Effects of enteric neural defunctioning on small bowel motility

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FRANTZIDES, CONSTANTINE T., ROBERT E. CONDON, BASIL T. DOUMAS, AND JOHN C. GARANCIS. *Effects of enteric neural defunctioning on small bowel motility.* Am. J. Physiol. 259 (Gastrointest. Liver Physiol. 22): G226–G232, 1990.—In this study, we investigated the role of intrinsic nerves of the small intestine on phase III migration of the migrating myoelectric complex. Fasting myoelectric activity was recorded from the small bowel in chronically instrumented dogs. Once control experiments were completed, the animals were divided into two groups and were reoperated. In the first group of five dogs, a 1.5-g/dl aqueous solution of cobaltous chloride (shown to induce degeneration of intestinal intrinsic nerves) was infused close intra-arterially to perfuse a 15-cm segment of jejunum. In the second group of dogs, a catheter was implanted in a branch of the superior mesenteric artery supplying a 15-cm segment of intestine. Tetrodotoxin (0.3–1 µg/kg) was infused through the catheter just before the arrival of phase III activity in the perfused segment. Subsequent to the fifth postcobalt perfusion day, phase III traversed but did not occur in the cobalt-treated segment. When tetrodotoxin was injected through the catheter, spontaneous phasic myoelectric and contractile activities in the perfused jejunal segment were inhibited, but phase III migration was not blocked. These findings suggest *1*) acute or chronic defunctioning of enteric nerves does not interrupt phase III migration, but *2*) phase III expression is dependent on the integrity of intrinsic nerves.

migrating myoelectric complex; intestinal nerves; denervation; phase III; cobalt; tetrodotoxin

THE ROLE of enteric nerves in the control of small intestinal motility is incompletely understood. A major limitation has been the inability to induce selective permanent defunctioning of these nerves. Several reports have suggested that the myenteric and submucosal plexi play important roles in the regulation of migrating myoelectric complexes (MMCs) and intestinal motility in general (11, 13, 20). MMCs continue to cycle after truncal vagotomy, splanchnicectomy, and celiac and superior mesenteric ganglionectomy. The timing of the complexes and the length of the different phases of the cycle, however, may be changed after these procedures (1, 10, 18). The understanding of the role of intrinsic nerves of the small bowel on phase III migration has been based on studies involving transection of bowel (6, 13) or close intra-arterial injections of pharmacological agents blocking neuronal transmission (14).

We have recently shown that the perfusion of a 15-cm segment of jejunum with a cobalt chloride solution (0.75–1.5 g/dl) causes degeneration of both the myenteric and submucosal plexi (3, 5). At these concentrations, cobalt chloride is selectively neurotoxic while sparing the intestinal smooth muscle. The objective of the present study was to compare the effects of chronic (cobalt) and acute [tetrodotoxin (TTX)] defunctioning of enteric nerves on migration and expression of phase III of the MMC.

METHODS

Five conditioned male dogs (30–40 kg) each had six bipolar electrodes and two electrode-strain gauge pairs implanted on the small intestine as illustrated in Fig. 1. Fasting myoelectric and contractile activities were recorded from the small bowel (7 h/day) for up to 15 days using a Grass recorder (model 7) with lower and upper cutoff frequencies set at 0.1 and 30 Hz, respectively. At least two consecutive MMC cycles were recorded each day. Bethanechol (20 µg/kg) was administered intravenously at 60% of the MMC cycle. Once control experiments were completed, the animals were reoperated and a segment (15 ± 5 cm) of jejunum was perfused with 1.5 g/dl of cobaltous chloride hexahydrate in an aqueous solution of 0.068 mol/l of trisodium citrate dihydrate. The cobalt solution was infused through a jejunal branch of the superior mesenteric artery in a volume of 200 ml over a period of 20 min. The animals were allowed to recover for 3 days, then recordings were resumed for 15 additional days.

Myoelectric and contractile activities from cobalt-treated intestine were compared with those of control tracings. Recordings were examined for alterations in the amplitude, duration, or frequency of contractions and spike bursts. The tracings were examined for the presence and duration of all phases of the MMC cycle and the extent of propagation of phase III migration along the small bowel. The recordings also were examined for the presence of ectopic phase III migrations arising distal to the denervated segment and the relative cycle periodicity above and below the denervated segment. In addition, the responses of the denervated segment to the intravenous administration of bethanechol (20 µg/kg) were compared with those of the control tracings using as parameters the amplitude, duration, and frequency of contractions. All animals were killed after completion of all experiments, and tissue samples were taken from the cobalt-perfused segment and from normal intestine (10 cm proximal and distal to the cobalt-perfused segment) for light and electron microscopic examination.

In another group of four dogs, in addition to the motility recording devices described above, a T-shaped silastic tube was implanted in a jejunal branch of the
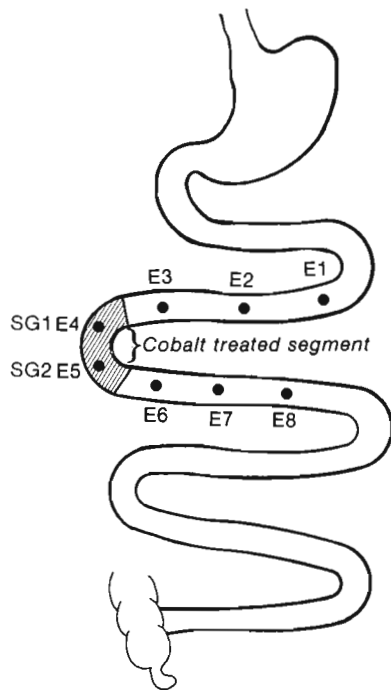
FIG. 1. Diagram of the arrangement of strain gauges (SG) and electrodes (E) in cobalt-injected animals.

superior mesenteric artery (4) supplying a segment (15 ± 5 cm) of jejunum (Fig. 2). The long limb of the catheter was tunneled subcutaneously to the subscapular region and protected by a jacket. The abdomen was closed, and the animals were permitted to recover for 3 days. The catheter was kept patent by flushing with heparinized saline (1,000 U) three times daily. TTX (0.3–1 µg/kg) was given through the T tube or through a peripheral vein in a volume of 20 ml during a 5-min period, just before the arrival of phase III activity in the perfused segment of intestine or at 60% of the MMC cycle. At least two consecutive MMC cycles were recorded as controls before the administration of TTX.

The study was designed to use each dog as its own control. The data representing change from the control period to the postperfusion period were analyzed by the paired t test. A two-tailed probability of 0.05 or smaller was accepted as indicating a significant change.

All procedures described above were approved by the Animal Welfare Committee of the Medical College of Wisconsin.

RESULTS

Spontaneous MMCs cycled normally in the small intestine beginning on the fourth day after cobalt perfusion. Phase III apparently traversed, but did not occur in the cobalt-treated segment (Fig. 3). The timing of the occurrence of phase III in the intestine distal to the cobalt-treated segment (E_6, see Figs. 1 and 2), in relation to the phase III occurring proximal to the segment (E_3), was identical to that obtained in control tracings (Fig. 4, Table 1). Phase III activity always first appeared at electrode E_1 and migrated to electrode E_8. No ectopic phase III migrations arose distal to the cobalt-treated segment. The total MMC cycle duration was not signif-
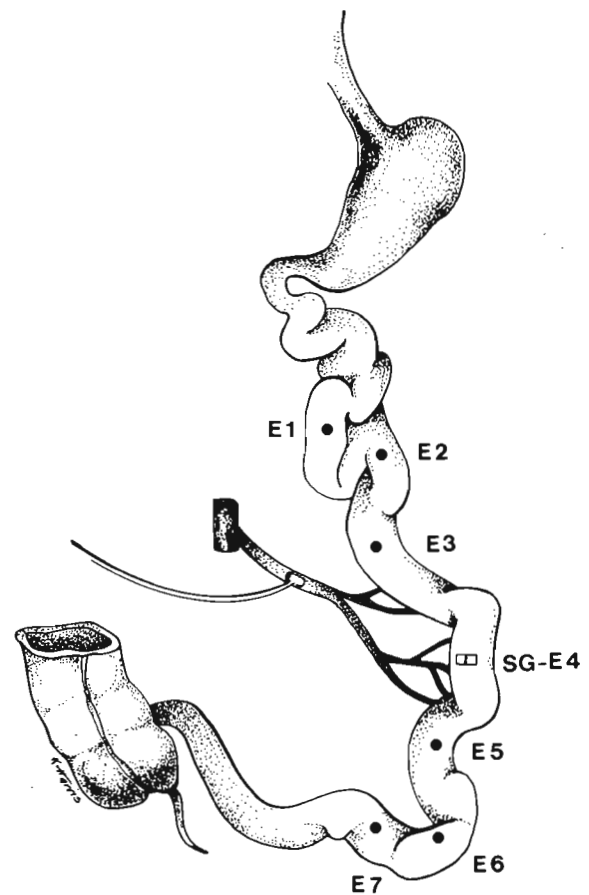

FIG. 2. Diagram showing arrangement of 6 bipolar electrodes (E), one combination strain gauge-bipolar electrode (SG-E), and a Silastic T-shaped tube for experiments involving close intra-arterial injections of TTX.

icantly different from controls in postcobalt-perfusion tracings (Table 1).

All phases of the MMC cycle were observed in the intestine proximal and distal to the cobalt-treated segment. The duration of phase II in the proximal segment, however, was significantly longer after cobalt treatment than in the control tracings (Table 1).

The cobalt-treated jejunal segment did not display the characteristic cyclical myoelectric and contractile activities (phases I, II, and III) seen in the normal small intestine of fasted dogs. Instead, spike bursts and contractions were occurring at random without any pattern, periodicity, or organization. Spike bursts and their associated contractions were of longer duration and higher amplitude but of lower frequency compared with the control tracings (Table 2). Contractions were characterized by a tonic component on which phasic contractions were superimposed.

The intravenous administration of bethanechol initiated spike bursts in the entire small intestine, including the cobalt-treated segment. The spike bursts and associated contractions induced by bethanechol in the cobalt-treated segment were of higher amplitude and longer duration (Fig. 5, Table 2) compared with the phasic contractions induced by bethanechol during control experiments. The frequency, however, of these contractions
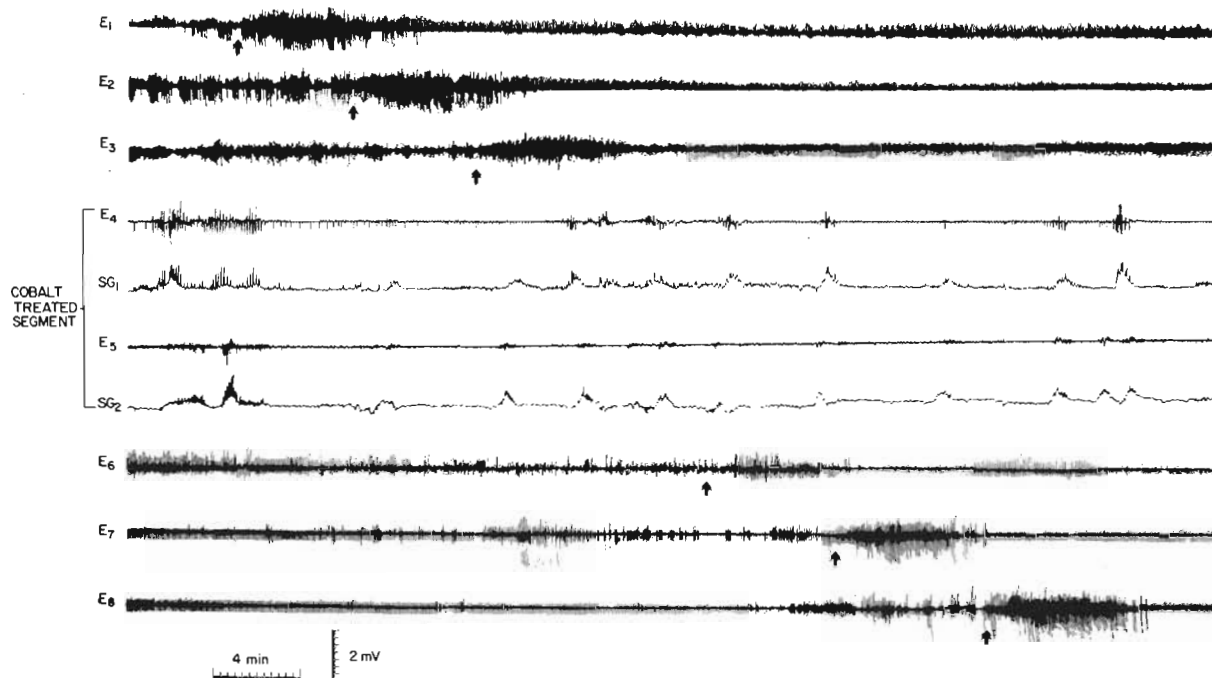
FIG. 3. Phase III migration in the small intestine after cobalt perfusion. Note that cobalt-treated segment does not display phase III activity. Phase III, however, reappears at electrode E_6 and migrates distally.
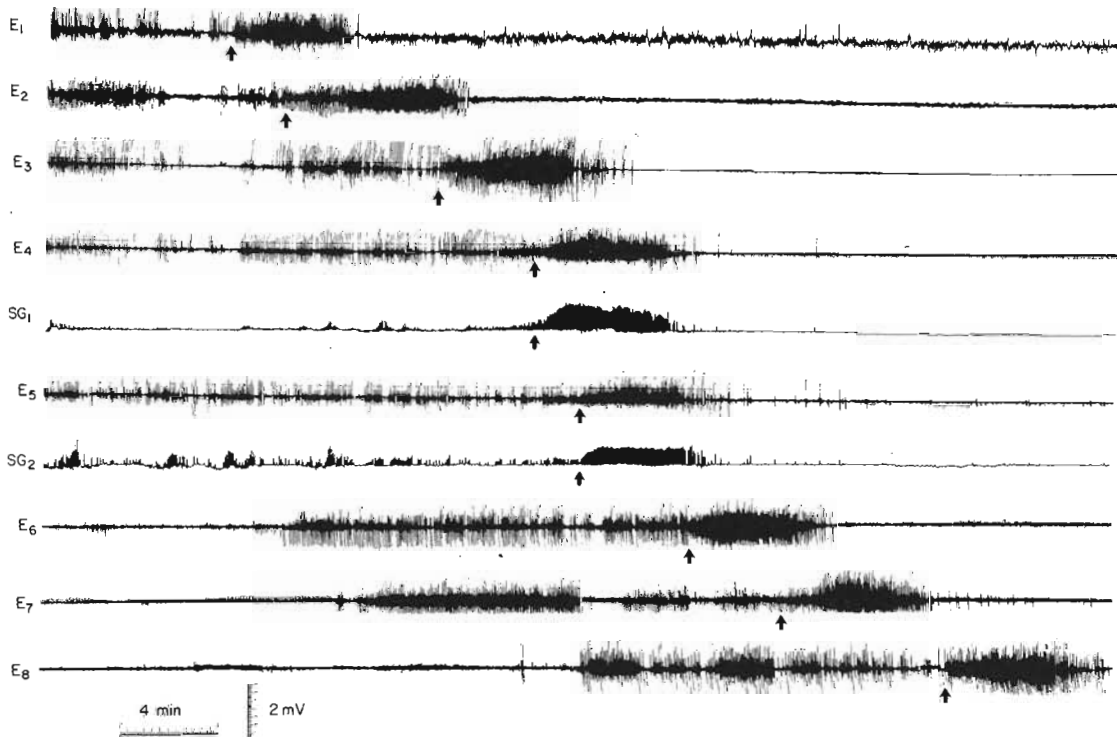

FIG. 4. Phase III migration in the small intestine before cobalt perfusion.

was lower than that of the phasic contractions of the control recordings (Table 2).

Three of five animals treated with cobalt chloride showed signs of incomplete bowel obstruction after the eighth postoperative day. These animals were well maintained, however, by a specially processed blended meat diet instead of their usual dry kennel diet. At completion of our experimental observations, autopsy demonstrated

a grossly normal appearance of the perfused bowel segment.

Electron microscopy of tissues from the cobalt-treated segment revealed severe degenerative changes in all nerve cells and axons of the myenteric and submucosal plexi (3, 5). All nerve cells examined showed a marked increase of autophagic lysosomes, extensive cytoplasmic necrosis, and condensation of nuclear chromatin. Micro-

TABLE 1. *Effects of CoCl₂ perfusion on the MMC*

	n	MMC Cycle Duration, min	Time Lag of Phase III Between E₃ and E₆, min	Duration of Phase II in Proximal Bowel Segment, min
Control	5	98.4±6.5	6.5±0.3	26.2±2.7
CoCl₂ perfused	5	102.3±8.8	6.7±0.3	55.4±6.5*

Values are means ± SE; n, no. of dogs. * $P < 0.05$.

TABLE 2. *Effect of cobalt treatment on contraction frequency, duration, and amplitude*

	n	Number of Contractions/h	Contraction Duration, s	Contraction Amplitude, cm
Control	5	231±18.7	4.06±0.039	0.65±0.04
Cobalt treated	5	80±5.7*	15.3±1.48*	1.83±0.06*
Control-bethanechol	5	572±27	4.04±0.42	1.01±0.04
Cobalt treated-bethanechol	5	141±8.2†	19.55±1.94†	2.25±0.07†

Values are means ± SE; n, no. of dogs. * $P < 0.05$ compared with control. † $P < 0.05$ compared with control-bethanechol.

tubules, neurofilaments, and mitochondria were completely lost from neuraxons. Necrotic nerve cells were observed both in the myenteric and submucosal plexi, and both plexi were equally affected. In contrast, the smooth muscle and the mucosa showed no abnormalities. Sections from control bowel showed no pathological alterations. The morphological changes reported in these animals have been described in detail elsewhere (5).

TTX at doses of 0.3 and 0.5 µg/kg administered through the T tube just before the arrival of phase III at electrode E₄ inhibited the spontaneous phasic myoelectric and contractile activities of the perfused jejunal segment (Fig. 6). Phase III activity did not occur in the TTX-perfused segment but appeared beyond the perfused segment (electrode E₅) in all 16 observations (Fig. 6); the periodicity of the MMC cycle was not affected (Fig. 7, Table 3). The timing of the occurrence of phase III activity below the TTX-perfused intestinal segment

was identical to that obtained in control recordings (Table 3). The same dose of TTX (0.3 and 0.5 µg/kg) had no effect on the myoelectric and motor activity when given through a peripheral vein.

When TTX at doses of 0.8 or 1.0 µg/kg was given through the T tube just before the arrival of phase III activity in the perfused segment (electrode E₄), myoelectric and contractile activities in the perfused segment were blocked, but bursts of retching and vomiting were initiated. Phase III activity did not appear in the perfused segment but occurred at electrode E₅ just after vomiting stopped (Fig. 8). The timing of the occurrence of phase III at electrode E₅ (in relation to phase III at electrode E₃) was significantly prolonged (Table 3). These doses of TTX (0.8 and 1.0 µg/kg) induced retching, vomiting, and delayed phase III migration when administered through a peripheral vein.

None of the doses of TTX initiated a phase III below the perfused segment when administered through the T tube at 60% of the MMC cycle.

DISCUSSION

Acute or chronic defunctioning of intestinal intrinsic nerves by perfusion of a bowel segment with cobalt chloride or TTX abolished the expression of phase III activity within the involved segment of bowel. However, both acute and chronic segmental defunctioning of enteric intrinsic nerves did not alter the appearance or characteristics of phase III migration in small bowel distal to the denervated segment. The enteric intrinsic nerves, therefore, appear to be essential to expression but not propagation of the MMC.

Other studies involving transection of the bowel (6, 13) or close intra-arterial injection of pharmacological agents that block neuronal transmission (14) have suggested an essential role for intrinsic nerves in the mechanism of phase III migration. Our observations, however,
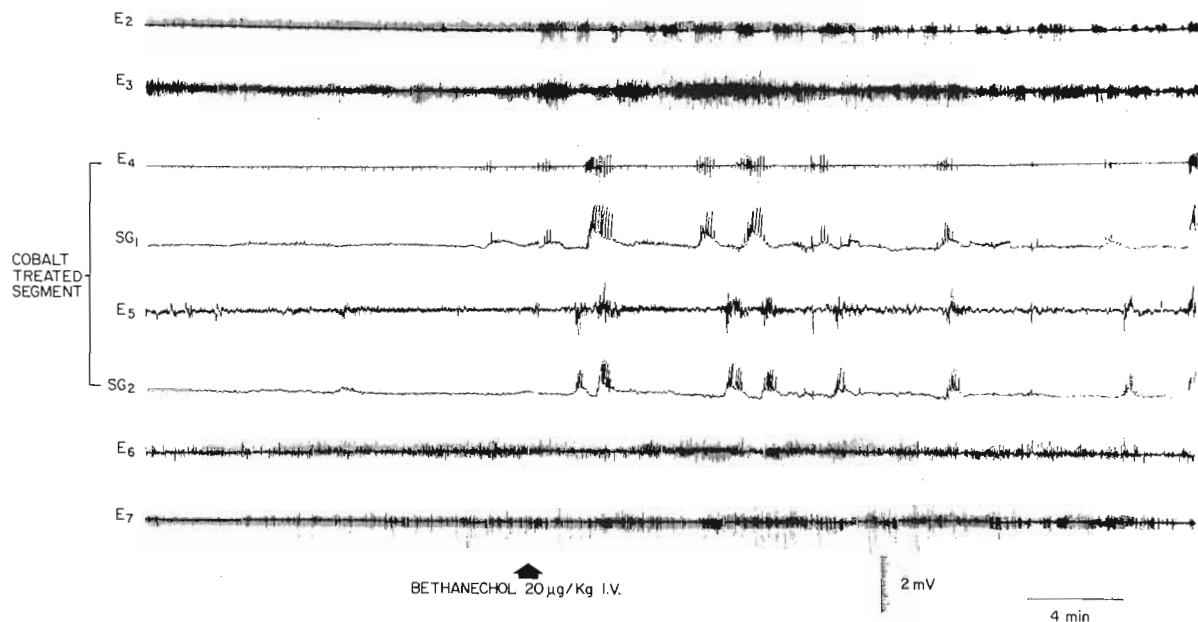

FIG. 5. Bethanechol (20 µg/kg iv) initiated spike bursts in the entire small intestine, including cobalt-treated segment. Contractions induced by bethanechol in the cobalt-treated segment are of longer duration and higher amplitude compared with the control tracings.
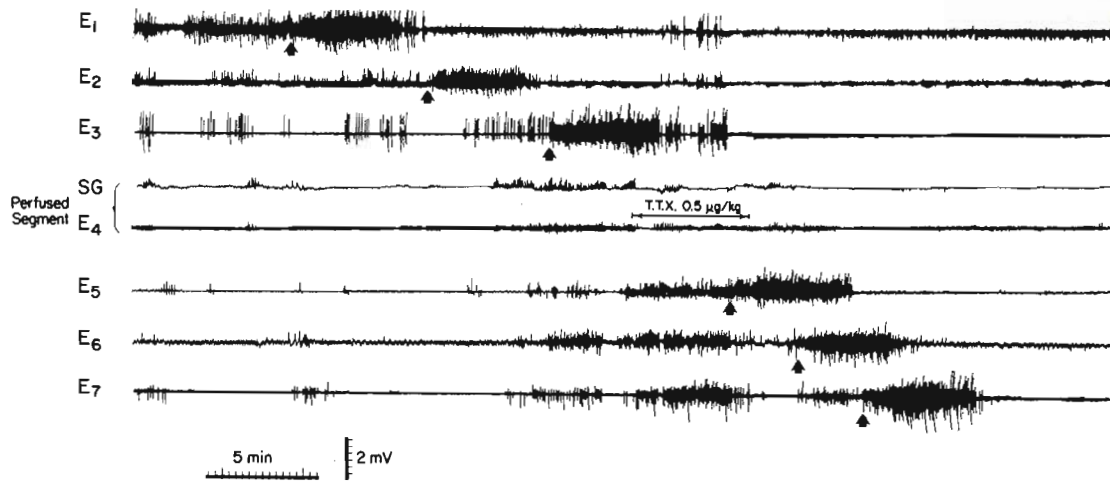
FIG. 6. TTX (0.5 µg/kg), administered through the T tube just before the arrival of phase III activity in the perfused segment, inhibits expression of phase III in this segment of bowel but does not block its distal propagation.
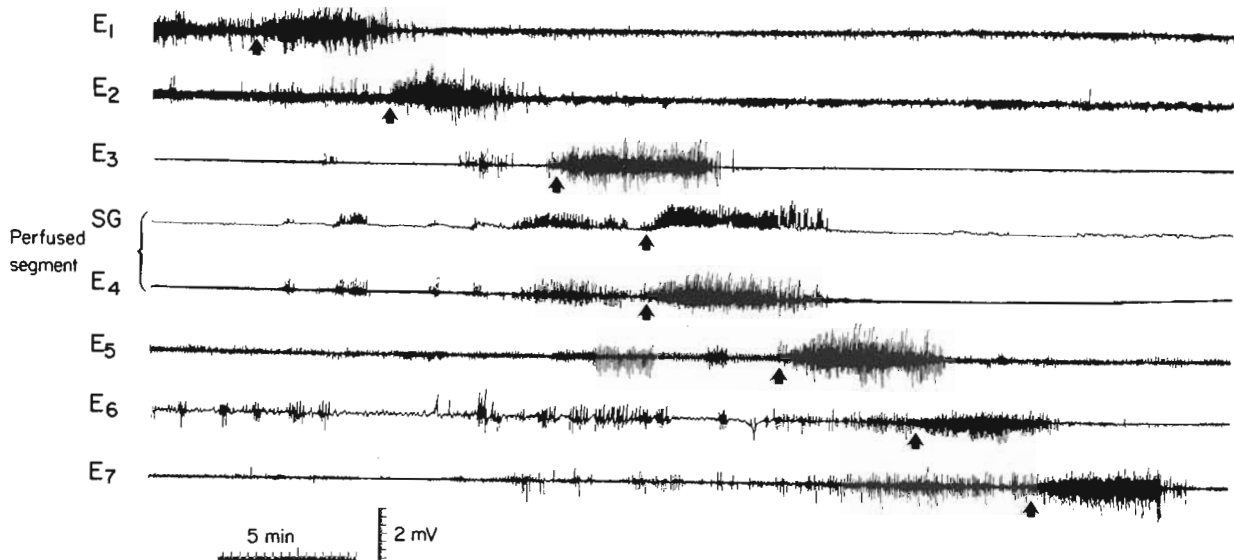

FIG. 7. Phase III migration in the small intestine before TTX perfusion.

TABLE 3. *Effects of TTX on MMC cycle and phase III migration*

	n	MMC Cycle Duration, min	Time Lag of Phase III Between E_3 and E_5, min
TTX (0.3 µg/kg)			
Control	4	120.8±12.3	10.8±0.9
TTX injected	4	122.6±17.7	10.8±1.0
TTX (1.0 µg/kg)			
Control	4	115.0±14.8	10.8±1.0
TTX injected	4	120.2±19.4	17.5±1.6*

Values are means ± SE; n, no. of dogs. * $P < 0.05$.

as well as those of Fox and Bass (2) in chronically denervated intestine, appear to contradict such a hypothesis. The difference between our findings and those of studies involving intestinal transection and reanastomosis may be due to the fact that transection disrupts not only enteric nerves but also smooth muscle continuity. The possible role of smooth muscle in the mechanism of phase III migration is not known. It has been

shown that phase III migration is usually reestablished across an anastomosis 45–60 days after transection (13). The explanation suggested for this phenomenon was that intrinsic nerves regenerated across the anastomosis, reestablishing phase III migration, but confirmation of this hypothesis by morphological or physiological studies has not yet been possible.

In our studies, acute blockade of intrinsic nerves with TTX, using doses that had only a local action, also had no effect on phase III migration. In contrast, when TTX was administered at larger doses that induced vomiting, a delay in the appearance of phase III below the TTX-perfused segment resulted. It appeared that the phase III activity that occurred below the perfused segment (at electrode E_5) was related to the migrating complex above the blocked segment and probably was not initiated by TTX, since administration of this compound through the T tube at 60% of the MMC cycle failed to initiate a phase III activity below the perfused segment.
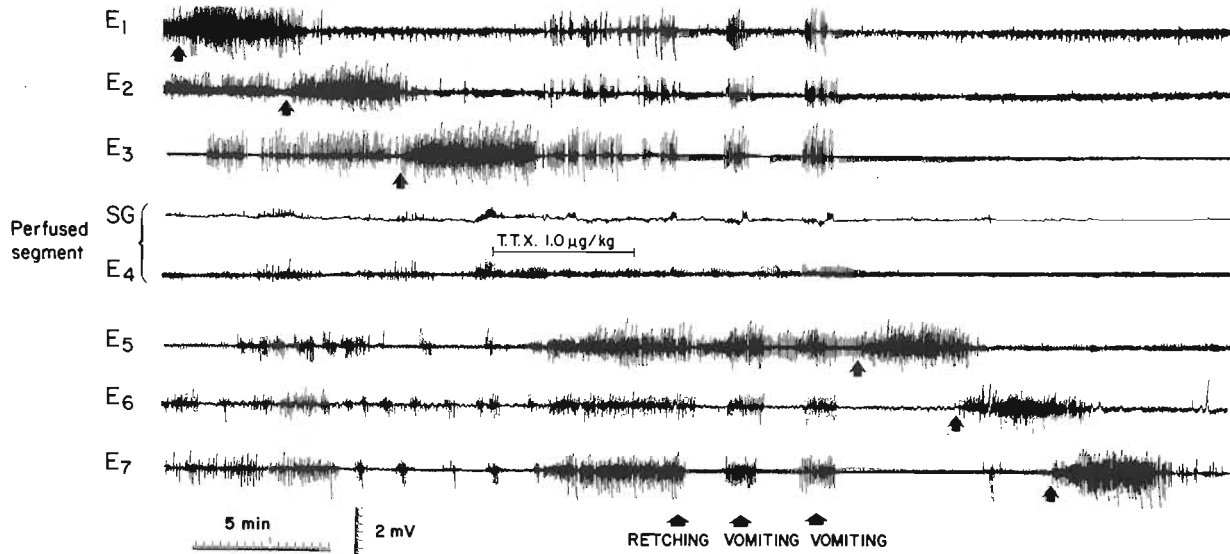
The maximum dose of TTX that can be administered

FIG. 8. TTX at higher doses (1 μg/kg) given through the T tube blocked the occurrence of phase III in the perfused segment and initiated retching and vomiting. Furthermore, the time lag between the appearance of phase III at E_3 and E_5 was prolonged.

close intra-arterially without causing a systemic effect is 0.5 μg/kg (17.5 μg in a 35-kg dog). We have, however, administered higher doses (1.0 μg/kg), which had a systemic effect, to make sure that high local tissue concentrations could be achieved. At these doses, the ~80 g of intestinal tissue perfused by the cannulated artery should have a calculated tissue concentration of 1.8×10^{-6} M, which is comparable to the concentrations of TTX used in muscle bath preparations (5×10^{-6} to 5×10^{-7} M). More importantly, however, in our preparation the TTX is infused through the vascular system and should obviously be distributed more uniformly throughout the tissue than in tissue suspended in a muscle bath preparation. The retching, vomiting, and delayed transmission of phase III seen with higher doses of TTX (1.0 μg/kg) are due to a systemic effect of the drug, since the intravenous administration of TTX at this dose had the same effects, and certainly this is not an indication of a more complete blockade of nerves at a higher dose.

Ablation of the myenteric plexus of dog small intestine by anoxia (16) and rat small intestine by surfactant (2) have been shown to cause a reduction of contractile frequency and spike bursts in the denervated bowel. These findings are in agreement with our observations that the overall number of spike bursts and associated contractions in the denervated segment were reduced after segmental administration of cobalt or TTX compared with controls. The mean amplitude and duration of contractions occurring in the denervated segment, however, was significantly higher and longer than in controls. Intrinsic denervation of the small intestine also induced distortion of the cyclical myoelectric and contractile activities in the involved segment of jejunum. The poor coordination of the denervated segment with the rest of the small intestine may be responsible for the obstructive symptoms exhibited by three of five dogs having chronic segmental intrinsic nerve blockade induced by cobalt perfusion. These symptoms were pre-

sumably due to a functional derangement of bowel motility, since there was no scarring or stenosis in the denervated segment at autopsy.

Much controversy exists concerning the response of aganglionic segments of the gastrointestinal tract to exogenous pharmacological agents. The response of the colon in congenital megacolon, for example, appears to be different than that of the esophagus in cardiospasm. Patients with achalasia may have an exaggerated response to bethanechol, acetylcholine, pentagastrin, edrophonium, or ergonovine (9, 12, 15, 19). The sensitivity of the aganglionic colon to cholinergic stimulation has been found to be decreased, normal, or increased (7, 8, 17, 21). In our studies, the administration of bethanechol caused sustained tonic contraction in the denervated segment of small bowel. The contractile frequency of the denervated segment, however, was significantly lower than that of the response of the normal bowel (control) to bethanechol.

Our observations suggest that this model of acute or chronic chemical defunctioning of the intrinsic nerves of the small intestine allows a functional differentiation between the role of intrinsic nerves and smooth muscle in the expression, organization, and propagation of the MMC. Specifically, once enteric neural defunctioning has been accomplished, the intestinal smooth muscle may serve as a cable to transmit phase III activity to the intestinal area located distal to the denervated segment. At present, however, we have no evidence that the interstitial cells of Cajal were destroyed by cobalt or affected by TTX, and thus their role in the mechanism of phase III migration remains a distinct possibility. Furthermore, although transection studies of small bowel (6, 13) demonstrate that extrinsic innervation is not essential for MMC propagation, in our model the intestine remains in continuity, and mechanisms involving the extrinsic innervation may influence and secure phase III migration.

Previous morphological studies completed in our laboratory have shown that cobalt chloride induces chronic degeneration of intrinsic intestinal nerves (5). Our present functional studies in cobalt-treated intestine demonstrate alterations of myoelectric and contractile activities compatible with previous observations in denervated intestine (2). Furthermore, TTX-treated segments of intestine exhibited identical functional alterations. These findings, however, do not eliminate the possibility that some resistant enteric nerves may still be intact and functional.

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