Disappeared	Disappeared	Disappeared
Space size	Tended to be wide	Widened	Tended to be wide
Collagen fibrils	Increased	Increased	Tended to increase
Thick extraneous nerve bundle	Absent	Rare	Abundant
Circular muscle layer			
Nerve fibres	Decreased or disappeared	Decreased or disappeared	Increased
Swelling of axons	Present	Present	Present
Large cored vesicles in axonal enlargement	Occasionally present	Occasionally present	Tended to increase
Small cored vesicles in axonal enlargement	Rare	Rare	Few
Agranular vesicles in axonal enlargement	Rare	Rare	Inconsistent
Collagen fibrils	Increased	Markedly increased	Markedly increased
Contour of smooth muscle cells	Contracting	Tended to contract	Contracting



a The circular muscle layer of the descending colon of a rat, treated with BC solution 7 months before examination. A Schwann cell (SW) with vacuoles (VA) is surrounded by collagen fibrils. A cell containing heterogenous dense bodies (DB) is also discernible. Normal axons have disappeared completely. SM: smooth muscle cells. Bar: 1 µm. *b* The circular muscle layer of the descending colon of a rat, treated with BC solution 14 months before. A small nerve bundle (N) is discernible in the vicinity of a capillary (CA). FB: fibroblast. SM: smooth muscle cell. Bar: 1 µm. *c* The space between the muscle layers of an aganglionic ileum, obtained from an 8-month-old female case of extensive aganglionosis. Marked increase in collagen fibrils (CO) is discernible. CM: circular muscle layer. FB: fibroblast. LM: longitudinal muscle layer. Bar: 1 µm. *d* The circular muscle layer of an aganglionic descending colon, obtained from a 5-year-old male case of long segment aganglionosis. Several small nerve bundles and collagen fibrils are surrounded by a fibroblast (FB). A: axons. SM: smooth muscle cells. SW: Schwann cells. Bar: 1 µm.

Comment and conclusion. It was described that, in Hirschsprung's disease, development of the intramural nervous elements, which normally proceed from the esophagus to the rectum, was arrested before reaching the rectum[1]. The cause of this arrest is obscure. It is true that there are rats in which aganglionosis has been genetically produced[7]. However, it is not probable that all the cases of Hirschsprung's disease have a purely genetic origin. In view of the fact that there was a discordant occurrence of the disease in monozygotic twins, following post-natal enteritis[8]; that there was a case of the disease probably due to destruction of intramural ganglion cells by intrauterine viral infection[9], and that there were cases in which the disease was accompanied by atresia of the ileum or the colon, suggesting intrauterine vascular accident[10–12], it seems very probable that there are cases in which the disease has been produced by the addition of such environmental factors as anoxia or inflammation to genetic factors. The fact that, in the present investigation, fairly close resemblance in fine structure was observed between the experimental aganglionosis, produced by external noxa, and the aganglionic descending colon or more proximal segments in the cases of long segment and extensive aganglionoses (table, columns a and b), lends support to the above consideration. On the other hand, a separate explanation, e.g. an explanation from the standpoint of the development of the sacral parasympathetic system[13], will be necessary to find the cause of the marked proliferation of extraneous nerve fibres, reminiscent of stump neuroma, in the distal segment in Hirschsprung's disease. In conclusion, a possible participation of environmental factors in the pathogenesis of some cases of Hirschsprung's disease was assumed on the basis of the results of the present investigation.

1 E. Okamoto, Med.J.Osaka Univ. *13*, 285 (1961).
2 E. Passarge, New Engl.J.Med. *276*, 138 (1967).
3 T. Ehrenpreis, Hirschsprung's Disease, p. 48–50. Year Book Medical Publishers, Chicago 1970.
4 R.J. Earlam, Am.J.dig.Dis. *17*, 255 (1972).
5 A. Sato, M. Yamamoto, K. Imamura, Y. Kashiki, T. Kunieda and K. Sakata, J.pediat.Surg. *13*, 399 (1978).
6 K. Sakata, T. Kunieda, T. Furuta and A. Sato, Experientia *35*, 1611 (1979).
7 P.W. Lane, J.Hered. *57*, 29 (1966).
8 T.C. Moore, D.B. Landers, R.S. Lachman and M.E. Ament, J.pediat. Surg. *14*, 158 (1979).
9 C.W. Chow, W.C. Chan and P.C.K. Yue, J. pediat. Surg. *12*, 675 (1977).
10 J.H.Louw, J.pediat.Surg. *1*, 8 (1966).
11 H.H. Nixon, Arch.Dis.Child. *30*, 13 (1955).
12 G.A. Hyde, Jr and A.A. deLorimer, Surgery *64*, 976 (1968).
13 W. Meier-Ruge, O. Hunziker, H.-J. Tobler and Ch. Wallister, Beitr.Path. *147*, 228 (1972).