Digestive Diseases and Sciences, Vol. 38, No. 6 (June 1993), pp. 1040–1045

Impaired Colonic Motor Response to Cholinergic Stimulation in Patients with Severe Chronic Idiopathic (Slow Transit Type) Constipation

GABRIO BASSOTTI, MD,[1,4] GIUSEPPE CHIARIONI, MD,[2] BRUNO PIETRO IMBIMBO, PharmD,[3] CESARE BETTI, MD,[1] FABRIZIO BONFANTE, MD,[2] ITALO VANTINI, MD,[2] ANTONIO MORELLI, MD,[1] and WILLIAM E. WHITEHEAD, PhD[4]

Chronic idiopathic constipation, especially the slow transit type, is a troubling problem often afflicting young women. The pathophysiological basis for this entity is unknown, although a defective cholinergic innervation has been postulated. We tested the hypothesis that cholinergic colonic innervation is deranged in this condition by studying colonic motor activity after strong cholinergic stimulation with edrophonium chloride in 14 women complaining of slow transit constipation. Unlike healthy subjects, constipated patients showed minimal or no response to edrophonium injection. It is concluded that in slow transit constipation there is an important alteration of colonic cholinergic activity and that edrophonium chloride may represent a useful test drug for colonic pathophysiological investigations.

KEY WORDS: cholinergic; colon; constipation; edrophonium; motility.

Severe idiopathic chronic constipation is a troubling problem often afflicting young women (1, 2). The most disruptive symptoms are chronic tenesmus, straining with defecation, bloating, and occasionally pain. The severity of symptoms and the lack of efficacy of medical therapeutic measures may eventually lead to colonic resection in these subjects (3–10). The major motor abnormalities described in this condition consist of significantly reduced numbers of forceful propulsive contractions (mass movements) (11, 12) and defective motor activity of the rectum (13, 14). The enteric neural alterations producing these abnormalities are unknown, although histologic and immunohistochemical studies have shown morphological changes of the myenteric plexus and abnormalities in the distribution of some neurotransmitters (vasoactive intestinal peptide, serotonin, 5-hydroxyindolacetic acid) in resected colonic specimens of patients with chronic constipation (15–20).

Cholinergic innervation normally plays a pivotal role in regulating colonic motility (21–24), and some *in vitro* data in patients with severe chronic consti-

Manuscript received May 6, 1992; revised manuscript received November 2, 1992; accepted January 5, 1993.

From the [1]Laboratorio di Motilità Intestinale, Istituto di Gastroenterologia ed Endoscopia Digestiva, Università di Perugia; [2]Divisione di Riabilitazione Gastroenterologica, Ospedale Clinicizzato di Valeggio sul Mincio, Università di Verona; [3]Direzione Medica, Mediolanum Farmaceutici, Milano, Italy; and [4]Center for Study of Functional and Motility Disorders of the Gut, Departments of Psychiatry and Medicine, The Johns Hopkins University School of Medicine and Francis Scott Key Medical Center, Baltimore, Maryland.

Address for reprint requests: Dr. Gabrio Bassotti, Laboratorio di Motilità Intestinale, Istituto di Gastroenterologia ed Endoscopia Digestiva, Policlinico Monteluce, 06100 Perugia, Italy.

0163-2116/93/0600-1040$07.00/0

pation have shown a reduced activity of cholinergic nerves (25). A recent study described the possible usefulness of strong cholinergic stimulation of distal colonic motility by edrophonium chloride in patients with chronic constipation (26), and another study showed that this compound vigorously stimulates the whole colon of healthy subjects (27).

In the present study we tested the hypothesis that patients with slow transit constipation present a severe derangement of colonic cholinergic innervation by assessing the effect of a powerful stimulating cholinergic compound, edrophonium chloride.

MATERIALS AND METHODS

Patients. Fourteen women (ages 21–48 years) complaining of severe chronic idiopathic (slow transit type) constipation entered the study. Inclusion criteria were: (1) long-standing (more than nine months) history of constipation; (2) one or fewer evacuations per week; (3) no secondary causes of constipation, as determined by drug history, physical examination, and laboratory screening (blood chemistry, thyroid hormones and, where necessary, oral glucose tolerance test, sex hormone profile, antinuclear antibodies); (4) no previous abdominal surgery except appendectomy or cholecystectomy; and (5) failure to respond to medical treatment and high-fiber diet. To exclude organic disease or mechanical causes of constipation, each patient underwent double-contrast barium enema (showing no evidence of megacolon or megarectum), colonoscopy, and abdominal ultrasound scans. Upper gastrointestinal radiological series and upper panendoscopy were carried out if upper gastrointestinal symptoms were reported. Absence of Hirschsprung's disease was demonstrated by normal relaxation of the internal anal sphincter at anorectal manometry (see 28 for method). All patients also had a normal manometric response to straining and were able to expel a 50-ml air-filled balloon per anus, thereby excluding pelvic floor dyssynergia (29, 30).

Slow transit constipation was documented by a simplified intestinal transit time technique (11). All patients retained >80% of 40 ingested radiopaque markers within the colon after 96 hr, that is the mean + 2 SD for normal controls studied in our laboratory.

Ethical Considerations. After careful explanations about the aims of the investigation, each patient gave informed consent, and the studies were carried out in accordance with the principles of the Declaration of Helsinki.

Methods. After an overnight fast, an eight-lumen manometric probe with side holes spaced 12 cm apart (Arndorfer Medical Specialties, outer diameter 4.5 mm, inner diameter 0.8 mm for each lumen) was introduced within the colon by the aid of a colonoscope, according to a previously described technique (11, 31–33). Bowel cleansing was obtained through a semiliquid diet for two days and magnesium sulfate (30 g *per os* 36 hr before colonoscopy) and tap water enemas (12 hr before the procedure). Premedication for colonoscopy consisted of 5–10 mg diazepam through a venous cannula inserted in the left antecubital vein. The cannula was left in place for subsequent pharmacologic stimulation. The probe was positioned by advancing it together with the endoscope, the tip of the probe being fixed to the tip of the colonoscope by a silk thread held by biopsy forceps inside the operative channel of the endoscope. Once the desired portion (at least the splenic flexure) was reached, the forceps were opened and the colonoscope gently withdrawn while aspiring air as completely as possible. The probe was connected to external physiologic pressure transducers (Bell & Howell, 4-327-I) and to a low-compliance pneumohydraulic system (Arndorfer Medical Specialties) perfusing bubble-free distilled water at a constant rate of 0.2 ml/min. At this perfusion rate, the system yields a pressure rise to distal occlusion of more than 100 mm Hg/sec. Intraluminal pressures were recorded by a Beckman R-611 Dynograph Recorder coupled to the transducers (paper speed: 0.5 mm/sec).

Experimental Procedure. After colonoscope withdrawal, a fluoroscopic check with injection of 2 ml radiopaque contrast medium through the first channel of the probe was obtained to ascertain its position. The most proximal recording site was always located at least in the proximal descending colon (splenic flexure), and the other 12-cm-spaced ports spanned from the descending colon to the rectum. The subjects then rested for an average of 3 hr to recover from the endoscopic procedure. Therefore, baseline activity was recorded for 30 min, followed by intravenous injection of placebo (10 ml saline), and a further 30-min recording. Finally, edrophonium chloride, 10 mg intravenously, was injected and recording continued for 30 min, after which the study session was stopped. Patients were blind to when the drug vs the placebo (which appeared identical to the drug) were given. Throughout the study, one of the investigators closely monitored the subjects for the presence of side effects.

Data Analysis. All tracings were analyzed manually. A motility index (MI)/5 min was calculated separately for the descending colon, the sigmoid colon, and the rectum during placebo injection and after edrophonium chloride administration, by measuring the amplitude and duration of each pressure wave and then multiplying half the mean amplitude of pressure waves by the sum of their duration (27) (this formula estimates the area under the curve by assuming that pressure waves are triangular in shape). In order to avoid erroneous conclusions due to respiratory artifacts, only pressure waves of amplitude >10 mm Hg were taken into account for calculations. Due to their spike-like appearance at all recording ports, movement artifacts were also easily recognized and discarded.

Control Group. Data from descending and sigmoid colon were compared with those of a group of eight healthy volunteers (studied with the above described technique) who participated in a previous study on the effects of edrophonium chloride on colonic motility (27).

Statistical Analysis. In order to satisfy the requirements for applying analysis of variance, for each colonic segment MI were transformed [ln $(x + 1)$] to obtain homoscedasticity and normalize the data (34). Then, these

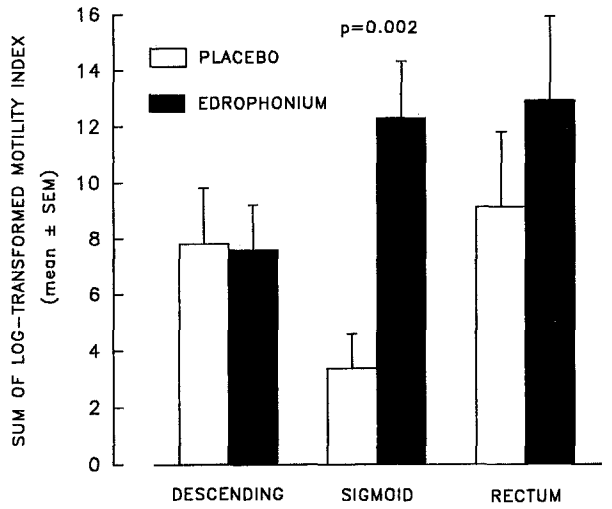

Fig 1. Effects of edrophonium chloride injection on the motility index (mean ± SEM) in different colonic segments of patients with slow transit constipation.

values were summed to obtain a single MI for the placebo and edrophonium chloride 30-min periods. These summary scores were calculated for each region of the colon studied. Placebo and edrophonium sums of MI of the constipated subjects were then compared with the analysis of variance for a randomized block design with two treatment factors (treatment and location within the viscus). Comparison of placebo and edrophonium sums of MI within each portion of the viscus were made with the Student's t test for paired data. Colonic motor response to the drug of patients and controls was analyzed with the analysis of variance for a randomized block design with three factors (disease, treatment, and location within the viscus). Significance of factors and their interactions (disease × treatment and disease × treatment × portion of the viscus) were tested with a multivariate least-squares analysis procedure. Values of $P < 0.05$ (two-tailed tests) were chosen for rejection of the null hypothesis. Calculations were done with the SYSTAT statistical package (35). Data are presented as means ± SEM.

RESULTS

In constipated patients (Figure 1), edrophonium chloride did not stimulate increased motor activity in the descending colon (MI 7.8 ± 2.0 for placebo vs 7.5 ± 1.6 for edrophonium chloride, NS) or in the rectum (MI 9.0 ± 2.2 for placebo vs 12.8 ± 3.0 for edrophonium chloride, NS). In the sigmoid colon, however, motility increased significantly following edrophonium injection (MI 3.3 ± 1.2 vs 12.3 ± 2.0, $P = 0.002$) (Figure 1).

Analysis of variance comparing control subjects to patients showed that (Figures 2 and 3): overall, MI of the patients was significantly ($P = 0.006$) less with respect to that of controls. The MI of the controls after edrophonium was significantly ($P < 0.001$) greater than that of constipated subjects. In the latter group, also, the effect of the drug was significantly different ($P < 0.05$) among the two colonic segments.

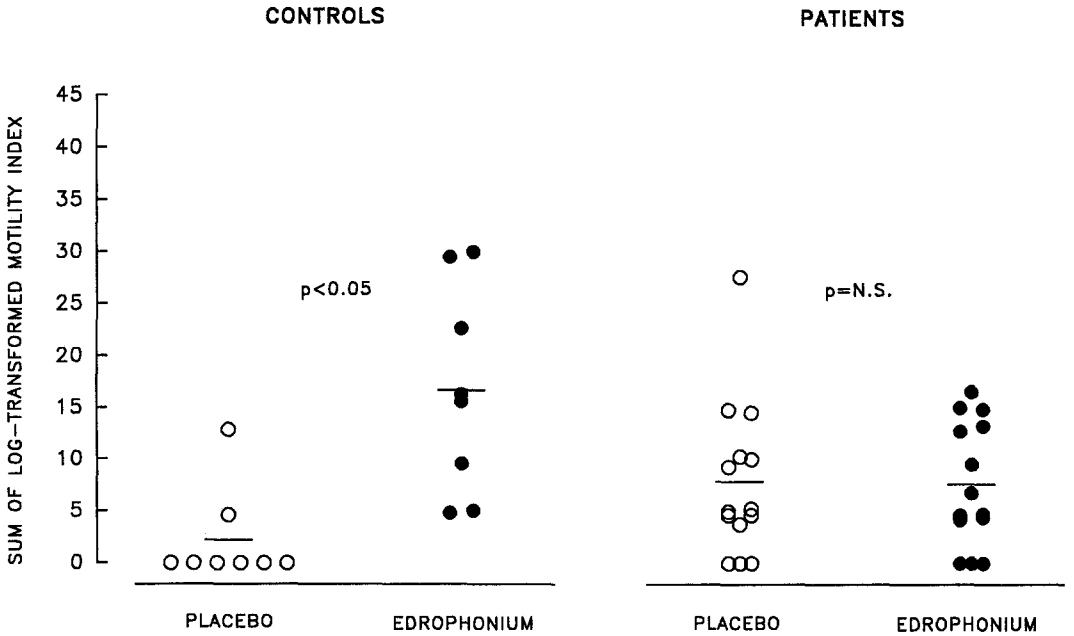

Fig 2. Effects of edrophonium chloride on individual motility indices of descending colon in controls and patients with slow transit constipation. Mean values are also indicated.

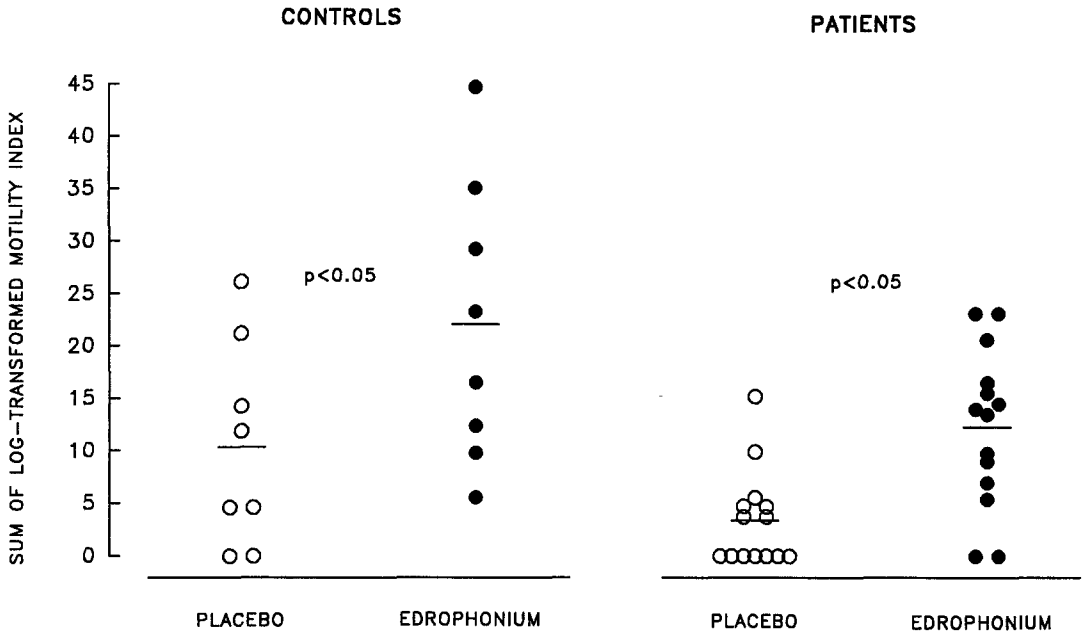

Fig 3. Effects of edrophonium chloride on individual motility indices of sigmoid colon in controls and patients with slow transit constipation. Mean values are also indicated.

The motor response elicited by edrophonium chloride always consisted of bursts of low-amplitude pressure waves. No grossly propagating motor phenomena, ie, the high-amplitude propagated contractions, the manometric equivalent of mass movements (11, 31, 33), were documented in any of the recording sessions.

Within 10 min from injection of the drug, four subjects complained of mild abdominal cramping sensation. No other side effects were reported.

DISCUSSION

The present study showed that, unlike healthy subjects (27), patients with slow transit constipation display an impaired colonic motor response to strong cholinergic stimulation in the descending colon, thereby confirming data obtained *in vitro* (25). Reynolds et al (26) also administered edrophonium chloride during a myoelectrical rectosigmoid study to 25 chronically constipated patients and reported absence of response in three (12%). In our series, a complete absence of motor response was observed in four constipated subjects (28%), and the motor responses that were observed in constipated patients were significantly attenuated relative to controls. Differences in outcome are probably explained by the inclusion of patients with diverse etiologies for their constipation in the Reynolds study, whereas we excluded patients with causes of constipation other than the slow transit type.

The rectosigmoid region appeared to respond differently from the descending colon; edrophonium stimulated increased motility in both constipated patients and controls. The explanation for these apparent regional differences is unknown. However, other investigators have also found this region of the distal colon to be hyperreactive to stimulation (36). These findings are also supported by histological evidence using silver staining techniques, which showed abnormalities of the myenteric plexus in all segments of colons resected for severe chronic constipation (16).

Abnormal cholinergic innervation in severely constipated subjects was anticipated because the role of acetylcholine as a neurotransmitter at neuroneuronal and neuroeffector synaptic junctions within the enteric nervous system is well established (25, 37–39). The release of acetylcholine from the enteric postsynaptic cholinergic neurons, in fact, is the primary stimulant for spontaneous contractile activity of colonic smooth muscle (40).

Colonic myoelectrical and motor activity is strongly stimulated by anticholinesterase agents, particularly neostigmine (41–43). However, neostigmine has a relatively long half-life for research purposes after intravenous administration (52 min)

(44), and such investigations have usually been carried out in the very distal segments of the viscus. The need for a short-lived, potent compound with strong efficacy on the motor activity of the entire colon prompted us to employ edrophonium chloride (27) to test cholinergic stimulation of the viscus in patients with slow transit constipation.

Edrophonium has several advantages as a test substance: (1) its effect, after intravenous administration, begins within 30–60 sec and lasts 5–10 min (43); (2) it is an effective stimulant for human smooth muscle contractility (27, 45–47); (3) experience in a large number of subjects has shown the safety of this compound as a routine pharmacological agent (37, 48–50); (4) side effects, which consist chiefly of light headedness, nausea, abdominal cramps, and transient bradycardia, are infrequent and are less severe than with neostigmine, because of the short duration of action of the drug (43); and (5) serious side effects are nearly always associated with existing cardiac dysfunction (47, 51).

The pathophysiological mechanisms leading to chronic constipation are largely unknown (1, 26, 52–56), and the findings of the present study may prove useful, especially considering that we investigated a homogeneous group of patients, ie, subjects with slow transit constipation.

The demonstration of a defective response to edrophonium chloride in these subjects might find clinical application as a practical, relatively safe test to identify those patients unlikely to respond to medical treatment and to propose them for a surgical option with a reasonable expectation of success (10, 57).

ACKNOWLEDGMENTS

We are indebted to Drs. Carla Fusaro and Adolfo Quintieri for useful technical assistance, and to Giuseppe Piferotti, RN, and Goffredo Pompei, RN, for the invaluable assistance in nursing the patients under investigation. This study constitutes part of Dr. Bassotti's fourth-year research project for a PhD in Experimental Surgery.

REFERENCES

1. Preston DM, Lennard Jones JE: Severe chronic constipation of young women: Idiopathic slow transit constipation. Gut 27:41–48, 1986
2. Read NW, Timms JM, Barfield KJ, Donnelly TC, Bannister JJ: Impairment of defecation in young women with severe constipation. Gastroenterology 90:53–56, 1986
3. Fasth S, Hedlund G, Svanninger G, Oreland T, Hultén L: Functional results after subtotal colectomy and caecorectal anastomosis. Acta Chir Scand 149:623–627, 1983
4. Klatt GR: Role of subtotal colectomy in the treatment of incapacitating constipation. Am J Surg 145:623–625, 1983
5. Poisson J, Devroede G: Severe chronic constipation as a surgical problem. Surg Clin North Am 63:193–217, 1983
6. Kamm MA, Hawley PR, Lennard-Jones JE: Outcome of colectomy for severe idiopathic constipation. Gut 29:969–973, 1988
7. Vasilevsky CA, Nemer FD, Balcos EG, Christenson CE, Goldberg SM: Is subtotal colectomy a viable option in the management of chronic constipation? Dis Colon Rectum 31:679–681, 1988
8. Yoshioka K, Keighley MRB: Clinical results of colectomy for severe constipation. Br J Surg 76:600–604, 1989
9. Christiansen J: Surgical treatment of chronic constipation. Scand J Gastroenterol 26:225–230, 1991
10. Bassotti G, Betti C, Pelli MA, Morelli A: Extensive investigation on colonic motility with pharmacological testing is useful for selecting surgical options in patients with inertia colica. Am J Gastroenterol 87:143–147, 1992
11. Bassotti G, Gaburri M, Imbimbo BP, Rossi L, Farroni F, Pelli MA, Morelli A: Colonic mass movements in idiopathic chronic constipation. Gut 29:1173–1179, 1988
12. Bazzocchi G, Ellis J, Villanueva-Meyer J, Jing J, Reddy SN, Mena I, Snape WJ: Postprandial colonic transit and motor activity in chronic constipation. Gastroenterology 98:686–693, 1990
13. Waldron DJ, Kumar D, Hallan RI, Wingate DL, Williams NS: Evidence for motor neuropathy and reduced filling of the rectum in chronic intractable constipation. Gut 31:1284–1288, 1990
14. Bassotti G, Betti C, Pelli MA, Morelli A: Prolonged (24 hour) manometric recording of rectal contractile activity in patients with slow transit constipation. Digestion 49:72–77, 1991
15. Preston DM, Butler MG, Smith B, Lennard-Jones JE: Neuropathology of slow transit constipation. Gut 24:A997, 1983
16. Krishnamurti S, Schuffler MD, Rohrmann CA, Pope CE: Severe idiopathic constipation is associated with a distinctive abnormality of the colonic myoenteric plexus. Gastroenterology 88:26–34, 1985
17. Krishnamurti S, Schuffler MD: Pathology of neuromuscular disorders of the small intestine and colon. Gastroenterology 93:610–639, 1987
18. Koch TR, Carney JA, Go VLW: Idiopathic chronic constipation is associated with decreased colonic vasoactive intestinal peptide. Gastroenterology 94:300–310, 1988
19. Lincoln J, Crowe R, Kamm MA, Burnstock G, Lennard-Jones JE: Serotonin and 5-hydroxyindolacetic acid are increased in the sigmoid colon in severe idiopathic constipation. Gastroenterology 98:1219–1225, 1990
20. Milner P, Crowe R, Kamm MA, Lennard-Jones JE, Burnstock G: Vasoactive intestinal polypeptide levels in sigmoid colon in idiopathic constipation and diverticular disease. Gastroenterology 99:666–675, 1990
21. Ek B: Studies on mechanisms for beta-adrenoceptor mediated inhibition of colon motility. Acta Physiol Scand 125 (suppl 546):1–39, 1985
22. Narducci F, Bassotti G, Daniotti S, Del Soldato P, Pelli MA, Morelli A: Identification of muscarinic receptor subtype mediating colonic response to eating. Dig Dis Sci 30:124–128, 1985

23. Roman C: Commande nerveuse de la motricité digestive. Gastroenterol Clin Biol 11:27B–34B, 1987
24. Burks TF: Actions of pharmacological agents on gastrointestinal functions. *In* An Illustrated Guide to Gastrointestinal Motility. D Kumar, S Gustavsson (eds). Chichester, John Wiley & Sons Ltd, 1988, pp 272–289
25. Burleigh DB: Evidence for a functional cholinergic deficit in human colonic tissue resected for constipation. J Pharm Pharmacol 40:55–57, 1988
26. Reynolds JC, Ouyang A, Lee CA, Baler L, Sunshine AG, Cohen S: Chronic severe constipation. Prospective motility studies in 25 consecutive patients. Gastroenterology 92:414–420, 1987
27. Bassotti G, Imbimbo BP, Betti C, Erbella GS, Pelli MA, Morelli A: Edrophonium chloride for testing colonic contractile activity in man. Acta Physiol Scand 141:289–293, 1991
28. Bassotti G, Gaburri M, Clausi GG, Pelli MA, Morelli A: Can idiopathic megacolon cause functional motor abnormalities in the upper gastrointestinal tract? Hepato-Gastroenterol 4:186–189, 1987
29. Preston DM, Lennard-Jones JE: Anismus in chronic constipation. Dig Dis Sci 30:413–418, 1985
30. Whitehead WE, Chaussade S, Corazziari E, Kumar D: Report of an international workshop on management of constipation. Gastroenterol Int 4:99–113, 1991
31. Narducci F, Bassotti G, Gaburri M, Morelli A: Twenty four hour manometric recording of colonic motor activity in healthy man. Gut 28:17–25, 1987
32. Bassotti G, Imbimbo BP, Gaburri M, Daniotti S, Morelli A: Transverse and sigmoid colon motility in healthy humans: Effects of eating and of cimetropium bromide. Digestion 37:59–64, 1987
33. Bassotti G, Gaburri M: Manometric investigation of high-amplitude propagated contractile activity of the human colon. Am J Physiol 255:G660–G664, 1988
34. Bartlett MS: The use of transformations. Biometrics 3:39–52, 1947
35. Wilkinson, L: SYSTAT: The System for Statistics. Evanston, Illinois, Systat, Inc, 1986
36. Chowdhury AR, Dinoso VP, Lorber SH: Characterization of a hyperactive segment in the rectosigmoid junction. Gastroenterology 71:584–588, 1976
37. Kosterlitz HW, Lees GM: Pharmacological analysis of intrinsic intestinal reflexes. Pharmacol Rev 16:301–339, 1964
38. Furness JB, Costa M: Identification of gastrointestinal neurotransmitters. *In* Mediators and Drugs in Gastrointestinal Motility, Vol I. G Bertaccini (ed). Berlin, Springer-Verlag, 1982, pp 383–462
39. North RA: Electrophysiology of the enteric nervous system. Neuroscience 7:315–327, 1982
40. Sarna SK: Physiology and pathophysiology of colonic motor activity (part one). Dig Dis Sci 36:827–862, 1991
41. Misiewicz JJ, Connell AM, Pontes FA: Comparison of the effect of meals and prostigmine on the proximal and distal colon in patients with and without diarrhoea. Gut 7:468–473, 1966
42. Snape WJ, Carlson GM, Cohen S: Human colonic myoelectric activity in response to prostigmine and the gastrointestinal hormones. Am J Dig Dis 22:881–887, 1977
43. Sarna S, Latimer P, Campbell D, Waterfall WE: Effect of stress, meal and neostigmine on rectosigmoid electrical control activity (ECA) in normals and in irritable bowel syndrome patients. Dig Dis Sci 27:582–591, 1982
44. American Hospital Formulary Service: Drug Information 87. American Society of Hospital Pharmacists, Inc., Bethesda, 1987
45. Taylor P: Anticholinesterase agents. *In* The Pharmacological Basis of Therapeutics, 7th ed. AG Gilman, LS Goodman, TW Rall, F Murad (eds). New York, Macmillan, 1985, pp 110–129
46. Hollis JB, Castell DO: Effects of cholinergic stimulation on human esophageal peristalsis. J Appl Physiol 40:40–43, 1976
47. London RL, Ouyang A, Snape WJ, Goldberg S, Hirschfeld JW, Cohen S: Provocation of esophageal pain by ergonovine or edrophonium. Gastroenterology 81:10–14, 1981
48. Richter JE, Hackshaw BT, Wu WC, Castell DO: Edrophonium: A useful provocative test for esophageal chest pain. Ann Intern Med 103:14–21, 1985
49. Van Dyk HJL, Florence L: The Tensylon test. A safe office procedure. Ophthalmology 87:210–212, 1980
50. Katz PO, Dalton CB, Richter JE, Wu WC, Castell DO: Esophageal testing in patients with noncardiac chest pain or dysphagia: Results of three years' experience with 1161 patients. Ann Intern Med 106:593–597, 1987
51. Atienza P, Chaussade S, Hiltgen M, Couturier D, Guerre J: Danger du test a l'edrophonium au cors du syndrome d'hypertonie vagale. Gastroenterol Clin Biol 12:180–181, 1988
52. Read NW, Timms JM: Defecation and the pathophysiology of the constipation. Pathophysiology of non-neoplastic colonic disorders. Clin Gastroenterol 15:937–965, 1986
53. Kamm MA, Lennard-Jones JE, Thompson DG, Sobnack R, Garvie NW, Granowska M: Dynamic scanning defines a colonic defect in severe idiopathic constipation. Gut 29:1085–1092, 1988
54. Devroede G: Constipation. *In* Gastrointestinal Disease, 4th ed. MH Sleisenger, JS Fordtran (eds). Philadelphia, WB Saunders, 1989, pp 331–368
55. Sarna SK: Physiology and pathophysiology of colonic motor activity (part two). Dig Dis Sci 36:998–1018, 1991
56. Kamm MA: Pathophysiology of constipation. *In* The Large Intestine: Physiology, Pathophysiology, and Disease. SF Phillips, JH Pemberton, RG Shorter (eds). New York, Raven Press, 1991, pp 709–726
57. Williams NS, Womack NR: Surgical therapy of constipation and incontinence. *In* The Large Intestine: Physiology, Pathophysiology, and Disease. SF Phillips, JH Pemberton, RG Shorter (eds). New York, Raven Press, 1991, pp 757–774