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Review Article

Sporadic Visceral Neuropathy

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Abstract: We encountered three cases of chronic functional colonic obstruction caused by intramural ganglion cell death. Morphologic and pharmacological studies were performed using resected specimens. The patients included a 59-year-old man, a 72-year-old woman, and a 28-year-old man. Barium enema studies revealed segmental stenosis in their left colon. A mecholyl test was positive in all three cases and was useful in diagnosing this disorder. Histopathologic and cytometric examinations disclosed both degeneration and the disappearance of intramural ganglion cells. The number of muscarinic acetylcholine receptors was observed to increase in the muscle layers of the stenotic portion. In addition, the muscle of the affected region showed hypersensitivity to the muscarinic agonist (oxotremorine). These results seem to suggest that this disease is caused by a noncongenital injury to the intramural ganglion cells while the resulting stenosis is considered to reflect the degeneration of the ganglion cells. The etiology of ganglion cell death still remains to be clarified; however, we propose that patients with this disorder may represent a subset of patients with sporadic visceral neuropathy.

Key Words: sporadic visceral neuropathy, degeneration of intramural ganglion cells, muscarinic acetylcholine receptor, esophageal achalasia

Introduction

Hirschsprung's disease (H disease) and esophageal achalasia are typical diseases associated with an abnormality of the intramural plexus. H disease, which is caused by the congenital absence of ganglion cells, is characterized by its consistent onset at the end of the rectum followed by continuous spreading over various lengths in an oral direction.[1] Esophageal achalasia is characterized by: (1) the selective degeneration and disappearance of intramural ganglion cells in the lower esophagus, (2) the development of reflective opening insufficiency (achalasia) of the lower esophagus, and (3) a sensitive response to mecholyl, a muscarinic agonist. We encountered three cases of segmental, inorganic colonic stenosis in patients with acquired degeneration of intramural ganglion cells in their left colons. In these three patients, degeneration of the intramural ganglion cells could be diagnosed prior to surgery based on the results of a mecholyl test, and were confirmed postoperatively by pathologic and pharmacological examinations of the resected specimens.

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Materials and Methods

Cases

Case 1 was a 59-year-old man with a chief complaint of constipation and abdominal pain. At the age of 59, he developed numbness in the distal regions of his extremities. The patient was diagnosed with multiple neuritis at a clinic and subsequently underwent germanium treatment for approximately 3 years. His family history was unremarkable. A barium enema revealed stenosis of the descending colon with dilatation of the transverse colon. No abnormality of the mucosal surface was found by colonic fiberscopy. Upper gastrointestinal series revealed no remarkable findings. In viral antibody assays, only the parainfluenza type 3 viral antibody titer was abnormally high. A barium enema with administration of 0.1 mg/kg of mecholyl (prepared at our pharmacy) showed that the stricture had narrowed but demonstrated no changes in the transverse or lower part of the sigmoid colon. The administration of 0.5 mg of atropine sulfate rapidly reversed the mecholyl-intensified stenosis, and the

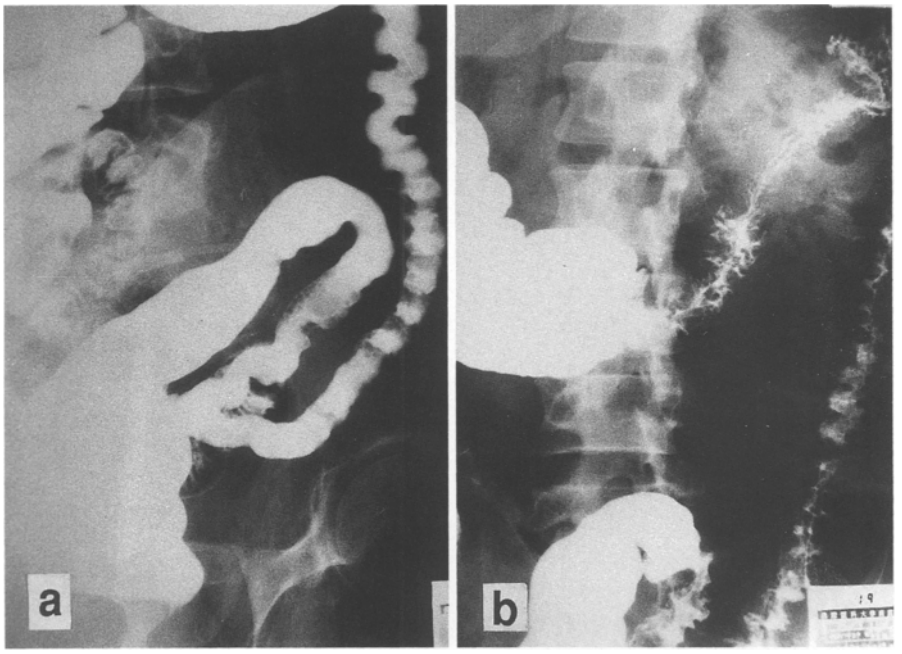

Fig. 1. a Barium enema in case 1 showing marked narrowing from the splenic flexure to the middle of the sigmoid colon. **b** After the administration of mecholyl, the stenotic portion was more distinct

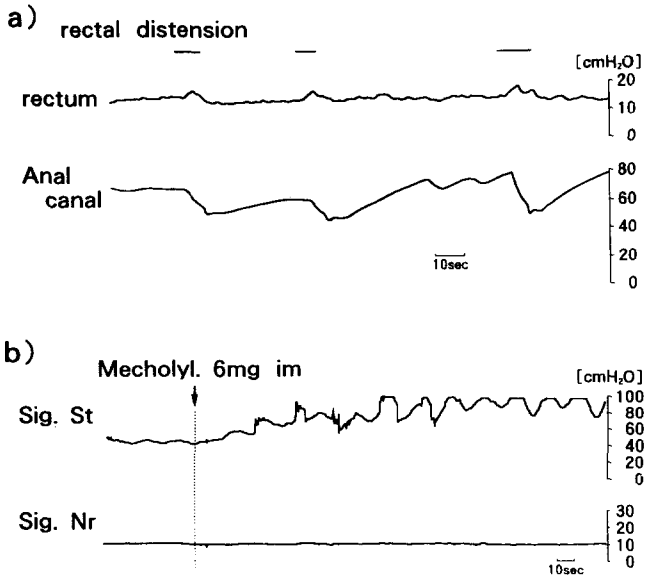

Fig. 2. a The anorectal reflex was positive and the resting pressure was normal. **b** In the stenotic portion, the intraoperative mecholyl test was positive *Sig*, sigmoid colon; *St*, stenotic portion; *Nr*, normal portion

patient was judged to have a positive response to the mecholyl test (Fig. 1). In anorectal manometry, the anorectal reflex was positive and the resting pressure was normal (Fig. 2a). Laparotomy was performed and the narrowing of the descending colon was macroscopically detected. The serosa and mesenterium of the descending colon showed no abnormalities. An intramuscular injection of mecholyl for intraoperative manometry induced no reaction in the lower part of the sigmoid colon, although it elevated the resting pressure and produced high-amplitude contraction waves (Fig. 2b). The case was managed surgically by left hemicolectomy. Four years have passed since the operation, and the postoperative course has been uneventful.

Case 2 was a 72-year-old woman with a chief complaint of left lower abdominal pain. The patient had no noteworthy disease history and an unremarkable family history. She developed intermittent colicky pain in her left lower abdomen 4 years before the present study. Because the pain gradually worsened, a barium enema revealed stenosis extending from her sigmoid colon to the upper part of her rectum. A viral antibody assay in hematological studies showed a high parainfluenza type 3 viral antibody titer. Another barium enema done in conjunction with the mecholyl test disclosed stenosis which intensified after the administration of mecholyl (Fig. 3). The stenotic portion could be detected macroscopically at the time of laparotomy and was managed surgically by anterior resection. The patient's postoperative course has been uneventful for 3 years.

Case 3 was a 28-year-old man with a chief complaint of constipation and nausea. The patient suffered a hard blow to his abdomen in a traffic accident 1 year prior to this study. The abdominal pain had disappeared quickly and no defecation trouble ensued. However, approximately half a year posttrauma, constipation and nausea appeared, and he was referred to us after the condition worsened. A barium enema

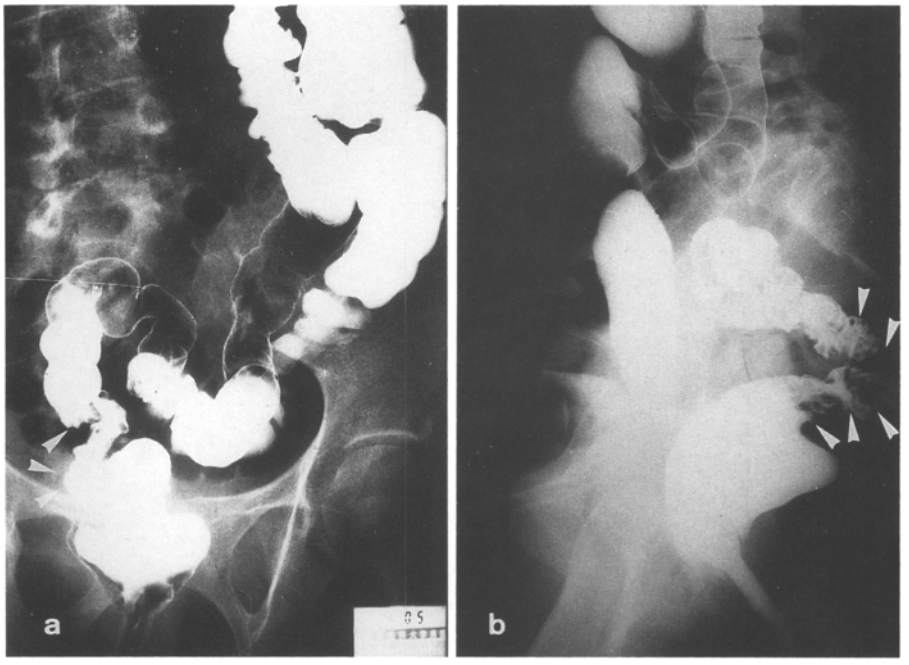

Fig. 3. a Barium enema in case 2 showing stricture from the sigmoid colon to the upper part of the rectum. **b** The stenosis narrowed after the administration of mecholyl. *Arrowheads* show the stenotic portion

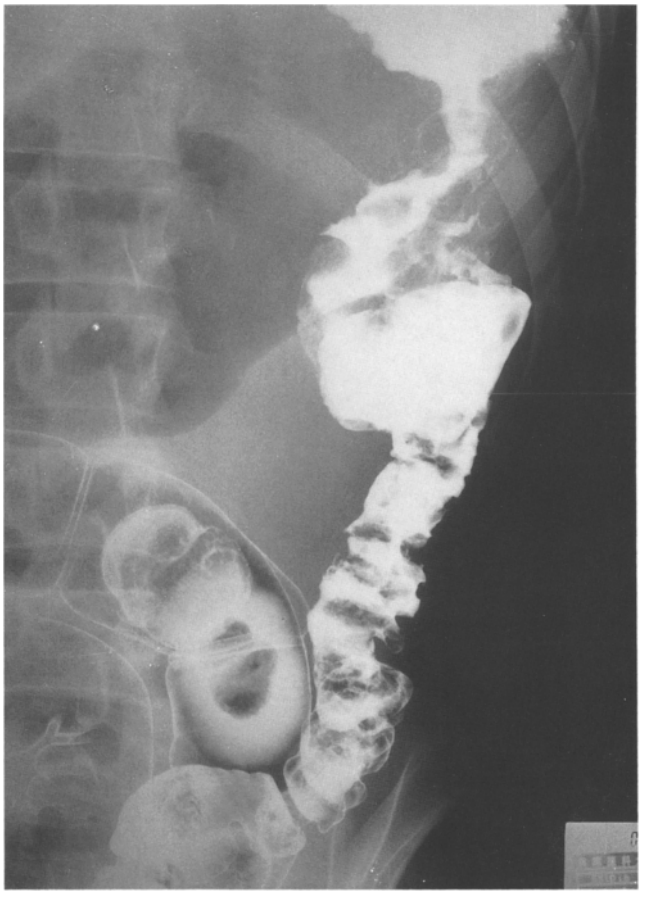

Fig. 4. Barium enema in case 3 showing stricture of the entire descending colon

revealed a megacolon due to stenosis of the descending colon (Fig. 4). The viral antibody levels, antibody titers to mumps, cytomegalovirus and parainfluenza type 3 virus were all high.

The reaction of the patient's colon to mecholyl was positive, but the reaction was milder than the response in the two previous patients. Laparotomy was performed which consisted of a resection of the descending colon and colostomy of the transverse colon. The stoma was closed after 6 months. The patient's postoperative course has been uneventful for 2 years.

Histopathological Examination

Specimens resected from the colon were fixed in 10% formalin and evaluated histopathologically with hematoxylin and eosin (H&E) staining and neuron-specific enolase (NSE) staining.

Cytometrical Study

Transverse paraffin-embedded sections encircling the whole circumference were prepared from resected specimens of the colon. The number of Auerbach's ganglion cells per intestinal circumference was counted. Specimens of normal tissue from five patients with early colon cancer who had undergone a colectomy were used as controls.

Muscarinic Acetylcholine Receptor Binding Assay

A [^{3}H]quinuclidinyl benzilate (QNB) binding assay was performed on the smooth muscle by a modification

of the method described by Yamamura and Snyder.[2] Fresh muscles were obtained from the three present patients and from five control patients. After stripping the mucosa, each tissue specimen was homogenized with a polytron (Kinematica, type PT20, ODS, 22000, Kinematica, Switzerland). The assay for the binding of [^{3}H]QNB (New England Nuclear, Boston, MA, USA; 33.1 Ci/mmol) was carried out at 37°C for 30 min in the reaction medium (2 ml) containing the Tris-HCl buffer (pH 7.4), 100 µl of tissue homogenate, and various concentrations of [^{3}H]QNB (0.1–1.6 nM). Bound [^{3}H]QNB was separated from the medium by filtration on a Whatman GF/F filter (Whatman International, Maidstone, UK) and counted in a liquid scintilation spectrometer (Packard 460 Meriden, CT, USA). Specific binding was defined as the difference between total binding and binding in the presence of 10 µM of atropine. Protein was measured by the method of Lowry et al.[3]

Contractile Response to Oxotremorine

A 1.5-cm strip of fresh muscle was suspended at 37°C in a 10-ml organ bath containing Krebs solution (pH 7.4) gassed with a mixture of 95% O_2 and 5% CO_2. The isotonic contraction with a 1-g load was recorded with an electric DC transducer (FD hip up TB-612T, carrier amplifier AP-612, recticorder RJG-4124, Nihon Kohden, Tokyo, Japan). Oxotremorine, a muscarinic agonist (0.5 nM-20 µM), was added to the organ bath. The maximum contraction of the muscle was defined as the peak value in each dose-response curve to oxotremorine.[4] The contraction is expressed as a percentage of the maximum contraction.

Statistical Analysis

Statistical analysis was performed using Student's *t*-test. The results are expressed as the mean ± standard deviation.

Results

Histopathological Findings

On histopathological examination, the mucosa was free from ischemic colitis changes such as ulcer formation, bleeding, or pseudomembrane formation. The extraserosal vessels showed no evidence of atherosclerosis or thrombosis.

The muscle layer was thickened at the stenotic portion. In Auerbach's and Meissner's plexuses at the stenotic portion, the ganglion cells were observed to have markedly decreased. In case 1, the glial cells had proliferated and some of the residual ganglion cells had degenerated (Fig. 5). In NSE staining, the number of positively stained ganglion cells decreased in the stenotic portion compared to that in the normal portions (Fig. 6).

Cytometrical Finding

The number of Auerbach's ganglion cells per circumference at various points of the resected colon are shown in Fig. 7. The cell count was 39.4 ± 9.5 in the stenotic portion, 150.0 ± 23.8 in the nonstenotic portion, and 273.9 ± 67.4 in the control. The number of ganglion cells in these three patients were significantly ($P < 0.01$) less than those of the control patients.

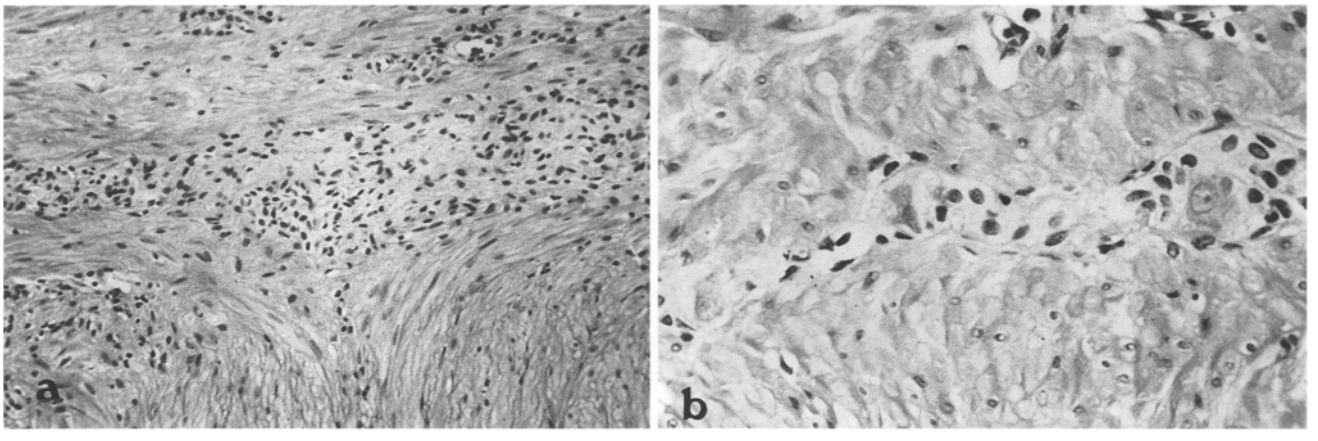

Fig. 5. **a** Auerbach's plexus of case 1 showing marked proliferation of glial cells (H&E, ×140). **b** Some of the residual ganglion cells were degenerated (H&E, ×280)

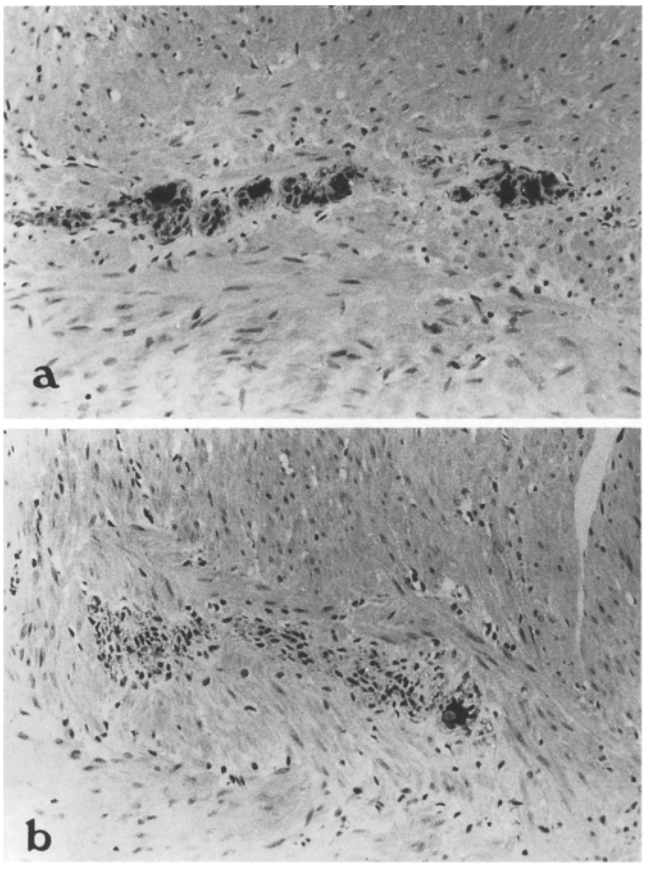

Fig. 6a,b. Neuron-specific enolase staining showing a striking difference in immunoreactivity between the stenotic portion (**b**) and the normal portion (**a**)

Muscarinic Acetylcholine Receptor Binding Assay

The specific binding in the colonic tissue from the stenotic portion in the three present patients was higher in all plots than the specific binding in the tissue specimens from both the nonstenotic portion and those from the control patients. However, the nonspecific binding was not saturable and increased linearly with the [^{3}H]QNB concentration. These values represented less than 10% of the total binding. Figure 8 shows the Scatchard[5] analyses of specific-bound [^{3}H]QNB. The line showing the binding of the tissue from the stenotic portion shifted to the right compared with the line representing the binding in the tissue specimens from both the nonstenotic portion and from the control patients. The results of the [^{3}H]QNB assay are summarized in Table 1. The value of the specific maximal bindings (Bmax) were 289.5 ± 48.6 fmol/mg protein in the control tissue, 419.6 ± 64.4 fmol/mg protein in the tissue specimens from the nonstenotic portion, and 654.0 ± 265.6 fmol/mg protein in those from the stenotic portion of the patients' colon. The Bmax in the tissue from the stenotic portion was significantly ($P < 0.05$) higher than the Bmax in the tissue from the nonstenotic portion and control tissue, but no differences were observed in either the dissociation constant (KD) or the protein content.

Contractile Response to Oxotremorine

Figure 9 shows the dose-response curves to oxotremorine in the smooth muscle of cases 2 and 3. The curve of the muscle from the stenotic portion shifted to the left in comparison with the curve of the muscle from the nonstenotic portion. The 50% effective dose was estimated to be 1.0×10^{-6} M in the tissue specimens from the stenotic portion and 8.3×10^{-6} M in those from the nonstenotic portion of the colon in case 2; and 0.4×110^{-6} M in the tissue specimens from the stenotic portion, and 5.0×10^{-6} M in those from the nonstenotic portion of the colon in case 3. The colonic

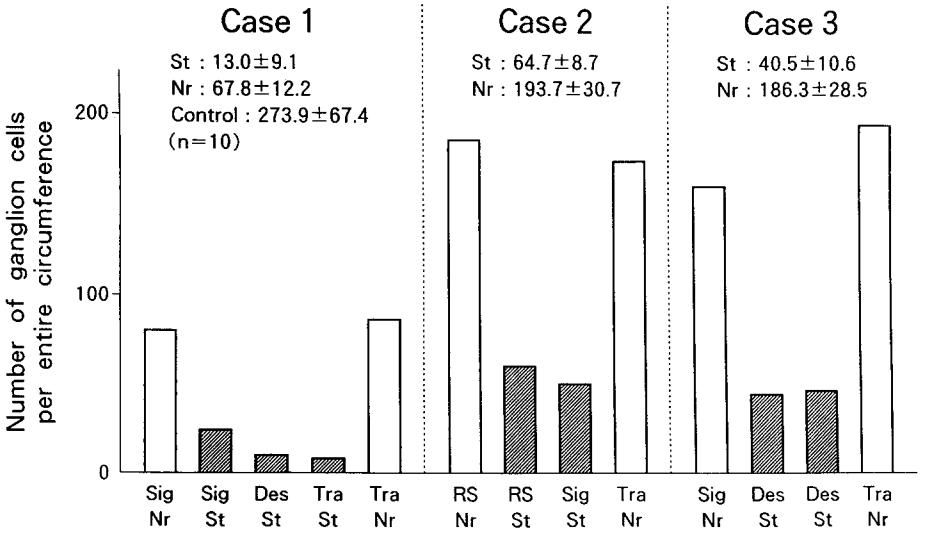

Fig. 7. The number of Auerbach's ganglion cells per intestinal circumference. *Sig*, sigmoid colon; *Tra*, transverse colon; *Des*, descending colon; *RS*, rectosigmoid colon; *St*, stenotic portion; *Nr*, normal portion

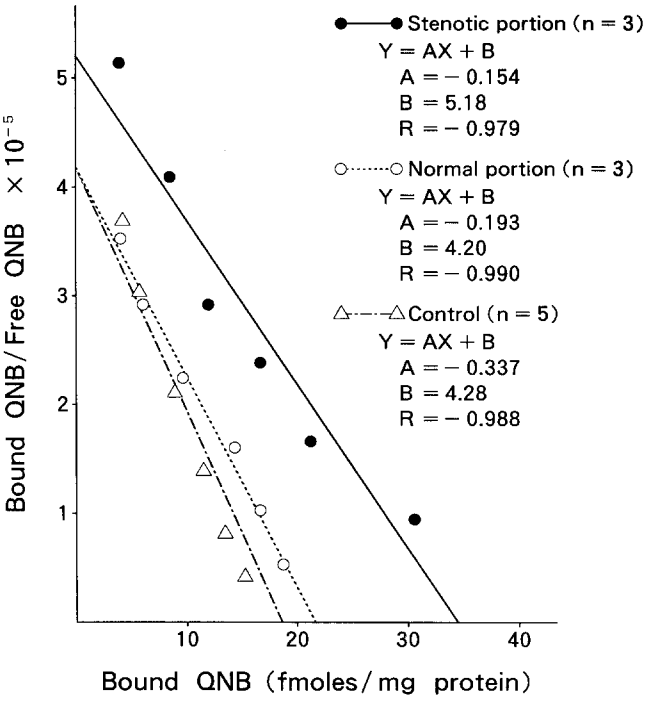

Fig. 8. Scatchard analyses of specific [^{3}H]quinuclidinyl benzilate (QNB) binding

smooth muscles from the affected sites were more sensitive to oxotremorine.

Discussion

The aganglionic segments of the colon in H disease and of the lower esophagus in esohageal achalasia are both strictured when visualized using contrast radiography, despite the lack of stromal stenosis in these regions. A normoganglionic intestine on the oral side is considerably dilated due to the retention of the intestinal contents. In the aganglionic segments, the smooth muscle is denervated and falls into a state of muscle-characteristic tonus which eventually results in narrowing of the intestine. H disease and esophageal achalasia share common features: peristalsis is disturbed due to the absence of intramural ganglion cells and the sphincter muscles are achalasic. The high sensitivity to mecholyl in patients with esophageal achalasia has been clinically explained by "Cannon's law of denervation".[6] This reaction which is not induced in patients with H disease, can be attributed to differences in the mechanism involved in the absence

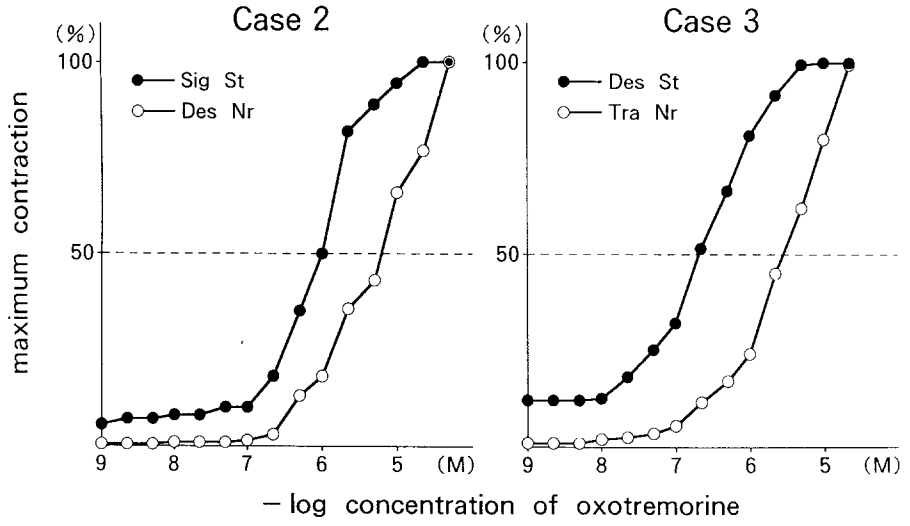

Fig. 9. Dose-response curves to oxotremorine in the smooth muscle of cases 2 and 3. *Sig*, sigmoid colon; *Des*, descending colon; *Tra*, transverse colon; *St*, stenotic portion; *Nr*, normal portion

Table 1. Muscarinic acetylcholine receptor binding

	Case 1		Case 2		Case 3		
QNB binding	St	Nr	St	Nr	St	Nr	Control $n = 5$
Bmax (fmol/mg protein)	959.3	492.8	526.9	394.2	475.8	371.8	289.5 ± 48.6
KD (nM)	0.83	0.63	0.71	0.63	0.37	0.38	0.44 ± 0.26
Protein contents (μg/mg wet tissue)	54	56	52	52	48	50	65 ± 13

Bmax, maximum specific binding; KD, dissociation constant; St, stenotic portion; Nr, normal portion; QNB, quinuclidinyl benzilate

of intramural ganglion cells. In other words, intramural ganglion cells are congenitally absent in H disease, whereas these cells are lost by noncongenital degenerative destruction in esophageal achalasia. In an attempt to verify this difference, Ueki et al.[7] quantified the muscarinic acetylcholine receptor (mAch-R) level in smooth muscles, and thus found that the mAch-R levels in aganglionic segments of the colon from patients with H disease were lower than the mAch-R levels from normoganglionic colon segments. Conversely, in a chemically induced experimental model produced by the intraperitoneal injection of mice with hexamethonium (C_6), the mAch-R level was found to be significantly higher than in the control group. Inoue et al.[8] pharmacologically confirmed that selective injury to the colonic intramural ganglion cells in rats by treatment with benzalkonium chloride (BC) increased the rats' mAch-R levels and induced hypersensitivity to oxotremorine. These findings led them to the conclusion that the mAch-R levels were increased in the intestinal segments from the subjects whose ganglion cells were destroyed by either C_6 or BC. In other words, Cannon's "law of denervation" can apply only to acquired denervation and the denervation results from the increase in mAch-R. The muscle tissue from the stenotic portion in the present study, in which the colonic intramural ganglion cells were segmentally and selectively degenerated, was found to react sensitively to mecholyl. The dose-response curve of the muscle to oxotremorine was found to shift to the left side, thus indicating that hypersensitivity to Ach could be induced in vitro. Furthermore, the mAch-R level was increased in tissue from the stenotic portion. These results seem to suggest that: (1) this disease is caused by the noncongenital injury of intramural ganglion cells, (2) strictured segments are in a denervated state, and (3) the disease may be interpreted as having an esophageal achalasia-like pathology which affects the colon. However, this disease is essentially diferent from esophageal achalasia because no sphincter exists in the colon.

Chronic idiopathic intestinal pseudo-obstruction (CIIP) is known to present with symptoms of ileus due to intestinal peristaltic insufficiency, despite the absence of any organic disorder. In CIIP, however, the intramural ganglion cells are free from morphological change.[9] An acute dilatation of the colon occurs in Ogilvie's syndrome.[10] The colon is markedly inflated with gas, and the disease is characterized by nonorganic obstruction of the colon. The disease in the three patients presented, where the colon is segmentally affected in adulthood and is strictured due to a decrease in intramural ganglion cells, had a different pathology from either CIIP or Ogilvie's syndrome. In addition, it is also important to differentiate this disease from small left colon syndrome. Small left colon syndrome is a distal colon obstruction with a meconium in the newborn. However, only the left colon exhibits a caliber change.[11] Other pathologies that need to be considered include inflammatory bowel disease such as ischemic colitis which can be excluded because of the absence of mucosal changes, and the positive reaction of tissue specimens to the mecholyl test. Another disease that should be differentiated from such patients is Chagas' disease. In Chagas' disease, the intramural plexus is destroyed by an infection with *Trypanosoma cruzi*, which leads to the development of either megaesophagus or megacolon. This disease has not previously been documented in the Japanese literature and its clinical features differ completely from those of the disease presented in this paper. We further investigated the possible etiologies of this disease. Germanium therapy was given in case 1, but no relation between the germanium and the patient's colonic symptoms was identifed. Kidney injury has been reported as a side effect of germanium, but constipation and injury of the colonic intramural plexus as side effects have not been previously described.[12] In case 3, abdominal distension occurred following abdominal trauma. It is presumed that, although all the layers of the left colon were injured due to the ischemic change caused by the trauma, only the ganglion cell injury failed to recover while the mucosa and muscle layers all recovered to their normal conditions. However, there was no history of abdominal trauma in either case 1 or case 2. In all three cases presented, only the left colon was involved and ischemic colitis has been found mostly in the left colon. This suggests that circulatory abnormalities are associated. Matsui et al.[13] reported a disease that is considered to be identical to the one presented in this paper which either occurs in patients during pregnancy or is secondary to rubella. Varicella-zoster antibody titers were normal and the parainfluenza type 3 antibody titer was high in all of our cases. Thus, the involvement of a virus cannot be excluded.

Six cases, including ours, have been reported describing pathology with features similar to those described in this paper.[14] The mecholyl test has produced positive results in all five cases in which this test has been carried out and the test is useful for the establishment of a definitive diagnosis. All cases were managed by surgery. This disease might be irreversible, because treatment by 6 months of total parenteral nutrition was not effective in case 3.

We thus propose that this disease should be categorized as a subset of visceral neuropathy. Approximately 300 years have passed since the initial discovery of esophageal achalasia, but the reason why only the intramural ganglion cells are selectively injured is still

unknown. The etiology of diseases associated with functional abnormality of the digestive tract thus also remains obscure in many respects. Further investigations are therefore required to determine the etiology of both esophageal achalasia the disease discussed in this paper.

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