Filling an Unmet Need

Tom R. DeMeester, M.D.

Just as there are indisputable observations of nature that water wets, fire burns, and stones drop, so too is there evidence that the natural consequence of an expanding body of knowledge is specialization. To say this is not so is like wishing bricks would fly. A result of this natural phenomenon toward specialization is that pockets of ignorance develop between boundaries of specialties. In the medical world, if a patient experiences problems in one of these pockets, it is difficult for that patient to find knowledgeable medical talent to focus on the complaint. Esophageal disease has fallen into such a pocket in that it crosses several medical specialties such as otorhinolaryngology from above, gastroenterology and general surgery from below, and general thoracic surgery and pulmonology from the sides. Each of these specialties understands a particular dimension of the problem, but none has complete comprehension of it. As a consequence, bits and pieces of the literature on the esophagus are found in the journals pertaining to each of these fields.

In this issue of JOURNAL OF GASTROINTESTINAL SURGERY, there are three reports on the esophagus and in the four previous issues there were eight. Dr. Salminen and colleagues report that both fundoplication and Roux-en-Y duodenal diversion remove the symptoms of gastroesophageal reflux and heal esophagitis. The surprising observation is that the Roux-en-Y procedure does so without reducing esophageal acid exposure to normal. This is strong evidence that duodenal contents have a major role in the pathogenesis of esophagitis when in an acid milieu. Dr. Patti and colleagues report that up to one third of patients with achalasia who are treated with pneumatic dilatation have increased esophageal acid exposure and in several this exposure was not related to the presence of symptoms. Increased esophageal acid exposure was uncommon in patients who were untreated. Consequently pneumatic dilatation, contrary to many of the reports in the literature that indicate otherwise, is not free of the complications of reflux. Dr. Crooks and colleagues reported, in deference to what others have suggested, that incomplete lower esophageal sphincter relaxation is not necessary for effective functioning of a Nissen fundoplication. Rather, the failure of normal sphincter relaxation after surgery is a result of constructing the fundoplication too tight or placing it under too much tension or torsion. With experience this tendency is reduced, and residual pressure during relaxation falls within the normal range. In contrast to the analyses of others, this indicates that an elevated residual pressure is not necessary for an effective fundoplication. Therefore it is not something to be sought but to be avoided.

These observations provide significant insight into the physiology, pathology, and therapy of esophageal diseases. These reports could have been scattered among three different journals, for example, one devoted to surgery, another to gastroenterology, and yet a third journal devoted to motility disorders. This all adds up to poor communication with the consequence of persistent controversy and slow growth in the understanding of esophageal disease. This situation exists despite the fact that gastroesophageal reflux disease is the most common upper gastrointestinal disease in present-day Western civilization. Herein lies an opportunity for our JOURNAL. I propose that our JOURNAL become the organ to disseminate information on the foregut, that is, on the physiology, pathology, and treatment of diseases of the pharynx, esophagus, and stomach. Here is an opportunity to fill a need that has not yet been met, and JOURNAL OF GASTROINTESTINAL SURGERY is in the best position to fill it.

From the Department of Surgery, University of Southern California School of Medicine, Los Angeles, Calif.
Reprint requests: Tom R. DeMeester, M.D., Department of Surgery, University of Southern California School of Medicine, 1510 San Pablo St., Ste. 514, Los Angeles, CA 90033-4612.

pH-Metric Analysis After Successful Antireflux Surgery: Comparison of 24-Hour pH Profiles in Patients Undergoing Floppy Fundoplication or Roux-en-Y Duodenal Diversion

Jukka T. Salminen, M.D., Jarmo A. Salo, M.D., Ph.D., Juha A. Tuominen, M.D., O. Juhani Rämö, M.D., Ph.D., Martti Färkkilä, M.D., Ph.D., Severi P. Mattila, M.D., Ph.D.

Fundoplication is the most widely used antireflux method, whereas Roux-en-Y duodenal diversion (partial gastrectomy, vagotomy, and Roux-en-Y reconstruction) has been used in fewer patients with more complicated gastroesophageal reflux disease. Abnormal esophageal pH values are normalized after successful fundoplication. However, very little is known about possible changes in the pH profile after successful Roux-en-Y duodenal diversion. A total of 37 patients with severe gastroesophageal reflux disease were treated by fundoplication (n = 22) or Roux-en-Y duodenal diversion (n = 15). Postoperatively all patients in both groups were symptom free and healing of esophagitis was verified endoscopically. After fundoplication, the 24-hour esophageal acid exposure decreased significantly ($P = 0.03$) and the pH profile normalized (pH <4 in 5.8% ± 2.4% of the recorded time). However, the decrease in esophageal acid exposure was not significant ($P = 0.77$) after successful Roux-en-Y reconstruction and the pH profile remained abnormal (pH <4 in 15.1% ± 4.3%). It was concluded that 24-hour esophageal pH monitoring is a reliable means of assessing the results of fundoplication, but the current test criteria should be reexamined in evaluating the results of Roux-en-Y duodenal diversion. Healing of esophagitis after Roux-en-Y duodenal diversion despite abnormal acid reflux, as shown by 24-hour pH measurements, suggests that duodenal contents also have a role in the pathogenesis of esophagitis in an acid milieu. (J GASTROINTEST SURG 1997;1:494-498.)

The principal method used in the surgical treatment of gastroesophageal reflux disease (GERD) and its complications (e.g., Barrett's esophagus) is fundoplication. Roux-en-Y duodenal diversion (partial gastrectomy, vagotomy, and Roux-en-Y reconstruction) has been used in fewer patients with more complicated disease and after failed initial antireflux surgery. The well-documented clinical effect of fundoplication is based on the formation of an antireflux valve,[1] whereas the Roux-en-Y procedure decreases gastric acid secretion and diverts the duodenal contents to the jejunum.[2]

In 1969 Spencer[3] first described a method of prolonged esophageal pH monitoring for detecting GERD. Ambulatory 24-hour esophageal pH monitoring enables detection of GERD with a high degree of sensitivity and specificity.[4-7] Normal individuals maintain an esophageal pH above 4 for a median of 98.5% of the time.[7] A drop in pH to below 4 in the distal esophagus is indicative of an episode of acid gastroesophageal reflux.[5-8]

The percentage of time that the pH is below 4 is a simple and widely used means of expressing the results of 24-hour esophageal pH monitoring.[6-8] A patient is considered to have increased esophageal acid exposure if the percentage of total time that the pH is below 4 is greater than 4.2% to 7.0%.[6-8] The greatest prevalence of mucosal damage has been found in patients with increased esophageal exposure to pH 0 to 2 and also to pH above 7.[9] However, acid exposure is obviously not the only factor; that is, the etiology of mucosal damage is multifactorial. Different

From the Department of Thoracic and Cardiovascular Surgery (J.T.S., J.A.S., O.J.R., and S.P.M.) and the Second Department of Medicine (J.A.T. and M.F.), Helsinki University Central Hospital, Helsinki, Finland.
Reprint requests: Jarmo A. Salo, M.D., Department of Thoracic and Cardiovascular Surgery, Helsinki University Central Hospital, Haartmaninkatu 4, FIN-00290 Helsinki, Finland.

components of the gastroduodenal contents (e.g., pepsin and conjugated bile salts in an acid milieu and trypsin in an alkaline milieu) play an important role in the development of severe esophagitis.[10-14] When the pH in the distal esophagus increases over 7, this is considered alkaline reflux containing a mixture of gastric and duodenal juices.[15-18] Stein et al.[17] reported that reflux of acid gastric juice contaminated with duodenal contents plays the most important role in the development of mucosal injury in GERD and it occurs more frequently in patients with stricture, especially patients with Barrett's esophagus, than in patients with GERD without complications.[16-18] Patients with Barrett's esophagus are also at increased risk of developing esophageal adenocarcinoma.[19,20] The role of duodenal contents in the pathogenesis of esophageal adenocarcinoma has also been stressed in experimental studies in which adenocarcinoma developed in cases of surgically induced duodenoesophageal reflux.[21,22]

It has been shown that abnormal esophageal pH values are normalized after successful fundoplication.[23,24] However, very little is known about possible changes in the pH profile following successful Roux-en-Y duodenal diversion. Therefore we analyzed the pre- and postoperative 24-hour esophageal pH measurements after clinically and endoscopically successful fundoplication and Roux-en-Y diversion and compared them. Our preliminary results were published in 1996.[25]

MATERIAL AND METHODS

Thirty-seven consecutive patients seen between 1992 and 1995 with severe GERD were included in this study. The protocol was approved by the Ethics Committee of Helsinki University Central Hospital. All patients had the typical symptoms of heartburn and regurgitation, and ulcerative esophagitis was verified endoscopically before surgery. Twenty-two patients underwent fundoplication and 15 underwent Roux-en-Y duodenal diversion (partial gastrectomy, selective gastric vagotomy, and Roux-en-Y reconstruction). Patients in the Roux-en-Y group had esophagitis that was clinically and endoscopically more severe, as well as decreased esophageal body function as determined by standard manometry. The demographic data on these patients are presented in Table I. Abnormal 24-hour pH esophageal profiles[7] were confirmed preoperatively. All patients had a history of medical treatment with H_2 blockers or proton pump inhibitors.

Floppy fundoplication was performed according to a standard open technique consisting of total fundic mobilization with ligation of short gastric vessels, 360-degree anterior wall fundoplication, and a sutured repair of the distal esophageal diaphragmatic hiatus. Apposition of the 2 cm long fundic wrap was accomplished with two U-shaped Teflon patch sutures and one suture fixed it to the lesser curvature.[23,26] The operator's index finger and an 18 F nasogastric tube in the esophagus were used to confirm the absence of esophageal narrowing. Roux-en-Y duodenal diversion consisted of selective gastric vagotomy, a one-half to two-thirds gastrectomy, retrocolic end-to-end gastrojejunostomy, fixation of the gastric remnant with mesocolon, and fixation of the efferent loop in the retroperitoneum. The distance between the gastrojejunostomy and the jejunojejunostomy was 50 cm.[27]

Table I. Demographic data on 37 patients with GERD

Method	No.	Male	Female	Age (yr)	Range (yr)
F	22	16	6	50.1	22-67
RY	15	13	2	59.4	44-72
TOTAL	37	29	8	54.0	22-72

F = fundoplication; RY = Roux-en-Y reconstruction.

pH measurements were performed using an antimony pH catheter, a silver–silver chloride-electrocardiographic-type cutaneous reference electrode, and an external portable digital data logger (Synectics Medical, Stockholm, Sweden). A pH electrode was passed transnasally and placed 5 cm above the upper border of the lower esophageal sphincter, which had been previously located by manometry. The reference electrode was placed on the chest skin. pH values in the distal esophagus were recorded at 4-second intervals for 24 hours. All medications that interfered with gastric acid secretion and gastrointestinal activity (particularly H_2 blockers and proton pump inhibitors) were discontinued at least 1 week before the preoperative testing was begun.[28] Postoperatively patients were not given any medications that would interfere with gastric acid secretion or gastrointestinal activity. Patients were instructed to continue with their normal diet and daily activities. The stored data were analyzed using a commercial software program (Gastrosoft, Inc., Irving, Tex.). The mean postoperative follow-up time was 6 months. The results are expressed as mean values ± standard error of the mean. Statistical analyses were performed using the Wilcoxon-Pratt test and the Mann-Whitney U test. *P* values less than 0.05 were considered significant.

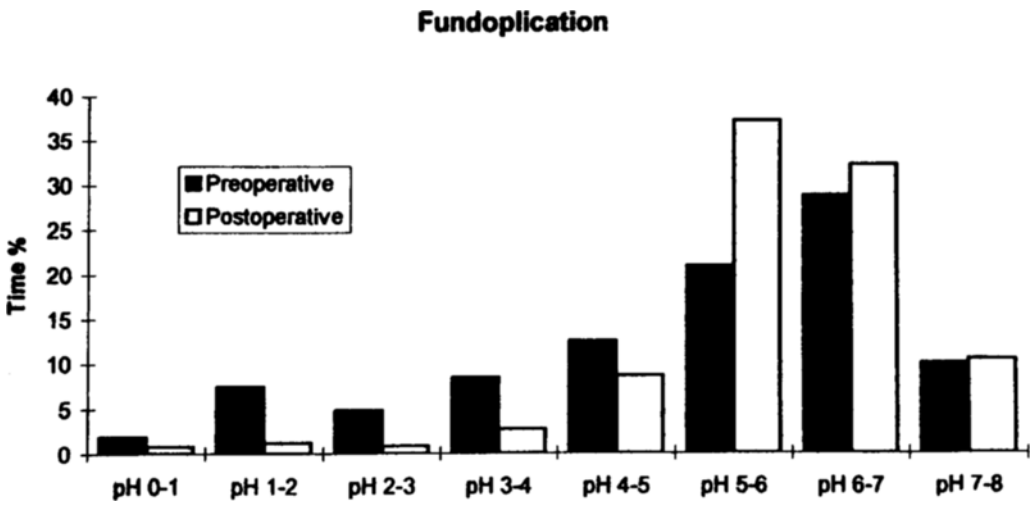


Fig. 1. Distribution of percentages of total time pre- and postoperatively at various pH intervals in 22 patients who underwent fundoplication. $P < 0.05$ for pH intervals 0 to 1, 1 to 2, 2 to 3, and 3 to 4.

RESULTS

All patients in both groups became free of symptoms (heartburn and/or regurgitation), and healing of esophagitis was verified endoscopically. In addition, patients did not complain of any of the typical side effects such as bloating or stasis. Esophageal acid exposure at various pH intervals pre- and postoperatively in the fundoplication group is shown in Fig. 1. The esophageal acid exposure in this group diminished significantly in comparison to preoperative values, and the postoperative pH profile normalized (pH <4 for 21.7% ± 6.0% of the total time preoperatively and pH <4 for 5.8% ± 2.4% of the total time postoperatively; $P = 0.03$). Also, the number of reflux episodes lasting longer than 5 minutes decreased significantly (10.4 ± 3.0 preoperatively and 2.3 ± 1.0 postoperatively; $P = 0.02$). In the Roux-en-Y group, the pH profile remained abnormal and neither the esophageal acid exposure nor the number of reflux episodes was significantly decreased (pH <4 for 18.7% ± 6.4% of the total time preoperatively and pH <4 for 15.1% ± 4.3% postoperatively; $P = 0.77$), and the number of reflux episodes lasting longer than 5 minutes was 8.5 ± 2.8 preoperatively and 5.7 ± 1.6 postoperatively ($P = 0.93$). However, the reflux time in the most acidic milieu decreased significantly. At the pH 0 to 1 interval, the reflux time was 3.9% ± 2.4% preoperatively vs. 0.04% ± 0.02% postoperatively ($P = 0.005$). Esophageal acid exposure at the pH 1 to 2 interval decreased from 4.4% ± 1.8% to 1.1% ± 0.8%; $P = 0.003$) (Fig. 2).

DISCUSSION

All patients were free of symptoms after undergoing either floppy fundoplication or Roux-en-Y duodenal diversion, and healing of esophagitis was verified endoscopically. Both techniques are usually effective in the treatment of severe GERD; when they are used for the proper indications and correct surgical technique is followed, 90% of patients are rendered asymptomatic. In this study, fundoplication resulted in normalization of preoperative pathologic 24-hour esophageal pH profiles, but 24-hour pH values remained abnormal after clinically and endoscopically successful Roux-en-Y reconstruction.

In the Roux-en-Y group, because of the vagotomy and partial gastrectomy, esophageal acid exposure in the most acidic milieu (pH 0 to 1 and 1 to 2) decreased significantly in spite of the fact that the total time the pH was below 4 was still abnormal. The importance of a low esophageal pH in the pathogenesis of esophageal mucosal damage is stressed by Bremner et al.,[9] who found the most severe cases of esophagitis among those patients with increased exposure to pH 0 to 2 and above 7. On the other hand, the high esophageal H^+ concentration in an acid milieu does not directly correlate with the severity of esophageal mucosal injury.[10] Pepsin and conjugated bile salts belong to the group of agents that are also active in an acid milieu and may therefore have a significant role as refluxate in the pathogenesis of esophageal mucosal damage.[10,29,30] There was no difference between the exposure time to the pH 7 to 8 interval pre- and post-

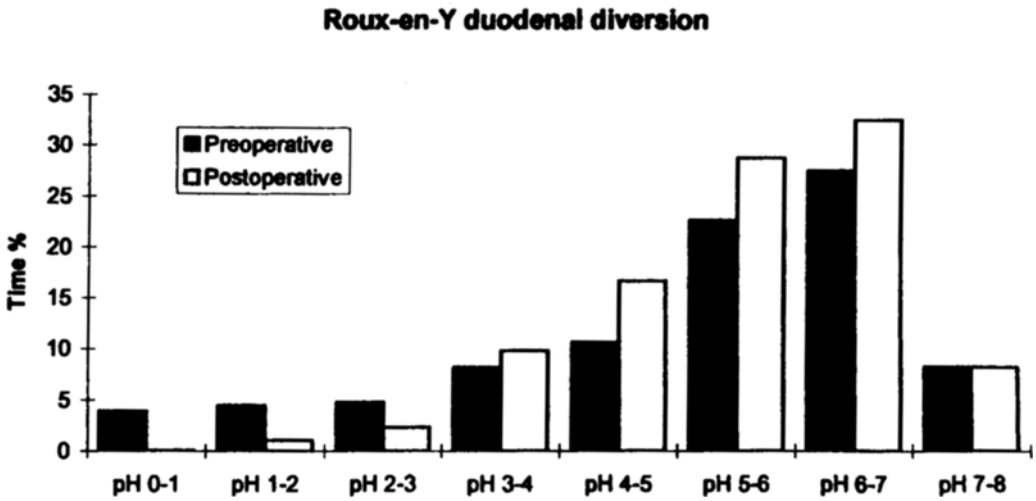


Fig. 2. Distribution of percentages total time pre- and postoperatively at various pH intervals in 15 patients who underwent Roux-en-Y duodenal diversion. $P < 0.05$ for pH intervals 0 to 1 and 1 to 2.

operatively (8.26% ± 3.05% vs. 8.23% ± 2.19%; $P = 0.99$) in the Roux-en-Y group. This suggests that a pH in this range is not solely due to refluxed duodenal contents and this method may yield data that are insufficient to make a diagnosis of alkaline duodenogastroesophageal reflux.[12,17,31] Also, the antimony probes are inaccurate in pH units in the alkaline range and can record values 2.1 units higher than would be recorded with glass electrodes.[32] This study supports the view that duodenogastroesophageal reflux is difficult to diagnose solely on the basis of esophageal pH monitoring. Therefore other methods such as 24-hour spectrophotometric measurements of the luminal bilirubin concentration[31] are needed to detect reflux of duodenal contents into the esophagus.

The present study and others[23,24] suggest that postoperative 24-hour esophageal pH monitoring is a reliable test for evaluating the results of fundoplication. However, the current test criteria should be reexamined if this test is to be used to assess the results of Roux-en-Y duodenal diversion. Possible reasons why acid reflux still occurs in the Roux-en-Y group may be the persistence of lower esophageal sphincter incompetence and the possibility of Roux stasis, both of which have been described previously.[33] This phenomenon may cause a predisposition to aspiration, but we have not encountered this problem in our patients.

We conclude that clinical and endoscopic healing of esophagitis following Roux-en-Y duodenal diversion, despite postoperative abnormal acid reflux as demonstrated by 24-hour esophageal pH monitoring, suggests that duodenal contents also have a role in the pathogenesis of GERD in an acid milieu. Since acid reflux still occurred in the Roux-en-Y group, the absence of duodenal contents may play a major role in the healing of esophagitis.

REFERENCES

1. Nissen R. Eine einfache Operation zur Beeinflussung de Refluxoesophagitis. Schweiz Med Wochenschr 1956;86:590-592.
2. Salo JA, Lempinen M, Kivilaakso E. Partial gastrectomy with Roux-en-Y reconstruction in the treatment of persistent or recurrent oesophagitis after Nissen fundoplication. Br J Surg 1985;72:623-625.
3. Spencer J. Prolonged pH recording in the study of gastro-oesophageal reflux. Br J Surg 1969;56:912-914.
4. Klaüser AG, Heinrich C, Schindlbeck NE, Müller-Lissner SA. Is long-term esophageal pH monitoring of clinical value? Am J Gastroenterol 1989;84:362-366.
5. Fuchs KH, DeMeester TR, Albertucci M. Specificity and sensitivity of objective diagnosis of gastroesophageal reflux disease. Surgery 1987;102:575-580.
6. Schindlbeck NE, Heinrich C, König A, Dendorfer A, Pace F, Müller-Lissner SA. Optimal thresholds, sensitivity and specificity of long-term pH-metry for the detection of gastroesophageal reflux disease. Gastroenterology 1987;93:85-90.
7. Jamieson JR, Stein HJ, DeMeester TR, Bonavina L, Schwizer W, Hinder RA, Albertucci M. Ambulatory 24-hour esophageal pH monitoring: Normal values, optimal thresholds, specificity, sensitivity, and reproducibility. Am J Gastroenterol 1992;87:1102-1111.
8. Johnson LF, DeMeester TR. Twenty-four-hour pH monitoring of the distal esophagus. Am J Gastroenterol 1974;62:325-332.

9. Bremner RM, Crookes PF, DeMeester TR, Peters JH, Stein HJ. Concentration of refluxed acid and esophageal mucosal injury. Am J Surg 1992;164:522-527.
10. Salo JA, Kivilaakso E. Role of luminal H^+ in the pathogenesis of experimental esophagitis. Surgery 1982;92:61-68.
11. Salo JA, Kivilaakso E. Role of bile salts and trypsin in the pathogenesis of experimental alkaline esophagitis. Surgery 1983;93:525-532.
12. Gotley DC, Morgan AP, Cooper MJ. Bile acid concentrations in the refluxate of patients with reflux oesophagitis. Br J Surg 1988,75:587-590.
13. Lillemoe KD, Johnson LF, Harmon JW. Role of the components of gastroduodenal contents in experimental acid esophagitis. Surgery 1982;92:276-284.
14. Lillemoe KD, Johnson LF, Harmon JW. Alkaline esophagitis: A comparison of the ability of components of gastroduodenal contents to injure the rabbit esophagus. Gastroenterology 1983;85:621-628.
15. Pellegrini CA, DeMeester TR, Wernly JA, Johnson LF, Skinner DB. Alkaline gastroesophageal reflux. Am J Surg 1978; 135:177-184.
16. Stein HJ, Feussner H, Kauer W, DeMeester TR. Alkaline gastroesophageal reflux: Assessment by ambulatory esophageal aspiration and pH monitoring. Am J Surg 1994;167:163-168.
17. Stein HJ, Barlow AP, DeMeester TR, Hinder RA. Complications of gastroesophageal reflux disease. Role of the lower esophageal sphincter, esophageal acid and acid/alkaline exposure, and duodenogastric reflux. Ann Surg 1992;216:35-43.
18. Attwood SEA, DeMeester TR, Bremner CG, Barlow AP, Hinder RA. Alkaline gastroesophageal reflux: Implications in the development of complications in Barrett's columnar-lined lower esophagus. Surgery 1989;106:764-770.
19. Cameron AJ, Beverly JO, Payne WS. The incidence of adenocarcinoma in columnar-lined (Barrett's) esophagus. N Engl J Med 1985;313:857-859.
20. Spechler JS, Robbins AH, Rubins HB, Vincent ME, Heeren T, Doos WG, Colton T, Schimmel EM. Adenocarcinoma and Barrett's esophagus. An overrated risk? Gastroenterology 1984;87:927-933.
21. Attwood EA, Smyrk TC, DeMeester TR, Mirvish SS, Stein HJ, Hinder RA. Duodenoesophageal reflux and the development of esophageal adenocarcinoma in rats. Surgery 1992; 111:503-510.
22. Pera M, Trastek VF, Carpenter HA, Fernandez PL, Cardesa A, Mohr U, Pairolero P. Influence of pancreatic and biliary reflux on the development of esophageal carcinoma. Ann Thorac Surg 1993;55:1386-1393.
23. DeMeester TR, Bonavina L, Albertucci M. Nissen fundoplication for gastroesophageal reflux disease. Ann Surg 1986;204:9-20.
24. Johnsson F, Joelsson B, Gudmundsson K, Floren C-H, Walther B. Effects of fundoplication on the antireflux mechanism. Br J Surg 1987;74:1111-1114.
25. Salo JA, Nemlander A, Tuominen J, Salminen J, Rämö OJ, Färkkilä M, Kivilaakso E. pH-metric analysis after successful surgery for severe reflux esophagitis: Comparison of 24-hour pH profiles in patients operated on using floppy fundoplication or Roux-en-Y duodenal diversion. Gastroenterology 1996;110:A1415.
26. Donahue PE, Samuelson S, Nyhus LM, Bombeck T. The floppy Nissen fundoplication. Arch Surg 1985;120:663-668.
27. Salo JA, Ala-Kulju KV, Heikkinen LO, Kivilaakso EO. Treatment of severe peptic esophageal stricture with Roux-en-Y partial gastrectomy, vagotomy, and endoscopic dilation. J Thorac Cardiovasc Surg 1991;101:649-653.
28. Marks IN, Young GO, Winter T, Louw JA, Zak J, Johnston DA, Tigler-Wybrandi NA, Bridger SA. Duration of acid inhibition after withdrawal of omeprazole treatment in DU patients in remission. S Afr Med J 1992;82:42A.
29. Zaninotto G, Di Mario F, Constantini M, Baffa R, Germanà B, Dal Santo PL, Rugge M, Bolzan M, Naccarato R, Ancona E. Oesophagitis and pH of refluxate: An experimental and clinical study. Br J Surg 1992;79:161-164.
30. Johnson LF, Harmon JW. Experimental esophagitis in a rabbit model. Clinical relevance. J Clin Gastroenterol 1986;8: 26-44.
31. Kauer WKH, Burdiles P, Ireland AP, Clark GWP, Peters JH, Bremner CG, DeMeester TR. Does duodenal juice reflux into the esophagus of patients with complicated GERD? Evaluation of a fiberoptic sensor for bilirubin. Am J Surg 1995;169:98-104.
32. Sjöberg F, Gustafsson U, Tibbling L. Alkaline oesophageal reflux—An artefact due to oxygen corrosion of antimony pH electrodes. Scand J Gastroenterol 1992;27:1084-1088.
33. Cullen JJ, Kelly KA. Gastric motor physiology and pathophysiology. Surg Clin North Am 1993;73:1145-1161.

Static and Dynamic Function of the Lower Esophageal Sphincter Before and After Laparoscopic Nissen Fundoplication

Peter F. Crookes, M.D., Manfred P. Ritter, M.D., William E. Johnson, M.D., Cedric G. Bremner, M.D., Jeffrey H. Peters, M.D., Tom R. DeMeester, M.D.

The means by which fundoplication protects against reflux is disputed. We studied the resting and dynamic features of the lower esophageal sphincter (LES) and 24-hour pH monitoring in 26 patients before and after laparoscopic Nissen fundoplication. Resting features were LES pressure, abdominal length, and total length. Dynamic function was assessed by the residual pressure in the LES during a swallow measured as the bolus flowed though the LES. All patients experienced near-total relief of heartburn and all but one had normal postoperative acid scores. Resting LES characteristics were restored to normal. Mean residual pressure on swallowing was 7.1 ± 3.2 mm Hg in the patients postoperatively compared with 1.2 ± 1 mm Hg preoperatively and 4.0 ± 2.4 mm Hg in normal subjects. Eighteen of 26 patients had residual LES pressure within the normal range (<8.2 mm Hg). There was a tendency for residual pressures to be lower as experience with the procedure was gained. Incomplete LES relaxation is not necessary for effective functioning of a Nissen fundoplication. In construction of a Nissen fundoplication, creating a large retroesophageal window and deliberate dissection of the back of the posterior fundus from the left crus allows the creation of an effective antireflux procedure with restoration of static LES parameters to normal and minimal limitation of LES relaxation. (J GASTROINTEST SURG 1997;1:499-504.)

The mechanism of action of fundoplication is disputed. Several groups have demonstrated that Nissen fundoplication restores the resting mechanical characteristics of the lower esophageal sphincter (LES) to normal.[1,2] A different explanation was suggested by Ireland et al.,[3] namely, that fundoplication works by abolishing the ability of the LES to relax completely. According to these investigators, the fundoplication is therefore believed to reduce the frequency and extent of transient LES relaxations, and consequently to diminish the opportunity for episodes of acid reflux. Such a view also implies that the antireflux effect of fundoplication is inseparable from the tendency to dysphagia, since both rely on the same phenomenon. Furthermore, there is a perception that dysphagia is a more frequent problem after laparoscopic fundoplication than after open fundoplication.[4] The aim of this study was to compare both the resting characteristics and the ability to relax of the LES before and after laparoscopic Nissen fundoplication, in an attempt to shed light on the mechanism of action of fundoplication, and to determine whether improvements in postoperative function were related to the surgeon's experience with this technique.

PATIENTS AND METHODS

Beginning in July 1992, patients undergoing laparoscopic Nissen fundoplication were prospectively studied clinically and physiologically before and after surgery. Twenty-six consecutive patients (17 men and 11 women; median age 49 years; IQ range 39 to 57) in whom postoperative motility studies were conducted form the basis of this report. Symptoms were scored using a structured questionnaire administered by a nurse, who questioned patients specifically about the presence and severity of heartburn, regurgitation, dysphagia, chest pain, pulmonary symptoms, and lower gastrointestinal symptoms. Patients underwent upper gastrointestinal endoscopy with biopsy, video

From the Department of Surgery, University of Southern California School of Medicine, Los Angeles, Calif.
Reprint requests: Tom R. DeMeester, M.D., Department of Surgery, University of Southern California School of Medicine, 1510 San Pablo St., Suite 514, Los Angeles, CA 90033-4612.

esophagogram, esophageal manometry, and 24-hour esophageal pH monitoring before surgery. Other physiologic tests such as ambulatory manometry or bilirubin monitoring were selectively employed. Postoperatively, regular clinical follow-up was maintained by clinic visits, and physiologic tests were scheduled from 6 to 18 months postoperatively. The 26 patients who agreed to undergo postoperative manometry and 24-hour esophageal pH monitoring represent a subgroup from a group of 158 patients in whom laparoscopic Nissen fundoplications have been performed since July 1992.

Esophageal Manometry

Manometry was performed as previously described using a low-compliance pneumohydraulic perfused system consisting of an eight-lumen catheter with four circumferential side holes at 90 degrees and four additional side holes at 5 cm intervals along the catheter.[5] The specific parameters studied included LES pressure, measured at the respiratory inversion point, overall length, and the length exposed to intra-abdominal pressure (abdominal length).

Relaxation of the LES in response to swallowing was measured by positioning the catheter so that the four circumferential side holes were located in the upper part of the high-pressure zone. This prevented the catheter from recording intragastric pressure secondary to upward movement of the LES during the swallow-induced esophageal shortening. Five swallows of 5 cc water were given. To assess the resistance to the flow of liquid through the LES, the residual pressure in the LES was measured by the four circumferential side holes at the moment when the bolus was judged to the flowing through the sphincter. This moment was determined as follows: the upstroke of the peristaltic wave in the transducer 5 cm proximal to the sphincter was recorded. This point indicates luminal closure and consequently the bolus is distal to this proximal transducer. Combined radiologic and manometric studies indicate that a 5 cc bolus has reached the sphincter at that point.[6] Identification of the upstroke of the peristaltic wave 5 cm proximal to the LES is an objective and readily identifiable moment at which to measure the residual pressure in the LES. The residual pressure was thus measured by each of the four circumferential side holes during five swallows, and the final value was the mean of these 20 readings. The results were compared with those of 45 recently studied normal volunteers. The technique is illustrated in Fig. 1.

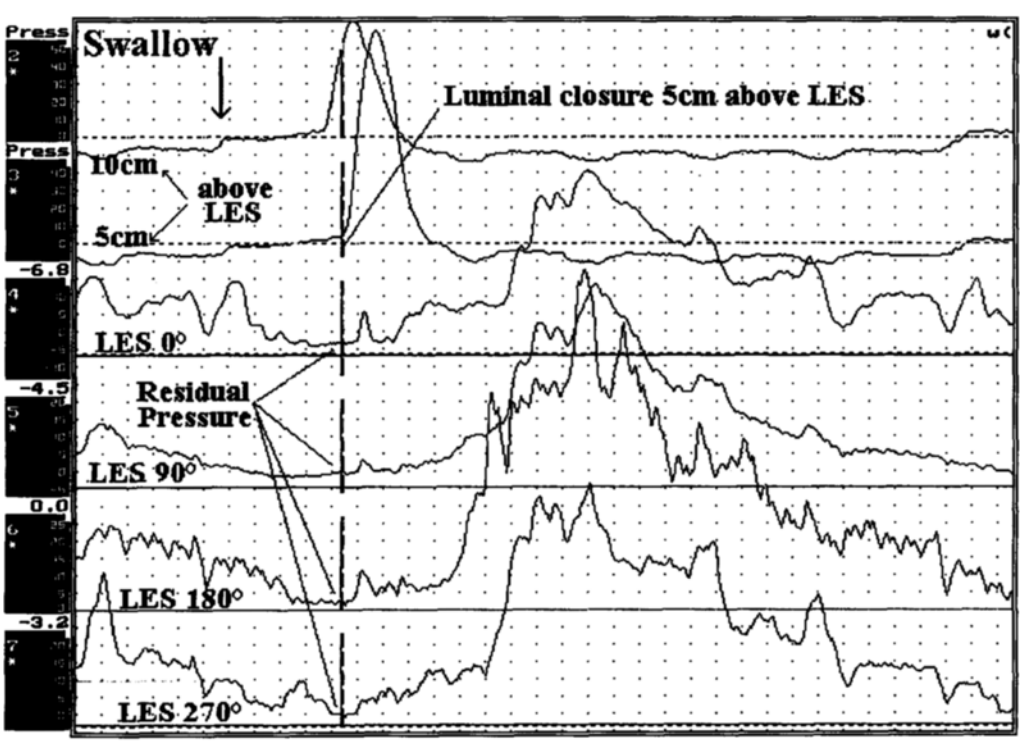


Fig. 1. Tracing demonstrating relaxation of the LES in response to a swallow. The residual pressure is measured at the point where the upstroke of the peristaltic wave in the channel 5 cm above the LES indicates luminal closure.

Esophageal pH Monitoring

Twenty-four-hour pH monitoring was performed as previously described by positioning a glass pH electrode (Mui Scientific, Toronto, Ontario, Canada) 5 cm above the manometrically measured upper border of the LES.[7] The electrode was connected to a digital recording device (Microdigitrapper, Synectics Medical, Inc., Irving, Tex.) and pH was continually monitored for 24 hours. The patient's diet was limited to foods having a pH in the range 5 to 7. The stored data were transferred to a personal computer and analyzed according to our standard protocol, which includes measurement of the percentage of time the pH is less than 4 for the total monitored period, the upright period, and the supine period. The total number of reflux episodes, the number of episodes longer than 5 minutes, and the duration of the longest episode were also calculated by the standard software analysis (Multigram, Gastrosoft, Inc., Irving, Tex.).

Surgical Technique

Laparoscopic Nissen fundoplication was performed as previously described and included closure of the crura and division of the short gastric arteries.[8] In all patients an attempt was made to reproduce precisely the steps previously shown to be important in the postoperative outcome after open fundoplication.[1] These included thorough mobilization of the fundus by division of the short gastric vessels, closure of the crura, sizing the fundoplication with an intraesophageal bougie of 60 F, so as to create a tension-free fundus cradling the esophagus, with its anterior and posterior lips united by a horizontal mattress suture reinforced by Teflon pledgets. The nasogastric tube was removed the next morning and liquids were allowed by mouth. The patients were discharged on postoperative day 2 with instructions to remain on soft foods for 3 to 4 weeks. As experience was gained, minor modifications were introduced into the routine technique to take account of the effect of magnification. These included creating a larger retroesophageal window and a more deliberate dissection of the back of the cardia off the left crus.

RESULTS

All patients had total or near-total relief of heartburn. Three complained of mild symptoms but had normal scores on pH monitoring. Four patients had persistent dysphagia, which was mild in three. The one patient with severe dysphagia had a nonspecific esophageal motility disorder, which became more pronounced postoperatively.

The resting parameters of the LES were restored to normal values (Fig. 2). Esophageal acid exposure was brought into the normal range in all but one patient in whom the percentage of time the pH was less than 4 decreased from 43.9% to 8.9% (Fig. 3). Preoperatively the mean residual LES pressure in the patients was 1.1 ± 1.0 mm Hg, rising to a mean of 7.1 ± 3.2 mm Hg postoperatively. Mean residual pressure

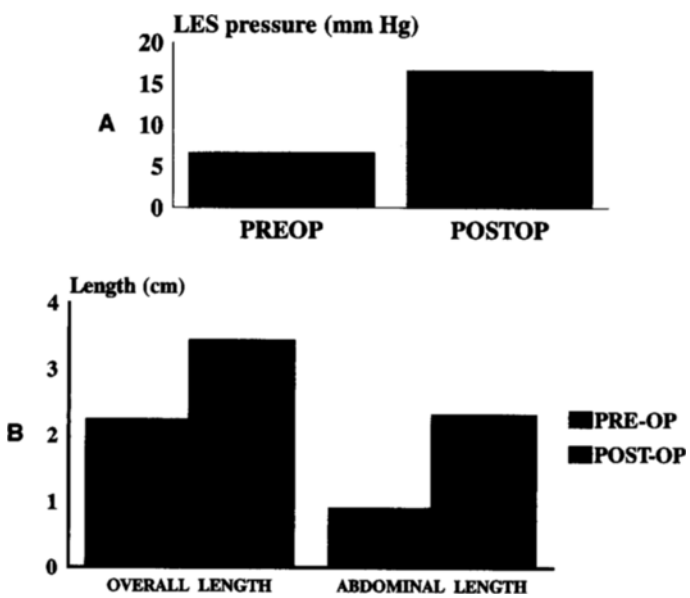


Fig. 2. LES pressure **(A)** and overall length and total length **(B)** before and after laparoscopic Nissen fundoplication.

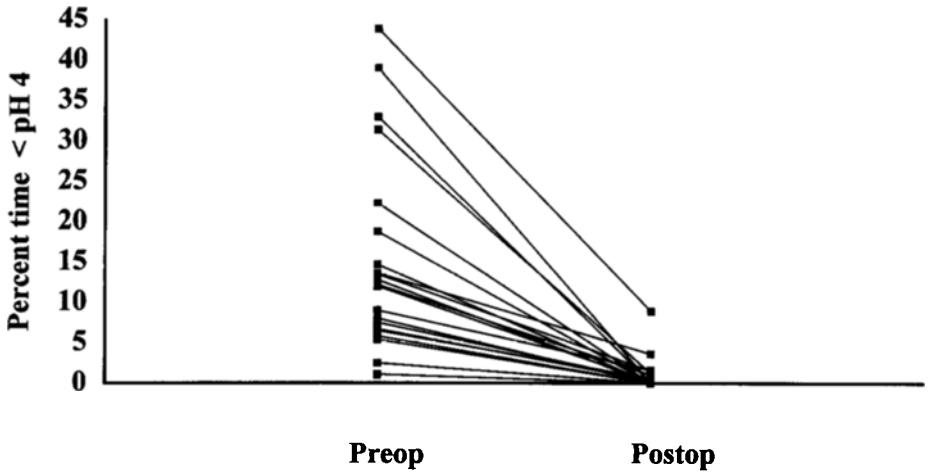


Fig. 3. Twenty-four-hour esophageal pH before and after laparoscopic Nissen fundoplication.

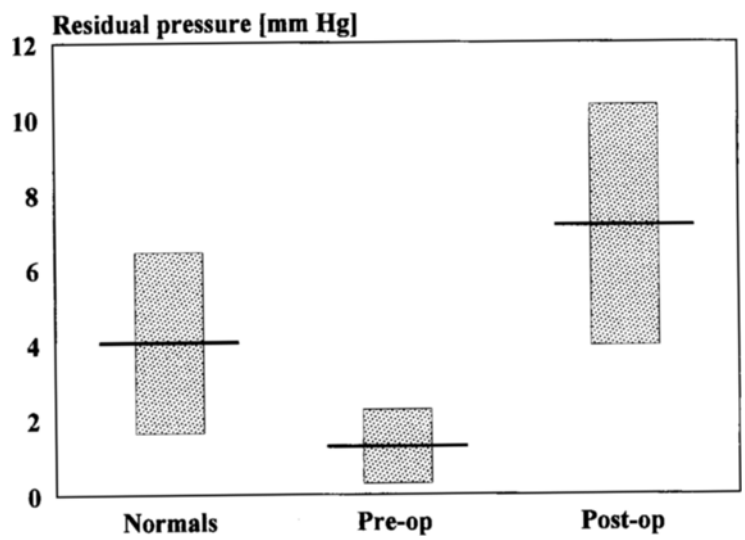


Fig. 4. LES residual pressure in normal control subjects and in patients before and after laparoscopic Nissen fundoplication.

in control subjects was 4.2 ± 2.4 mm Hg. Both preoperative and postoperative pressures in the patients differed significantly from values in control subjects ($P < 0.001$; Fig. 4). The ninety-fifth percentile for the normal subjects was 8.2 mm Hg: 18 of 26 patients had residual pressures within the normal range. LES residual pressure was negatively correlated with the surgeon's level of experience ($r = 0.52$; $P = 0.009$), with the higher values tending to occur in the early patients (Fig. 5). There was no significant correlation between residual pressure and esophageal acid exposure ($r = -0.14$; $P = 0.19$).

DISCUSSION

We have shown that laparoscopic Nissen fundoplication effectively controls reflux both objectively and symptomatically. It not only restores the resting characteristics of the LES to normal but also increases the mean LES residual pressure during swallowing by approximately 6 mm Hg. In only one third of the patients does this residual pressure exceed the ninety-fifth percentile of normal. As experience with the procedure was gained, a conscious attempt was made to create a larger retroesophageal window for the fundus to pass through and to ensure that the plicated

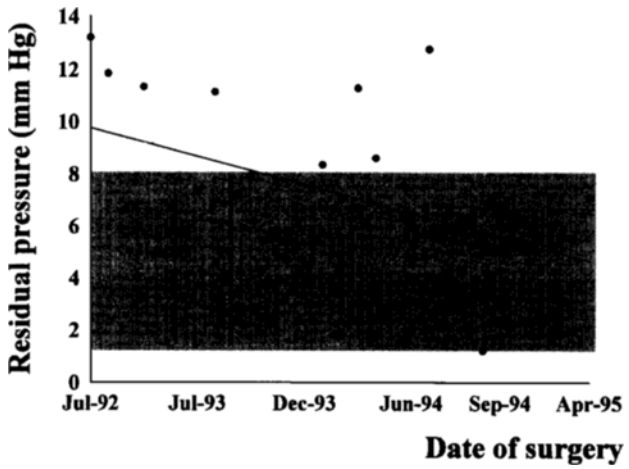


Fig. 5. The trend toward decreasing LES residual pressure as experience in laparoscopic fundoplication increased.

fundus lay comfortably without tension or torsion, cradling the lower esophagus. This was reflected in the tendency for the more recent patients to have a lower residual pressure. It may be argued that the 26 patients we studied are not representative of the whole group, since those agreeing to postoperative studies may include a higher proportion of symptomatic patients. However, all patients are offered clinical and physiologic follow-up, and the percentage who can be persuaded to undergo postoperative physiologic studies is comparable to other investigations.

Measurement of LES function during relaxation is difficult, and there is no unanimity as to the best manometric criterion for adequate relaxation.[9-12] Most investigators have used the Dentsleeve, but measurement of the resting parameters of the LES such as overall length and intra-abdominal length cannot be achieved with this catheter. Using a catheter with four circumferential side holes at the same level gives information from different segments of the LES, but inaccurate placement of the catheter is possible especially when the LES is short. This problem rarely arises after fundoplication, and accurate positioning of the catheter was possible in 25 of the 26 postoperative patients. Studies employing both manometric assessment of residual pressure combined with simultaneous radiologic assessment of bolus transit through the LES would obviously provide stronger evidence that our measure of residual pressure reflects LES relaxation, but such studies are difficult in routine clinical practice. Previously reported studies using this combination of methods show that luminal closure 5 cm above the LES best corresponds to the time of bolus transit through the LES.[6] In practical terms this is a simple manometric measurement to obtain and it is not dependent on the experience or judgment of the person reading the tracing, since the point at which the LES pressure is measured is so clearly discernible from the manometric tracing.

It is important to understand that the residual pressure detected by this technique is merely a marker of outflow resistance and is not itself the cause of the resistance. Similarly, it is not possible to make a direct link between the residual pressure and the symptom of dysphagia, as the subjective sensation of dysphagia is related to the interaction of many other factors such as esophageal body peristalsis and afferent sensitivity. In our small group of patients, only four had any significant dysphagia and the patient with severe dysphagia had an esophageal motility disorder.

Based on this measurement, patients after laparoscopic Nissen fundoplication, as a group, have higher residual pressures than normal subjects. However, since in most patients the residual pressure falls within the normal range and yet reflux is effectively abolished, the creation of an elevated residual pressure is not necessary for the effective functioning of the fundoplication.

That this method of assessment is of surgical relevance is demonstrated by the gradual reduction in postoperative residual pressure as experience was gained with laparoscopic fundoplication. It is more difficult to judge the adequacy of the retroesophageal window during Nissen fundoplication when the procedure is performed laparoscopically, because of the tendency for structures to be magnified and because the ability to widen the aperture digitally is lost. A conscious effort must be made to dissect the posterior part of the fundus and cardia off the left crus to allow the fundus to be passed behind the esophagus and lie comfortably without tension. Our experience shows that such diligence has a tendency to result in lower residual pressures on swallowing without loss of antireflux protection. Although it is not possible in this small series of patients to correlate a low residual pressure with freedom from dysphagia, it seems only logical to perform the operation in such a way as to maintain dynamic function of the LES as close to normal as possible without compromising reflux protection.

REFERENCES

1. DeMeester TR, Bonavina L, Albertucci M. Nissen fundoplication for gastroesophageal reflux diseases. Evaluation of primary repair in 100 consecutive patients. Ann Surg 1986;204:9-20.

2. Johnsson J, Johnsson F, Joelsson B, Floren C-H, Walther B. Outcome 5 years after 360° fundoplication for gastro-oesophageal reflux disease. Br J Surg 1993;80:46-49.
3. Ireland AC, Holloway RH, Toouli J, Dent J. Mechanisms underlying the antireflux action of fundoplication. Gut 1993;34:303-308.
4. Peters JH, Heimbucher J, Kauer WKH, Incarbone R, Bremner CG, DeMeester TR. Clinical and physiologic comparison of laparoscopic and open Nissen fundoplication. J Am Coll Surg 1995;180:385-393.
5. Crookes PF, Peters JH, DeMeester TR. Physiology of the antireflux barrier and diagnostic tests of foregut function. Semin Laparosc Surg 1995;2:10-26.
6. Kahrilas PJ, Dodds WJ, Hogan WJ. The effect of peristaltic dysfunction on esophageal volume clearance. Gastroenterology 1988;94:73-80.
7. DeMeester TR, Wang C-I, Wernly JA, Pellegrini CA, Little AG, Klementschitch P, Bermudez G, Johnston LF, Skinner DB. Technique, indications, and clinical use of 24-hour pH monitoring. J Thorac Cardiovasc Surg 1980;79:656-670.
8. Peters JH, DeMeester TR. Technique of laparoscopic Nissen fundoplication. Semin Laparosc Surg 1995;2:27-44.
9. Dent J. A new technique for continuous sphincter pressure measurement. Gastroenterology 1976;71:263-267.
10. Castell JA, Dalton CB, Castell DO. On-line computer analysis of human lower esophageal sphincter relaxation. Am J Physiol 1988;255 (Gastrointest Liver Physiol 18):G794-G799.
11. Aliperti G, Clouse RE. Incomplete lower esophageal sphincter relaxation in subjects with peristalsis: Prevalence and clinical outcome. Am J Gastroenterol 1991;86:609-614.
12. DiMarino AJ, Cohen S. Characteristics of lower esophageal sphincter function in symptomatic diffuse esophageal spasm. Gastroenterology 1974;66:1-6.

Importance of Preoperative and Postoperative pH Monitoring in Patients With Esophageal Achalasia

Marco G. Patti, M.D., Massimo Arcerito, M.D., Jenny Tong, M.D., Mario De Pinto, M.D., Mario de Bellis, M.D., Anne Wang, M.D., Carlo V. Feo, M.D., Sean J. Mulvihill, M.D., Lawrence W. Way, M.D.

Gastroesophageal reflux (GER) can develop in patients with esophageal achalasia either before treatment or following pneumatic dilatation or Heller myotomy. In this study we assessed the value of pre- and postoperative pH monitoring in identifying GER in patients with esophageal achalasia. Ambulatory pH monitoring was performed preoperatively in 40 patients with achalasia (18 untreated patients and 22 patients after pneumatic dilatation), 27 (68%) of whom complained of heartburn in addition to dysphagia (group A), and postoperatively in 18 of 51 patients who underwent a thoracoscopic (n = 30) or laparoscopic (n = 21) Heller myotomy (group B). The DeMeester reflux score was abnormal in 14 patients in group A, 13 of whom had been treated previously by pneumatic dilatation. Two types of pH tracings were seen: (1) GER in eight patients (7 of whom had undergone dilatation) and (2) pseudo-GER in six patients (all 6 of whom had undergone dilatation). Therefore 7 (32%) of 22 patients had abnormal GER after pneumatic dilatation. Postoperatively (group B) seven patients had abnormal GER (6 after thoracoscopic and 1 after laparoscopic myotomy). Six of the seven patients were asymptomatic. These findings show that (1) approximately one third of patients treated by pneumatic dilatation had GER; (2) symptoms were an unreliable index of the presence of abnormal GER, so pH monitoring must be performed in order to make this diagnosis; and (3) the preoperative detection of GER in patients with achalasia is important because it influences the choice of operation. (J GASTROINTEST SURG 1997;1:505-510.)

At the time they present for surgical care, approximately half of patients with esophageal achalasia complain of heartburn in addition to dysphagia.[1] This paradoxical situation can result from gastroesophageal reflux (GER)[2,3] or from stasis and fermentation of food in the esophagus.[3,4] Most often, GER is a complication of treatment, which is not surprising since pneumatic dilatation and Heller myotomy intentionally disrupt the lower esophageal sphincter.[5-12]

In this study we assessed in patients with esophageal achalasia the following: (1) whether the diagnosis of GER could be made reliably based on symptoms alone; (2) the incidence of GER before and after operative treatment; and (3) the effects of this information on the choice of operation.

PATIENTS AND METHODS

Between October 1989 and November 1995, a total of 1200 patients were referred to the Swallowing Center of the University of California, San Francisco, for evaluation of foregut symptoms. Esophageal achalasia was diagnosed in 87 patients (7%) by esophageal manometry, upper gastrointestinal series, and endoscopy. Ambulatory pH monitoring was performed in 40 of these patients (group A). Eighteen patients (45%) had had either no previous treatment or medications alone, and 22 patients (54%) had been treated by balloon dilatation. Fig. 1 shows the demographic data and severity of symptoms. All patients had dysphagia, and 27 patients (68%) complained of discomfort that was thought by the treating physicians to be heartburn.

From Department of Surgery, University of California, San Francisco, San Francisco, Calif.
Presented at the Thirty-Seventh Annual Meeting of The Society for Surgery of the Alimentary Tract, San Francisco, Calif., May 19-22, 1996.
Reprint requests: Marco G. Patti, M.D., Department of Surgery, University of California, San Francisco, 533 Parnassus Ave. (U-122), San Francisco, CA 94143-0788.

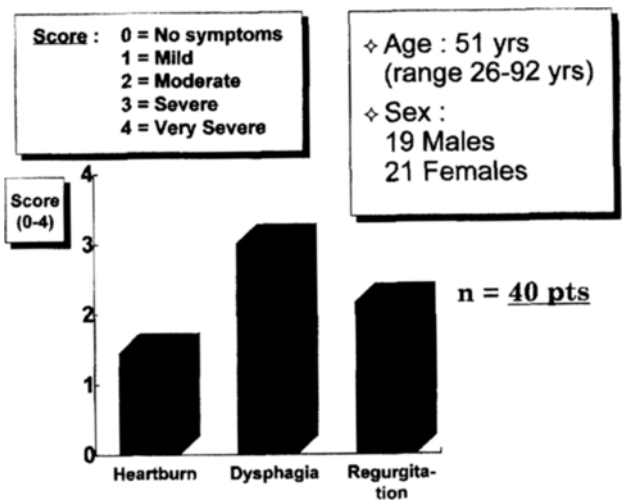


Fig. 1. Demographic data and severity of symptoms in 40 patients with esophageal achalasia.

Esophageal manometry and ambulatory pH monitoring were repeated postoperatively in 18 of 51 patients who underwent a thoracoscopic or laparoscopic Heller myotomy between January 1991 and August 1995 (group B).

Esophageal Manometry

Patients were studied in the morning after an overnight fast. All medications that could interfere with esophageal motor function (e.g., calcium channel–blocking agents, nitrates, and prokinetic agents) were discontinued at least 24 hours before the study. The manometry catheter was placed under fluoroscopic guidance in 16 patients in group A (40%) and in all 18 patients in group B. The study was performed according to previously described techniques.[13]

Ambulatory pH Monitoring

Esophageal acid exposure was measured using a probe with an antimony sensor positioned 5 cm above the upper border of the manometrically located lower esophageal sphincter.[13] Acid-suppressing medications were discontinued 3 days (H_2 blockers) or 14 days (proton pump inhibitors) before the study. A chest radiograph was obtained to confirm that the probe was properly positioned in the esophagus. The data were analyzed using a commercial software program (Gastrosoft, Synectics Medical, Irving, Tex.).

The tracing of patients with abnormal DeMeester scores (i.e., score >15)[14] were examined to distinguish between bona fide acid reflux (GER) and acid pH resulting from food stasis in the esophagus (pseudo-GER). Although a certain amount of GER is normal (i.e., DeMeester score <15), the term gastroesophageal reflux in this report is used exclusively to refer to abnormal amounts of reflux (i.e., DeMeester score >15).

Thoracoscopic Myotomy

Thirty patients underwent myotomy through a left chest approach using previously described techniques.[11,15] The myotomy was 7 cm long and extended 0.5 cm onto the gastric wall. No antireflux procedure was included.

Laparoscopic Myotomy

In 21 patients the myotomy was performed through an abdominal approach. The myotomy was 7 cm long and extended 1.0 to 1.5 cm beyond the lower esophageal sphincter onto the stomach. In addition to the myotomy, a Dor fundoplication was performed in all 21 of these patients using techniques similar to those described by Rosati et al.[16]

Statistical Analysis

Student's *t* test and the Mann-Whitney U test were used for statistical evaluation of the data. All results are expressed as mean ± standard error of the mean. Differences were considered significant at $P < 0.05$.

RESULTS

Group A

The DeMeester score was normal (<15) in 26 patients (65%) (average 2 ± 1; range 0.2 to 14) and abnormal (>15) in 14 patients (35%) (mean 32 ± 5; median 25; range 16 to 76). Fig. 2 shows the severity of the symptoms in patients with normal and abnormal DeMeester scores. The nature and severity of symptoms were similar in the two groups (P = NS). Table I shows the treatment modalities used in these patients before they were tested.

There were two types of pH monitoring tracings (Fig. 3) among the 14 patients with abnormal scores: (1) true GER (n = 8), characterized by intermittent drops of the pH below 3 with subsequent clearance and return of the pH to above 5, and (2) pseudo-GER, or false GER (n = 6), characterized by a slow downward drift of the pH curve below 4 without a return to higher levels. The nature and severity of symptoms were similar in these two groups (P = NS) (Fig. 4). Seven patients with true GER and all patients

Table I. Treatment modalities used in patients with normal (n = 26) and abnormal (n = 14) DeMeester scores prior to testing

Treatment	Normal DeMeester score No. (%)	Abnormal DeMeester score No. (%)
Medications		
Nitrates	3 (12)	0
Calcium channel blockers	8 (31)	3 (21)
Prokinetic agents	2 (8)	2 (14)
H_2 blockers	5 (19)	4 (28)
Proton pump inhibitors	5 (19)	7 (50)
Botulinus toxin injection	0	1 (7)
Dilatation	9 (35)	13 (93)
No. of dilatations/patient	2 (range 1-3)	1.7 (range 1-4)

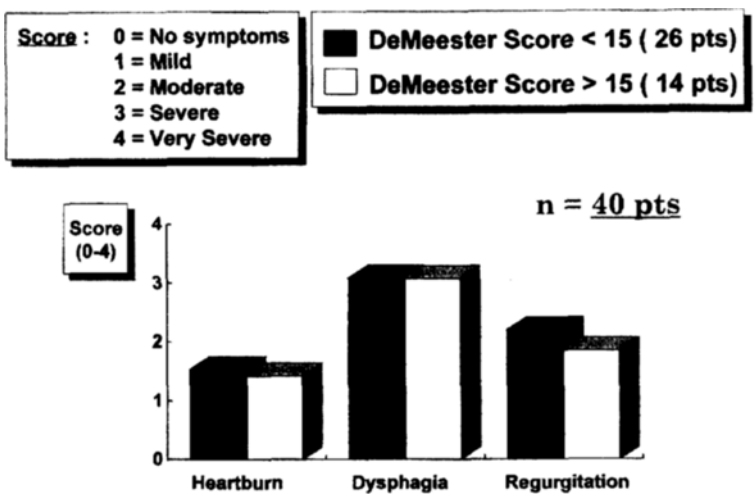


Fig. 2. Severity of symptoms in patients with normal and abnormal DeMeester scores. There was no difference in the severity of symptoms between the two groups.

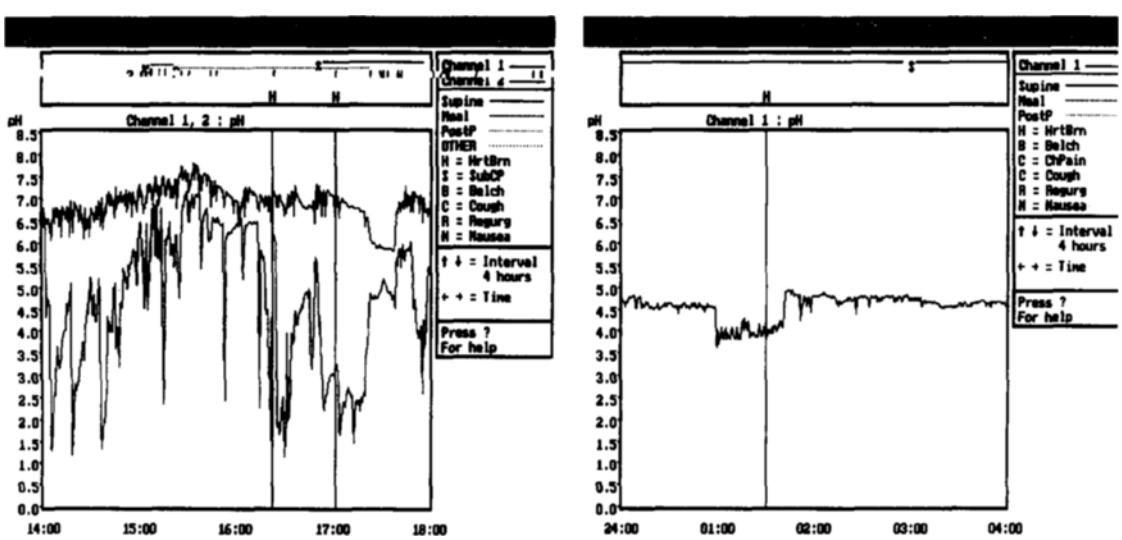


Fig. 3. Types of pH tracings in patients with esophageal achalasia. GER = gastroesophageal reflux.

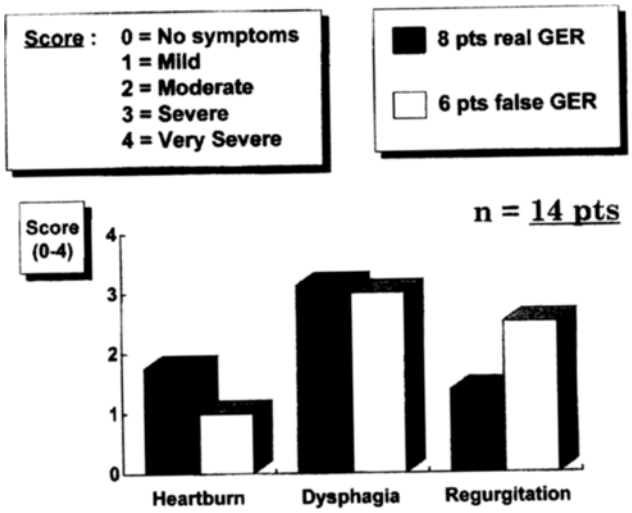


Fig. 4. Severity of symptoms in patients with gastroesophageal reflux (GER) and pseudo-GER. There was no difference in the severity of symptoms between the two groups.

with pseudo-GER had undergone dilatations in the past (1.7 dilatations/patient; range 1 to 4). Therefore 7 (32%) of 22 patients who had undergone dilatation had true GER. The remaining patient with GER (DeMeester score 21) had had no previous invasive treatment.

Group B

Postoperative pH monitoring (n = 18 patients) showed abnormal (real) GER in 6 of 10 patients after thoracoscopic myotomy (average DeMeester score 38 ± 10; median score 24; range 21 to 77) and in one of eight patients following laparoscopic myotomy and Dor fundoplication (DeMeester score 19). Only one of these seven patients with GER (DeMeester score 61) complained of heartburn. None of these patients had abnormal (real) GER preoperatively, and therefore the postoperative abnormal reflux discovered by pH monitoring was attributed to the operation. One patient had GER and dysphagia after dilatation but before the Heller myotomy, and in this patient laparoscopic Heller myotomy and Dor fundoplication eliminated both.

Among these 18 patients, good to excellent results in the relief of dysphagia were obtained in nine patients following thoracoscopic myotomy (90%) and in all patients following laparoscopic myotomy (100%). Overall, 17 patients (94%) had good to excellent results. Six patients (33%) who experienced heartburn-like discomfort postoperatively had normal DeMeester scores on ambulatory pH monitoring.

DISCUSSION

Preoperative Assessment

Symptoms are rarely specific enough by themselves to diagnose esophageal functional disorders.[17,18] For example, only two thirds of those who are thought to have gastroesophageal reflux disease based on a complaint of heartburn have abnormal reflux on 24-hour pH monitoring.[17,18] Only one third of patients with dysphagia who are initially thought to have an esophageal motor disorder ultimately are found to have abnormal motility when tested.[18] Therefore treatment should rarely be instituted on the basis of symptoms alone, inasmuch as esophageal function tests are necessary to make an accurate diagnosis.[17-19]

In patients with achalasia, heartburn can result from GER or stasis and fermentation of food within the esophagus.[1-4] Ambulatory pH monitoring yields abnormal scores in both cases, but a distinction between the two is possible by examining the tracings (Fig. 3). In previously untreated patients with achalasia, heartburn is most often caused by slow esophageal emptying, for GER is uncommon in this situation. On the other hand, GER is relatively common following esophageal balloon dilatation. Even if our study was performed on a selected group of patients (i.e., patients with residual dysphagia after pneumatic dilatation), it is noteworthy that one third of those who had undergone esophageal dilatation had abnormal esophageal acid exposure on 24-hour pH monitoring, and only 70% of them experienced heartburn. The latter shows that the sensitivity as well as the specificity of symptoms is poor in reflecting the status of esophageal functional disease. Sixty-seven percent of the patients who had undergone dilatation and were found to have pseudo-GER had heartburn in addition to dysphagia, both symptoms being indications that the dilatations had not decreased the outflow resistance sufficiently; slow emptying persisted.

The identification of patients with GER after dilatation is important for the following reasons: (1) reflux is often clinically silent, and untreated patients are at risk of developing a stricture or Barrett's esophagus; (2) regardless of the effect on dysphagia, additional dilatations can only worsen the reflux; and (3) if surgery is performed, it must include an antireflux feature (i.e., fundoplication) in addition to the myotomy. Thus we recommend routine preoperative pH monitoring in any patient with achalasia who has symptoms suggestive of heartburn or a history of previous balloon dilatations.

Postoperative Assessment

GER was present in 6 (60%) of 10 patients tested after thoracoscopic myotomy and was marginally

present in one (13%) of eight patients after laparoscopic myotomy and Dor fundoplication. None of these patients had abnormal GER preoperatively. Six of these seven patients were asymptomatic. Streitz et al.[24] reported that when heartburn appeared following a myotomy for achalasia, the average time of onset was about 10 years postoperatively. Taken together, these observations suggest that the silent phase can be quite long, since it seems more likely that the sphincter incompetence occurred as an immediate effect of the myotomy rather than something that developed years later.

The best operation for esophageal achalasia has been debated for years. Traditionally, the results of surgery have been based on symptomatic assessment, which is now known to be less reliable than originally thought.[7-9] Because a myotomy eliminates or largely eliminates the antireflux barrier in patients who have no esophageal peristalsis, and because reflux is often asymptomatic, it may be important to identify by pH monitoring which patients have reflux postoperatively before a stricture or Barrett's esophagus develops.[20-22] These patients, in fact, can be treated by H_2 blockers or proton pump inhibitors. Alternatively, if reflux has occurred after thoracoscopic myotomy, a partial fundoplication could be added laparoscopically.

Ambulatory pH monitoring has recently been used to assess the frequency of GER after a Heller myotomy, whether performed as an open or minimally invasive procedure.[10,23-27] When the operation was performed by laparotomy, approximately 10% of patients had reflux,[10,23] and when it was performed through a left thoracotomy, 29% of patients had reflux.[24] In contrast, fewer patients had reflux following a laparoscopic Heller myotomy with a Toupet (0% of patients)[25] or a Dor (6% of patients) fundoplication.[26,27] Furthermore, a laparoscopic Heller myotomy with fundoplication is preferred for patients who have dysphagia and reflux following dilatation because it corrects both abnormalities.

These findings show that (1) symptoms are not reliable to diagnose GER; (2) preoperative pH monitoring is essential to identify those patients with achalasia who have GER; (3) approximately one third of our patients who had been treated by dilatation had GER; and (4) GER is uncommon in patients with this disease who have not undergone dilatation or surgery.

REFERENCES

1. Spechler SJ, Souza RF, Rosenberg SJ, Ruben RA, Goyal RK. Heartburn in patients with achalasia. Gut 1995;37:305-308.
2. Shoenut JP, Micflikier AB, Yaffe CS, Den Boer B, Teskey JM. Reflux in untreated achalasia patients. J Clin Gastroenterol 1995;20:6-11.
3. Smart HL, Foster PN, Evans DF, Slevin B, Atkinson M. Twenty-four hour oesophageal acidity in achalasia before and after pneumatic dilatation. Gut 1987;28:883-887.
4. Crookes PF, Corkill S, DeMeester TR. When is "reflux" in achalasia really reflux? [abstr] Gastroenterology 1994;106:65.
5. Vantrappen G, Hellemans J. Treatment of achalasia and related motor disorders. Gastroenterology 1980;79:144-154.
6. Sauer L, Pellegrini CA, Way LW. The treatment of achalasia. Arch Surg 1989;124:929-932.
7. Okike N, Payne WS, Neufeld DM, Bernatz PE, Pairolero PC, Sanderson DR. Esophagomyotomy versus forceful dilation for achalasia of the esophagus: Results in 899 patients. Ann Thorac Surg 1979;28:119-125.
8. Ferguson MK. Achalasia: Current evaluation and therapy. Ann Thorac Surg 1991;52:336-342.
9. Ellis FH, Gibb SP, Crozier RE. Esophagomyotomy for achalasia of the esophagus. Ann Surg 1980;192:157-161.
10. Bonavina L, Nosadini A, Bardini R, Baessato M, Peracchia A. Primary treatment of esophageal achalasia. Arch Surg 1992;127:222-227.
11. Pellegrini C, Wetter LA, Patti M, Leichter R, Mussan G, Mori T, Bernstein G, Way L. Thoracoscopic esophagomyotomy. Initial experience with a new approach for the treatment of achalasia. Ann Surg 1992;216:291-299.
12. Wehrmann T, Jacobi V, Jung M, Lembcke B, Caspary WF. Pneumatic dilatation in achalasia with a low-compliance balloon: Results of a 5-year prospective evaluation. Gastrointest Endosc 1995;42:31-36.
13. Patti MG, Debas HT, Pellegrini CA. Clinical and functional characterization of high gastroesophageal reflux. Am J Surg 1993;165:163-168.
14. Jamieson JR, Stein HJ, DeMeester TR, Bonavina L, Schwizer W, Hinder RA, Albertucci M. Ambulatory 24-hour esophageal pH monitoring: Normal values, optimal thresholds, specificity, sensitivity, and reproducibility. Am J Gastroenterol 1992;87:1102-1111.
15. Patti MG, Pellegrini CA, Arcerito M, Tong J, Mulvihill SJ, Way LW. Comparison of medical and minimally invasive surgical therapy for primary esophageal motility disorders. Arch Surg 1995;130:609-616.
16. Rosati R, Fumagalli U, Bonavina L, Segalin A, Montorsi M, Bona S, Peracchia A. Laparoscopic approach to esophageal achalasia. Am J Surg 1995;169:424-427.
17. Patti MG, Bortolasi L, Arcerito M, Tong J, Murgia AP, Way LW. Clinical and radiographic findings are unreliable to diagnose gastroesophageal reflux disease [abstr]. Gastroenterology 1995;108:1238.
18. Costantini M, Crookes PF, Bremner RM, Hoeft SF, Ehsan A, Peters JH, Bremner CG, DeMeester TR. Value of physiologic assessment of foregut symptoms in a surgical practice. Surgery 1993;114:780-787.
19. Fuchs KH, DeMeester TR, Albertucci M. Specificity and sensitivity of objective diagnosis of gastroesophageal reflux disease. Surgery 1987;102:575-580.
20. Ellis FH Jr. Oesophagomyotomy for achalasia: A 22-year experience. Br J Surg 1993;80:882-885.
21. Jaakkola A, Reinikainen P, Ovaska J, Isolauri J. Barrett's esophagus after cardiomyotomy for esophageal achalasia. Am J Gastroenterol 1994;89:165-169.
22. Jaakkola A, Ovaska J, Isolauri J. Esophagocardiomyotomy for achalasia. Eur J Surg 1991;157:407-410.
23. Parrilla Paricio P, Martinez de Haro L, Ortiz A, Aguayo JL. Achalasia of the cardia: Long-term results of esophagomyotomy and posterior partial fundoplication. Br J Surg 1990;77:1371-1374.

24. Streitz JM, Ellis FH Jr, Williamson WA, Glick ME, Aas JA, Tilden RL. Objective assessment of gastroesophageal reflux after short esophagomyotomy for achalasia with the use of manometry and pH monitoring. J Thorac Cardiovasc Surg 1996;11:107-113.
25. Swanstrom LL, Pennings J. Laparoscopic esophagomyotomy for achalasia. Surg Endosc 1995;9:286-292.
26. Mitchell PC, Watson DI, Devitt PG, Britten-Jones R, MacDonald S, Myers JC, Jamieson GG. Laparoscopic cardiomyotomy with a Dor patch for achalasia. Can J Surg 1995;38: 445-448.
27. Ancona E, Anselmino M, Zaninotto G, Costantini M, Rossi M, Bonavina L, Boccu C, Buin F, Peracchia A. Esophageal achalasia: Laparoscopic versus conventional open Heller-Dor operation. Am J Surg 1995;170:265-270.

Comparison of Ischemic and Reperfusion Injury in Canine Bowel Viability Assessment

Robert E. Brolin, M.D., Christopher Bibbo, D.O., Andrew Petschenik, B.S., Michael T. Reddell, B.S., John L. Semmlow, Ph.D.

The purpose of these experiments was to evaluate two methods of bowel viability assessment in two distinct models of intestinal ischemia. Bowel viability was assessed in 32 dogs by means of three methods: (1) a probe that quantified the intestinal electromyographic (EMG) measurements in millivolts (mV), (2) Doppler ultrasonography, and (3) perfusion fluorometry, which quantified serosal blood flow in indexed dye fluorescence units (dfi). Ischemia was created using one of two methods: (1) a chronic model in which the blood supply to 40 cm of ileum was ligated and viability assessed 24 hours later, or (2) an acute model in which the main superior mesenteric artery was occluded for 3½ hours and then released. Viability parameters were assessed every 5 minutes for 30 minutes after release. After viability assessment was completed, the ischemic bowel was resected and anastomosed at the site where the EMG measurements approximated 50% of the values obtained in normal bowel. In the chronic group 3 of 20 dogs died of necrosis in contrast to none of 12 dogs in the acute reperfusion group. In the acute model EMG values steadily increased after reperfusion, stabilizing by 15 minutes after release. Mean EMG values at 15 through 30 minutes after release were significantly greater than the 5- and 10-minute postrelease and prerelease values, suggesting that the electromyogram is affected by reperfusion. Conversely, postrelease fluorometry measurements rapidly increased to levels that exceeded measurements obtained in normal bowel. There was a significant difference in the number of audible Doppler signals in the marginal artery of survivors of the acute vs. the chronic model. Fluorometry measurements in survivors of the acute model (99 ± 9 dfi) were significantly greater than measurements in the chronic model (54 ± 4 dfi, $P \leq 0.004$). Conversely, intermodel differences in the EMG measurements were not significant. These results show significant differences in the magnitude of ischemic damage induced by reperfusion vs. mesenteric ligation, which had a significant impact on the objective blood flow measurements that were used to predict bowel viability. The results also suggest that intestinal reperfusion injury in dogs has a negligible impact on bowel survival. (J GASTROINTEST SURG 1997;1:511-516.)

There is currently no reliable objective method for intraoperative assessment of bowel viability in the clinical setting. Diagnosis of bowel viability primarily depends on interpretation of subjective criteria such as presence of visible peristalsis, visual assessment of bowel color, bleeding from the cut edge, or presence of pulsatile arterial blood flow as determined by Doppler ultrasound imaging.[1-3] Although Doppler ultrasound is probably the most common objective method of intestinal viability assessment currently used in the clinical setting, our previous experimental studies yielded conflicting results with the use of this approach in a chronic canine model of mesenteric ligation.[4,5]

We have designed a probe that is capable of quantifying both intestinal electromyogram (EMG) and smooth muscle contractile activity in the ischemic small bowel.[5,6] In our previous studies with this probe, we used a chronic canine model of ischemia that consistently produced a gradation of ischemic damage in a 40 cm length of ileum. In those experiments the myoelectric measurements were compared with Doppler ultrasound and quantitive perfusion fluorometry as determinants of long-term bowel survival.

From the Departments of Surgery (R.E.B., C.B., A.P., and M.T.R.) and Biomedical Engineering (J.L.S.), University of Medicine and Dentistry of New Jersey—Robert Wood Johnson Medical School, New Brunswick, N.J.
Supported by grant 91-6-052 from the American Heart Association, New Jersey Affiliate.
Reprint requests: Robert E. Brolin, M.D., Department of Surgery, Robert Wood Johnson Medical School, New Brunswick, NJ 08903.

The primary purpose of this series of experiments was to determine whether the predictive accuracy of either the quantified myoelectric measurements or the objective blood flow measurements is affected by reperfusion injury in the bowel. Toward that end, our chronic canine model was compared with an acute model of ischemia followed by reperfusion.

MATERIAL AND METHODS

Viability Assessment Parameters

Parameters used in this study included subjective visual assessment of bowel color, two objective measurements of intestinal blood flow (i.e., perfusion fluorometry and Doppler ultrasonography), and quantified electromyography using a specially designed probe, the electronic contractility meter (ECM). Bowel color was arbitrarily graded by one observer according to the following criteria: pink/viable bowel showed no change in color in comparison with adjacent normal bowel outside of the 40 cm ischemic segment; slightly cyanotic (dusky) bowel appeared hemorrhagic and darker in color than normal bowel; moderately cyanotic bowel was blue but judged as possibly viable; and deep blue or black bowel was judged as nonviable. Doppler ultrasound data were also recorded by one observer as the presence or absence of an audible pulsatile signal in the marginal artery.

An illustration of the ECM is shown in Fig. 1. The electromyogram (EMG) is recorded by means of two 3.0 mm diameter, nonpenetrating silver electrodes located on the top arm of the probe. A computer algorithm quantifies the primary components of the EMG, the basal electrical rhythm (slow waves) and contractile (spike) activity in millivolts (mV). After a response time of 15 to 20 seconds, the quantified EMG is displayed by the ECM. The electrical circuitry of the probe has been described in detail elsewhere.[7]

Intestinal blood perfusion was measured on the serosal surface using a perfusion fluorometer (model PF4, Diversatonics Inc., Broomall, Pa.). The fluorometer uses fiberoptics to noninvasively quantify the fluorescence of sodium fluorescein in tissue. Each animal was given 15 μg/kg sodium fluorescein intravenously. Ten minutes following injection of the dye, fluorometry measurements were recorded at each 2 cm interval within the ischemic segment. Measurements are expressed in indexed dye fluorescence units (dfi).

Experimental Models

Both experimental groups underwent laparotomy for creation of intestinal ischemia under general anesthesia. Anesthesia was induced with sodium thiamylal, 8 mg/kg, and maintained with a 50% mixture of 1.0% to 1.5% halothane and nitrous oxide. During each laparotomy all dogs received lactated Ringer's solution intravenously at a rate of ≥100 ml/hr. No heparin or antibiotics were used.

The chronic model of ischemia shown in Fig. 2 was

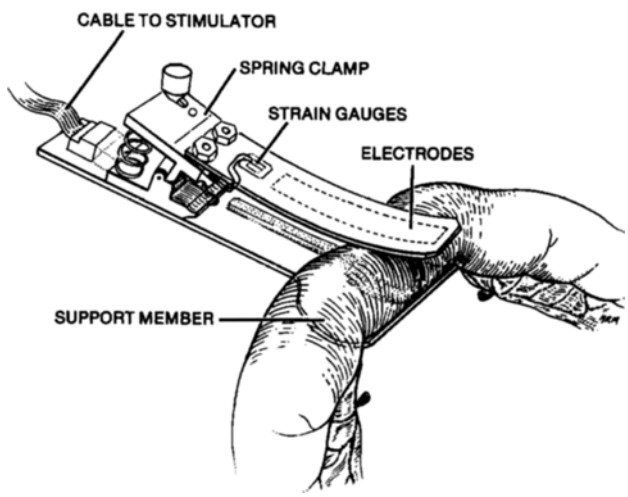


Fig. 1. Electronic contractility meter probe as it is clamped on the bowel. A spring clamp allows for precise adjustment of the flexible support arms to provide good contact with the bowel. Prior to each measurement, the intraelectrode resistance is checked to ensure good contact between the electrodes and the bowel wall. The small silver electrodes used to record the EMG are not shown. (From Brolin RE, Semmlow JL, Koch RA, et al. Myoelectric assessment of bowel viability. Surgery 1987;102:32-38.)

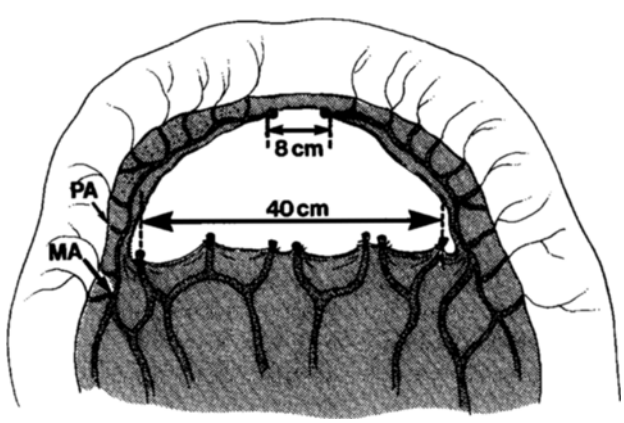


Fig. 2. The mesenteric blood supply to a 40 cm length of ileum was ligated. The marginal artery *(MA)* was also ligated for a distance of 8 cm in the center of the ischemic segment, thus depriving that length of bowel of all collateral flow. (PA = peripheral arterioles). (From Brolin RE, Semmlow JL, Koch RA, et al. Myoelectric assessment of bowel viability. Surgery 1987;102:32-38.)

used in 20 dogs. This model consistently produces a gradation of ischemic damage ranging from normal-appearing bowel at the periphery to grossly gangrenous bowel at the center of the 40 cm ischemic segment. A second laparotomy was performed 24 hours later, at which time the five viability assessment parameters were evaluated at 2 cm intervals within the 40 cm ischemic segment. EMG and fluorometry measurements were also recorded in normal bowel proximal and distal to the boundaries of the ischemic segment.

In the acute model 12 dogs underwent a 3½-hour period of ischemia followed by reperfusion. The main superior mesenteric artery was isolated and occluded at its base with a vascular snare and an atraumatic vascular clamp (Baxter CV No. 5114, Applied Medical Resources, Laguna Hills, Calif.). The marginal artery and vein at the outer boundaries of the ischemic bowel were similarly clamped en mass with Baxter clamps. The superior mesenteric vein was not occluded. Absence of arterial inflow was confirmed by Doppler ultrasonography after all clamps were applied. The length of ischemic bowel was then measured and the midpoint was marked with a serosal suture of 3-0 silk. The bowel was then covered with warm, saline-soaked towels for 3½ hours. Following the period of ischemia, the clamps were released and bowel viability was assessed using the parameters previously described. Bowel color was visually assessed before injection of the fluorescein. EMG and fluorometry measurements were recorded every 5 minutes at 20 cm intervals along the entire length of ischemic bowel.

After viability assessment was completed, resection of ischemic bowel was performed using the EMG to determine the site of resection and anastomosis. The site of resection and anastomosis in both models was predetermined at approximately 50% of the EMG value obtained in normal bowel. This value was chosen because in previous experiments using the chronic model an indexed EMG value of 0.50 was associated with problematic survival.[5,6] In the reperfusion group the bowel was merely transected and reanastomosed because the EMG values invariably increased above the 0.50 threshold 30 minutes after the clamps were released. All anastomoses were performed using an inner layer of continuous 3-0 polyglycolic acid sutures (Vicryl, Ethicon, Inc., Somerville, N.J.) and an outer layer of interrupted seromuscular 3-0 silk sutures.

Postoperatively all dogs were closely monitored according to Institutional Animal Care and Use Committee (IACUC) guidelines. Any dog that exhibited signs of sepsis was immediately killed using a mixture of potassium chloride and pentobarbital given intravenously. Dogs that recovered uneventfully were killed 10 days after resection and anastomosis. Autopsy was performed immediately after the animals were killed in all cases. At the time the animals were killed each anastomosis was inspected in situ for evidence of anastomotic leaks.

Analysis of Data

EMG and fluorometry data were analyzed using both raw and indexed (normalized) measurements according to methodology described in detail elsewhere.[5] Because our chronic model consistently produced necrosis in the center of the ischemic segment, indexing of the EMG was performed to account for the predictably lower level of myoelectric activity that is recorded in normal bowel located distal to an area of severe ischemic damage.[8,9]

All numeric data are expressed as mean values ± standard error of the mean (SEM). Comparative statistics were performed using chi-square analysis, two-tailed paired and unpaired Student's *t* tests, and two-way analysis of variance.

RESULTS

There were three deaths from anastomotic leaks in the chronic model and none in the acute model. All leaks were fatal and resulted from necrosis at the anastomotic site. There were no anastomotic leaks in either group when the EMG measurement at the resection margin was ≥60% of the EMG measurement in normal bowel.

Comparison of Experimental Models

The ischemic segments of all dogs contained regions of pink/viable bowel. Grossly gangrenous bowel was noted in all but 2 of the 20 dogs in the chronic ischemia group. Conversely, none of the 12 dogs in the acute reperfusion group had visual evidence of irreversible ischemia. Table I shows the viability assessment data obtained at the resection margin in the two models. Bowel color at the resection margin was significantly different between the two models. Dusky-appearing bowel in the reperfusion group became pink 5 to 10 minutes after the clamps were released. Bowel color in the chronic group did not change during the period of observation. In the chronic model Doppler signals were audible in the marginal artery at 17 of the 40 resection margins. Conversely, in the acute model Doppler signals were audible in the marginal artery throughout the entire small bowel immediately after the vascular clamps were released.

There were no differences in the EMG or fluorometry measurements obtained in normal bowel of

Table I. Comparison of methods of viability assessment in two experimental models

Viability parameters	Chronic model (n = 40)	Acute model (n = 24)	Difference
Intestinal color			
Pink	9	20	$X^2 = 21.0$; $P < 0.0001$*
Dusky	28	4	
Blue	3	0	
Doppler signal in marginal artery			
Present	18	24	$X^2 = 20.1$; $P < 0.0001$*
Absent	22	0	
Indexed fluorometry	54 ± 4 dfi	99 ± 9 dfi	$P \leq 0.004$†
Indexed EMG	48 ± 6 mV	49 ± 5 mV	$P = 0.91$†

N = number of observations in each model (two resection margins per dog). Data are presented as mean ± SEM. Data from the acute model were obtained immediately after reestablishment of arterial inflow. Mean EMG measurements in normal bowel were 97 ± 18 mV and 95 ± 11 mV in the chronic and acute groups, respectively. dfi = indexed dye fluorescence units; mV = millivolts.
*Difference between models computed using chi-square analysis.
†Difference between models computed using unpaired Student's *t* test.

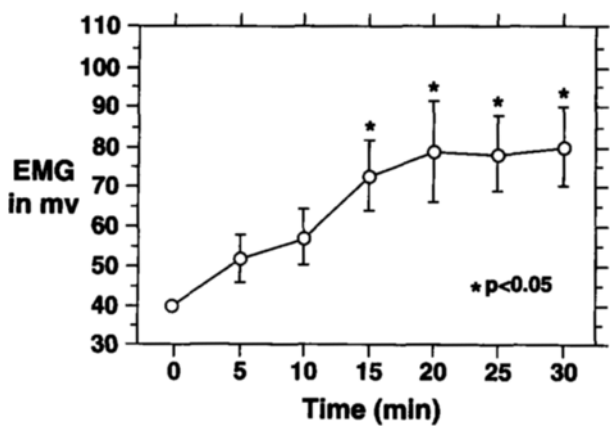


Fig. 3. EMG measurements recorded at the resection margin of the acute reperfusion model. Time zero is immediately prior to release of the clamps. Subsequent measurements were obtained at 5-minute intervals and recorded as mean ± SEM. * = significant difference vs. time zero measurement by paired Student's *t* test.

the acute vs. the chronic model. However, at the resection margin the mean indexed fluorometry measurements of dogs in the chronic model that survived were significantly less than the mean indexed measurements at the cut point of survivors in the acute reperfusion group (Table I). Conversely, the mean indexed EMG measurements obtained at the cut point of survivors in the acute model shortly after release of the clamps were no different from the corresponding measurements obtained at the resection margin from survivors in the chronic model.

The fluorometry measurements were less affected by reperfusion than the EMG measurements. After release of the clamps, fluorometry measurements increased rapidly to levels that often exceeded the measurements obtained in normal bowel. Conversely, EMG measurements increased more slowly after reperfusion as shown in Fig. 3. Mean EMG values at 15 through 30 minutes after release were significantly greater than the 5- and 10-minute postrelease and prerelease measurements.

DISCUSSION

Reperfusion injury is defined as the damage that occurs in tissue during the resumption of blood flow following an acute episode of ischemia. The injury to tissue resulting from reperfusion is first evident in the tips of the mucosal villi and is caused by toxic metabolites such as oxygen free radicals. Conversely, ischemic injury is caused by tissue hypoxia. The mucosal damage induced by reperfusion injury is usually reversible. Although the mucosa is particularly sensitive to reperfusion injury, there is little histologic evidence of submucosal damage in the vast majority of studies of intestinal reperfusion injury. Several investigators have

suggested that the submucosal layer is protected from ischemic damage by a countercurrent exchange mechanism that deprives the mucosa of oxygen during periods of reduced blood flow.[10,11]

There is a finite period of time during which tissue damage can occur as a direct consequence of reperfusion. It is generally acknowledged that at least 15 minutes of ischemia is necessary to produce any tissue damage in the gut.[12,13] However, tissue damage following mesenteric occlusion exceeding 4 hours is usually a consequence of hypoxia rather than reperfusion injury.[11-13]

There are conflicting data regarding the duration of ischemia that is necessary to produce irreversible damage in the bowel. These discrepancies are probably due to differences in both the capability of various species to withstand intestinal ischemic injury and variations in methodologies used to induce ischemia. Although Robinson and Mirkovitch[14] induced extensive mucosal damage in dogs after only 60 minutes of mesenteric occlusion followed by reperfusion, histologic and metabolic studies performed in the same animals 24 hours later showed complete structural and functional recovery. Conversely, other investigators have shown that rat intestine is vulnerable to irreversible ischemic injury after occlusion times of less than 90 minutes.[15-17] Administration of fluids and antibiotics may prolong the interval between the initial ischemic insult and transmural infarction. Amano et al.[18] have shown that rat intestine will maintain its integrity after 4 hours of total ischemia when the animal is supported systemically by intravenous fluids and antibiotics. However, ischemic times approaching 8 hours usually resulted in transmural necrosis despite adequate hemodynamic support. In the present study liberal intraoperative administration of lactated Ringer's solution may have attenuated the magnitude of ischemic damage in the reperfusion group.

The primary goal of these experiments was to compare several objective bowel viability assessment parameters in two distinct models of intestinal ischemic disease. We were particularly interested in whether reperfusion injury could be detected by either the surface EMG or the two objective blood flow measurements. The reperfusion model used in our study was designed in an attempt to produce tissue damage exclusively from reperfusion rather than from hypoxic injury. The duration of occlusion (3½ hours) used in this model was based on results of experiments by Boras et al.[19,20] and Grogaard et al.,[12] which showed that reperfusion injury in canine small bowel is consistently produced after 120 to 180 minutes of arterial occlusion. Conversely, our chronic model was designed to create a "steady state" of ischemic damage that would be typical of the pattern encountered by surgeons during emergency operations performed for intestinal ischemic disease.

The data in Table I clearly show the difference in magnitude of ischemic changes produced by the two models used in these experiments. In the reperfusion model there were only subtle changes in bowel color. Doppler pulses returned in the marginal artery immediately after release of the occlusion clamps. However, 3½ hours of ischemia resulted in a transient decrease in intestinal myoelectric activity since mean EMG values during the first 10 minutes after reperfusion were significantly lower than measurements obtained between 15 and 30 minutes after restoration of blood flow (see Fig. 3). These results suggest that the myenteric plexus is affected by reperfusion injury. Conversely, the fluorometry measurements increased to normal or supranormal levels immediately after release of the clamps, suggesting that capillaries in the muscularis and serosa remained open throughout the period of ischemia. These results suggest that the quantified EMG may be a more sensitive indicator of the magnitude of ischemic damage than either Doppler ultrasonography or perfusion fluorometry. These observations also suggest that the duration of ischemia has a significant impact on objective blood flow measurements used for assessment of bowel viability.

Although the experimental protocol was designed so that ischemic bowel was resected and anastomosed at the EMG measurement that most closely approximated 50% of the measurement obtained in normal bowel, there were five cases in the reperfusion group in which the lowest indexed EMG value obtained throughout the entire length of ischemic bowel was $\geq$60 mV. Moreover, no bowel was actually resected in the reperfusion group because the EMG measurements invariably increased above the 50 mV threshold by 30 minutes after release of the clamps. The ischemic insult caused by reperfusion did not adversely affect intestinal anastomotic healing since all of the anastomoses in this group were intact at the time the animals were killed. Clearly, the 3½-hour occlusion time used in these experiments was insufficient to produce irreversible ischemic damage in canine small bowel.

CONCLUSION

These experiments show significant differences in the degree of intestinal ischemic damage induced by 24 hours of segmental mesenteric occlusion as compared to 3½ hours of ischemia followed by reperfusion. Although reperfusion injury did not result in irreversible intestinal ischemic damage in these experiments, reperfusion has a subclinical but measurable

effect on the submucosal neuromuscular plexus, which was detected by the ECM probe. Because extensive mucosal damage does not consistently result in bowel infarction, measurements used in bowel viability assessment should probably be obtained from the serosal surface. This study also suggests that bowel survival may be an inappropriate end-point measurement of reperfusion injury in dogs.

REFERENCES

1. Tepperman BL. Measurement of gastrointestinal blood flow. Annu Rev Physiol 1982;44:71-82.
2. Bulkley GB, Zuidema GD, Hamilton SR, et al. Intraoperative determination of small intestinal viability following ischemic injury. Ann Surg 1980;193:628-637.
3. Wright CB, Hobson RW Jr. Prediction of intestinal viability using Doppler ultrasound techniques. Am J Surg 1975; 129:642-645.
4. Brolin RE, Semmlow JL, Mackenzie JW, et al. Quantitative myoelectric determination of bowel viability. J Surg Res 1986;41:557-562.
5. Orland PJ, Cazi G, Semmlow JL, et al. Determination of small bowel viability using quantitative myoelectric and color analysis. J Surg Res 1993;55:581-587.
6. Brolin RE, Orland PJ, Bibbo C, et al. Comparison of blood flow and myoelectric measurements in two chronic models of mesenteric ligation. Arch Surg 1995;130:147-152.
7. Semmlow JL, Brolin RE. Instrumentation for quantitative assessment of intestinal viability. IEEE Trans Biomed Eng 1988;35:888-892.
8. Cabot RM, Kohatsu S. The effects of ischemia on the electrical and contractile activities of the canine small intestine. Am J Surg 1978;136:342-346.
9. Code CF, Szurszewski JH. The effect of duodenal and mid small bowel transection on the frequency gradient of the pacesetter potential in the canine small intestine. J Physiol (Lund) 1970;207:287-299.
10. Parks DA, Granger DN. Contributions of ischemia and reperfusion to mucosal lesion formation. Am J Physiol 1986; 250:G749-G753.
11. Haglund U, Bulkley GB, Granger DN. On the physiology of intestinal ischemic injury. Acta Chir Scand 1987;153:321-324.
12. Grogaard G, Parks DA, Granger DN, et al. Effects of ischemia and oxygen radicals on mucosal albumin clearance in intestine. Am J Physiol 1982;242:6448-6454.
13. Parks DA, Grogaard B, Granger DN. Comparison of partial and complete arterial occlusion models for studying intestinal ischemia. Surgery 1982;92:896-901.
14. Robinson RWL, Mirkovitch V. The recovery of function and microcirculation in small intestinal loops following ischemia. Gut 1972;13:784-789.
15. Dalsing MC, Grosfeld JL, Shiffler MA, et al. Superoxide dismutase: A cellular protective enzyme in bowel ischemia. J Surg Res 1983;34:589-596.
16. Caty MG, Guice KS, Oldham KT, et al. Evidence for tumor necrosis factor–induced pulmonary microvascular injury after intestinal ischemia–reperfusion injury. Ann Surg 1990;212: 694-700.
17. Lelli JL, Pradhan S, Cobb LM. Prevention of postischemic injury in immature intestine by deferoxamine. J Surg Res 1993;54:34-38.
18. Amano H, Bulkley GB, Gorey T, et al. The role of microvascular patency in the recovery of small intestine from ischemic injury. Surg Forum 1980;31:157-159.
19. Boros M, Karacsony G, Kaszaki J, et al. Reperfusion mucosal damage after complete intestinal ischemia in the dog: The effects of antioxidant and phospholipase A_2 inhibitor therapy. Surgery 1993;113:184-191.
20. Boros M, Kaszaki J, Ordogh R, et al. Intramucosal pH changes following complete segmental small intestinal ischemia, as compared with the effects of superior mesenteric artery occlusion. Eur Surg Res 1994;26:76-86.

Conversion of Proximal to Distal Gastric Bypass for Failed Gastric Bypass for Superobesity

Harvey J. Sugerman, M.D., John M. Kellum, M.D., Eric J. DeMaria, M.D.

The purpose of this study was to analyze outcome following malabsorptive distal gastric bypass (D-GBP) in superobese patients who were reoperated for recurrent obesity comorbidity after a failed standard gastric bypass (S-GBP). Twenty-seven formerly superobese patients with a failed S-GBP converted to a D-GBP were studied. The small bowel was anastomosed 250 cm from the ileocecal valve to the disconnected Roux limb; the bypassed small intestine was connected to the ileum 50 cm from the ileocecal valve in five patients between 1985 and 1986 and 150 cm from the ileocecal valve in 22 patients thereafter. Comorbidity was reassessed yearly following conversion to D-GBP. Malnutrition occurred in all five patients with a 50 cm "common tract"; all required further revision and two died of hepatic failure. Three of 22 patients with a 150 cm common tract were reoperated with bowel lengthening because of malnutrition. Initial body mass index was 57 ± 2 kg/m^2 and fell from 46 ± 2 kg/m^2 before revision to 37 ± 2 kg/m^2 at 1 year and 32 ± 2 kg/m^2 at 5 years after revision; the percentage of excess weight lost went from 30 ± 4% to 61 ± 4% at 1 year and 69 ± 5% at 5 years after revision. Preoperative comorbidity in patients undergoing revision included 14 with insulin-dependent type II diabetes mellitus, 11 with sleep apnea, 14 with hypoventilation, 13 with hypertension, and two with venous stasis ulcers. Obesity comorbidity was corrected within 1 year in all but two patients with hypertension and remained stable in all patients followed for 5 years. Revision of a failed S-GBP to a 150 cm common tract D-GBP corrects failed weight loss and severe obesity comorbidity but requires nutritional support to prevent protein-calorie malnutrition, iron and fat-soluble vitamin deficiencies, and further revision in some patients to correct malnutrition. A 50 cm common tract has an unacceptable morbidity and mortality. (J GASTROINTEST SURG 1997;1:517-525.)

A standard gastric bypass (S-GBP) with a 45 cm Roux limb, a 15 ml gastric pouch, and a 1 cm gastrojejunostomy has been found to produce a significantly greater weight loss than a banded gastroplasty in both randomized prospective studies[1-4] and retrospective studies.[5] The S-GBP provides the loss of 66% excess weight (%EWL), defined as the loss of weight in excess of ideal body weight (IBW) according to the 1983 Metropolitan Life Insurance tables,[6] at 2 years, 60% at 5 years, and 50% at 10 years after surgery.[7-9] However, in 15% of patients this procedure will fail through inadequate weight loss or weight regain from the excessive ingestion of high-fat "junk" foods, which crumble and pass through the stoma without resistance, or through the loss of or failure to develop symptoms of dumping syndrome with the ingestion of high-calorie liquids and sweets, the so-called "soft" calories.[1-5,7-11] This is associated with inadequate control or recurrence of preoperative obesity comorbid conditions such as severe hypertension, diabetes, hypoventilation, sleep apnea, or venous stasis ulcers. Restapling a disrupted staple line will often produce a return to the patient's previous weight loss curve; this can usually be detected by the history of a marked increase in the ability to eat high-fiber foods and an upper gastrointestinal radiographic series demonstrating early filling of the distal bypassed stomach with contrast material. Revision of a dilated stoma is almost always unsuccessful.[12]

It seemed reasonable to evaluate a more aggressive approach using a malabsorption procedure that might be warranted in particularly severe cases. We chose to use a modification of the partial biliopancreatic bypass procedure, which differed from the original op-

From the Division of General and Trauma Surgery, Department of Surgery, Medical College of Virginia, Virginia Commonwealth University, Richmond, Va.
Presented at the Thirty-Seventh Annual Meeting of The Society for Surgery of the Alimentary Tract, San Francisco, Calif., May 19-22, 1996.
Reprint requests: Harvey J. Sugerman, M.D., Box 980519 MCV Station, Richmond, VA 23298-0519.

eration proposed by Scopinaro et al.[13] in that it involved a small 30 ml gastric pouch without resection of the distal stomach and a 150 cm "common absorptive limb," we have termed this procedure distal gastric bypass (D-GBP). The Scopinaro partial biliopancreatic bypass involves the creation of a 200 ml gastric pouch in superobese patients and a 400 ml pouch in those who are not superobese, with resection of the distal stomach. The small bowel is transected 250 cm from the ileocecal valve and the distal end is anastomosed to the stomach pouch. The bypassed small bowel (the so-called "biliopancreatic limb") is 200 to 400 cm in length and is anastomosed to the ileum 50 cm from the ileocecal valve, creating a 200 cm "alimentary limb" through which food passes with little absorption and a 50 cm "common limb" where bile and pancreatic juices permit digestion and nutrient absorption.

When we performed the D-GBP procedure with a 200 ml gastric pouch and a 50 cm common limb in superobese (body mass index [BMI] $\geq$50 kg/m^2) American patients who ate large quantities of high-fat junk food, a large percentage of them developed severe protein-calorie malnutrition and fat-soluble vitamin deficiencies.[14] We then conducted a randomized prospective trial of S-GBP vs. D-GBP with a small 30 ml gastric pouch in superobese patients where the bypassed small bowel was connected to the ileum 150 cm from the ileocecal valve, creating a 100 cm alimentary limb, a 200 to 300 cm biliopancreatic limb, and a 150 cm common limb.[15] The D-GBP was associated with a significantly greater weight loss than the S-GBP at 2 to 4 years after surgery (Table I) but required lengthening of the common tract in 25% of patients because of protein-calorie malnutrition, fat-soluble vitamin deficiency, and hypocalcemia with osteoporosis. Two D-GBP patients have required monthly intramuscular injections of 50,000 IU vitamin D and six 600 mg calcium tablets per day for the treatment of severe osteoporosis associated with bone pain. Three patients have required intermittent courses of metronidazole (250 mg twice a day) to treat symptoms of bacterial overgrowth (fever, diarrhea, malaise) in the "biliopancreatic tract." We have been unable to identify determinants (age, sex, preoperative weight, or eating habits) of unfavorable clinical outcomes after D-GBP.

Although we recognized the potential problems associated with D-GBP, we initially thought it was appropriate to use a 50 cm common absorptive intestinal tract in superobese patients in whom S-GBP had failed, based on the data from the Italian experience with partial biliopancreatic diversion.[13] Because most of the patients with a 50 cm common tract developed severe malnutrition and fat-soluble vitamin deficiencies, we thought it reasonable to convert superobese patients with a failed S-GBP without staple line disruption, and severe obesity comorbidity, to a less malabsorptive 150 cm common tract D-GBP when no other treatment has proved effective, recognizing that there would still be a risk of protein-calorie malnutrition, fat-soluble vitamin deficiencies, and osteoporosis. This decision was based on data from our randomized prospective trial of S-GBP vs. 150 cm D-GBP for superobesity.[15]

METHODS

Superobese patients with a BMI of 50 kg/m^2 or greater were considered to have a failed S-GBP if they had lost less than 40% of their excess weight at 3 or more years after surgery and if the procedure had

Table I. Weight loss in S-GBP vs. D-GBP: A randomized prospective trial

	S-GBP Preop	D-GBP Preop	S-GBP 2 yr	D-GBP 2 yr	S-GBP 4 yr	D-GBP 4 yr
No. of patients	26	27	25	15*	21	12†
Weight (pounds)	361 ± 11	379 ± 10	225 ± 8	201 ± 11	242 ± 11	212 ± 16‡
Weight loss (pounds)	—	—	133 ± 10	174 ± 9	111 ± 11	163 ± 13‡
%WL	—	—	36 ± 2	44 ± 3	31 ± 3	44 ± 3‡
%EWL	—	—	60 ± 3	75 ± 4‡	51 ± 5	70 ± 5‡
%IBW	262 ± 5	272 ± 10	164 ± 6	145 ± 8‡	177 ± 7	152 ± 11‡
BMI (kg/m^2)	59 ± 1	61 ± 1	37 ± 1	32 ± 2‡	40 ± 2	35 ± 3‡

S-GBP = standard gastric bypass; D-GBP = distal gastric bypass; BMI = body mass index; %WL = percentage weight loss; %EWL = percentage excess weight loss; %IBW = percentage ideal body weight.

*One S-GBP patient was lost to follow-up; seven D-GBP patients required conversion to S-GBP because of protein-calorie malnutrition and five were lost to follow-up.

†An additional four S-GBP and three D-GBP patients were lost to follow-up.

‡P <0.01 D-GBP vs. S-GBP.

failed to correct, or patients had a recurrence of their original severe (sleep apnea, hypoventilation, diabetes, or hypertension difficult to control with medication) preoperative comorbidity. They were also considered for revisional surgery if they were regaining weight despite intensive dietary counseling and they had severe obesity comorbidity that could be predicted to recur if they continued to gain weight. Twenty-seven patients were offered conversion of a D-GBP after a thorough discussion of the risks and benefits of the procedure based on data from the randomized prospective trial. The operation (Fig. 1) consisted of transection of the Roux limb at the jejunojejunostomy using a GIA-60 stapler (Autosuture, U.S. Surgical Corp., Norwalk, Conn.) with 2.5 mm staples (white cartridge). The small bowel was then transected 250 cm from the ileocecal valve using another GIA-60 stapler and anastomosed to the Roux limb as a functional end-to-end anastomosis, using a side-to-side anastomosis with a third GIA-60 application and a PI-55 horizontal stapler (U.S. Surgical Corp.) with medium-sized (3.5 mm) staples (blue cartridge). The bypassed small bowel was then anastomosed to the ileum 50 cm from the ileocecal valve in five patients between June 1986 and July 1988, creating a 50 cm common tract. Because of the development of severe protein-calorie malnutrition in all five patients with the 50 cm common tract, this operation was discontinued. Subsequently the ileum was transected at 250 cm from the ileocecal valve and the bypassed small bowel was anastomosed to the ileum at 150 cm from the ileocecal valve in 22 patients, creating a 150 cm common tract, a 145 cm alimentary tract, and a bypassed biliary tract, which was between 200 and 400 cm (not measured in most patients). Five of these 22 patients had a previous failed horizontal gastroplasty prior to their S-GBP. All patients had an upper gastrointestinal radiographic examination prior to revisional surgery to rule out gastric staple line disruption.

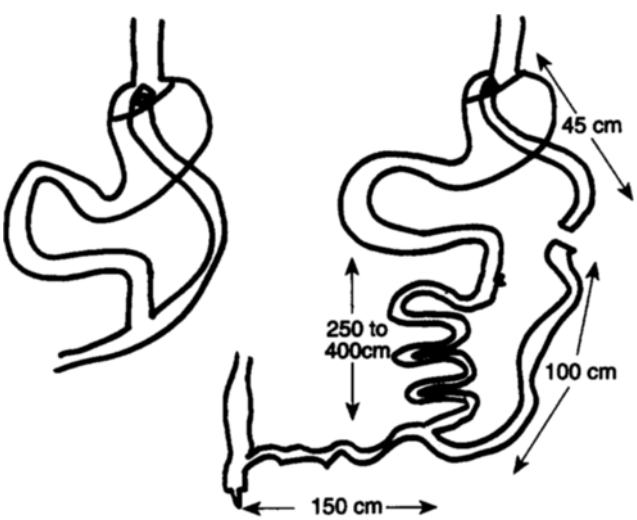


Fig. 1. Schematic of conversion of S-GBP to 150 cm D-GBP. Distal small bowel transected 250 cm from the ileocecal valve and proximal end anastomosed to the disconnected 45 cm Roux limb. Bypassed small bowel, or "biliopancreatic limb," anastomosed to the ileum at 150 cm from the ileocecal valve. This creates a 145 cm "alimentary limb," a 150 cm "common limb," and a 250 to 400 cm "biliopancreatic limb."

In these 27 patients, severe obesity comorbidity included type II diabetes, sleep apnea, hypoventilation, venous stasis ulcers, and hypertension. All patients with type II diabetes were taking 20 or more units of insulin per day. Hypoventilation was defined as a PaO_2 of 55 mm H or less and/or a $PaCO_2$ of 47 mm Hg or more. Room air arterial blood gases were obtained before S-GBP and before revisional D-GBP, as well as 1 year following conversion to D-GBP in all patients with clinically obvious shortness of breath. All patients with symptoms of sleep apnea (restless sleep, severe snoring, multiple episodes of awakening, daytime somnolence) underwent sleep polysomnography and the sleep apnea index (No. of apneas + No. of hypopneas/hour of sleep) was found to be 20 or more (moderate to severe sleep apnea) prior to both their original and prerevisional gastric bypasses. The presence of sleep apnea was based on the continued need for treatment with either nasal continuous positive airway pressure (CPAP) or tracheostomy. Patients with hypertension had to be taking at least one antihypertensive medication not including a diuretic. The diagnosis of venous stasis ulcers required the presence of a nonhealing pretibial ulcer despite the use of rigid medicated compression boots or split-thickness skin grafts.

All patients who underwent the procedure were advised to take a multivitamin, three 500 mg calcium tablets or extra-strength TUMS, 500 μg of vitamin B_{12}, 10,000 units of vitamin A, 400 units of vitamin D, and 400 units of vitamin E daily. If hypoproteinemia (albumin $\leq$3 gm/dl) developed, they were also advised to take three noncoated pancreatic enzymes (Viokase or pancrealipase, not Pancrease) three times a day with meals. All menstruating women were advised to take one 325 mg iron tablet daily unless they developed chemical evidence of iron deficiency anemia, in which case they were asked to increase their oral iron medication to three tablets daily. Laboratory tests to determine values for sodium, potassium, chloride, carbon dioxide, iron, calcium, magnesium, blood urea nitrogen, creatinine, bilirubin, alkaline phosphatase, oxaloacetic acid and glucose-pyruvate transferases, hemoglobin, hematocrit, vitamin B_{12}, and fat-

soluble vitamins were performed at 6 months, 1 year, 18 months, and yearly thereafter after revision to D-GBP. Patients were contacted regarding abnormal values and advised to alter their medication supplements accordingly. Patients with low levels of calcium, iron, hemoglobin, albumin, or fat-soluble vitamins were seen at 3-month intervals until these values returned to normal. Patients who had a persistently low serum albumin level that could not be corrected by dietary manipulation (high-protein supplements such as Ensure-Plus, Sustacal, or Carnation Liquid Breakfast) and oral pancreatic enzymes (three with each meal three times a day and two with snacks twice a day) or who did not comply with dietary recommendations were converted to a less malabsorptive procedure by adding length to the common tract. Early and late postoperative complications were compiled by reviewing the outpatient charts.

BMI, %EWL, and %IBW were determined for all patients before S-GBP, before conversion to D-GBP, and yearly thereafter. Data are expressed as means ± standard error of the mean and were analyzed by paired Student's *t* test with Fischer's exact modification and analysis of variance when appropriate; $P < 0.05$ was considered significant.

RESULTS

Conversion from a failed S-GBP to D-GBP was performed in 27 (2.2%) of 1231 patients who had undergone a S-GBP, or 5.6% of 485 superobese patients who underwent S-GBP from 1981 through 1994. The time between S-GBP and conversion ranged from 2 to 12 years (mean 5.6 ± 0.6 years). Average preoperative weight before the initial GBP in these 27 patients was 367 ± 13 pounds, BMI 57 ± 2 kg/m^2, and %IBW 264 ± 11 (Table II). At the time of conversion, in these same patients BMI was 46 ± 2 kg/m^2 and %IBW 201 ± 7, which is consistent with morbid obesity. They weighed 296 ± 10 pounds and their % EWL was only 30 ± 4. One or more serious comorbidity factors were present in each of them (Table III). Nineteen (70%) of these patients were women and eight (30%) were men in contrast to 18% of the 1231 S-GBP patients or 22% of the 485 superobese S-GBP patients who were male; 14 (52%) were black and 13 (48%) were white as compared to 28% of the overall S-GBP population and 39% of our superobese S-GBP patients who were black.

One year after conversion to D-GBP, the average BMI of 25 patients (2 were lost to follow-up) was 37 ± 2 kg/m^2, %EWL was 61 ± 4, and %IBW was 162 ± 7 (see Table II). The weight loss remained relatively stable after conversion in 10 of the 12 patients who had the operation five or more years who were available for follow-up. Obesity comorbidity had resolved in all except two patients who had persistent hypertension (see Table III). No patients with an intact D-GBP have regained more than 5% of their lowest weight up to 9 years after conversion.

Table II. Weight loss following conversion from S-GBP to D-GBP

	No.	Weight (pounds)	BMI (kg/m^2)	%EWL	%IBW
Before S-GBP	27	367 ± 13	57 ± 2	—	264 ± 11
Before D-GBP	27	296 ± 10*	46 ± 2*	30 ± 4	201 ± 7*
1 year after	25/27	232 ± 9†	37 ± 2†	61 ± 4†	162 ± 7†
3 yr after	13/16	216 ± 15†	35 ± 2†	67 ± 5†	152 ± 10†
5 yr after	11/12	211 ± 14†	33 ± 2†	69 ± 5†	144 ± 7†

S-GBP = standard gastric bypass; D-GBP = distal gastric bypass; BMI = body mass index; %EWL = percentage excess weight loss; %IBW = percentage ideal body weight.
*$P < 0.01$ S-GBP compared to preoperative values.
†$P < 0.01$ D-GBP vs. S-GBP.

Table III. Obesity comorbidity before and after conversion to D-GBP

	Before S-GBP	Before D-GBP	1 yr after D-GBP
Type II diabetes mellitus	14	6	0
Sleep apnea	11	7	0
Hypoventilation	14	5	0
Hypertension	13	11	2
Venous stasis	2	2	0

All five patients who had a 50 cm common tract developed severe protein-calorie malnutrition that was refractory to high-protein liquid supplements as well as non-enteric-coated pancreatic enzymes. They were given total parenteral nutrition (TPN), which transiently corrected their malnutrition; malnutrition recurred, however, following discontinuation of TPN. After another course of TPN, these patients were converted to a less malabsorptive procedure by moving the entero-enterostomy and extending the common tract to 150 cm in three patients, 200 cm in one patient, and 250 cm in the remaining two patients. Two of these patients died of hepatic failure 1 to 3 years after their second revision despite TPN and aggressive medical management. These deaths occurred prior to our ability to provide liver transplantation. Twenty-two patients underwent revisional D-GBP with a 150 cm common tract; two of them subsequently required revision to a "long-limb" gastric bypass[16] because of refractory protein-calorie malnutrition and one other patient underwent revision to a 250 cm common tract. The three patients who underwent further revision were given TPN to correct their malnourished state prior to revision. One patient had a gastrostomy tube placed percutaneously under fluoroscopic guidance into her bypassed stomach for supplemental enteral nutrition to correct protein-calorie malnutrition, which developed shortly after she underwent conversion to a 150 cm D-GBP. The supplemental feedings were able to be discontinued 1 year after conversion and the feeding tube was removed.

Laboratory data at the most recent follow-up (within the past 12 months in all but two patients) were frequently abnormal despite micronutrient supplementation. All patients had a least one abnormal laboratory value at some time following conversion. Hemoglobin was less than 11 g/dl in 21% with hematocrit less than 30% in 7% of patients. Several of these patients had normal serum iron and vitamin B_{12} levels suggesting an anemia of chronic disease. Albumin was less than 3.6 g/dl in 56% and less than 3.0 g/dl in 12% of patients; 23% had hypocalcemia after correction for hypoalbuminemia, 63% had decreased vitamin D-25, and 38% had vitamin E-alpha. Two patients developed intermittent symptoms of bacterial overgrowth in the bypassed intestine (increased diarrhea, fever, and malaise), which responded to repetitive 2-week courses of metronidazole (250 mg per orem twice a day).

Early postoperative complications included one wound seroma, one wound hematoma, one wound infection, two intra-abdominal abscesses, four small bowel obstructions requiring laparotomy and adhesiolysis, two staple line disruptions, and two anastomotic ulcers. Late complications included 12 incisional herniorrhaphies and two anal fissures secondary to frequent diarrhea. Eight of the 27 patients had a prior cholecystectomy before revision to a D-GBP; seven underwent cholecystectomy at the time of revision and six after revision secondary to the development of cholecystitis, leaving only 6 of the 27 patients who did not undergo cholecystectomy. The potential benefits of conversion to D-GBP are exemplified by the following case report.

Case Report

A 28-year-old black operating room transportation employee suffered from severe hypertension (blood pressure 190/120 mm Hg), while taking clonidine, and type II diabetes mellitus, for which he was taking 15 units of regular insulin and 64 units of NPH insulin every morning and 64 units of NPH insulin at night. He had severe obstructive sleep apnea with a sleep apnea index of 57, for which he required nocturnal nasal CPAP. He was 67 inches tall and weighed 428 pounds, with a BMI of 67 kg/m^2 and 286% IBW; he underwent a S-GBP on February 22, 1988. At 1 year after surgery he had lost 146 pounds which was 58% EWL, reaching a BMI of 44 kg/m^2 and 188% IBW. At that time his sleep apnea had resolved clinically, he no longer required insulin or other hypoglycemic medications to maintain his blood sugar under 150 mg/dl or any medication to control his blood pressure, which was 168/89 mm Hg.

Unfortunately he began to regain weight during the second year. Dietary evaluation revealed that he was ingesting approximately 2250 calories/day, 25% of which was in the form of high-fat foods such as potato chips, corn chips, popcorn, bacon, sausage, and biscuits. Despite attempts at behavioral modification under the guidance of a dietician, he continued to gain weight. An upper gastrointestinal study revealed an intact gastric staple line and approximately 1 cm gastrojejunal stoma. At 4 years after his S-GBP, his weight had increased from 282 to 330 pounds with a BMI of 52 kg/m^2 and 252% IBW. He redeveloped severe hypertension (blood pressure 192/117 mm Hg), which was refractory to medication (50 mg metoprolol and two 10 to 25 mg enalapril tablets daily) and associated with an increase in blood urea nitrogen to 25 mg/dl and creatinine to 2.6 mg/dl, as well as recurrence of type II diabetes mellitus, which necessitated 35 units of NPH insulin every morning for control of his hyperglycemia. After discussing the risks and benefits of the malabsorptive procedure, his S-GBP was converted to a 150 cm D-GBP. One year later, he had lost 78 pounds or 63% EWL after the surgical revision, reaching a BMI of 39 kg/m^2, which corresponded to 168% IBW. Four years after his conversion to a D-GBP, he weighed 222 pounds, which was 148% IBW, and his BMI was 35 kg/m^2 for a 71% EWL. He no longer requires nasal CPAP for the control of sleep apnea symptoms nor does he require any hypoglycemic agents to maintain his blood glucose below 150 mg/dl or medication for control of blood pressure, which is currently 154/80 mm Hg. Because of mild hypoalbuminemia (3.0 g/dl) he has to take pancreatic en-

zymes, which have raised his albumin to 3.2 g/dl. He also takes fat-soluble vitamins, vitamin B_{12}, and calcium with normal annual vitamin A, D, E, B_{12}, and calcium levels.

DISCUSSION

Previous studies have shown that a significantly greater weight loss is achieved with S-GBP than with banded gastroplasty.[1-5,10,11] Most patients who undergo a gastric bypass find that they lose their desire to eat high-density carbohydrates or develop persistent dumping syndrome symptoms with their ingestion.[1-5,10,11] Unfortunately, S-GBP will fail in approximately 15% of these patients, primarily because of ingestion of excessive quantities of high-fat foods, which crumble easily and pass through the stoma without providing a feeling of satiety.[10,11] Even if the stoma is found to be dilated on upper gastrointestinal radiography, revision of the stoma has not been associated with resumption of significant weight loss.[12] Revision of a failed S-GBP secondary to a disrupted staple line[17] or conversion of a failed vertical banded gastroplasty to a S-GBP[18] can be performed with low morbidity and mortality. The current study evaluated conversion of superobese patients (BMI $\geq$50 kg/m^2) in whom S-GBP had failed and who had redeveloped obesity comorbidity in the absence of staple line disruption to a malabsorptive D-GBP. This represented approximately 2% of our S-GBP patients and was therefore restricted to a small percentage of those patients in whom S-GBP had failed. Revision to D-GBP was performed in a higher percentage of males (30%) than our overall S-GBP population (18%) or superobese S-GBP patients (22%), but these differences were not significant. There were more black patients who underwent conversion to D-GBP (52%) than our total S-GBP group (28%; $P < 0.02$) or superobese S-GBP patients (39%).

The data in this study demonstrated that a 50 cm common tract in which the food from the alimentary tract meets the bile and pancreatic juices from the biliary tract is much too short and is associated with an unacceptable incidence of protein-calorie malnutrition. Most patients did well with a 150 cm common tract, although three patients still required revision to a longer common tract because of malnutrition. In a previous randomized prospective trial in which superobese patients underwent a 150 cm D-GBP with a small gastric pouch and stoma, 25% developed severe protein-calorie malnutrition and required operative revision.[15] It was not possible to differentiate between patients who did poorly in this randomized study and the majority who did well when the data were retrospectively analyzed with regard to age, sex, preoperative weight, or eating habits. Based on the currently available data, it still is not possible to predict who will do well and who will develop nutritional problems following conversion to the malabsorptive procedure. It is also not possible to predict the length of common tract needed to maintain adequate protein and fat-soluble vitamin absorption.

The D-GBP is a fat malabsorptive operation that is invariably associated with foul-smelling stools that float. It is mandatory that these patients take supplemental fat-soluble vitamins and calcium to prevent night blindness and osteoporosis and that these levels, as well as the serum albumin level, be evaluated at least once a year. If abnormalities are noted, correction with additional supplements (e.g., fat-soluble vitamins, calcium, magnesium) are necessary with reevaluation on a quarterly basis until these values return to normal. Mild protein-calorie malnutrition will require protein supplements and oral non–enteric-coated pancreatic enzymes to improve absorption. Patients can develop symptoms of bacterial overgrowth (i.e., diarrhea, fever, malaise) as a result of the overgrowth of bacteria in their bypassed intestine (the biliary tract); the incidence of this appears to be much lower than in the previous jejunoileal intestinal bypass operation, as there is still bile and pancreatic juice flowing through this limb. Intermittent courses of metronidazole, 250 mg per orem twice a day, are usually effective. The additional tests, supplements, and antibiotics can be costly. Occasionally patients who undergo this radical procedure fail to return for follow-up, which can lead to serious medical problems necessitating rehospitalization for intravenous nutrition and further surgical revision.

In another recent report, 80 patients with an average BMI of 40 kg/m^2 prior to obesity surgery underwent conversion to a 100 cm D-GBP for failed weight loss following various bariatric surgical procedures (i.e., gastroplasty, gastric banding, gastric bypass). Protein-calorie malnutrition developed in 28 patients (35%), one of whom died of malnutrition.[19] Other major problems in this study included iron deficiency anemia (35%), intractable diarrhea (18%), and prolonged, frequent vomiting (4%). The authors concluded that the success of the procedure depended on patient compliance with proper nutrition, supplements, and regular follow-up with laboratory monitoring. However, the nutrient supplements, office visits, and laboratory tests can be very expensive and patients may not be able to afford to be compliant. It is probable that a 100 cm D-GBP is too radical a procedure for patients who are not superobese.

All of the patients in this study who underwent conversion of D-GBP were superobese and had asso-

ciated severe obesity comorbidity. At this time there is no other effective treatment. Obesity hypoventilation syndrome, severe hypertension, and diabetes are life-threatening conditions. Chronic venous stasis ulcers that fail to heal with the use of skin grafts, medicated rigid compression boots, and elevation are severely disabling. Although sleep apnea responds to tracheostomy or nasal CPAP, both are unpleasant and the latter is often unused. The additional weight loss that occurred with conversion to D-GBP was associated with resolution of severe obesity comorbidity in almost all of these patients. These patients were all superobese and had failed a S-GBP with a 45 cm Roux limb. It is possible that the long-limb gastric bypass with a 150 cm Roux limb,[16] which we are currently using for superobese patients, will have better long-term efficacy, obviating the need to convert as many patients to the malabsorptive D-GBP. Long-term results of this procedure are not yet available.

The D-GBP procedure differs from partial biliopancreatic bypass in that it involves a much smaller gastric pouch than the operation devised by the Italian group,[13] and it is performed without a gastrectomy. In a previous study we found that the partial biliopancreatic bypass for superobese patients with a 200 ml gastric pouch and a 50 cm common tract was associated with an unacceptably high incidence of protein-calorie malnutrition, fat-soluble vitamin deficiencies, and osteoporosis.[14] A high incidence of these complications has been reported by other investigators from the United States,[19-22] in contrast to studies from Italy.[13] Severely obese Americans consume a diet that is much higher in fat compared to Italians whose diet has a high starch content; therefore Americans may have more steatorrhea producing severe fat-soluble vitamin, calcium, and protein deficiencies with a radical fat malabsorptive procedure. In our opinion, a D-GBP with a 50 cm common tract should not be used at all in severely obese American patients and a 150 cm common tract should be considered only for patients who are superobese and who also have severe comorbidity. It is possible that an even longer common limb of 200 to 250 cm may be used to reduce the risk of protein-calorie malnutrition, steatorrhea, and associated calcium and fat-soluble vitamin deficiencies. It is also possible that a long biliopancreatic limb might obviate the problems associated with malnutrition but may again be associated with an inadequate weight loss.

CONCLUSION

Superobese patients who have a failed S-GBP and who also have severe comorbidity should be considered candidates for conversion to a D-GBP with a 150 cm common limb, as the problems associated with obesity comorbidity are almost uniformly reversible with weight loss. The operation is potentially dangerous, mandates long-term follow-up, and is entirely dependent on the willingness and ability of the patient to adhere to follow-up monitoring and treatment. It may require further conversion to a less malabsorptive procedure. A 50 cm common limb has an unacceptable morbidity and mortality in our population.

REFERENCES

1. Sugerman HJ, Starkey JV, Birkenhauer R. A randomized prospective trial of gastric bypass versus vertical banded gastroplasty for morbid obesity and their effects on sweets versus non-sweets eaters. Ann Surg 1987;205:613-624.
2. Hall JC, Watts JM, O'Brien PE, et al. Gastric surgery for morbid obesity. The Adelaide study. Ann Surg 1990;211:419-427.
3. McLean LD, Rhode BM, Sampalis J, Forse RA. Results of the surgical treatment of obesity. Am J Surg 1993;165:155-160.
4. Agren G. Naslund I. A prospective, randomized comparison of vertical banded gastroplasty (VBG), loop gastric bypass (GBP) and gastric banding (GB). Presented at the Fourth International Symposium on Obesity Surgery, August, 1989, London, England.
5. Yale CE. Gastric surgery for morbid obesity. Complications and long-term weight control. Arch Surg 1989;124:941-946.
6. Metropolitan height and weight tables. Stat Bull Metrop Insur Co 1983;64:2-9.
7. Sugerman HJ, Kellum JM, Engle KM, et al. Gastric bypass for treating severe obesity. Am J Clin Nutr 1992;55:560S-566S.
8. Pories WJ, Macdonald KG Jr, Morgan EJ, et al. Surgical treatment of obesity and its effect on diabetes: 10-yr follow-up. Am J Clin Nutr 1992;55:582S-585S.
9. Pories WJ, Swanson MS, MacDonald KG, et al. Who would have thought it? An operation proves to be the most effective therapy for adult-onset diabetes mellitus? Ann Surg 1995;222: 339-350.
10. Sugerman HJ, Londrey GL, Kellum JM, et al. Weight loss with vertical banded gastroplasty and Roux-Y gastric bypass for morbid obesity with selective versus random assignment. Am J Surg 1989;157:93-102.
11. Brolin RE, Robertson LB, Kenler HA, Cody RP. Weight loss and dietary intake after vertical banded gastroplasty and Roux-en-Y gastric bypass. Ann Surg 1994;220:782-790.
12. Schwartz RW, Strodel WE, Simpson WS, Griffen WO Jr. Gastric bypass revision: Lessons learned from 920 cases. Surgery 1988;104:806-812.
13. Scopinaro N, Gianetta E, Adami F, et al. Biliopancreatic diversion for obesity at eighteen years. Surgery 1996;119:261-268.
14. Liszka TG, Sugerman HJ, Kellum JM, et al. Risk/benefit considerations of distal gastric bypass. Int J Obesity Metab Relat Disord 1988;12:604(A).
15. Sugerman HJ, Kellum JM, Rothrock MK, et al. Proximal vs. distal gastric bypass in the superobese patient: A randomized study. Poster presentation, The Society for Surgery of the Alimentary Tract, Boston, Mass., April 17, 1993.
16. Brolin RE, Kenler HA, Gorman JH, Cody RP. Long-limb gastric bypass in the superobese. A prospective randomized study. Ann Surg 1992;215:387-395.
17. Benotti PN, Forse A. Safety and long-term efficacy of revisional surgery in severe obesity. Am J Surg 1996;172:232-235.

18. Sugerman HJ, Kellum JM Jr, DeMaria EJ, Reines HD. Conversion of failed or complicated vertical banded gastroplasty to gastric bypass in morbid obesity. Am J Surg 1996;171:263-269.
19. Fox SR, Fox KM, Oh KH. The gastric bypass for failed bariatric surgical procedures. Obesity Surg 1996;6:145-150.
20. Clare MW. An analysis of 37 reversals on 504 biliopancreatic surgeries over 12 years. Obesity Surg 1993;3:169-173.
21. Clare MW. Equal biliopancreatic and alimentary limbs: An analysis of 106 cases over 5 years. Obesity Surg 1993;3:289-295.
22. Cates JA, Drenick EJ, Abedin MZ, et al. Reoperative surgery for the morbidly obese. A university experience. Arch Surg 1990;125:1400-1404.

Discussion

Dr. J.G. Kral (Brooklyn, N.Y.). This report demonstrates that we are recovering from the backlash that followed the mismanagement of patients undergoing intestinal bypass, when for many years nobody considered performing malabsorptive operations. The perspective is the realization that we are dealing with a disease that has all the hallmarks of malignancy—not only is there hyperplasia of tissue, but there is an odds ratio of mortality between two- and 37-fold for conditions such as sudden death, hormonal and nutritional cancer, diabetes, and stroke. This report describes a staged approach to this virtually malignant disease, which is quite logical.

My questions are based on two of the soft points in this report—that is, the criteria for progressing to reoperation using a more aggressive procedure in cases where there is no manifest recurrence of serious comorbidity. There is no question that patients with recurrence of any serious comorbid conditions need more aggressive operations with a staged strategy. What are your criteria for patients who did not have any recurrence of severe mortality-inducing complications of their obesity.

You mention that laboratory test results were obtained "when possible." I think it is ethically imperative for surgeons involved in this type of surgery to do everything to guarantee follow-up. Such factors as the patient's inability to pay for laboratory tests or the prescribed medications pose a serious ethical dilemma in the management of these often poor patients. How do you select your patients? Did you analyze whether sex is of any importance in the likelihood of requiring aggressive reoperation?

Dr. H. Sugerman. There were more male than female patients who needed reoperation, but in terms of the ones who had complications following the distal bypass, we could find no clues relating to sex, weight, preoperative weight, and so forth. Regarding the decision as to when to reoperate, they all had to have severe comorbidity at one time or another. If they were rapidly regaining weight and there appeared to be no sign that this was going to stop, then even though their comorbidity had not yet recurred, we thought it was appropriate to proceed with revisional surgery.

Dr. K. MacDonald (Greenville, N.C.). This concept is intriguing to all of us who perform this type of surgery, as we are all plagued by what to do for those patients who fail to lose weight. Certainly we do not have very good luck with revised gastrojejunostomies and sometimes even staple line breakdown occurs. As the superobese patients frequently fail to lose the weight considered acceptable in terms of reduced health risks, would you recommend this type of operation as a primary procedure for those superobese patients?

Dr. Sugerman. The answer to the latter question is no. I think it is a very high-risk procedure and I do not think it should be done as a primary operation. Many people believe that if the patient has not done well after a gastroplasty or gastric bypass, it is the patient's own fault and that is all these people are willing to do for them. It has been our belief and policy that if patients have significant medical problems related to their obesity, and they have not lost a significant amount of weight, then we would perform the procedure to treat comorbidity or prevent its recurrence, but only under those circumstances. This is a very radical operation with some significant problems.

We have had two patients lost to follow-up. We know where they are, but we have not been able to get them to return for laboratory studies. Whether this is an economic consideration, we do not really know. Obviously the hospital charges them for their laboratory tests. I think it is obvious that the surgeons performing these malabsorptive procedures have a very serious follow-up problem.

Dr. R. Brolin (New Brunswick, N.J.). Regarding the two patients who developed liver failure. Did you perform liver biopsies at any point in time in any of these patients? Did either of the two patients who died have evidence of preexisting liver disease at any time prior to the revisional procedure?

I wonder if you considered the use of parenteral hyperalimentation as a treatment adjunct prior to surgical revision in some of these cases. Certainly that has been described by the Italian investigators and others in the treatment of the protein-calorie malnutrition associated with this particular operation.

I have had some experience with a similar procedure, both as a revision procedure and as a primary operation for superobese patients. What I have found is that the anemia that is seen in conjunction with these distal gastric bypasses or biliopancreatic-like procedures tends to be a normocytic, normochromic anemia, and is not associated with either iron deficiency or B_{12} deficiency. I wonder if your experience in that regard is similar.

I would like you to comment on the dietary evaluations that are performed in these patients at the time you see them postoperatively. As I suspect you know, Scopinaro's group reports the so-called postcibal effect where the calorie intake during the first year after their procedure is substantially reduced in comparison with both the preopera-

tive intake and the intake after the first year. They attribute this to many of the metabolic complications that are seen with these procedures.

I would like your opinion regarding a group of patients who simply are not candidates for revision. Your emphasis on follow-up with this type of operation is exceedingly important, and in my opinion there is a group of patients who, for a variety of reasons, when they fail a primary procedure probably should not be considered for revision.

Dr. Sugerman. With regard to liver biopsies, you and I have both seen patients with idiopathic obesity-related cirrhosis. The two patients who died of liver failure did not have liver biopsies, but they had no gross evidence of cirrhosis before either their primary gastric bypass or their revisional procedure.

All of the patients who developed malnutrition following conversion to the distal gastric bypass were given TPN prior to their revisional procedure, and in some of them the TPN was then boosted as necessary. One patient had a percutaneous G tube inserted in the distal stomach, and after about a year her body became acclimated to her bypass procedure.

In terms of normocytic, normochromic anemia, that has occurred in some of our patients.

There was no apparent significant decrease in caloric intake. There did seem to be a decrease in fat intake after conversion to the malabsorptive procedure, but I think this is because patients were counseled that the more fat they consumed, the more bowel movements they were going to have, the more foul-smelling they would be, the more unpleasant their convalescence, and the higher the risk of osteoporosis. I do not think there was any really major change in the volume of food they ingested. It did change the choices they made.

I would agree that patients who have not returned for follow-up, and then return after having regained their weight are unreliable and should not be offered this operation.

Dr. J. Sonneland (Spokane, Wash.). Bariatric surgery has been on the agenda for approximately 25 years. Either in your own series or in any other series that you know of, can you give us figures as to their employability and their longevity?

Dr. Sugerman. I think that all of us would like to see randomized prospective data; however, there will never be randomized data with respect to employability and longevity. There is a study being conducted in Sweden that hopefully will provide some answers. In that study 10,000 Swedish patients are being entered into a dietary program, 2000 of whom will undergo surgery. Already we know that in the dietary arm, there are patients who have lost no weight and they have progressively increasing comorbidity. Those in the surgical arm have lost a third of their weight, on average, with a progressive decrease in comorbidity. We anticipate a significant decrease in mortality in the surgical group.

Commentary

John G. Kral, M.D., Ph.D.

Controversy and Obesity Surgery—What Else Is New?

The billion dollar weight loss industry targeting the pleasantly plump has done nothing to educate the public about the disease of obesity, the plight of the obese in a society obsessed by anorexic Calvin Klein models, or the contribution of fatness to escalating medical costs. Miraculously, coincident with FDA approval of the first new drugs for obesity in decades (dexfenfluramine and tetrahydrolipstatin), a former Surgeon General has spoken out about the disease, talk shows have taken an interest, and as surgeons are advertising for obese patients, societies of the surgical establishment have begun to accept papers on the topic for presentation and even publication!

The most important message of the preceding article by Sugerman et al. is that severe obesity and its surgical treatment is very serious business, indeed. The glass is half full when we note that 1231 patients undergoing standard Roux-en-Y gastric bypass between 1981 and 1994 have shed pounds, stopped taking medications, and improved their quality of life to a degree unsurpassed by any treatment of any disease. It is half empty, however, when we consider that 10% to 15% (my estimate) of these 1231 patients might be medical failures that we are unable to "weed out" preoperatively or salvage postoperatively.

If one denies that obesity is a legitimate disease, encompassing individuals abused as children, as well as those with "thrifty genes," defective leptin receptors, or those who are simply too poor to avoid high-fat Western diets that can lead to obesity (blame the patient for gluttony), it is very difficult to accept, let alone appreciate, the extraordinary efforts by these investigators to help the subset of patients described in this article. During the "dark era" of jejunoileal bypass (blame the operation not the medical profession for ignorance about how best to manage it), some of us were willing to make similar efforts to make the operation work in selected cases. The backlash after blatant mismanagement of jejunoileal bypass patients led to ever smaller, ever more proximal constrictions of the gastrointestinal tract (from gastroplasty to subcricoid esophageal banding[1]) in attempts to foil the "soft calorie syndrome."[2]

This report by Sugerman et al. exemplifies a significant recent trend in obesity surgery—that is, the resurgence of increasingly malabsorptive operations.

tive intake and the intake after the first year. They attribute this to many of the metabolic complications that are seen with these procedures.

I would like your opinion regarding a group of patients who simply are not candidates for revision. Your emphasis on follow-up with this type of operation is exceedingly important, and in my opinion there is a group of patients who, for a variety of reasons, when they fail a primary procedure probably should not be considered for revision.

Dr. Sugerman. With regard to liver biopsies, you and I have both seen patients with idiopathic obesity-related cirrhosis. The two patients who died of liver failure did not have liver biopsies, but they had no gross evidence of cirrhosis before either their primary gastric bypass or their revisional procedure.

All of the patients who developed malnutrition following conversion to the distal gastric bypass were given TPN prior to their revisional procedure, and in some of them the TPN was then boosted as necessary. One patient had a percutaneous G tube inserted in the distal stomach, and after about a year her body became acclimated to her bypass procedure.

In terms of normocytic, normochromic anemia, that has occurred in some of our patients.

There was no apparent significant decrease in caloric intake. There did seem to be a decrease in fat intake after conversion to the malabsorptive procedure, but I think this is because patients were counseled that the more fat they consumed, the more bowel movements they were going to have, the more foul-smelling they would be, the more unpleasant their convalescence, and the higher the risk of osteoporosis. I do not think there was any really major change in the volume of food they ingested. It did change the choices they made.

I would agree that patients who have not returned for follow-up, and then return after having regained their weight are unreliable and should not be offered this operation.

Dr. J. Sonneland (Spokane, Wash.). Bariatric surgery has been on the agenda for approximately 25 years. Either in your own series or in any other series that you know of, can you give us figures as to their employability and their longevity?

Dr. Sugerman. I think that all of us would like to see randomized prospective data; however, there will never be randomized data with respect to employability and longevity. There is a study being conducted in Sweden that hopefully will provide some answers. In that study 10,000 Swedish patients are being entered into a dietary program, 2000 of whom will undergo surgery. Already we know that in the dietary arm, there are patients who have lost no weight and they have progressively increasing comorbidity. Those in the surgical arm have lost a third of their weight, on average, with a progressive decrease in comorbidity. We anticipate a significant decrease in mortality in the surgical group.

Commentary

John G. Kral, M.D., Ph.D.

Controversy and Obesity Surgery—What Else Is New?

The billion dollar weight loss industry targeting the pleasantly plump has done nothing to educate the public about the disease of obesity, the plight of the obese in a society obsessed by anorexic Calvin Klein models, or the contribution of fatness to escalating medical costs. Miraculously, coincident with FDA approval of the first new drugs for obesity in decades (dexfenfluramine and tetrahydrolipstatin), a former Surgeon General has spoken out about the disease, talk shows have taken an interest, and as surgeons are advertising for obese patients, societies of the surgical establishment have begun to accept papers on the topic for presentation and even publication!

The most important message of the preceding article by Sugerman et al. is that severe obesity and its surgical treatment is very serious business, indeed. The glass is half full when we note that 1231 patients undergoing standard Roux-en-Y gastric bypass between 1981 and 1994 have shed pounds, stopped taking medications, and improved their quality of life to a degree unsurpassed by any treatment of any disease. It is half empty, however, when we consider that 10% to 15% (my estimate) of these 1231 patients might be medical failures that we are unable to "weed out" preoperatively or salvage postoperatively.

If one denies that obesity is a legitimate disease, encompassing individuals abused as children, as well as those with "thrifty genes," defective leptin receptors, or those who are simply too poor to avoid high-fat Western diets that can lead to obesity (blame the patient for gluttony), it is very difficult to accept, let alone appreciate, the extraordinary efforts by these investigators to help the subset of patients described in this article. During the "dark era" of jejunoileal bypass (blame the operation not the medical profession for ignorance about how best to manage it), some of us were willing to make similar efforts to make the operation work in selected cases. The backlash after blatant mismanagement of jejunoileal bypass patients led to ever smaller, ever more proximal constrictions of the gastrointestinal tract (from gastroplasty to subcricoid esophageal banding[1]) in attempts to foil the "soft calorie syndrome."[2]

This report by Sugerman et al. exemplifies a significant recent trend in obesity surgery—that is, the resurgence of increasingly malabsorptive operations.

It also exemplifies a new concept in antiobesity surgery—a *staged approach*[3] instead of the "one-stop shopping" or "one-size-fits-all" mentality of super aggressive malnutritional operations as primary procedures for superobesity.[4,5] In the absence of outcome predictors, one might have to accept a primary gastric restriction operation as a test of the severity of the eating disorder or the comorbidity. Therapeutic failure of the relatively safe and simple gastric restriction would "qualify" the patient for a more aggressive, riskier malabsorptive operation.

There is no "free lunch" in the treatment of obesity. Effective operations come with penalties. In this series, there were at least two deaths among the 27 patients studied, and anywhere from one to three were lost to follow-up. (In Table I, among 53 patients, 13 [25%] were lost to follow-up.) Twenty percent of the patients in this series had serious malnutrition, three required gut lengthening operations, and one needed 1 year's worth of supplemental jejunostomy feeding. The tragedy of obesity and its surgical treatment is that the patients almost universally prefer postoperative medical failure to being fat in a lean world.[6]

The article by Sugerman et al. raises several issues. Although the growing epidemic of obesity is creating new markets endearing gastrointestinal surgeons to their managed care employers, the simplicity of obstructive antiobesity operations bears no resemblance to the complex management of the superobese or the 40% or more of patients with inadequate gastric restrictive operations beyond 5 years postoperatively. Malabsorptive operations titrating proportions of stomach and stoma size to small intestinal limb lengths should be considered human experimentation, as evidenced by Sugerman's 30 ml (reference 15 in their report) to 200 ml (just how they are measured is uncertain) gastric pouches attached to 50 to 150 cm common limbs with bypassed bowel varying by 100%, that is, 200 to 400 cm. Poverty, expense, or a "United States managed care environment" are not excuses for failing to investigate, prevent, or treat the effects of these types of operations. Beyond the mandated educated informed consent, there should be a requirement for the surgeon to have ascertained, with a reasonable degree of certainty, that each patient has the minimum socioeconomic resources needed to guarantee the costly follow-up that is called for.

Even as new drugs become available, antiobesity surgery is needed to provide cost-effective (economic parity with nonoperative methods after 4 to 5 years[7]), sustained, curative treatment of severe comorbidity. Realization of the powerful preventive potential of surgical treatment should lead to earlier operations in patients younger than the current mean age of approximately 38 years and below the current "approved" weight levels for a BMI of 35 to 40 kg/m^2. This might prevent progression of the eating disorder (at least 40% of severely obese patients fulfill DSM IV criteria[8] for binge eating disorder) and establishment of irreversible severe comorbidity. Nevertheless, surgical treatment of obesity should not be taken lightly!

REFERENCES

1. Peitersen E, Quaade F, Breum L, Olesen HP. Esophageal banding: Pilot study of a new operation. Surg Res Commun 1990;9: 177-182.
2. Kral JG, Kissileff HR. Surgical approaches to the treatment of obesity. Ann Behav Med 1987;9:15-19.
3. Kral JG. Surgical treatment of obesity. Med Clin North Am 1989;73:251-264.
4. Scopinaro N, Gianetta E, Adami GF, et al. Biliopancreatic diversion for obesity at eighteen years. Surgery 1996;119:261-268.
5. Liszka TG, Sugerman HJ, Kellum JM, Birkenhauer R, Starkey J. Risk/benefit considerations of distal gastric bypass. Int J Obesity 1988;12:604(A).
6. Rand CSW, MacGregor A. Successful weight loss following surgery and the perceived liability of morbid obesity. Int J Obesity 1991;15:577-579.
7. Martin LF, Tan T-L, Horn J, et al. Comparison of the costs associated with medical and surgical treatment of obesity. Surgery 1995;118:599-607.
8. Diagnostic and Statistical Manual of Mental Disorders, version IV. Washington, D.C.: American Psychiatric Association.

Development of Systemic Immunologic Responses Against Hepatic Metastases During Gene Therapy for Peritoneal Carcinomatosis With Retroviral HS-tk and Ganciclovir

Takeyuki Misawa, M.D., Mimi H. Chiang, Ph.D., Lalita Pandit, M.D., Erlinda M. Gordon, M.D., W. French Anderson, M.D., Dilip Parekh, M.D.

Gene therapy with retroviral mediated gene transfer of the herpes simplex thymidine kinase (HS-tk) gene into a tumor mass confers sensitivity of the tumor cells to ganciclovir (GCV). Tumor-specific immunologic responses may develop following treatment of the primary tumor with retroviral HS-tk and GCV. In the present study we assessed whether GCV treatment of HS-tk transduced colon cancer (TK^+) implanted in the peritoneal cavity induced a systemic antitumor response that would inhibit growth of a second wild-type (TK^-) tumor implanted in the liver. DHDK12 rat colon cancer cells were transduced in vitro with the retroviral HS-tk vector and established as a permanent cell line (TK^+ cells). TK^+ or TK^- DHDK12 cells (6×10^6 cells) were injected intraperitoneally on day 0 into BD-IX rats. On day 10, TK^- cells (3×10^6 cells) were injected into the liver in all the groups. The animals were then treated with GCV (150 mg/kg) for 13 days. TK^+ peritoneal tumors underwent significant regression during therapy with GCV (0.05 ± 0.004 g; n = 7) compared to wild-type (TK^-) tumors (2.2 ± 0.7g; n = 6) ($P < 0.05$). The volume of TK^- tumors in the liver was significantly lower in GCV-treated rats with TK^+ peritoneal tumors (12.5 ± 8.3 mm^3) compared to rats with TK^- peritoneal tumors (96.7 ± 18.1 mm^3) ($P < 0.05$). Histology of the liver tumors in the TK^+ groups showed a dense monocytic infiltrate with fibrosis and only occasional viable tumor cells. Gene therapy with retroviral HS-tk vectors may provide a novel approach to treatment of gastrointestinal cancer by both direct cytotoxicity and an indirect mechanism that may include enhanced immunologic responses against disseminated disease. (J GASTROINTEST SURG 1997;1:527-533.)

Metastatic gastrointestinal cancer is associated with a dismal prognosis.[1,2] The median survival of patients with gastric, biliary tract, or pancreatic cancer metastatic to the peritoneum or liver is less than 6 months and most patients die within a year.[1,2] Patients with colon cancer have a somewhat longer median survival; however, there are virtually no 5-year survivors from metastatic gastrointestinal cancer.[1,2] Chemoradiation has not been shown to be very effective for metastatic gastrointestinal cancer.[1,2] Although intraperitoneal chemotherapy has been shown to be of some benefit in ovarian cancer, this modality has not been shown to be very effective in gastrointestinal cancer metastatic to the peritoneal cavity.[1,2] Metastatic pancreatic cancer has a particularly dismal prognosis, since an active regimen against this disease has not been found despite numerous clinical trials.[2] Clinicians who frequently deal with patients with metastatic gastrointestinal cancer clearly recognize the need for development of effective alternative therapeutic approaches.

Gene therapy has developed as a consequence of the tremendous increase in the information available on the biology of cancer and the development of gene transfer technology.[3-6] The transfer of drug susceptibility genes into malignant tumors by retroviral and adenoviral vectors is a novel approach for treatment of cancer.[3-5] Transfer of the herpes simplex thymidine kinase (HS-tk) gene into tumor cells renders the transduced cells susceptible to the viral drug ganciclovir

From the Department of Surgery (T.M., M.H.C., L.P., and D.P.) and Gene Therapy Laboratories (E.M.G. and W.F.A.), University of Southern California School of Medicine, Los Angeles, Calif.
Presented at the Thirty-Seventh Annual Meeting of The Society for Surgery of the Alimentary Tract, San Francisco, Calif., May 19-22, 1996.
Reprint requests: Dilip Parekh, M.D., 1510 San Pablo St., Suite 430, Los Angeles, CA 90033.

(GCV).[3-5] This is the most promising gene therapy approach currently under investigation that uses a suicide gene transfer strategy.

Disseminated gastrointestinal cancer is often metastatic only to the peritoneal cavity and to the liver. We have previously demonstrated that intraperitoneal injections of a concentrated supernate of the HS-tk retroviral vector with ganciclovir treatment lead to inhibition of metastatic peritoneal cancer.[7] Successful therapy for peritoneal metastases may, however, have no effect on disease-free survival since many of the patients will return with metastatic disease in the liver. Recent reports have suggested that immune responses develop during retroviral HS-tk/GCV treatment of the primary cancer that limit subsequent growth of a distant naive nontransduced tumor.[8,9] As a first step toward developing a protocol for managing metastatic gastrointestinal cancer, we attempted to determine in this study whether treatment with GCV of peritoneal implants transduced with the HS-tk retrovirus (TK^+) leads to development of systemic antitumor responses that inhibit growth of a second naive nontransduced (TK^-) distant tumor implanted in the liver. We hypothesized that if systemic antitumor responses develop during GCV treatment of HS-tk transduced peritoneal carcinomatosis, then this would provide a rationale for treatment of metastatic gastrointestinal cancer with intraperitoneal gene therapy.

MATERIAL AND METHODS

Cell Lines

The DHDK12 cell line is an established colon carcinoma cell line originating from a 1,2-dimethylhydralazine-induced colon adenocarcinoma in syngeneic BD-IX rats.[10,11] The DKDK12 cells were cultured as monolayers in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum under humidified conditions in the presence of 5% carbon dioxide.

Retroviral Mediated Gene Transfer

The G1TklSvNa.7 vector was provided by Genetic Therapy, Inc. (Gaithersburg, Md.). The G1 backbone of this vector is derived from the Moloney murine leukemia virus. The G1TklSvNa.7 vector contains the HS-tk gene just downstream from the 5′ long terminal repeat sequence and uses this long terminal repeat sequence as its promoter. The SV40 early promoter serves as an internal promoter for the neomycin phosphotransferase gene (NeoR), which confers resistance to the neomycin analogue geneticin (G418). The HS-tk vector is packaged by the amphotrophic retroviral producer cell line, PA317, which was derived from NIH 3T3 cells. The G1TkSvNa.7/PA317 producer cell line generates a supernatant fluid with a titer of 4.9×10^6 colony-forming units/ml. The DHDK12 cells were transduced with the HS-tk vector supernatant (G1Tk1SvNa.7) in the presence of protamine (10 μg/ml) at 37° C for 2 hours. After 2 hours, fresh tissue culture growth medium was added, and the transduced cells were then selected in G418 (0.4 mg/ml). The selected NeoR clones were subcultured and established as a permanent HS-tk transduced DHDK12 cell line (TK^+ cell line).

In Vivo Experimental Protocol

Male BD-IX rats (7 weeks old; National Cancer Institute, Bethesda, Md.) were used. Animals were given free access to standard laboratory chow and water throughout the experiment. TK^+ and TK^- DHDK12 cells were grown to near-confluence in 175 cm^2 flasks, washed twice with phosphate-buffered saline solution, and dispersed by incubation at 37° C with 0.05% trypsin-ethylenediametetraacetic acid for 8 minutes. The cells were washed with DMEM plus 10% fetal bovine serum to inactivate trypsin, and aliquots of the cell suspensions were stained with trypan blue, counted in a hemocytometer, and resuspended at 6×10^6 viable cells/ml. TK^+ or TK^- DHDK12 cells (6×10^6 cells/animal) dispersed in 1.0 ml Hanks' balanced salt solution (HBSS) were injected intraperitoneally. The rats were then observed for 10 days for establishment of peritoneal tumors. On day 10, rats were anesthetized with pentobarbital (35 to 50 mg/kg body weight), and a laparotomy was performed to select the animals with established intraperitoneal tumor deposits as assessed by visual examination for inclusion into the study. Fourteen (74.0%) of 19 rats injected with TK^- cells and 15 (75%) of 20 rats injected with TK^+ cells developed macroscopic peritoneal tumors. The tumor foci were scattered over the omentum. We estimated the number of foci, and the animals were then randomized to the different treatment groups (Table I) after being stratified for the number of foci. TK^- DHDK12 cells (3×10^6 cells/animal) dispersed in 0.05 ml HBSS were inoculated under the liver capsule in all the groups. Treatment with GCV (150 mg/kg/day) was begun 3 days after the laparotomy and continued for 13 days (Fig. 1). There were no deaths from GCV treatment in any of the groups. The tumors in the peritoneal cavity and liver were harvested on day 30 of

Table I. Treatment groups

Group	No.	Peritoneal tumor	Treatment	Liver tumor
$TK^-/G-$	7	TK^-	PBS	TK^-
$TK^-/G+$	6	TK^-	GCV	TK^-
$TK^+/G-$	7	TK^+	PBS	TK^-
$TK^+/G+$	7	TK^+	GCV	TK^-

TK^+ = HS-tk transduced cells; TK^- = wild-type nontransduced cells; PSB = phosphate-buffered saline solution; GCV = ganciclovir (150 mg/kg).

Table II. Effects of treatment of TK^+ or TK peritoneal tumors with GCV on growth of TK^- liver tumors

Group	No.	Peritoneal tumor (g)	Liver volume (mm^3)
$TK^-/G-$	7	9.9 ± 3.5	255.1 ± 66.2
$TK^+/G-$	6	3.4 ± 2.0	195.2 ± 43.6
$TK^-/G+$	7	2.2 ± 0.7	96.7 ± 18.1
$TK^+/G+$	7	0.05 ± 0.004*	12.5 ± 8.8*

*P <0.05 vs. all control groups.

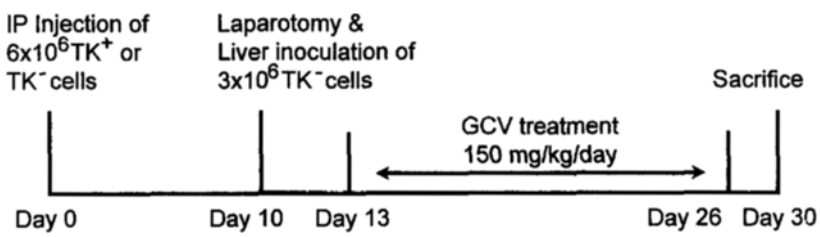


Fig. 1. Experimental protocol for the in vivo study.

the experiment. Peritoneal tumor weight and liver tumor volume were then measured, and the liver tumors were fixed in formalin for histologic analysis. The experimental protocol is summarized in Fig. 1.

RESULTS

Peritoneal Tumors

Extensive spread of peritoneal tumors was found throughout the entire peritoneal cavity, the abdominal wall, and the retroperitoneum in the $TK^-/G-$, $TK^-/G+$, and $TK^+/G-$ groups. In contrast, in four (57%) of the seven animals in the $TK^+/G+$ group, complete regression of the peritoneal tumors was found and only tiny foci (<1 mm in diameter) limited to within the omentum were found in the other three animals (Fig. 2). The mean weight of the peritoneal tumor was significantly less in the $TK^+/G+$ group (0.05 ± 0.004 g) compared to the other treatment groups ($TK^-/G-$ = 9.9 ± 3.5 g; $TK^-/G+$ = 2.2 ± 0.7g; and $TK^+/G-$ = 3.4 ± 2.0 g; P <0.05) (Table II). The marked increase in sensitivity in vivo of TK^+ transduced tumor cells to GCV is demonstrated by the 40-fold reduction in mass of the tumors in the $TK^+/G+$ group compared to the $TK^-/G+$ treatment group (Fig. 3, *A*). A tendency toward a reduction in the tumor weight of the $TK^+/G-$ group was seen in comparison to the $TK^-/G-$ group; however, this difference failed to reach statistical significance.

Liver Tumors

Macroscopic tumor in the liver was found in all three control groups ($TK^-/G-$, $TK^-/G+$, and $TK^+/G-$). The mean volume of the hepatic tumors in the $TK^+/G+$ group (12.5 ± 8.8 mm^3) was significantly less compared to the control groups ($TK^-/G-$ = 255.1 ± 66.2 mm^3; $TK^-/G+$ = 96.7

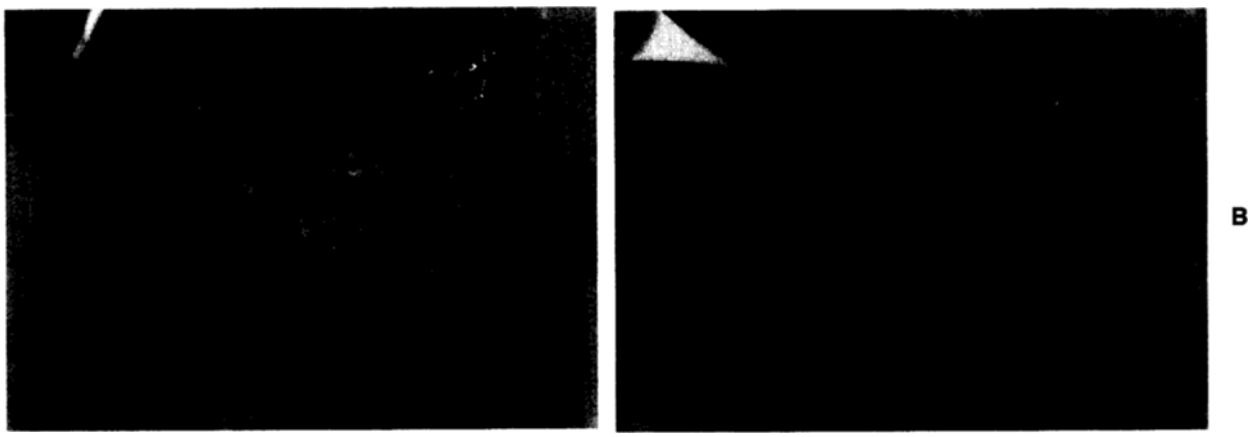


Fig. 2. Effects of GCV treatment on TK^+ and TK^- peritoneal tumors in BD-IX rats. Peritoneal tumor on day 30 after implantation of 6×10^6 cells and treatment with GCV (150 mg/kg daily) in the $TK^+/G+$ group **(A)** and the $TK^-/G+$ group **(B)**. The marked sensitivity of TK^+ tumors to GCV is clearly demonstrated by the dramatic difference in the amount of peritoneal tumors between the two groups.

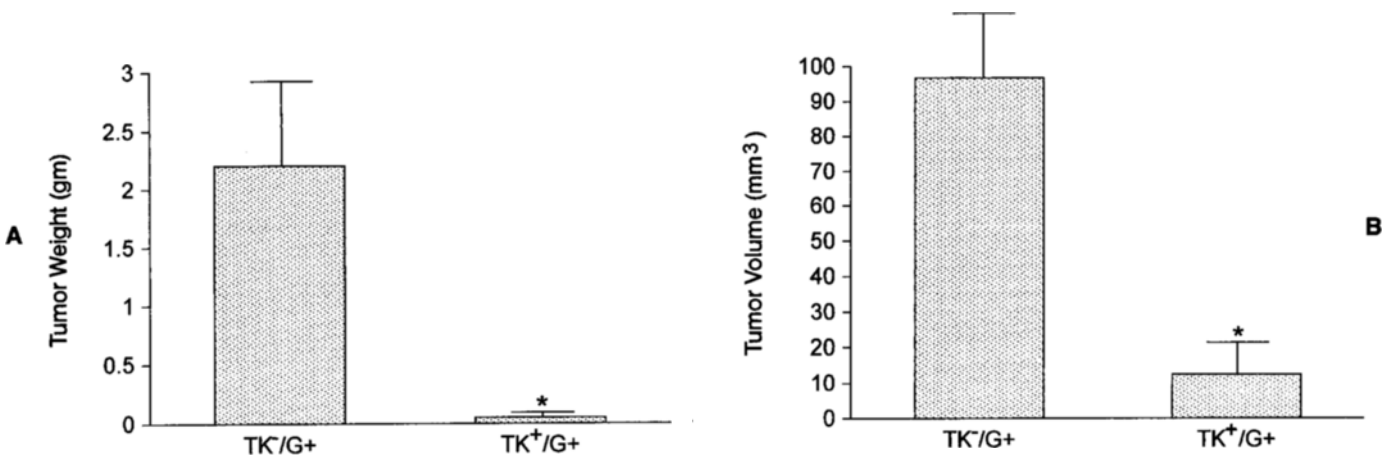


Fig. 3. Growth of TK^- liver tumors during treatment of TK^+ or TK^- peritoneal tumors with GCV. **A,** Intraperitoneal tumor. A highly significant reduction of TK^+ peritoneal tumors ($TK^+/G+$ group) is found after treatment with GCV compared to TK^- tumors ($TK^-/G+$ group). **B,** Liver tumor (TK^-). Growth of TK^- liver tumors was significantly inhibited during treatment of TK^+ peritoneal tumors ($TK^+/G+$ group) with GCV compared to TK^- peritoneal tumors ($TK^-/G+$ group; * = $P < 0.05$).

$\pm$ 18.1 mm^3; and $TK^+/G- = 195.2 \pm 43.6$ mm^3; $P < 0.05$) (Table II and Fig. 3, *B*). The liver tumors were macroscopically visible in all the control animals; however, in the $TK^+/G+$ groups, three of seven rats did not show any tumor at the injection site. Gross inspection of the hepatic tumors in the control animals showed that the tumors were composed of solitary, well-defined, grayish white nodules. In the $TK^+/G+$ animals, the hepatic tumors were rather ill defined and much smaller compared to the control groups. Microscopically, in control animals, the hepatic tumors formed distinct nodules, displacing the hepatocytes, and showed evidence of a poorly differentiated adenocarcinoma consisting mainly of tumor cells organized into lobules or several thick cell cords (Fig. 4). In the $TK^+/G+$ group, the residual tumor consisted of fibrotic scars with a few scattered tumor cells broken up into islands and located within dense fibrotic tissue with a significant infiltration of mononuclear inflammatory cells (see Fig. 4).

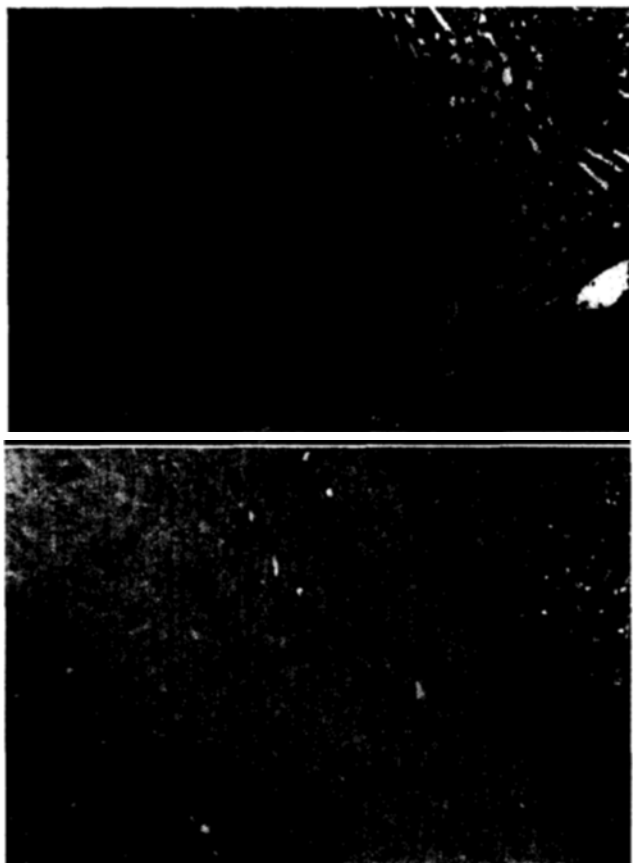


Fig. 4. Histology of liver tumors. **A,** Liver tumors in the $TK^-/G+$ group showing a well-formed adenocarcinoma in the liver. **B,** TK^- liver tumor in the $TK^+/G+$ group demonstrating a dense fibrosis and inflammatory infiltrate with only a few scattered tumor cells (original magnification ×40).

DISCUSSION

Moolten[12] first demonstrated that the transfer of the HS-tk gene into tumor cells renders the cells susceptible to the viral drug ganciclovir. Transfection of tumor cells is significantly better with viral vectors than with physical methods, and therefore the development of viral vectors has made clinical gene therapy possible.[13] Both retroviral and adenoviral vectors have been used for efficient transfer of the HS-tk gene into tumor cells and at present 11 clinical trials are in progress evaluating this approach for cancer.[3-6] The enhanced susceptibility of tumor cells transduced with the HS-tk gene to the viral drug ganciclovir is clearly illustrated in our study, which demonstrates that TK^+ peritoneal implants were almost completely eliminated by 13 days of treatment with ganciclovir, and the amount of peritoneal disease in the $TK^+/G+$ group was 40-fold less than that in the $TK^-/G+$ group.

Gastrointestinal cancers frequently metastasize to the peritoneal cavity and/or the liver. Peritoneal metastatic disease may be a target for gene therapy since locoregional treatment is possible by intraperitoneal administration of retroviral or adenoviral vectors. We have previously demonstrated that retroviral vectors efficiently transduce gastrointestinal tumor cells in the presence of human ascitic fluid suggesting that the vector will be active in the milieu of the peritoneal cavity.[7] We have also demonstrated that intraperitoneal injections of a concentrated retroviral vector supernate lead to significant and profound inhibition of pancreatic cancer metastatic to the peritoneal cavity, suggesting that intraperitoneal gene therapy with HS-tk vectors may provide a novel approach to the treatment of metastatic gastrointestinal cancer.[7] Treatment of peritoneal metastases alone, however, may not lead to improved survival because of the high probability of development of metastatic disease in the liver and elsewhere.

Barba et al.[8] first suggested that a systemic tumor-specific immune response develops during GCV treatment of 9L glioma xenografts transduced by in situ gene transfer after intratumoral injection of the retroviral TK producer cell line. Twenty-two percent of the animals treated by gene therapy with the TK producer cells in their study demonstrated long-term survival. Rechallenge of the long-term survivors with TK^- 9L cells at a second site in the brain or in the subcutaneous tissue at 90 days after treatment of the primary tumor was associated with significant growth inhibition of the implanted tumors at the secondary sites compared to naive rats not previously exposed to 9L cells. Tapscott et al.[9] compared the in vivo growth of TK^+ and TK^- 9L glioma tumor cells in F344 rats and found that significantly smaller tumors were formed after implantation of TK^+ tumor cells compared to TK^- tumor cells. It was suggested in this study that the growth of the genetically modified TK^+ tumor cells was inhibited by a systemic antitumor response mounted against the foreign viral TK protein in the cytoplasm of the TK^+ 9L tumor cells. Our study provides further evidence that a systemic antitumor response develops during treatment of TK^+ tumors with GCV. We found significantly smaller TK^- liver tumors in the group treated with $TK^+/G+$ compared to the $TK^-/G+$ group. The only difference between the groups was the injection of TK^+ cells into the peritoneal cavity in the experimental group and naive TK^- cells into the control group; the liver tumor in both groups was from

TK^- cells. The almost complete regression of peritoneal disease in the $TK^+/G+$ group was associated with significant reductions in the size of the TK^- liver tumors.

The mechanisms for the systemic antitumor responses seen during retroviral mediated gene therapy with the HS-tk vector are not clear.[8,9] It has been suggested that development of an immune response during HS-tk/GCV treatment is stimulated by the foreign viral HS-tk protein in the cytoplasm of the transduced host target tumor cell.[9] Our results suggest that this mechanism, if present, accounts only to a small extent for the systemic antitumor effects seen in our study, since only a trend toward smaller tumors in the peritoneal cavity was found in the $TK^+/G-$ group compared to the $TK^-/G-$ group. Furthermore, there were no differences in the size of the liver tumors in the $TK^-/G-$ and $TK^+/G-$ groups. Since growth inhibition of the liver tumors was found only in the $TK^+/G+$ group, our results suggest that cell killing of the transduced TK^+ tumors is necessary for the development of systemic responses that inhibit growth of distant TK^- tumors.

In our study the dose of GCV used was similar to that previously reported by Caruso et al.[14] with the use of DHDK12 cells during gene therapy experiments for treatment of liver metastases. Although these investigators did not find a direct toxic effect on DHDK12 tumor cells at a GCV dose of 150 mg/kg, some direct toxicity was found in our study since the tumors treated with $TK^-/G+$ tumors were smaller compared to $TK^-/G-$ tumors. The inhibition of growth of the liver and peritoneal tumors in the $TK^+/G+$ group, however, is not a GCV-mediated nonspecific toxic effect since the magnitude of growth inhibition of the tumors in the $TK^+/G+$ group was at least 40-fold greater for the peritoneal tumors and 20-fold greater for the liver tumors compared to the $TK^-/G+$ group, suggesting that a specific antitumor response is seen in the tumors treated with $TK^+/G+$.

Our finding that an immune response appears to develop during GCV treatment of TK^+ peritoneal implants provides support for further manipulation of the immune system for maximum antitumor effect. Transduction of mouse colon cancer cells with adenoviral vectors that incorporate both the HS-tk gene and a cytokine gene such as interleukin-2 leads to an augmented antitumor effect compared to treatment with a HS-tk vector alone.[15] Dranoff et al.[16] have shown that gene transfer of several immunostimulatory cytokines such as interleukin-2 and GM-CSF into tumor cells leads to significant immune-mediated antitumor effects. Our data clearly show that although there was significant reduction of liver tumors in the $TK^+/G+$ animals, viable tumor cells were still present in the liver suggesting that the observed systemic antitumor effect was only partial. These results provide a rationale for further study as to whether this partial antitumor effect could be further augmented either by simultaneous intratumoral injections of a second vector carrying genes for immunologically active cytokines or by using a combination vector carrying genes for both HS-tk and an immunostimulatory cytokine. Successful gene therapy may change the present concepts of management of patients with metastatic gastrointestinal cancer. If the experimental approaches described herein are successful in the patient care setting, then this may provide novel options for treatment of gastrointestinal cancer both in the adjuvant setting and for established metastatic gastrointestinal cancer.

The DHDK12 cell line was a gift from Dr. Seiichiro Ishii, Department of Surgery, Keio University School of Medicine, Tokyo, Japan.

REFERENCES

1. Baker AR, Weber JS. Treatment of malignant ascites in cancer. In DeVita VT Jr, Hellman S, Rosenberg SA, eds. Principles and Practice of Oncology, vol. 2, 4th ed. Philadelphia: JB Lippincott, 1993, pp 2255-2261.
2. Klapdor R. Perspectives in chemotherapy of pancreatic cancer. Eur J Surg Oncol 1991;17:153-166.
3. Culver KW, Ram Z, Wallbridge S, et al. In vivo gene transfer with retroviral vector-producer cells for treatment of experimental brain tumor. Science 1992;256:1550-1552.
4. Moolten FL, Wells JM. Curability of tumors bearing herpes thymidine kinase genes transferred by retroviral vectors. J Natl Cancer Inst 1990;82:297-300.
5. Smythe RW, Hwang HC, Elshami AA, et al. Treatment of experimental human mesothelioma using adenovirus transfer of the herpes simplex thymidine kinase gene. Ann Surg 1995;222:78-86.
6. Marshall E. Gene therapy's growing pains. Science 1995; 269:1050-1055.
7. Yang L, Hwang R, Pandit L, Gordon EM, Parekh D. Gene therapy of metastatic pancreas cancer with intraperitoneal injections of concentrated retroviral HS-tk vector supernatant and ganciclovir. Ann Surg 1996;224:405-417.
8. Barba D, Hardin J, Sadelain M, Gage FH. Development of anti-tumor immunity following thymidine kinase-mediated killing of experimental brain tumors. Proc Natl Acad Sci USA 1994;91:4348-4352.
9. Tapscott SJ, Miller AD, Olson JM, et al. Gene therapy of rat 9L gliosarcoma tumors by transduction with selectable genes does not require drug selection. Proc Natl Acad Sci USA 1994;91:8185-8189.
10. Martin MS, Bastien H, Martin F, et al. Transplantation of intestinal carcinoma in inbred rats. Biomed 1973;19:555-558.
11. Dunnington D, Burscarino C, Gennaro E, Greig R, Poste G. Characterization of an animal model of metastatic colon carcinoma. Int J Cancer 1987;39:248-254.
12. Moolten FL. Tumor chemosensitivity conferred by inserted herpes thymidine kinase genes. Paradigm for a prospective cancer control strategy. Cancer Res 1986;46:5276-5281.

13. Anderson FW. Human gene therapy. Science 1992;256:808-813.
14. Caruso M, Panis Y, Gagnadeep S, et al. Regression of established macroscopic liver metastases after in situ transduction of a suicide gene. Proc Natl Acad Sci USA 1993;90:7024-7028.
15. Chen S, Li Chen XH, Kosai K, Wang Y, Finegold MJ. Combination gene therapy for liver metastases of colon carcinoma in vivo [abstr] Am Assoc Cancer Res, 1996.
16. Dranoff G, Jaffee E, Lazenby A, et al. Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulation factor stimulates potent, specific, and long-lasting anti-tumor immunity. Proc Natl Acad Sci USA 1993;90:3539-3543.

Duodenal Segment Complications in Vascularized Pancreas Transplantation

Robert J. Stratta, M.D., Rakesh Sindhi, M.D., Debra Sudan, M.D., John T. Jerius, M.D., Stanley J. Radio, M.D.

Bladder drainage by the duodenal segment (DS) technique is currently the preferred method of pancreas transplantation (PTX) but is associated with unique complications. Over a 7-year period, 191 diabetic patients underwent 201 whole-organ PTXs with bladder drainage using a 6 to 8 cm length of DS as an exocrine conduit. A retrospective chart review was performed to document all DS morbidity. DS complications occurred in 38 cases (19%). Twelve patients developed DS leaks and required operative repair. DS bleeding was documented in 26 cases, necessitating cystoscopy in 22 patients and open repair in eight patients for significant hematuria. Cytomegalovirus (CMV) duodenitis was diagnosed in seven cases, with four presenting as DS leaks and three with hematuria. Five patients experienced ampullary obstruction early after PTX. Rejection of the DS was confirmed by biopsy in 13 patients, including eight cases of acute and five cases of chronic rejection. Two patients had stone formation from the DS staple line. Enteric conversion was performed in five patients for DS abnormalities (leaks in 2 cases, bleeding in 2, and CMV duodenitis in 1). Among patients with DS complications, patient survival is 84% and pancreas graft survival is 68% after a mean follow-up of 44 ± 12 months. Complications related to the DS remain an important source of morbidity but rarely cause death after PTX. In spite of unique side effects, transplantation of the DS remains an acceptable alternative for exocrine drainage after PTX. (J GASTROINTEST SURG 1997;534-544.)

With improvements in organ retrieval technology, refinements in surgical techniques, and advances in clinical immunosuppression, success rates for vascularized pancreas transplantation (PTX) have improved dramatically.[1] As a result, PTX has become an accepted treatment option in appropriately selected diabetic patients.[2] The history of clinical PTX largely revolves around the development and application of various surgical techniques.[3] Lillehei et al.[4] initially described the technique of whole-organ pancreaticoduodenal transplantation in the late 1960s. Cook et al.[5] introduced the concept of urinary bladder drainage of the exocrine pancreas in the early 1980s. Nghiem and Corry[6] later modified these methods to incorporate whole-organ pancreaticoduodenal transplantation with anastomosis of a segment of duodenum to the urinary bladder.

Bladder drainage with a duodenal segment (DS) conduit is currently the preferred technique for managing exocrine secretions after vascularized PTX. According to United Network for Organ Sharing (UNOS) Registry data, more than 90% of PTXs are performed by this method.[1] The success of PTX is due in part to bladder drainage by the DS technique, which is not only safe but also allows monitoring of exocrine secretions in the urine and affords cystoscopic access to the allograft for visual inspection or biopsy.[7] However, this technique creates an unphysiologic connection with the duodenal conduit interposed between the allograft pancreas and the urinary bladder that may cause problems. Although well tolerated in the majority of PTX recipients, bladder drainage by the DS technique has been associated with metabolic and urologic complications resulting from altered physiology.[7-13] In most reports in the literature, there is a failure to differentiate complications specific to the DS from either urologic or intra-abdominal morbidity.[11-15] In only a few reports are DS

From the Department of Surgery and the Department of Pathology and Microbiology, University of Tennessee-Memphis (R.J.S.), Memphis, Tenn.; and the University of Nebraska Medical Center (R.S., D.S., J.T.J., and S.J.R.) and Clarkson Hospital, Omaha, Neb.
Presented at the Thirty-Seventh Annual Meeting of The Society for Surgery of the Alimentary Tract, San Francisco, Calif., May 19-22, 1996.
Correspondence: Robert J. Stratta, M.D., University of Tennessee-Memphis, Department of Surgery, Division of Transplantation, 956 Court Ave., Suite A202, Memphis, TN 38163-2116.

complications regarded as a separate entity.[16-18] Independent of the method of drainage (bladder vs. enteric), transplantation of the DS as an exocrine conduit is a paradigm of bowel transplantation. The purpose of this study was to review our experience and present a spectrum of morbidity unique to the DS in PTX recipients.

MATERIAL AND METHODS

During an 82-month period extending from April 1989 through February 1996, we performed 201 consecutive PTXs in 191 adult diabetic patients including 136 simultaneous kidney-pancreas transplantations (SKPT), 42 PTXs alone, 20 sequential pancreas transplantations after kidney transplantation (PAK), and three combined liver-pancreas transplantations (LP). The age of the recipients was 38 years with a mean duration of diabetes of 25 years. All patients underwent a comprehensive pretransplant evaluation as outlined previously.[19] In all cases the pancreas was procured from heart-beating cadaveric donors in conjunction with multiple-organ retrieval.[20] Combined hepatectomy and pancreaticoduodenosplenectomy was performed by an en bloc technique. The organ donors ranged in age from 8 to 56 years (mean 25 years), and all donors weighed at least 28 kg (mean 68 kg). All organs were preserved in University of Wisconsin solution with a mean pancreaticoduodenal preservation time of 16.5 hours (range 8 to 28 hours).

All patients underwent whole-organ PTX with bladder drainage by the DS technique as described previously.[21] Prior to PTX, reconstruction of the DS was performed with the intention of fashioning a 6 to 8 cm length of the second portion of the duodenum attached to the head of the pancreas without opening the duodenum. A gastrointestinal stapler was used to isolate the DS, taking care not to compromise the integrity of the ampulla of Vater (Fig. 1). The proximal and distal duodenal staple lines were then inverted with 3-0 monofilament suture. After revascularization of the allograft, pancreaticoduodenocystostomy was performed as a side-to-side anastomosis of the DS to the urinary bladder. A 3 to 4 cm longitudinal incision was made along the antimesenteric surface of the DS followed by a cystotomy similar in length in an appropriate area on the posterior aspect of the bladder. Depending on the orientation of the pancreas in the iliac fossa, the cystotomy was either transverse or vertical relative to the patient. A two-layer hand-sewn duodenocystostomy was then performed with a running inner layer and an interrupted outer layer of absorbable monofilament suture (Fig. 2). In some cases the lateral umbilical ligaments were "rolled up" and loosely wrapped around the duodenocystostomy to help prevent leaks from a potentially ischemic DS.

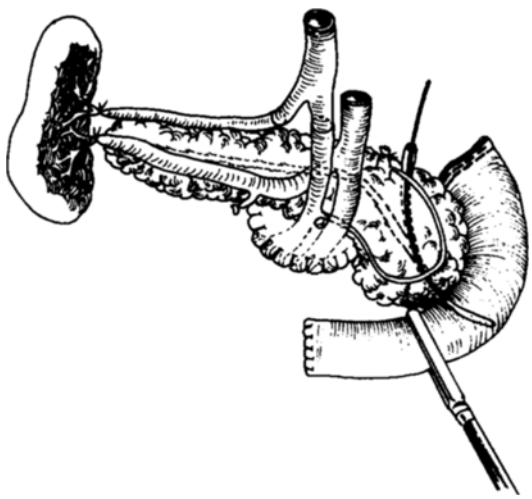


Fig. 1. Duodenal segment reconstruction of the pancreas allograft showing dissection and stapled division of the distal duodenum after identification of the ampulla by probing the common bile duct.

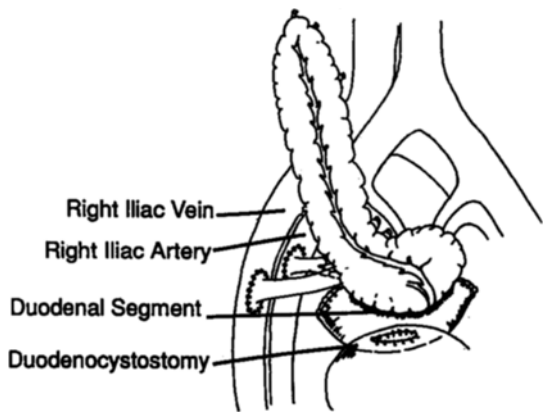


Fig. 2. Method of whole-organ pancreas transplantation with bladder drainage by the duodenal segment technique.

Patients were selected for transplantation based on ABO blood type compatibility, degree of sensitization, period of time on the waiting list, a negative T-lymphocytotoxic crossmatch, medical urgency, and human leukocyte antigen (HLA) matching in accordance with UNOS guidelines.[22] The mean HLA-ABDR match was 1.4 ± 0.3 for SKPT and 2.65 ± 0.6 for PTX-alone and PAK recipients. Perioperative antibiotic prophylaxis consisted of a preoperative, an intraoperative, and three postoperative doses of cefazolin (1 gm intravenously). Patients also received a single

postoperative dose of vancomycin (1 gm intravenously). Antifungal prophylaxis consisted of daily doses of ketoconazole (400 mg orally) or fluconazole (200 mg orally) for 2 to 3 months. All patients also received trimethoprim/sulfamethoxazole prophylaxis (for *Pneumocystis*) twice weekly for 12 months. Antiviral prophylaxis included intravenous ganciclovir for 2 weeks followed by oral acyclovir for 3 months.[23] Antiplatelet therapy consisting of oral aspirin (80 mg/day) was administered to all patients. In addition, solitary PTX-alone recipients received 5000 units of intravenous heparin intraoperatively with subcutaneous heparin (5000 units twice daily) continued postoperatively for 5 to 7 days until the patient was fully ambulatory.

All patients were managed with triple or quadruple immunosuppression with either OKT3 (Orthoclone, Ortho Biotech Inc., Raritan, N.J.) or antithymocyte globulin (ATGAM, The Upjohn Company, Kalamazoo, Mich.) induction for 10 to 14 days in combination with cyclosporine, steroids, and azathioprine.[24] Urethral catheter drainage was maintained for 7 to 10 days postoperatively. Low-pressure cystography with demonstration of DS reflux and documentation of the absence of an anastomotic leak was usually performed prior to catheter removal (Fig. 3). Initial rejection episodes were treated with pulsed or recycled corticosteroids. OKT3 or ATGAM was employed for the treatment of steroid-resistant rejection, rejection occurring during induction therapy, or biopsy-proved moderate-to-severe rejection. The diagnosis of rejection was based on clinical criteria, allograft dysfunction, serum and urine amylase levels, serum anodal trypsinogen levels, and urine cytology and was confirmed by pancreaticoduodenal histopathology.[25,26]

After transplantation, duplex ultrasonography and radionuclide perfusion scanning were performed on postoperative day 1 and whenever clinically indicated to evaluate graft perfusion, anatomy, and fluid collections. DS leaks were confirmed by conventional cystography, CT scan-cystography, or radionuclide scanning. Hematuria was evaluated by conventional cystoscopy.[27] Ultrasound-guided, cystoscopically directed DS and pancreas allograft biopsies were performed for clinical indications such as hematuria or allograft dysfunction.[28] The diagnoses of duodenal rejection and cytomegalovirus (CMV) duodenitis were confirmed by DS histopathology.[29,30] The diagnoses of ampullary obstruction and foreign body (stone) formation were based on clinical presentation in the absence of other cystoscopic, histologic, or imaging findings.[31]

Enteric conversion was performed through a lower midline intraperitoneal approach and required mobilization and takedown of the duodenocystostomy followed by a three-layer closure of the bladder.[14] The DS and head of the pancreas were then carefully mobilized. In most cases, enteric drainage was established by side-to-side duodenoenterostomy (Fig. 4). This was performed by direct anastomosis of the graft duodenum to an intact, in-continuity loop of mid-small bowel that could be easily brought down to the DS without tension. In one patient, enteric drainage was performed between the DS and the efferent end of a defunctionalized Roux-en-Y limb of small bowel. Perioperative antibiotic prophylaxis consisted of a single preoperative, an intraoperative, and three postoperative doses of intravenous cefazolin. Nasogastric tube decompression was usually maintained for 3 days, and the cystotomy closure was protected with an indwelling urethral catheter for 5 days.

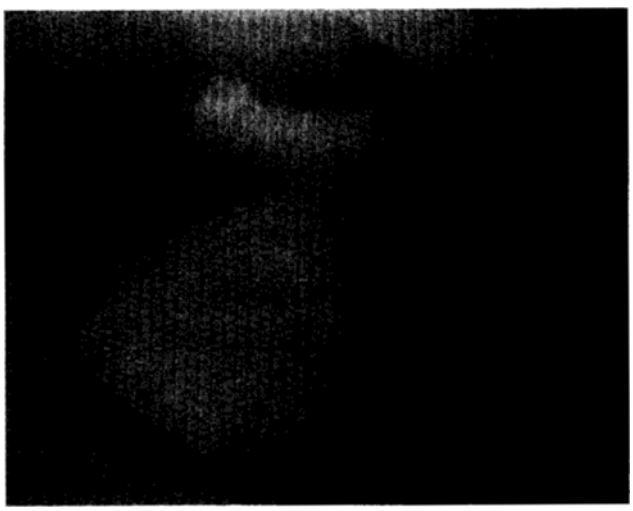


Fig. 3. Conventional retrograde cystogram showing reflux of contrast medium into the duodenal segment.

A retrospective chart review of hospital records was performed to document all morbidity related to the DS occurring at any time after PTX. Data were recorded as mean ± standard deviation and analyzed according to the type of transplant. Univariate analysis was performed by Student's *t* test for continuous variables, chi-square test for categoric variables, and Fisher's exact test when data were sparse. Actuarial patient and pancreas graft survival rates were computed by Wilcoxon life-table analysis. A P value of less than 0.05 was considered significant.

RESULTS

One or more DS complications occurred in 38 cases (19%) after PTX. The incidence of DS complications was similar according to the type of transplant:

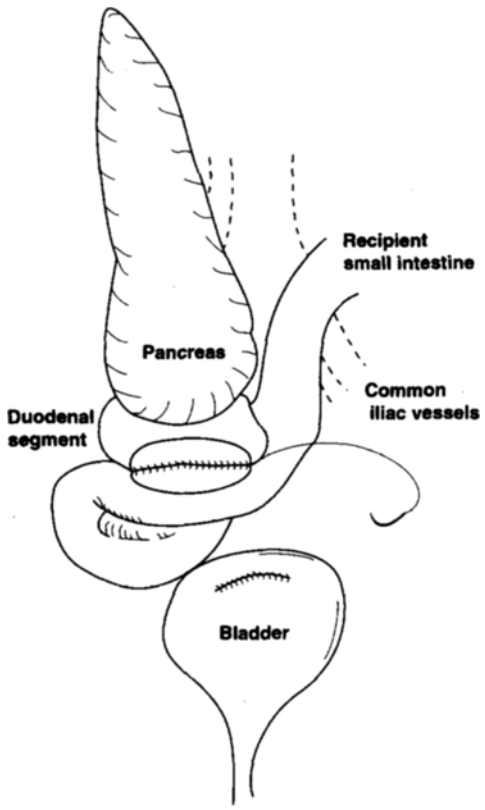


Fig. 4. Technique of exocrine drainage conversion from bladder to enteric drainage while maintaining the duodenal segment conduit.

Fig. 5. CT scan-cystogram demonstrating reflux of contrast medium into the duodenal segment with large leak and fluid collection between the bladder and rectum.

26 (19%) after SKPT, nine (21%) after PTX alone, three (15%) after PAK, and none after LP. The incidence of specific DS complications was as follows: 12 DS leaks (6%), 26 instances of DS bleeding (13%), seven cases of CMV duodenitis (3.5%), five ampullary obstructions (2.5%), 13 cases of DS rejection (6.5%), and two instances of stone formation in the DS (1%). Clinical presentation was variable but usually included fever, hyperamylasemia, allograft pancreatitis, lower abdominal pain, ileus with abdominal distention, leukocytosis, hematuria, or a reduction in urinary amylase. The diagnosis of DS complications was usually suggested by imaging studies and confirmed either by direct visualization (cystoscopic or open surgical) or histopathologic examination. A summary of specific DS complications follows.

DS Leaks

Twelve patients developed localized DS perforation or necrosis manifesting as a urine leak and pancreatic fistula. In nine cases (6.6%) these complications occurred after SKPT and in three cases (7%) after PTX alone. Clinical presentation usually included the sudden onset of lower abdominal pain and hyperamylasemia followed by fever, leukocytosis, ascites, and ileus. Antecedent hematuria occurred in six cases. Diagnosis was based on evidence of extravasation either by voiding cystography or CT scan-cystogram (Fig. 5). Nine leaks occurred early (within 6 months), at a mean of 56 days after PTX. The remaining three leaks occurred between 6 and 12 months after PTX. Specific DS pathology was documented in five patients including four cases of CMV duodenitis and one case of rejection. In the remaining seven cases (5 early and 2 late), a specific etiology was not determined but was presumed to be related to technical difficulties or ischemia.

Open surgical repair was performed in all 12 patients. Eleven of them underwent primary repair with bladder drainage, whereas the remaining patient underwent enteric conversion because the leak occurred 12 months after PTX. Seven patients (58%) developed peripancreatic sepsis, including four with recurrent leaks at a mean of 35 days after the initial repair. Six of these seven patients underwent a second operation, including one enteric conversion and one allograft pancreatectomy. Mean length of hospital stay for a DS leak was 18 days (range 5 to 48 days). Patient survival was 83% and pancreas graft survival was 75%. DS leak was responsible for the death of one patient following pancreas graft loss in an individual who developed two DS leaks as a result of CMV duodenitis 3.5 and 5 months after PTX alone. The initial leak was managed with open surgical repair, and the recurrent leak was treated with allograft pancreatec-

tomy. Eight days later, the patient suffered a massive pulmonary embolism and died.

DS Bleeding

Hematuria related to DS hemorrhage occurred in 26 patients including 15 (11%) after SKPT, eight (19%) after PTX alone, and three (15%) after PAK. Patients presented with persistent or recurrent hematuria with or without urinary retention. Initial management consisted of urethral catheter drainage in all cases. A total of 22 cases occurred early (within 6 months) at a mean of 53 days after PTX. The remaining four cases occurred late at a mean of 36 months after PTX. Etiology included rejection in 12 patients, DS leak in six, CMV duodenitis in three, foreign body (stone) formation in two and was unknown in three.

Therapy consisted of cystoscopy in 22 patients, open repair in three, and expectant management in one. Seven patients required repeat cystoscopy, five of whom subsequently underwent open repair. Two patients underwent enteric conversion, whereas another patient required allograft pancreatectomy for a mycotic pseudoaneurysm that had ruptured into the DS. Mean length of hospital stay for DS bleeding was 10 days (range 1 to 24 days). Patient survival was 81% and pancreas graft survival was 65%. DS bleeding was related to death in two patients and pancreas graft losses in three. One patient underwent enteric conversion for recurrent DS bleeding 67 months after SKPT. Histopathologic examination of the DS revealed chronic rejection and ischemia. The enteric conversion procedure was complicated by an anastomotic leak and breakdown leading to intra-abdominal sepsis and eventual death 2 months later. Another patient presented with severe hematuria 10 months after PTX alone and 8 months after open surgical repair of a DS leak from CMV duodenitis. Open surgical exploration revealed DS hemorrhage and bladder rupture. The patient subsequently died 18 days later from sepsis and aplastic anemia. The remaining graft loss related to DS bleeding occurred in a patient who developed duodenal rupture from severe rejection 6 weeks after PTX alone. Although the duodenal rupture was successfully repaired and the patient underwent revision duodenocystostomy, the solitary pancreas graft was lost to unrelenting rejection 3 months later.

CMV Duodenitis

Invasive CMV infection of the DS was documented in seven patients including four (3%) after SKPT and three (7%) after PTX alone. Six patients received organs from CMV-seropositive donors including two with primary CMV exposure. As noted previously, four presented with DS leaks and the remaining three had hematuria due to DS bleeding. The histopathologic diagnosis was confirmed by open biopsy in the four patients with DS leaks and by cytoscopic DS biopsy in the remaining three with hematuria (Fig. 6). All cases occurred between 2 and 4.5 months after PTX at a mean of 95 days.

Open surgical repair was performed in five cases of CMV duodenitis and cystoscopy alone in the remaining two. The mean length of hospital stay was 10 days (range 1 to 37 days). Two patients developed recurrent leaks as a result of biopsy-proved CMV duodenitis at a mean of 56 days after the initial repair. Both underwent reoperation, with one successful repair and one allograft pancreatectomy complicated by a fatal pulmonary embolus as previously discussed. Patient survival was 71% and pancreas graft survival was 57%. The other death occurred as a result of sepsis and aplastic anemia 8 months after open surgical repair of a DS leak without documentation of recurrent CMV infection as previously noted. The re-

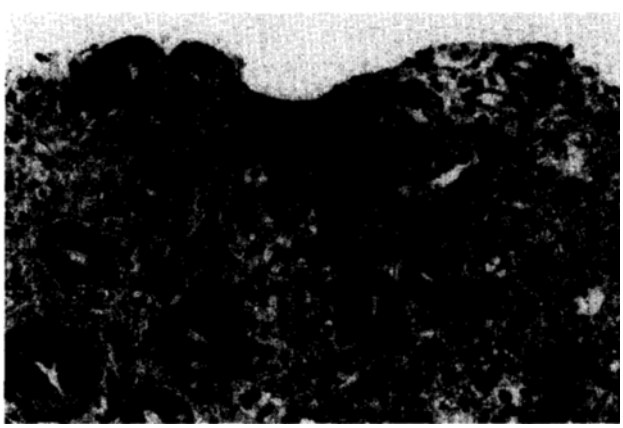


Fig. 6. Cystoscopically directed biopsy of duodenal segment mucosa with enteritis with villous blunting and characteristic nuclear and cytoplasmic inclusions of cytomegalovirus *(arrow)*. (Hematoxylin and eosin stain; ×200.)

maining pancreas graft loss occurred several years after successful open repair of a DS leak from CMV duodenitis and was due to chronic rejection.

Ampullary Obstruction

Five patients developed ampullary obstruction manifesting as a marked decrease in urinary amylase with or without pancreatitis or a pancreatic fistula in the absence of a documented urine leak or vascular thrombosis. Obstruction occurred after SKPT in two cases (2%), after PTX alone in two cases (5%), and after PAK in the remaining case (5%). All cases occurred early at a mean of 46 days (range 5 to 150 days), after PTX. The etiology was believed to be technical in two patients, rejection in two, and postbiopsy swelling in one. Initial management consisted of urethral catheter drainage and treatment with Sandostatin (Sandoz Pharmaceuticals, East Hanover, N.J.). Sphincterotomy was not attempted. Three cases subsequently resolved, one patient developed persistent pancreatitis and responded to open surgical drainage of a peripancreatic phlegmon, and the remaining case was complicated by the development of a pancreatic fistula, which resulted in allograft pancreatectomy due to necrotizing pancreatitis. The mean length of hospital stay was 21 days (range 7 to 44 days). Patient survival was 100% and pancreas graft survival was 20%. In contrast to the one pancreas graft loss due to allograft pancreatectomy, the three remaining graft losses were unrelated to ampullary obstruction and resulted from chronic rejection in PTX-alone recipients.

Duodenal Rejection

A total of 13 patients had biopsy-proved evidence of DS rejection including eight (6%) after SKPT, three (7%) after PTX alone, and two (10%) after PAK. Clinical presentation included hematuria (DS bleeding) in 12 and DS leak in one. The diagnosis was confirmed by cystoscopic DS biopsy in seven patients and open DS biopsy in six. Evidence of acute duodenal rejection was found in eight biopsies (Fig. 7) and chronic duodenal rejection in the remaining five (Fig. 8). Acute rejection was diagnosed at a mean of 4.7

Fig. 7. Explanted pancreas allograft with features of acute rejection in the duodenal segment mucosa including infiltrate of lymphocytes and eosinophils with intraepithelial lymphocytes, villous blunting, and crypt cell necrosis. (Hematoxylin and eosin stain; ×100.)

Fig. 8. Explanted pancreas allograft with features of chronic rejection in the duodenal segment mucosa including villous blunting and severe atrophy, gland disarray and dilatation, and mucosal fibrosis. Vascular changes of transplant arteriopathy were present in pancreas portion of the allograft. (Hematoxylin and eosin stain; ×40.)

months, whereas chronic rejection occurred at a mean of 46 months after PTX.

Five patients were treated with pulsed corticosteroids, four with antilymphocyte rescue therapy, and four with observation alone. Two patients underwent open surgical repair (one for a DS leak and one for persistent bleeding due to duodenal rupture). The mean length of hospital stay for duodenal rejection was 14 days (range 1 to 65 days). Patient survival was 69% and pancreas graft survival was 46%. Two deaths were temporarily related to duodenal rejection and resulted from intra-abdominal sepsis. One patient underwent allograft pancreatectomy for chronic rejection complicated by a DS fistula, whereas three other graft losses were due to acute and chronic rejection.

Enteric Conversion

Five patients (13%) underwent enteric conversion because of DS pathology. Indications for enteric conversion included the following: early DS leak in one, late DS leak in one, early DS bleeding in one, late DS bleeding in one, and CMV duodenitis in one. The mean length of stay after enteric conversion was 26 days (range 10 to 65 days). Patient survival was 80% and pancreas graft survival was 60%. In the two patients who had enteric conversions performed late (at 5.3 and 6 years after PTX), chronic rejection of the DS was documented by open biopsy at the time of the procedure. Both of these patients subsequently developed anastomotic leaks, with death from intra-abdominal sepsis in one and eventual allograft pancreatectomy in the other.

All DS Complications

The majority (79%) of the DS complications occurred within the first 6 months after PTX. The mean length of hospital stay associated with DS complications was 13.4 days. Open surgical repair was performed in 58%, cystoscopy in 63%, and enteric conversion in 13% of patients. Two deaths were directly attributed to DS complications. Although DS complications resulted in a 13% rate of allograft pancreatectomy, the presence of DS complications was not associated with either an increased risk of pancreatectomy or decreased pancreas graft survival when compared to patients without DS complications (Table I and Fig. 9).

Table I. Results

	Duodenal segment complications	
	Yes	No
Number	38	163 in 153 patients
Patient survival	32 (84%)	138 (90%)
Pancreas graft survival	26 (68%)	114 (70%)
Follow-up (mo)*	44 ± 12 (range 7-85)	39 ± 9 (range 3-81)
Graft pancreatectomy	5 (13%)	22 (13%)

*Values are expressed as mean ± standard deviation; P = not significant.

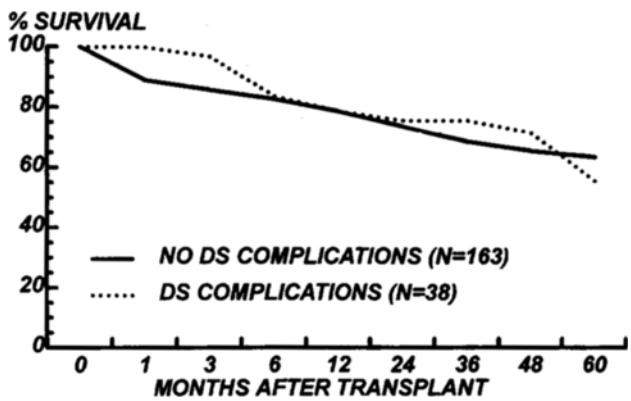


Fig. 9. Actuarial pancreas graft survival in patients with and without duodenal segment *(DS)* complications. No significant difference was noted.

DISCUSSION

Vascularized PTX has assumed an increasingly important role in the treatment of highly selected insulin-dependent patients with complications of diabetes.[2,3] However, the benefits of PTX must be weighed against the morbidity associated with the operative procedure and long-term immunosuppression. PTX is associated with a higher morbidity than kidney transplantation. In addition to rejection, the major causes of graft loss after PTX are vascular thrombosis, pancreatitis, and infection.[1-3] Because of the unforgiving nature of the pancreas as a parenchymal organ, the preferred method of transplantation has evolved into a whole-organ pancreaticoduodenal technique.[3] By incorporating a portion of the donor's duodenum into the pancreas allograft, exocrine drainage can be achieved without violating parenchymal integrity. However, transplantation of the DS as an exocrine conduit is a paradigm of bowel transplantation and may be associated with unique complications.

In 1984 Starzl et al.[32] reported on four cases of composite splanchnic organ grafts, which included the entire pancreas, the spleen, and variable amounts

of donor duodenum and jejunum. One graft was lost to early venous thrombosis. Two grafts transplanted with duodenojejunal limbs developed protein-losing enteropathy resulting in subsequent reoperation with bowel resection, leaving a proximal duodenal "bubble" that was anastomosed to the jejunum of the recipient. In the final case, a short segment of the duodenum closed at both ends was anastomosed side to side to the recipient jejunum without any subsequent complications. Based on this initial experience, the authors concluded that inclusion of the extrapancreatic organs (spleen and bowel) was responsible for much of the morbidity of this procedure. Subsequent to this report, bladder drainage by the duodenal segment technique became the method of choice for pancreas transplantation in the next decade.[32]

Most centers report an operative complication rate of 20% to 30% after PTX related to vascular problems, urologic problems, difficulties related to exocrine pancreatic drainage and allograft pancreatitis, wound or infectious complications, and miscellaneous problems.[3,11,13,32-38] In most of these reports there is a failure to differentiate complications specific to the DS from either urologic or intra-abdominal morbidity.[14] Only a few reports regard DS complications as a separate entity.[16-18] In 1990 Gruessner et al.[16] reported on 15 cases (10%) of duodenal complications requiring surgical repair after PTX. Eleven patients had DS leaks, three had DS hemorrhage, and one had DS necrosis. Twelve DS complications were believed to be technical in nature, two were due to CMV, and one was due to rejection. Four patients (26.7%) experienced graft loss due to allograft pancreatectomy, and another patient underwent allograft duodenectomy for severe DS rejection and necrosis with preservation of pancreas graft function.

In 1993 Elkhammas et al.[17] reported on 30 SKPT recipients (20%) with DS complications including 12 patients with urine leaks and 18 with hematuria. Seven patients with DS leaks were successfully treated nonoperatively with prolonged indwelling urethral catheter drainage and antibiotics. In addition, six patients with hematuria were successfully treated with urethral catheter drainage and continuous bladder irrigation, thus avoiding cystoscopy or open repair. One patient underwent enteric conversion in this series, and no graft losses resulted from either complication.

In an updated series from the University of Minnesota, Hakim et al.[40] reported on 42 patients with DS leaks, 26 with DS-related hematuria confirmed by cystoscopy, two with recurrent stone formation at the DS staple line, nine with recurrent urinary tract infections requiring cystoscopy and removal of suture or staple material in the DS as the focus of infection, and six with duodenal ulceration due to invasive CMV infection. The overall incidence of DS complications was 23%. Eight patients (9.4%) underwent enteric conversion, and the rate of pancreas graft loss directly related to DS complications was 8%. Isolated case reports of DS ulcer perforation and DS rupture due to rejection have also appeared in the literature.[40,41]

There have been a number of indirect studies of DS complications in PTX recipients reported either as the presenting symptom (hematuria, perforation, or "urologic" complication) or as the specific treatment (enteric conversion or "surgical" therapy).[3,11-15,32-38,42-47] Gross hematuria is the most frequent major urologic complication, occurring in approximately 10% of patients with bladder-drained PTXs.[48] Early hematuria can be minimized by careful preparation and handling of the DS to avoid devascularization or mucosal hemorrhage. Urethral catheter drainage is the mainstay of therapy, with most episodes being self-limited.[27] Cystoscopy is reserved for refractory cases. In our experience the DS was the most common site of bleeding resulting in significant hematuria. By following a systematic approach, gross hematuria was treated successfully with minimal morbidity or graft loss and only rarely required open surgical therapy.

Further review of the literature reveals that perforation of the DS and urinary extravasation occurs nearly as frequently as gross hematuria (4% to 14%) and, if unrecognized, may result in significant morbidity.[3,11-13,16,17,48] Most patients experience the sudden onset of lower abdominal pain and hyperamylasemia. Abdominal pain usually subsides with placement of an indwelling urethral catheter in the absence of peritonitis. A high degree of clinical suspicion is sometimes required because patients may present with only vague abdominal symptoms and fever in the setting of immunosuppression. The most common site of DS leak is nonanastomotic, usually at one of the staple lines. If a standard retrograde cystogram does not demonstrate the leak, a CT scan-cystogram or radionuclide voiding cystourethrogram can be helpful to confirm the presence of a perforation.[3,48-51] DS leaks may occur early or late, with early cases usually attributed to technical difficulties or ischemia and late cases resulting from rejection or infection. These leaks are usually treated by direct open repair, although some authors advocate nonoperative management with prolonged urethral catheter drainage in the absence of peritonitis.[44] Others perform an enteric conversion for DS leaks, particularly if these leaks are recurrent or associated with significant DS disease.[42-46] In our experience 9 of 12 DS leaks occurred early and the distal staple line was the most common site. All

patients underwent open repair including two in whom enteric conversion was performed.

For PTX recipients with bladder drainage, enteric conversion is recommended for refractory problems such as dehydration with intractable metabolic acidosis, chronic urethritis with urethral disruption or stricture, recurrent urine leaks with severe DS pathology, persistent hematuria, chronic urinary tract infections with foreign body formation or urosepsis, transitional cell dysplasia, and recurrent reflux pancreatitis.[14,42-46] Most authors recommend waiting 3 to 6 months before considering an enteric conversion because many of the previously mentioned problems are self-limited and improve with time and nonoperative management.[14] For persistent, refractory, or late cases, however, enteric conversion can be performed with low morbidity and can be effective in treating urologic, metabolic, or DS complications. Enteric conversion rates range from 10% to 20% in most large series.[3,14,42-46,48]

In our experience the rate of enteric conversion performed for urologic complications (3%) is low compared to rates in other published series (7% to 15%).[14] However, these series failed to distinguish between urologic and DS complications. In reviewing this literature we noted that DS pathology accounted for 40% to 50% of enteric conversions performed for so-called urologic complications.[3,14,42-46] We do not believe that it is appropriate to categorize DS problems with urologic complications because this may contribute to misrepresentation of the morbidity of bladder drainage.

In this descriptive study, DS complications occurred in 19% of PTX recipients, with bleeding and perforation being the two most common clinical presentations. Ampullary obstruction and foreign body formation were less common. Possible causes included rejection, invasive CMV infection, and technical difficulties leading to ischemia. Nearly 80% of DS complications occurred within the first 6 months after PTX, and most required operative intervention such as open surgical repair (58%), cystoscopy (63%), or enteric conversion (13%). Two deaths (1%) were directly related to DS complications, and the rate of allograft pancreatectomy (13%) was no different in patients with and without DS complications. Similarly, patient and pancreas allograft survival rates were similar in patients with and without DS complications. Based on this retrospective experience, we believe that whole-organ pancreaticoduodenal transplantation is associated with a finite risk of DS complications that are an important source of morbidity but rarely cause death. Analysis of DS complications may provide insight into morbidity associated with bowel transplantation. Early diagnosis and prompt intervention (open surgery or cystoscopy) can result in a high rate of graft salvage. In spite of unique side effects, transplantation of the DS as an exocrine conduit remains an acceptable alternative for either bladder or enteric drainage after PTX and is a paradigm of bowel transplantation.

We gratefully acknowledge the assistance and expertise of Jannene Sass and Deborah Aaron in preparation of the manuscript.

REFERENCES

1. Sutherland DER, Gruessner A. Pancreas transplantation in the United States of America (USA) as reported to the United Network for Organ Sharing (UNOS) and analyzed by the International Pancreas Transplant Registry. In Terasaki PI, Cecka JM, eds. Clinical Transplants 1995. Los Angeles: UCLA Tissue Typing Laboratory, 1996, pp 49-67.
2. Stratta RJ, Taylor RJ, Larsen JL, Cushing KA. Pancreas transplantation: State of the art. Int J Pancreatol 1995;17:1-13.
3. Stratta RJ, Taylor RJ, Gill IS. Pancreas transplantation: A managed cure approach to diabetes. In Wells SA, ed. Current Problems in Surgery, vol 33. St. Louis: Mosby, 1996, pp 711-808.
4. Lillehei RC, Simmons RL, Najarian JS. Pancreatico-duodenal allotransplantation: Experimental and clinical experience. Ann Surg 1970;172:405-436.
5. Cook K, Sollinger HW, Warner T, Kamps D. Belzer FO. Pancreaticocystostomy: An alternative method for exocrine drainage of segmental pancreatic allografts. Transplantation 1983;35:634-636.
6. Nghiem DD, Corry RJ. Technique of simultaneous renal pancreaticoduodenal transplantation with urinary drainage of pancreatic secretion. Am J Surg 1987;153:405-406.
7. Sollinger HW, Pirsch JD, D'Alessandro AM, Kalayoglu M, Belzer FO. Advantages of bladder drainage in pancreas transplantation: A personal view. Clin Transpl 1990;4:32-36.
8. Schang T, Timmermann W, Thiede A, Najarian JS, Sutherland DER. Detrimental effects of fluid and electrolyte loss from duodenum in bladder-drained pancreas transplants. Transplant Proc 1991;23:1617-1618.
9. Munda R, Tom WW, First MR, Alexander JW. Pancreatic allograft exocrine urinary tract diversion: Pathophysiology. Transplantation 1987;43:95-97.
10. Nghiem DD, Gonwa TA, Corry RJ. Metabolic effects of urinary diversion of exocrine secretions in pancreatic transplantation. Transplantation 1987;43:70-73.
11. Sollinger HW, Messing EM, Eckhoff DE, Pirsch JD, D'Alessandro AM, Kalayoglu M, Knechtle SJ, Hickey D, Belzer FO. Urological complications in 210 consecutive simultaneous pancreas-kidney transplants with bladder drainage. Ann Surg 1993;218:561-570.
12. Smith JL, See WA, Ames SA, Piper JB, Corry RJ. Lower urinary tract complications in patients with duodenocystostomies for exocrine drainage of the transplanted pancreas. Transplant Proc 1991;23:1611-1612.
13. Marsh CL, Forg P. The diagnosis and management of urologic complications in non-renal transplant recipients. Semin Urol 1994;12:233-250.
14. Sindhi R, Stratta RJ, Lowell JA, Sudan D, Cushing KA, Castaldo P, Jerius JT. Experience with enteric conversion after pancreas transplantation with bladder drainage. J Am Coll Surg 1997;184:281-289.

15. Burke GW, Sutherland DER, Najarian JS. Intra-abdominal fluid collections in pancreas transplant recipients: Bladder versus enteric drainage. Transplant Proc 1988;20:887-888.
16. Gruessner RWG, Dunn DL, Tzardis PJ, Nakhleh RI, Najarian JS, Sutherland DER. Complications occurring after whole organ duodenopancreatic transplantation: Relation to the allograft duodenal segment. Transplant Proc 1990;22:578-579.
17. Elkhammas EA, Henry ML, Tesi RJ, Ferguson RM. Duodenal segment-associated complications following combined kidney/pancreas transplantation. Transplant Proc 1993;25:2230-2231.
18. D'Alessandro AM, Sollinger HW, Stratta RJ, Kalayoglu M, Pirsch JD, Belzer FO. Comparison between duodenal button and duodenal segment in pancreas transplantation. Transplantation 1989;47:120-122.
19. Stratta RJ, Taylor RJ, Wahl TO, Duckworth WC, Gallagher TF, Knight TF, Fischer JL, Neumann TV, Miller S, Langnas AN, Ozaki CF, Bynon JS, Larsen JL, Weide LG, Cassling RS, Taylon AJ, Shaw BW Jr. Recipient selection and evaluation for vascularized pancreas transplantation. Transplantation 1993;55:1090-1096.
20. Stratta RJ, Taylor RJ, Spees EC, Langnas AN, Marujo WC, Li S, Ozaki C, Ranjan D, Duckworth RM, Shaw BW Jr. Refinements in cadaveric pancreas-kidney procurement and preservation. Transplant Proc 1991;23:2320-2322.
21. Stratta RJ, Taylor RJ, Lowell JA, Sindhi R, Sudan D, Castaldo P, Weide LG, Frisbie K, Cushing DA, Larsen JL, Grune M, Radio SJ. Pancreas transplantation: The Nebraska experience. In Terasaki PI, Cecka JM, eds. Clinical Transplants 1994. Los Angeles: UCLA Tissue Typing Laboratory, 1995, pp 265-281.
22. Stratta RJ, Taylor RJ. Kidney allocation in the 1990s: Progress and problems. Transplant Proc 1993;25:3065-3066.
23. Stratta RJ, Taylor RJ, Bynon JS, Lowell JA, Cattral MS, Frisbie K, Miller S, Radio SJ, Brennan DC. Viral prophylaxis in combined pancreas-kidney transplantation recipients. Transplantation 1994;57:506-512.
24. Stratta RJ, Taylor RJ, Weide IG, Sindhi R, Sudan D, Castaldo P, Cushing KA, Frisbie K, Radio SJ. A prospective randomized trial of OKT3 versus ATGAM induction therapy in pancreas transplant recipients. Transplant Proc 1996;28:927-928.
25. Stratta RJ, Sollinger HW, Perlman SB, D'Alessandro AM, Groshek M, Kalayoglu M, Pirsch JD, Belzer FO. Early diagnosis and treatment of pancreas allograft rejection. Transpl Int 1988;1:6-12.
26. Radio SJ, Stratta RJ, Taylor RJ, Linder J. The utility of urine cytology in the diagnosis of allograft rejection after combined pancreas-kidney transplantation. Transplantation 1993;55: 509-516.
27. Stratta RJ, Taylor RJ. Prevention and management of hematuria in combined pancreas-kidney transplant recipients with pancreaticoduodenocystostomy. Transplant Proc 1992;24: 788-790.
28. Lowell JA, Bynon JS, Nelson N, Hapke MR, Morton JJ, Brennan DC, Stratta RJ, Taylor RJ. Improved technique of transduodenal pancreas transplant biopsy. Transplantation 1994;57:752-753.
29. Nakhleh RE, Gruessner RWG, Tzardis PJ, Dunn DL, Sutherland DER. Pathology of transplanted human duodenal tissue: A histologic study, with comparison to pancreatic pathology, in resected pancreaticoduodenal transplants. Clin Transplant 1991;5:241-247.
30. Nakhleh RE, Benedetti E, Gruessner A, Troppmann C, Goswitz JJ, Sutherland DER, Gruessner RWG. Cystoscopic biopsies in pancreaticoduodenal transplantation: Are duodenal biopsies indicative of pancreas dysfunction? Transplantation 1995;60:541-546.
31. Wengrovitz M, Jarowenko MV, Gifford RRR, Sechtan AG, Mandell MJ, Yang HC. Stone formation as a cause of allograft pancreatitis in the recipient of a combined kidney and pancreas transplant. Clin Transpl 1990;4:117-119.
32. Starzl TE, Iwatsuki S, Shaw BW, Greene DA, Van Thiel DH, Nalesnik MS, Nusbacher J, Diliz-Perez H, Hakala TR. Pancreaticoduodenal transplantation in humans. Surg Gynecol Obstet 1984;159:265-272.
33. Stratta RJ, Taylor RJ, Bynon JS, Lowell JA, Sindhi R, Wahl TO, Knight TF, Weide LG, Duckworth WC. Surgical treatment of diabetes mellitus with pancreas transplantation. Ann Surg 1994;220:809-817.
34. Ozaki CF, Stratta RJ, Taylor RJ, Langnas AN, Bynon JS, Shaw BW Jr. Surgical complications in solitary pancreas and combined pancreas-kidney transplantation. Am J Surg 1992; 164:546-551.
35. Castoldi R, Staudacher C, Ferrari G, Cristallo M, Carlucci M, La Rocca E, Secchi A, Pozza G, Di Carlo V. Early postoperative surgical complications: Comparison of segmental duct-injected versus whole bladder-drained pancreas transplantation. Transplant Proc 1992;24:817-820.
36. Eckhoff DE, Sollinger HW. Surgical complications after simultaneous pancreas-kidney transplant with bladder drainage. In Terasaki PA, Cecka JM, eds. Clinical Transplants 1993. Los Angeles: UCLA Tissue Typing Laboratory, 1994, pp 185-191.
37. Douzdjian V, Abecassis MM, Cooper JL, Smith JL, Corry RJ. Incidence, management, and significance of surgical complications after pancreatic transplantation. Surg Gynecol Obstet 1993;177:451-456.
38. Troppmann C, Gruessner AC, Benedetti E, Papalois BE, Dunn DL, Najarian JS, Sutherland DER, Gruessner RWG. Vascular graft thrombosis after pancreatic transplantation: Univariate and multivariate operative and nonoperative risk factor analysis. J Am Coll Surg 1996;182:285-316.
39. Sutherland DER, Dunn DL, Goetz FC, Kennedy W, Ramsay RC, Steffes NW, Mauer SM, Gruessner R, Moudry-Munns KC, Morel P, Viste A, Robertson RP, Najarian JS. A ten-year experience with 290 pancreas transplants at a single institution. Ann Surg 1989;210:274-288.
40. Hakim NS, Benedetti E, Sutherland DER, Gruessner R. Duodenal complications after whole organ pancreas transplantation. HPB Surg 1996;9(Suppl 2):40.
41. Schleibner S, Theodorakis J, Illner WD, Leitl F, Abendroth D, Land W. Ulcer perforation in the grafted duodenal segment following pancreatic transplantation: A case report. Transplant Proc 1992;24:827.
42. Esterl RM, Stratta RJ, Taylor RJ, Radio SJ. Rejection with duodenal rupture after solitary pancreas transplantation: An unusual cause of severe hematuria. Clin Transplant 1995;9: 155-159.
43. Sollinger HW, Sasaki TM, D'Alessandro AM, Knechtle SJ, Pirsch JD, Kalayoglu M, Belzer FO. Indications for enteric conversion after pancreas transplantation with bladder drainage. Surgery 1992;112:842-846.
44. Stephanian E. Gruessner RWG, Brayman KL, Gores P, Dunn DL, Najarian JS, Sutherland DER. Conversion of exocrine secretions from bladder to enteric drainage in recipients of whole pancreaticoduodenal transplants. Ann Surg 1992; 216:663-672.
45. Elkhammas EA, Henry ML, Tesi RJ, Davies EA, Ferguson RM. Late urine leaks after combined kidney-pancreas transplantation. Transplant Proc 1994;26:453.

46. Ploeg RJ, Eckhoff DE, D'Alessandro AM, Stegall MD, Knechtle SJ, Pirsch JD, Sollinger HW, Belzer FO. Urological complications and enteric conversion after pancreas transplantation with bladder drainage. Transplant Proc 1994; 26:458-459.
47. Gruessner RWG, Stephanian E, Dunn DL, Gruessner AC, Najarian JS, Sutherland DER. Cystoenteric conversion after whole pancreaticoduodenal transplantation: Indications, risk factors, and outcome. Transplant Proc 1993;25:1179-1181.
48. See WA, Smith JL. Activated proteolytic enzymes in the urine of whole organ pancreas transplantation patients with duodenocystostomy. Transplant Proc 1991;23:1615-1616.
49. Taylor RJ, Bynon JS, Stratta RJ. Kidney/pancreas transplantation: A review of the current status. Urol Clin North Am 1994;21:343-354.
50. Eckhoff DE, Ploeg RJ, Wilson MA, D'Alessandro AM, Knechtle SJ, Pirsch JD, Belzer FO, Sollinger HW. Efficacy of 99MTc voiding cystourethrogram for detection of duodenal leaks after pancreas transplantation. Transplant Proc 1994; 26:462-463.
51. Rayhill SC, Odorico JS, Heisey DM, Wilson MA, Pirsch JD, D'Alessandro AM, Knechtle SJ, Eckhoff DE, Belzer FO, Sollinger HW. Clinical and laboratory features of pancreatic transplant bladder leaks. Transplant Proc 1995;27:3141-3142.

Use of an Ileal Roux Limb to Prevent the Roux Stasis Syndrome

Michinaga Takahashi, M.D., Bao-Lien Nguyen Tu, M.D., Edoardo Leombruni, M.D., Keith A. Kelly, M.D.

The aim of this study was to determine whether the use of an ileal Roux limb, rather than a jejunal Roux limb, would prevent the Roux stasis syndrome that can occur after Roux gastrectomy. An ileal Roux limb was constructed in eight dogs and anastomosed to the gastric remnant after distal hemigastrectomy. Flow of chyme through the jejunum was preserved via an ileojejunostomy and a jejunoileostomy. Six dogs with distal gastrectomy and a conventional Roux gastrojejunostomy served as a control group. Chronic enteric recording electrodes and intraluminal, open-tipped pressure catheters were implanted in all dogs. After recovery, the electrical activity and motility of the Roux limbs and the rates of gastric emptying of liquids and solids were measured. Dogs with a Roux gastroileostomy had a slower frequency of pacesetter potentials in the Roux limb, a greater Roux motility index, and a faster rate of gastric emptying of liquids and solids than did dogs with a Roux gastrojejunostomy. Stomal ulcers, however, developed in seven of the eight ileal Roux limbs but in none of the jejunal Roux limbs. It was concluded that Roux gastroileostomy does ameliorate the Roux stasis syndrome, but there is a greater risk of stomal ulceration in the limb. (J GASTROINTEST SURG 1997;1:545-553.)

Ectopic pacemakers appear in a jejunal Roux limb after Roux gastrectomy.[1-4] These pacemakers drive the proximal part of the limb in a retrograde direction and slow gastric emptying.[5,6] Nausea, vomiting, and pain may result.[7,8] Reoperation may then be necessary, but it may not alleviate the symptoms.[9,10]

Roux limbs are almost always fashioned from jejunum. The ileum, however, has electrical and motor properties that might favor its use as a Roux limb. The frequency of pacesetter potentials (PPs) is lower in the ileum than in the jejunum, the velocity of ileal PP propagation is slower, and the wavelength of ileal PPs is shorter.[11] The wavelength of a PP corresponds to the length of bowel whose motility is controlled by that PP. Thus ectopic pacemakers that might appear in an ileal Roux limb ought to disturb motility over shorter segments of bowel than would such pacemakers in a jejunal Roux limb. The ileum also has more clustered motor waves than does the jejunum.[12] We have shown in previous tests that clustered waves promote distal enteric transit, especially of solids, via a mechanism independent of the frequency or direction of propagation of the PPs.[13] These findings suggest that a Roux ileal limb might continue to propel content distally even though ectopic pacemakers were present in the limb.

Our specific aims, thus, were to assess the enteric electrical and motor patterns and the rates of gastric emptying in dogs with an ileal Roux limb after Roux gastrectomy, and to compare these patterns and the rates of gastric emptying to those of dogs with a jejunal Roux limb.

METHODS

Experimental Preparation

The experimental protocol was approved by the Animal Care and Use Committee of the Mayo Clinic on April 3, 1995. Surgical procedures and experiments were performed in accordance with the "Guidelines for the Care and Use of Laboratory Animals" (NIH publication No. 82-23, revised 1985).

Fourteen female mongrel dogs weighing between 16 and 22 kg were fasted overnight and given 100 mg

From the Department of Surgery, Mayo Clinic Scottsdale, Scottsdale, Ariz.
Supported by the Mayo Foundation, Tohoku University, and the Nigro Grant.
Presented at the Annual Meeting of the American Gastroenterological Association, May 22, 1996, San Francisco, Calif.
Reprint requests: Keith A. Kelly, M.D., Department of Surgery, Mayo Clinic Scottsdale, 13400 E. Shea Blvd., Scottsdale, AZ 85259.

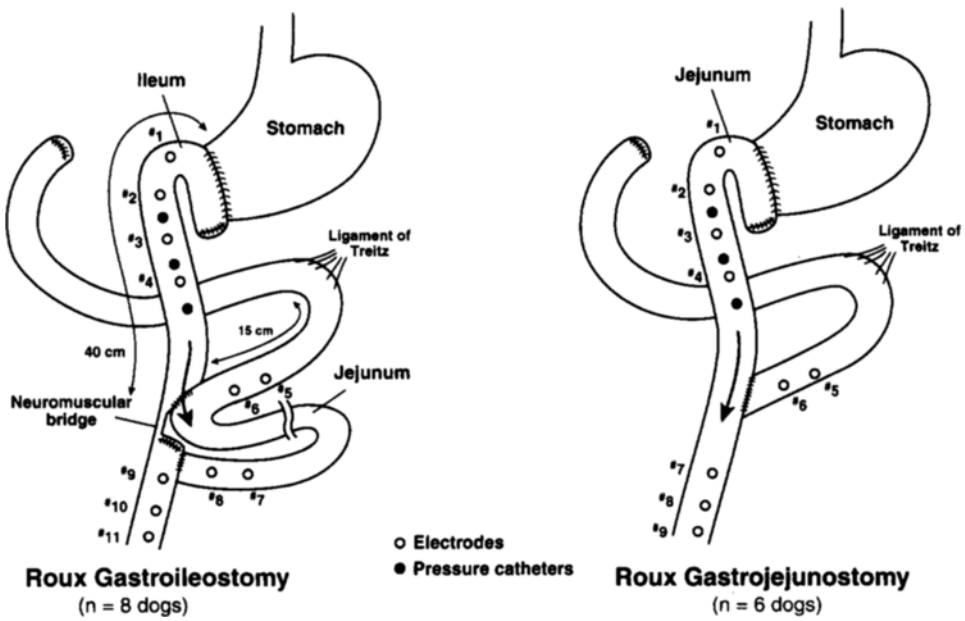


Fig. 1. Canine experimental preparations. After distal hemigastrectomy, gastrointestinal continuity was restored by Roux gastroileostomy in the experimental group *(left)* and Roux gastrojejunostomy in the control group *(right)*.

of cephalothin intravenously. General anesthesia was induced with methohexital sodium (12.5 mg/kg), 1.5% isoflurane, and atropine sulfate (0.04 mg/kg). Using sterile operative techniques, a midline celiotomy was made and a distal hemigastrectomy was accomplished.

An ileal Roux limb was fashioned in eight of the dogs. The small bowel was divided at a site one-half the distance between the ligament of Treitz and the ileocolic valve, the distal cut end was closed, and the gastric remnant was anastomosed end to side to the ileum just distal to the closure (Fig. 1). The proximal jejunum, 15 cm from the ligament of Treitz, was anastomosed side to end to the ileum at a site 40 cm distal to the gastroileostomy. The ileum was transected at the side-to-end jejunoileostomy, except for a neuromuscular bridge that was left intact to maintain pacesetter potential propagation from the Roux ileal limb to the more distal ileum. The lumen of the ileum distal to the bridge was sutured closed, while the distal cut end of divided jejunum was anastomosed to the ileum just distal to the bridge. The operation established a 40 cm Roux ileal limb and yet maintained transit through the same length of jejunoileum as does a conventional Roux jejunal limb. A second group of six dogs with conventional Roux gastrojejunostomies and 40 cm jejunal Roux limbs were used as controls (see Fig. 1). Vagotomy was not performed in either group of dogs, because we did not wish to obscure the cause of slow gastric emptying expected to be present, at least in the dogs with Roux jejunal loops, by adding an additional factor, postvagotomy gastric atony.

In both groups of dogs, enteric electrodes were applied at 4 cm intervals as in Fig. 1. All electrodes were monopolar Ag-AgCl recording electrodes. The electrodes were connected by insulated copper wires to a multioutlet connector embedded in a stainless steel cannula positioned in and anchored to the right anterior abdominal wall. In addition, a triple-lumen polyethylene catheter (internal diameter of each channel = 1 mm) was inserted 1 cm distal to the orad end of the Roux limb and its open side ports were positioned in the Roux limb 10 cm, 15 cm, and 20 cm distal to the gastroenterostomy. The external ends of the catheter were brought to the exterior via a metal connector in a stainless steel cannula implanted in the left anterior abdominal wall. The abdomen was sutured closed.

Analgesics (Butophin, 10 mg subcutaneously) were given every 4 hours after the operation for 2 days. The dogs were fasted for the first two postoperative days, during which time they received intravenous glucose, water, and electrolytes. Oral feedings were begun on the third postoperative day. Three weeks af-

ter surgery, when the animals had recovered from the operation, studies of myoelectric activity, motility, and gastric emptying were commenced.

Conduct of Experiments

Electrical Activity and Motility. Small intestinal electrical activity and motility were measured during fasting on three different days in each conscious animal. The dogs were starved for 18 hours prior to each daily study. They were placed fully conscious in a Pavlov stand, and the electrodes were connected to an electrical amplifier/computer acquisition system using the stainless steel cannula as a ground. The unipolar electrical signals were amplified by means of Mayo custom-built analogue amplifiers. The amplified analogue signals were then converted to digital signals sampled at 100 Hz, which were displayed in real time on an electronic monitor while simultaneously stored on magnetic media to be analyzed later. Roux limb motility was recorded concurrently with electrical activity. Motility was measured via the perfused side holes of the implanted polyethylene catheters using a low-compliance perfusion system connected to pressure transducers and a pressure-recording monitor for each channel.[14] The pressure data were also digitized and stored on disk for later computer analysis. Electrical activity and motility were measured for 4 hours during fasting.

On three different days after another 18-hour overnight fast, the dogs were given two cans (312 g, 394 cal: 45% protein, 28% fat, 17% carbohydrate) of canine a/d feline (Hill's Pet Nutrition, Topeka, Kan.) containing 8.5% protein, 5.25% fat, and 78% moisture. The dogs were placed in the Pavlov sling, and electrical activity and motility were recorded for 2 hours in the same manner as during the fasting studies.

Gastric Emptying. The rate of gastric emptying of a mixed meal was assessed in each dog on three other days. After an 18-hour overnight fast, the dogs were given a meal consisting of 200 ml 25% dextrose, 50 ml of ^{111}In-labeled beef broth (Campbell Soup Co., Paris, Tex.), and 50 g of ^{99m}Tc-labeled chicken liver chopped into 1 cm cubes, while electrical recordings were made. The liquid component of the meal contained 207 kcal (97% carbohydrate and 2.5% protein), while the solid component had 78 kcal (2% carbohydrate, 65% protein, and 33% fat). The chicken liver was prepared using an in vitro labeling technique; 1.0 mCi of ^{99m}Tc-sulfur colloid was injected at multiple sites into the parenchyma of 50 g of fresh chicken liver. The liver was then enclosed in a watertight bag and boiled for 15 minutes. The cooked liver was then cut into cubes 1 cm on a side. The liquid was prepared by injecting 0.10 mCi of ^{111}In-DTPA into the beef broth/dextrose solution. The liver cubes were placed in the beef broth, and the entire meal consumed by the dogs, usually within 2 minutes.

After the meal was consumed, the head of a gamma camera (model 409 [Elscint, Inc., Arlington Heights, Ill.], with a medium-energy collimator) was elevated to be in contact with the anterior surface of the dog's abdomen as the dog lay in the prone position in the Pavlov sling. Static anterior view scintigraphic images with 20% windows around the ^{111}In energy peak (247 keV) and around the ^{99m}Tc energy peak (140 keV) were obtained for 2.0 minutes every 10 minutes for the first 20 minutes, every 20 minutes for the next 100 minutes, and then every 30 minutes thereafter until at least 50% of the solid marker had left the stomach. Scintigraphic images were stored and later analyzed.

Analysis of Data

Electrical Activity. Digitized myoelectric signals were analyzed using a VAX/VMS platform by a previously described method developed in our laboratory.[15] The three basic analysis steps were as follows: (1) identification of the precise time (within 1/100th of a second) of each PP in all recording channels during each experiment; (2) using the PP event times in a single recording channel to calculate the instantaneous and the mean PP frequency in each channel during each experiment; and (3) using the PP event times in adjacent recording channels (spatially separated by 4 cm on the bowel wall) to determine the propagation direction of each PP detected in each channel. For example, in a typical 4-hour fasting study, assuming a PP frequency of approximately 20 cpm and 100% PP recognition, this analysis technique identified approximately 4800 PPs in each of nine channels for a total of 43,200 PPs over a 4-hour period. It also determined the PP propagation direction and velocity (4 cm/interelectrode time delay) for each of the pairs of adjacent electrodes for a total of 38,400 propagation events.

PP frequency and direction of propagation were examined during phase 1, phase 2, and phase 3 of the interdigestive migrating myoelectric complex (MMC), as identified by inspection of the tracings using the criteria of Code and Marlett[16] as well as during feeding.

Motility. The motility data were analyzed using programs developed in our laboratory.[15,16] Briefly, the programs allowed identification of single pressure waves and clustered pressure waves. The frequency, amplitude, area under the curve, direction of propa-

gation, and velocity of propagation of the waves were determined. A motility index was calculated as the amplitude × duration of clustered waves per 10 minutes.

Gastric Emptying. Scintigraphic images were analyzed using a Medical Data System A2 computer (Medical Data Systems, Ann Arbor, Mich.). For each static image, the gastric region of interest was interactively derived, and total counts ^{111}In raw (t) and ^{99m}Tc raw (t) were obtained at each time, t. Counts, corrected for background, crossover, and radioactive decay, were obtained using the following formulas:

$$^{111}\text{In cor(t)} = [^{111}\text{In raw(t)} - {}^{111}\text{In bkgrnd}] \times 2t/3888 \text{ min}$$

$$^{99m}\text{Tc cor(t)} = [^{99m}\text{Tc raw(t)} - {}^{99m}\text{Tc bkgrnd} - 0.55 \times {}^{111}\text{In raw(t)}] \times 2t/361 \text{ min}$$

The fraction of each marker remaining in the stomach at each time, t, was derived by dividing the corrected counts by the maximal value of corrected counts over the experiment. The fractions obtained were then used to fit a power exponential function as previously described[17]:

$$f(t) = 2 - (t/T_{1/2})b$$

where f = fraction of meal component remaining relative to 1.0 at time 0, $T_{1/2}$ = half emptying time, and b = the exponent power that describes delayed or accelerated early emptying. The use of anterior scintigraphic images alone produces an artifactual delay in the time of peak counts, which distorts the power exponential curve fitting the procedure for solid emptying. To partially compensate for this calculation of the $T_{1/2}$ and b parameters, only time points after the maximal observation were used in the solid curve fitting. The $T_{1/2}$, b and lag phase for gastric emptying of solids and liquids were assessed in each dog, as described above.

Statistical Analysis. Data from the two groups of dogs were compared using Student's *t* test for unpaired data and the Bonferroni correction where indicated.

Postmortem Examination

After completion of the experiments, all animals were killed with an overdose of pentobarbital sodium and the areas of operation inspected. In particular, the integrity of the luminal closure at the site of the neuromuscular bridge in the ileal Roux dogs was examined. The lumen of the bowel on one side of the closure was distended to 200 mm Hg pressure with a 1% solution of methylene blue, while the lumen on the other side was inspected for the appearance of the methylene blue.

RESULTS

Condition of the Dogs

The dogs with an ileal Roux limb were in fair or poor health after the operation. Three of the eight dogs died between 3 and 10 weeks after operation. None of the three contributed emptying data before death, but two of the three contributed electrical and motor data. The five dogs that survived had impaired nutrition and lost a mean ± standard error of the mean (SEM) of 0.66 ± 0.58 kg of body weight by 10 weeks after the operation. Among the five survivors, all contributed electrical, motor, and emptying data, but one developed gastric outlet obstruction 1 month after the operation. Only the emptying test performed 3 weeks after the operation was used to calculate the rate of gastric emptying in this dog. In total, six ileal Roux dogs contributed electrical data, six dogs provided motor data, and five dogs provided emptying data. In contrast to the ileal Roux dogs, the jejunal Roux dogs did well after the operation. None died, and no weight loss occurred. In fact, the jejunal Roux dogs gained 0.28 ± 0.71 kg during the course of the experiments. All six jejunal Roux dogs completed all electrical, motor, and emptying tests.

Electrical Activity

Pacesetter Potentials. The frequency of the PPs in the ileal Roux limb (mean ± SEM = 10 ± 1 cpm) was slower than that in the jejunal Roux limb (13 ± 1 cpm; $P < 0.05$) during phase 1 of the MMC, during phase 3 of the MMC, and in the fed state (Fig. 2 and Table I). In addition, the percentage of PPs that propagated aborally was less in the ileal Roux limbs than in the jejunal Roux limbs during phase 3 of the MMCs and during the fed state (see Table I) but not during phase 1 of the MMC. In contrast to the Roux limbs, the frequency of the PPs and the percentage of aborad propagation of PPs in the proximal jejunum were similar in the two groups of dogs (see Table I).

Migrating Myoelectric Complexes. The period of interdigestive MMCs, that is, the minutes from the end of one phase 3 of an MMC to the end of the next phase III, was longer in the ileal Roux limbs than in the jejunal Roux limbs, both in the Roux limbs themselves (103 ± 8.5 minutes vs. 67 ± 2.8 minutes; $P < 0.05$) and in the bowel distal to the Roux limbs (105 ± 7.8 minutes vs. 64 ± 2.6 minutes; $P < 0.05$). The period of the MMCs in the proximal jejunum (99 minutes), however, was similar in the two groups of dogs (Table II).

The time required for phase 3 of the MMC to travel from electrode 4 to electrode 10, a distance of

Table I. Enteric electrical activity after distal hemigastrectomy and reconstruction with an ileal Roux limb or a jejunal Roux limb

	Mean ± SEM frequency of PPs (cpm)			Mean ± SEM % aborad PP propagation		
	Fasting			Fasting		
Area of bowel	Phase 1 of MMC	Phase 3 of MMC	After feeding	Phase 1 of MMC	Phase 3 of MMC	After feeding
Roux limb						
Ileal Roux (n = 6 dogs)	10.4 ± 0.5	12.4 ± 0.2	11.2 ± 0.6	66 ± 3.4	52 ± 2.5	61 ± 2.6
Jejunal Roux (n = 6 dogs)	13.4 ± 0.4*	14.5 ± 0.5*	14.2 ± 0.4*	70 ± 4.0	68 ± 2.5*	77 ± 4.2*
Proximal jejunum						
Ileal Roux (n = 6 dogs)	18.9 ± 0.2	19.0 ± 0.3	18.8 ± 0.2	99 ± 0.5	91 ± 4.7	94 ± 4.9
Jejunal Roux (n = 6 dogs)	19.3 ± 0.2	19.3 ± 0.2	19.2 ± 0.5	84 ± 6.3	67 ± 9.2	79 ± 8.6

PP = pacesetter potential; MMC = migrating myoelectric complex.
*Values differ from those above ($P < 0.01$).

Table II. Period and propagation of interdigestive myoelectric complex in the Roux limb, in bowel distal to the Roux limb, and in the proximal jejunum after Roux gastroileostomy (ileal Roux) or Roux gastrojejunostomy (jejunal Roux)

	Mean ± SEM period of MMC (min)*			
Type of Roux limb	Roux limb	Bowel distal to Roux limb	Proximal jejunum	Mean ± SEM for MMC propagation (min)†
Ileal (n = 6 dogs)	103 ± 8.5	105 ± 7.8	98 ± 6.2	22 ± 1.7
Jejunal (n = 6 dogs)	67 ± 2.8‡	65 ± 2.6‡	100 ± 6.8	6.5 ± 0.7‡

*Period of migrating myoelectric complexes (MMC) represents the duration from the end of the one phase 3 to the end of the next phase 3.
†Minutes for phase 3 to propagate 35 cm from the Roux limb (electrode 4, see Fig. 1) to the bowel distal to the Roux limb (ileal Roux dogs, electrode 10; jejunal Roux dogs, electrode 8).
‡Values differ from those above ($P < 0.01$).

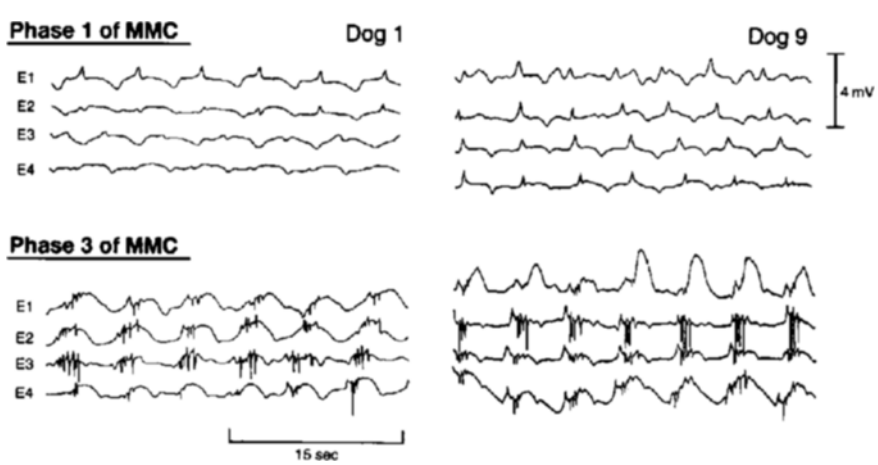


Fig. 2. Electrical tracings from a Roux limb during phases 1 and 3 of the interdigestive migrating myoelectric complex *(MMC)* in a dog with hemigastrectomy and a Roux gastroileostomy *(left)* or a Roux gastrojejunostomy *(right)*.

35 cm, in the ileal Roux dogs was 21.6 ± 1.7 minutes, whereas in the jejunal Roux dogs it was 6.5 ± 0.7 minutes ($P < 0.05$). Thus the velocity of propagation of phase 3 was slower in the ileal Roux dogs than the jejunal Roux dogs (1.6 cm/min vs. 5.4 cm/min, respectively; $P < 0.05$).

MMCs were abolished 15 to 30 minutes after feeding in both groups of dogs.

Roux Limb Motility

The motility index (MI) was greater during fasting and with feeding in the ileal Roux limb than in the jejunal Roux limb (phase 1 of fasting = 10.9 ± 0.9 vs. 8.0 ± 0.5; fed state = 12.0 ± 0.6 vs. 10.5 ± 0.6; $P < 0.05$; Table III). In contrast, the frequency and amplitude of clustered waves, the number of clusters that propagated aborally, and the duration of the clusters did not differ in the two groups of dogs (Table IV). However, the velocity of aborad propagation of clusters was faster after feeding in the ileal Roux limbs than in the jejunal Roux limbs (28.2 ± 2.9 cm/min vs. 19.8 ± 2.3 cm/min; $P < 0.05$), although the velocity of orad propagation was not.

Gastric Emptying

Liquids and solids emptied faster from the gastric remnant in the ileal Roux dogs than in the jejunal Roux dogs. The $T_{1/2}$ of liquid emptying of the ileal Roux dogs was 107 ± 9.2 minutes in the ileal Roux dogs and 166 ± 18.5 minutes in the jejunal Roux dogs ($P < 0.05$), whereas the $T_{1/2}$ of solid emptying was 164.6 ± 8.5 minutes in the ileal Roux dogs and 213.6 ± 18.3 minutes in the jejunal Roux dogs (Fig. 3; $P < 0.05$).

Postmortem Studies

In the three ileal Roux dogs that died, the cause of death was generalized peritonitis from perforated stomal ulcers in the ileal Roux limb. The ulcers were located at a site just distal to the gastroilestomy (Fig. 4). Of the five ileal Roux dogs that survived, four also had stomal ulcers, two of which were "kissing" ulcers. In one of these dogs the kissing ulcers had penetrated into the liver, whereas in the other dog the kissing ulcers had caused gastric outlet obstruction. The other two dogs with ulcers had small stomal ulcers that were not perforated or obstructing.

A patent enteric lumen between the ileal Roux limb

Table III. Motility index (MI = $\log_e$ [sum of the amplitude × number of pressure waves] + 1) in the Roux limb after Roux gastroileostomy (ileal Roux) or Roux gastrojejunostomy (jejunal Roux)

	Mean ± SEM motility index (units)		
Type of Roux limb	Phase 1 of MMC	Phase 3 of MMC	Fed state
Ileal (n = 6 dogs)	11 ± 0.9	12 ± 0.4	12 ± 0.6
Jejunal (n = 6 dogs)	8.0 ± 0.5*	11 ± 0.2*	11 ± 0.6*

MMC = migrating myoelectric complex.
*Values differ from those above ($P < 0.05$).

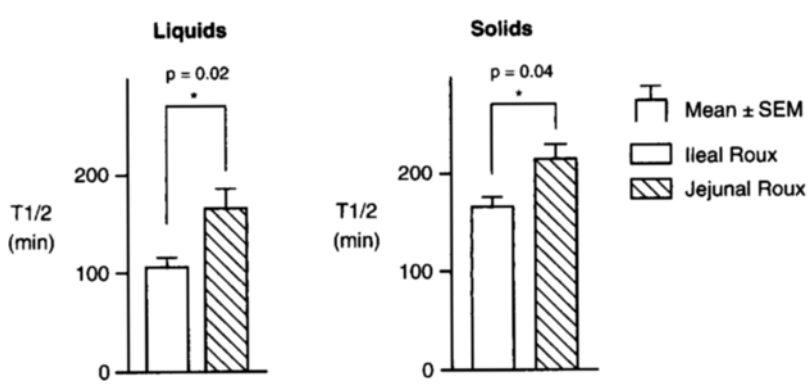


Fig. 3. Gastric emptying of liquids and solids after distal hemigastrectomy and Roux gastroileostomy (n = 5 dogs) or Roux gastrojejunostomy (n = 6 dogs). $T_{1/2}$ = minutes required for one-half of the liquid or solid component of the meal to empty.

Table IV. Mean ± SEM clustered wave characteristics in the Roux limb after Roux gastroileostomy (ileal Roux) or Roux gastrojejunostomy (jejunal Roux) during fasting and after feeding

Type of Roux limb	Frequency (No./hr)		Amplitude (mm Hg)		Duration (sec)		Aborad velocity (cm/min)		Orad velocity (cm/min)	
	Fasting	Fed	Fasting	Fed	Fasting	Fed	Fasting	Fed	Fasting	Fed
Ileal (n = 6)	15.3 ± 2.1	15.6 ± 1.4	12.1 ± 0.4	12.3 ± 0.5	64.9 ± 6.2	56.4 ± 4.4	19.6 ± 1.1	28.2 ± 2.9	18.8 ± 2.9	20.7 ± 1.3
Jejunal (n = 6)	12.8 ± 1.7	15.9 ± 1.9	11.5 ± 0.2	11.2 ± 0.2	56.5 ± 2.4	46.5 ± 4.2	16.8 ± 1.3	19.8 ± 2.3*	18.7 ± 2.0	20.3 ± 2.7

*Value differs from those above ($P < 0.05$).

and the more distal ileum at the site of the neuromuscular bridge was found in the three ileal Roux dogs that died. The enteric stream in these three dogs likely bypassed the jejunum, resulting in a "short bowel." A fourth dog had a small, 3 mm diameter fistula between the ileal Roux limb and the more distal ileum across the neuromuscular bridge. This fistula was associated with a stomal ulcer in the limb. The other four ileal Roux dogs had no fistulas, but three of them had stomal ulcers. No jejunal Roux dogs had fistulas or stomal ulcers.

DISCUSSION

Dogs with an ileal Roux limb emptied liquids and solids from their stomachs faster than dogs with a jejunal Roux limb. The better emptying was likely due to a slower frequency of PPs in the ileal Roux limb, a shorter PP wavelength, a greater motility index, and clustered waves that moved distally with greater velocity in the ileal Roux limb than in the jejunal Roux limb or a combination of these factors. Both limbs had similar clustered wave parameters otherwise, so that other changes in the patterns of clustered waves were unlikely to have led to more rapid gastric emptying in the ileal Roux dogs. These results suggest that subjects with ileal Roux limbs would be less likely to have the Roux stasis syndrome after the operation than would subjects with jejunal Roux limbs.

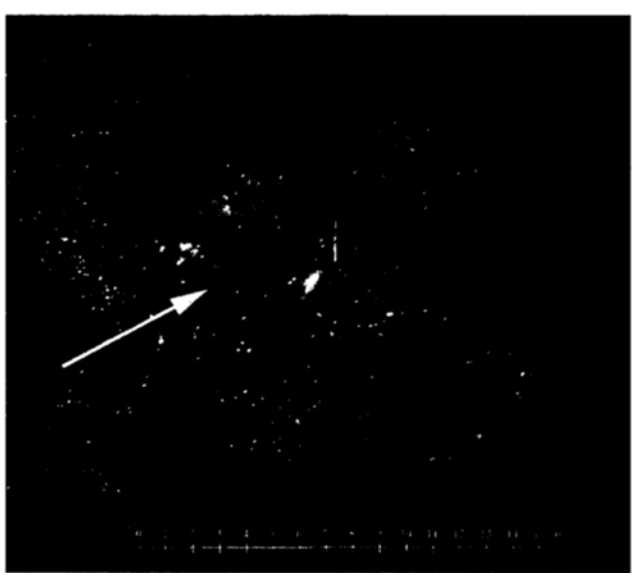


Fig. 4. Photograph of a perforated ileal ulcer *(arrow)* found at autopsy in a dog with an ileal Roux limb. The ulcer is located in the ileal limb just distal to the gastroileostomy. The forceps is retracting the gastric mucosa to expose the anastomosis.

The ileal Roux limbs also had interdigestive MMCs with a period similar to that of the intact proximal jejunum and a velocity of aborad migration similar to that found in intact ileum,[12] compared to the shorter period and more rapid velocity in the jejunal Roux limbs. Others have also found shorter MMC periods and more rapid MMC velocities in jejunal Roux limbs.[1,3,18,19] Whether the healthier pattern of MMCs in the ileal limbs contributed to better function and less stasis in these limbs is unknown.

Chen et al.,[5] emphasized the importance of maintaining the flow of bile and pancreatic juice through Roux limbs in preserving gastric emptying. In their tests, gastric emptying of solids was faster after Billroth II gastrojejunostomy, which maintains biliopancreatic flow through the efferent jejunum, than it was after Roux gastrojejunostomy, which diverts bile and pancreatic juice away from the Roux limb. This was true irrespective of the subsequent transection of the efferent jejunal limb in the Billroth dogs or division of a neuromuscular bridge to the limb in the Roux dogs. Nonetheless, our current experiments provide evidence that the electrical and motor properties of the Roux limb itself are also major determinants of the rate of gastric emptying, and that the ileal Roux limb has a more favorable pattern in this regard than the jejunal Roux limb.

The main problem with the ileal Roux limbs in our tests was that seven of eight of the dogs that had them developed stomal ulcers in the limbs. These ulcers seemed to be similar to Mann-Williamson ulcers.[20,21] Mann and Williamson anastomosed the distal jejunum to the proximal duodenum without gastric resection and diverted biliary and pancreatic juice to the distal ileum. Unbuffered gastric juice entered the proximal jejunum in their dogs and resulted in jejunal ulcers. We did not determine gastric acid secretion in our dogs, but it does seem that the mucosa of the ileal Roux limb was more susceptible to ulceration from gastric acid than was the jejunal mucosa. Nutritional deficits from a breakdown of the ileal-jejunal diversion and the resulting unintended ileal-ileal bypass of the jejunum in some of the dogs likely also had a role. The presence of stomal ulcers in the ileal Roux limbs may have altered the patterns of myoelectric activity and motor activity or the rates of gastric emptying, but this is unknown. The one dog who developed ileal stenosis at the site of an ileal ulcer had no further tests of gastric emptying once the obstruction developed.

In summary, dogs with hemigastrectomy and ileal Roux limbs had faster gastric emptying of liquids and solids than dogs with jejunal Roux limbs, and so would be less likely to develop the Roux stasis syndrome. However, seven of eight dogs with ileal Roux limbs developed stomal ulcers in the ileal Roux limb, whereas no ulcers developed in a jejunal Roux limb. Because of the ulcers, an ileal Roux limb cannot be recommended for clinical application after gastrectomy at this time. The use of drugs that suppress acid secretion or the use of vagotomy with a Roux ileal limb might make clinical application successful, but these approaches would need to be tested.

We thank S. Hart, T. Lombari, and S. Rosewell for technical assistance.

REFERENCES

1. Miedema BW, Kelly KA, Camilleri M, Hanson RB, Zinsmeister AR, O'Connor MK, Brown ML. Human gastric and jejunal transit and motility after Roux gastrojejunostomy. Gastroenterology 1992;103:1133-1143.
2. Karlstrom LH, Kelly KA. Ectopic jejunal pacemakers and gastric emptying after Roux gastrectomy: Effect of intestinal pacing. Surgery 1989;106:867-871.
3. Miedema BW, Kelly KA. The Roux Stasis syndrome. Treatment by pacing and prevention by use of an "uncut" Roux limb. Arch Surg 1992;127:295-300.
4. Mathias JR, Fernandez A, Sninsky CA, Clench MH, Davis RH. Nausea, vomiting and abdominal pain after Roux-en-Y anastomosis: Motility of the jejunal limb. Gastroenterology 1985;88:101-107.
5. Chen G, Vogel SB, Hocking MD. Efferent limb myoneural and luminal continuity and postgastrectomy gastric emptying. J Surg Res 1995;58:746-753.
6. Hocking MP, Vogel SB, Falasca CA, Woodward ER. Delayed gastric emptying of liquids and solids following Roux en Y biliary diversion. Ann Surg 1981;194:494-501.
7. Gustavsson S, Ilstrup DM, Morrison P, Kelly KA. Roux-Y stasis syndrome after gastrectomy. Am J Surg 1988;155:490-494.
8. Perino LE, Adcock KA, Goff JS. Gastrointestinal symptoms, motility and transit after the Roux en Y operation. Am J Gastroenterol 1988;83:380-385.
9. Eckhauser FE, Knol JA, Raper SA, Guice KS. Completion gastrectomy for postsurgical gastroparesis syndrome. Ann Surg 1988;208:345-353.
10. Hinder RA, Esser J, DeMeester TR. Management of gastric emptying disorders following the Roux-en-Y procedure. Surgery 1988;104:765-772.
11. Akwari OE, Kelly KA, Steinbach JH, Code CF. Electrical pacing of intact and transected canine small intestine and its computer model. Am J Physiol 1975;229:1188-1197.
12. Quigley EM, Phillips SF, Dent J. Distinctive patterns of interdigestive motility at the canine ileocolonic junction. Gastroenterology 1994;87:836-844.
13. Miedema BW, Sarr MG, Hanson RB, Kelly KA. Electric and motor patterns associated with canine jejunal transit of liquids and solids. Am J Physiol 1992;262:G962-G970.
14. Heddle R, Miedema BW, Kelly KA. Integration of canine proximal gastric, antral, pyloric and proximal duodenal motility during fasting and after a liquid meal. Dig Dis Sci 1993;38:856-869.
15. Cullen JJ, Eagon JC, Hould FS, Hanson RB, Kelly KA. Ectopic jejunal pacemakers after jejunal transection and their relationship to transit. Am J Physiol 1995;208:6859-6867.

16. Code CF, Marlett JA. The interdigestive myoelectric complex of the stomach and small bowel of dogs. J Physiol 1975;246: 289-309.
17. Elashoff JD, Reedy TJ, Meyer JH. Analysis of gastric emptying data. Gastroenterology 1982;83:1306-1312.
18. van der Mijle HCJ, Kleibeuker JH, Limburg AJ, Bleichrodt RP, Beekhuis H, van Schilfgaarde R. Manometric and scintigraphic studies of the relation between motility disturbances in the Roux limb and the Roux-en-Y syndrome. Am J Surg 1993;155:11-17.
19. Kruis W, Azpiroz F, Phillips SF. Contractile patterns and transit of fluid in canine terminal ileum. Am J Physiol 1985;249: G264-G270.
20. Mann FC, Williamson CS. The experimental production of peptic ulcer. Ann Surg 1923;77:409-422.
21. Richter HM, Kelly KA, Go VLW. Proximal gastric vagotomy and mucosal antrectomy: Effect on gastric acid secretion, plasma gastrin, and experimental ulcerogenesis in the dog. Surgery 1986;101:623-631.

Intestinal Flora and Nutrient Absorption After Intestinal Resection

Jon S. Thompson, M.D., Eamonn M. Quigley, M.D.

Intestinal resection results in loss of surface area, motor disruption, and an altered luminal milieu, all of which might influence bacterial growth. Our aim was to determine the effect of extensive intestinal resection in the dog on small intestinal bacterial flora and nutrient absorption. Ten dogs underwent 75% proximal intestinal resection and were killed at either 12 or 40 weeks. Five animals underwent transection alone and were killed at 12 weeks. Ileal aspirates were cultured. Nutritional status and nutrient absorption were measured every 4 weeks. Mean total and anaerobic ileal flora were increased after resection, significantly at 40 weeks. Overall, more cultures from resected animals had more than 5×10^6 total bacteria (6 of 10 vs. 0 of 10, $P < 0.05$) and more than 10^5 anaerobic bacteria (5 of 10 vs. 0 of 10, $P < 0.05$) than unoperated animals. Total but not anaerobic bacteria were increased after transection alone. Ingestion and absorption of carbohydrate decreased but absorption efficiency was maintained. Nitrogen intake decreased but excretion and absorption were unchanged. Fat intake decreased and excretion was unchanged resulting in decreased absorption. Mean intake, excretion, and absorption of nutrients were not influenced by the presence of significant growths of total ($>5 \times 10^6$/ml) or anaerobic ($>10^5$/ml) bacteria. It was concluded that (1) 75% proximal intestinal resection results in significantly more aerobic and anaerobic bacteria in the ileal remnant; (2) intestinal bacterial content does not correlate with absorption of nutrients; and (3) the colon, and particularly colonic bacteria, may have a more important role in nutrient absorption than luminal flora in the small intestine after resection. (J GASTROINTEST SURG 1997;1:554-560.)

Overgrowth of bacteria, especially anaerobes, in the small intestine occurs in a variety of disease states.[1] Bacterial overgrowth in the small intestine can result in malabsorption of nutrients including carbohydrate, fat, and protein. Bacteria alter intestinal absorption of nutrients by interacting with luminal nutrients and the intestinal mucosa. The degree of overgrowth correlates, to a large extent, with the severity of malabsorption.[1] Bacterial concentrations of greater than 10^5 organisms per milliliter in the small intestinal lumen have been associated with malabsorption.

Intestinal resection results in loss of intestinal absorptive area, transient motor disruption, and alteration in the luminal content of various secretions and nutrients.[2,3] These changes may have a profound effect on the quantity and type of bacterial flora present in the small intestine.[1] Bacterial overgrowth secondary to stasis clearly occurs in some patients with short-bowel syndrome. Although malabsorption of nutrients occurs after extensive resection, the role of altered bacterial flora in this impaired absorption, in the absence of significant stasis, is uncertain. Colonic bacterial mass and capacity to ferment nutrients are increased in patients with short-bowel syndrome.[4] However, it is not clear what changes in the bacterial flora occur in the small intestine after extensive intestinal resection.[3,5] The aims of the present study were to determine the effect of extensive intestinal resection in dogs on small intestinal bacterial flora and the effect of such alterations on nutrient absorption.

MATERIAL AND METHODS

Fifteen mongrel dogs (13 to 20 kg) were included in the study, which was approved by the Omaha Veterans Affairs Animal Research Committee. Ten animals underwent 75% proximal resection of the small intestine with maintenance of intestinal continuity.

From the Surgical Service, Omaha Veterans Affairs Medical Center, and the Departments of Surgery and Internal Medicine, Section of Gastroenterology, University of Nebraska Medical Center, Omaha, Neb.
Reprint requests: Jon S. Thompson, M.D., University of Nebraska Medical Center, Department of Surgery, 600 S. 42nd St., Omaha, NE 68198-3280.

These animals were killed either 12 weeks (n = 5) or 40 weeks (n = 5) postoperatively. Five animals underwent intestinal transection and reanastomosis alone and served as controls; they were killed at 12 weeks. All animals were fed standard dog chow and caloric intake was recorded daily. Intestinal fluid was aspirated for bacterial culture from the normal ileum prior to resection and from the ileal remnant distal to the anastomosis at death. Nutritional status was determined on the basis of body weight and serum albumin levels. Intestinal absorption was evaluated preoperatively and every 4 weeks after surgery by measuring stool weight and moisture and absorption of ingested carbohydrate, nitrogen, and fat.

Operative Procedures

After an overnight fast, the animals were anesthetized with intravenous thiamylal sodium and halothane by inhalation. Through a midline incision the small intestine was measured twice along the antimesenteric border from the duodenojejunal junction to the ileocecal junction. The average of these two values was used for intestinal length. Intestinal length ranged from 250 to 450 cm. Seventy-five percent of the measured intestinal length was resected beginning 10 cm distal to the duodenojejunal junction. Intestinal continuity was restored by a two-layer end-to-end anastomosis. Transection with end-to-end anastomosis was performed at a similar site in the control group. Sections of distal intestine were removed at the site of anastomosis for histologic examination.

Cefazolin (250 mg) was administered by intramuscular injection, one dose preoperatively and two doses postoperatively. The animals received intravenous fluid during the operation and took only water by mouth for 48 hours after the operation. On postoperative day 3 the dogs were offered 85 calories per kilogram of body weight per day of dog chow (Wayne Dog Food, St. Charles, Mo.) consisting of 25% crude protein, 8% fat, and 50% carbohydrate by weight. On completion of the study period the abdomen was reopened and the intestinal remnant length measured. Intestinal segments were removed just distal to the previous anastomosis for histologic studies. The animals were then killed with the use of T61 euthanasia solution.

Absorption Studies

Absorption studies were performed by measuring oral intake, in grams, and calculating nutrient components. The animals were housed in metabolic cages and stool was collected for 48 hours every 4 weeks. To calculate net absorption, measured fecal nutrients were subtracted from the amount ingested. Absorption was expressed as both grams per day and percentage absorption.

A D-xylose intestinal absorption test was performed the week before the dogs were killed on animals anesthetized after an overnight fast. An orogastric tube was positioned under fluoroscopic guidance in the antrum of the stomach; 250 ml of water containing 1 g of xylose per kilogram of body weight was instilled. Urine was collected by Foley catheter drainage, and urinary xylose content was determined in the 5-hour specimen by means of a colorimetric method with 2% *p*-bromoanaline.[6]

Analytic Procedures

Intestinal tissue specimens were placed in formalin and processed for histologic examination under light microscopy. Sections were stained with hematoxylin and eosin. Villus height was measured on at least 10 intact, axially oriented villi in each specimen with the aid of an ocular micrometer.

Serum albumin values were measured in the clinical laboratory at Omaha Veterans Affairs Hospital by means of standard automated laboratory techniques. Stool wet weight was measured and stool dry weight determined by freeze drying. Fecal fat was determined by the ether extraction technique.[7] Stool nitrogen was measured by the Kjeldahl method.[8] Fecal content of glucose equivalents was assessed by a spectrophotometric technique.[9]

Bacterial cultures were obtained from the lumen of the ileum just distal to the anastomosis after an overnight fast. Two rayon-tipped swabs were saturated with fluid and placed in an anaerobic transport device that was immediately sealed and activated. The swabs were rinsed thoroughly in 1 ml of prereduced chopped-meat broth. This initial processing was considered at 1:10 dilution. Serial dilutions were quantitatively plated on both aerobic and anaerobic media and incubated for 24 and 48 hours, respectively. Culture results were expressed as colonies per milliliter.

STATISTICAL ANALYSIS

All data are expressed as mean ± standard deviation. Measurements were analyzed by either one-way or repeated-measures analysis of variance and the Kruskal-Wallis test. Data comparisons were performed with a Bonferroni-corrected *t* test. Correlations were evaluated by linear regression analysis. Statistical significance was ascribed to P values <0.05.

RESULTS

Bacterial Cultures

Mean concentrations of both total and anaerobic bacterial flora were increased in the ileal remnant of resected animals compared to normal ileum; this difference achieved statistical significance at 40 weeks (Table I). Total bacteria were increased in the ileum of transected animals compared to unoperated values but mean concentrations of anaerobic bacteria were similar. Overall, significantly more cultures from resected animals had more than 5×10^6/ml total bacteria (6 of 10 vs. 0 of 10, $P < 0.05$) and more than 10^5/ml anaerobic bacteria than control animals (5 of 10 vs. 0 of 10, $P < 0.05$).

Nutrition and Adaptation

Mean caloric intake was 70 calories per kilogram per day. The resected animals maintained their body weight (99% ± 5% initial at 12 weeks and 95% ± 9% initial at 40 weeks) and serum albumin levels (3.3 ± 0.3 g/dl at 12 weeks and 3.1 ± 0.1 g/dl at 40 weeks vs. 3.3 ± 0.4 g/dl preoperatively) after resection. Intestinal remnant length increased by 19% at 12 weeks (85 ± 10 cm vs. 75 ± 25 cm preoperatively) and 32% at 40 weeks (103 ± 40 cm). Villus height increased by 8% at 12 weeks (303 ± 38 μm vs. 282 ± 37 μm preoperatively and 29% at 40 weeks (369 ± 79 μm, $P < 0.05$).

Absorptive Studies

Resected animals had initial clinical diarrhea, which resolved within 8 to 12 weeks. Stool weight did not increase significantly 4 and 8 weeks after resection compared to preoperative values (187 ± 64 g/day and 188 ± 65 g/day vs. 175 ± 123 g/day). Stool moisture was increased significantly at 4 weeks (74% ± 6%) but not at 8 weeks (70% ± 10%) compared to preoperative values (65% ± 8%).

Carbohydrate absorption was maintained at 97% to 99% of intake after 75% proximal resection (Table II). However, total grams of carbohydrate ingested and absorbed were significantly decreased through-

Table I. Comparison of ileal flora

			75% Proximal resection	
	Preoperative	Transection	12 wk	40 wk
Total bacteria				
Mean concentration (10^5/ml)	0.5(0.1-1.0)	3.6(2.2-4.8)*	51.3(0.5-200)	216.4(24-660)*
Number $>5 \times 10^6$	0/10	0/5	2/5	4/5
Anaerobic bacteria				
Mean concentration (10^5/ml)	0.2(0-0.6)	0.8(0-2.0)	31.8(0.3-140)	2.3(0-6)*
Number $>10^5$	0/10	2/5	2/5	3/5

*$P < 0.05$ vs. preoperative value (Kruskal-Wallis test).

Table II. Carbohydrate absorption following resection

Week	Intake (g/day)	Fecal excretion (g/day)	Absorption (g/day)	% Intake absorbed
Preop				
0	175 ± 0	0.8 ± 0.3	174 ± 0	99 ± 0
4	116 ± 65*	1.4 ± 0.7	114 ± 25*	97 ± 1
8	143 ± 38*	1.8 ± 0.8*	141 ± 28*	98 ± 1
12	135 ± 38*	1.6 ± 0.4*	133 ± 28*	99 ± 1
16	142 ± 29*	1.2 ± 0.3*	141 ± 29*	99 ± 1
20	153 ± 24*	1.4 ± 0.2*	151 ± 24	99 ± 1
24	158 ± 16*	1.5 ± 1.0	156 ± 16*	99 ± 1
28	154 ± 30	1.0 ± 0.7	153 ± 30	99 ± 1
32	161 ± 19*	1.2 ± 1.5	165 ± 18	99 ± 1
36	153 ± 25*	1.6 ± 0.7	149 ± 27	98 ± 1
40	139 ± 27*	0.6 ± 0.4	134 ± 13*	99 ± 1

*$P < 0.05$ vs. preoperative value.

out most of the study period. Carbohydrate excretion increased twofold between 8 and 24 weeks but remained quite small in relation to grams ingested.

Nitrogen absorption was maintained at 80% to 90% of intake after resection (Table III). Oral intake of nitrogen was significantly reduced during the first 24 postoperative weeks. Fecal excretion and calculated absorption of nitrogen, however, were unchanged during the study period.

Fat absorption ranged from 82% to 93% of intake up to 40 weeks after resection (Table IV). Fat intake was reduced during the first 24 postoperative weeks. Fat absorption in grams was significantly less compared to preoperative values during the first 8 weeks after resection. Fecal fat excretion was unchanged during the period of study.

Mean intake, excretion, and absorption of nutrients were similar in resected animals whether or not total flora exceeded 5×10^6/ml or there were more than 10^5 anaerobes in luminal cultures (Table V). Resected animals with more than 10^5 and less than 10^5 anaerobic bacteria had similar serum albumin levels (3.3 ± 0.3 g/dl vs. 3.3 ± 0.2 g/dl) and body weight (100% ±7% vs. 98% ± 5% initial weight) 12 weeks after resection. Fecal fat excretion tended to increase in animals with increased bacterial concentrations, but the difference did not achieve statistical significance.

Initial intestinal remnant length was similar in animals with more than 10^5 and less than 10^5 anaerobic bacteria (78 ± 23 cm vs. 73 ± 15 cm). There were no statistically significant correlations between either initial remnant length and carbohydrate ($r = 0$), nitrogen ($r = 0.011$), and fat ($r = -0.283$) absorption or remnant length at death and carbohydrate ($r = -0.016$), nitrogen ($r = 0.314$), and fat ($r = -0.369$) absorption.

Table III. Nitrogen absorption following resection

Week	Intake (g/day)	Fecal excretion (g/day)	Absorption (g/day)	% Intake absorbed
Preop	12.8 ± 0	2.3 ± 1.7	10.5 ± 1.7	82 ± 13
4	8.5 ± 4.8*	2.1 ± 1.0	8.7 ± 4.6	80 ± 11
8	10.0 ± 2.1*	2.1 ± 0.8	8.5 ± 2.3	83 ± 6
12	9.9 ± 2.0*	2.2 ± 0.8	8.2 ± 1.9	83 ± 4
16	10.4 ± 2.2*	1.9 ± 1.2	9.3 ± 2.3	89 ± 4
20	11.2 ± 1.7	1.3 ± 0.4	9.9 ± 1.9	88 ± 5
24	11.6 ± 1.2*	1.6 ± 1.3	10.0 ± 1.1	86 ± 11
28	11.3 ± 2.2	1.2 ± 0.9	10.0 ± 2.8	88 ± 11
32	11.8 ± 1.4	1.3 ± 1.0	10.7 ± 1.4	88 ± 8
36	10.3 ± 1.5*	1.4 ± 0.5	9.3 ± 2.2	87 ± 2
40	11.2 ± 2.2	0.8 ± 0.7	10.8 ± 1.8	93 ± 5

*$P < 0.05$ vs. preoperative value.

Table IV. Fat absorption following resection

Week	Intake (g/day)	Fecal excretion (g/day)	Absorption (g/day)	% Intake absorbed
Preop	25.6 ± 0.5	2.0 ± 1.7	23.6 ± 1.7	92 ± 7
4	16.9 ± 9.5*	2.0 ± 0.7	15.1 ± 0.9*	90 ± 7
8	20.4 ± 4.3*	2.2 ± 1.3	16.1 ± 5.3*	82 ± 18
12	19.7 ± 4.0*	2.3 ± 1.1	17.6 ± 3.8	90 ± 7
16	20.7 ± 1.3*	2.3 ± 1.2	19.2 ± 4.5	92 ± 4
20	22.2 ± 3.2*	2.1 ± 1.0	20.1 ± 3.5	91 ± 5
24	21.2 ± 23*	2.8 ± 1.5	20.5 ± 2.3	88 ± 6
28	22.5 ± 4.4	3.2 ± 3.4	19.3 ± 7.1	83 ± 12
32	23.7 ± 2.6	3.1 ± 2.8	21.2 ± 3.0	88 ± 11
36	23.1 ± 2.3	3.6 ± 3.0	17.2 ± 6.2	82 ± 17
40	21.5 ± 4.0*	1.6 ± 0.9	20.0 ± 3.0	93 ± 3

*$P < 0.05$ vs. preoperative value.

Table V. Bacterial overgrowth and absorption

	$<5 \times 10^6$ total (n = 4)	$>5 \times 10^6$ total (n = 6)	$<10^5$ anaerobes (n = 5)	$>10^5$ anaerobes (n = 5)
Glucose (g/day)				
Intake	132 ± 33	145 ± 19	134 ± 28	146 ± 3
Excretion	1.6 ± 2.6	1.4 ± 2.1	1.6 ± 0.2	1.3 ± 0.2
Absorbed	130 ± 33	143 ± 18	131 ± 27	145 ± 3
% Absorbed	98 ± 1	99 ± 1	98 ± 1	99 ± 1
Nitrogen (g/day)				
Intake	9.6 ± 2.4	10.7 ± 1.6	9.9 ± 2.1	10.6 ± 1.8
Excretion	1.8 ± 0.3	2.0 ± 0.7	1.7 ± 0.4	2.1 ± 0.7
Absorbed	8.1 ± 2.3	9.6 ± 1.5	8.6 ± 2.3	9.2 ± 1.7
% Absorbed	82 ± 4	87 ± 1	83 ± 4	86 ± 2
Fat (g/day)				
Intake	19.2 ± 4.7	21.1 ± 2.8	19.5 ± 4.1	21.2 ± 3.1
Excretion	1.7 ± 0.5	2.7 ± 1.1	1.8 ± 0.5	2.8 ± 1.3
Absorbed	16.8 ± 5.3	18.2 ± 3.4	17.0 ± 4.5	18.3 ± 4.4
% Absorbed	87 ± 7	86 ± 9	87 ± 6	85 ± 11

Urinary D-xylose excretion was 25% ± 6% of the amount ingested in control animals, which was similar overall to the values in the resected group (28% ± 4%). However, resected animals with more than 10^5 anaerobic bacteria excreted 13% ± 4% of ingested xylose, which was significantly less than the values in controls and resected animals with less than 10^5 anaerobic bacteria (35% ± 11%, $P < 0.05$).

DISCUSSION

In the present study we found that luminal content of total and anaerobic bacteria increased in the ileum after a 75% resection of the proximal small intestine. A significant growth of total ($>5 \times 10^6$) and anaerobic ($>10^5$) bacteria was present in the ileal remnant in 50% of resected animals but not in normal ileum. Myrvold et al.[5] found no significant difference in jejunal and proximal ileal flora after 40% distal resection in dogs compared to unoperated values. We previously found no difference in jejunal flora after transection alone and 50% distal resection.[3] This apparent discrepancy among studies may be related to regional differences in small intestinal flora or the response to intestinal resection or to differences in the extent of resection. Furthermore, differences in sampling techniques and location of samples within a given intestinal segment may also influence culture results.[10]

Several factors might influence bacterial flora after intestinal resection. Luminal content of bacteria in the small intestine was increased after both transection and proximal resection in the present study. We previously found similar bacterial content after both 50% distal resection and transection alone.[3] Thus changes in bacterial flora after resection may be related to disrupted motor patterns. Stasis secondary to anastomotic stenosis is less likely to be a factor inasmuch as changes in flora have been observed both proximal and distal to the anastomosis in animals undergoing transection alone. Myrvold et al.[5] found that loss of the ileocecal valve markedly increased flora in the distal small intestine. Our previous study confirmed this as well.[3] However, in the present study the ileocecal junction and the entire colon were maintained in situ. Changes in luminal nutrients and secretions and the hormonal response to resection may also influence enteric flora.

We found that animals undergoing 75% resection of the proximal intestine with an intact ileocecal junction and colon experienced minimal disturbances of absorption of carbohydrate, fat, and nitrogen. In fact they maintained fairly normal nutritional status for up to 40 weeks after resection. Although oral intake diminished, overall absorptive efficiency was maintained. However, because the total available absorptive surface area is reduced after extensive resection, these animals might be expected to be fairly sensitive to changes in intestinal flora. Nonetheless, we found no differences in mean intake, excretion, or absorption of nutrients when significant bacterial content ($>5 \times 10^6$ total bacteria or $>10^5$ anaerobes) was present. Whether or not small intestinal bacteria would play a more significant role with greater resection or colonic dysfunction is not clear.

D-xylose excretion was similar to preoperative values after resection. Thus intestinal resection does not appear to intrinsically alter intestinal absorption or

permeability of D-xylose. However, luminal bacterial growth in the small intestine diminished D-xylose absorption in the resected animals with more than 10^5 anaerobic bacteria. Because D-xylose is absorbed proximally in the small intestine and not in the colon, and is more sensitive to factors affecting intestinal absorption than glucose, these findings suggest that bacteria in the small intestine do impair absorption in the small intestine.[11] However, bacteria may also metabolize D-xylose so that diminished urinary excretion may not accurately reflect intestinal absorption of carbohydrates.

Carbohydrate absorption remained highly efficient after resection in the present study. Intake and absorption in grams per day were decreased for up to 9 months. Although fecal excretion increased significantly, it remained quite small relative to intake. Fecal carbohydrate excretion preoperatively was quite similar to the less than 1 g excreted normally by humans.[12] However, we did not observe the 20- to 40-fold increase in fecal excretion of carbohydrates reported in patients with short-bowel syndrome.[8,12] This discrepancy may be related, in part, to dietary differences since dietary composition can markedly influence nutrient excretion.[8] In addition, some undigested carbohydrate may not have been measured by our fecal assay of glucose equivalents. Furthermore, animals in the present study had an intact ileocecal junction and colon, which might improve carbohydrate absorption. Small intestinal absorption of nutrients was not measured directly.

The important role of the large intestine in absorption of carbohydrates after extensive intestinal resection has recently received emphasis. The colon is important for salvaging calories from malabsorbed carbohydrates, increasing absorption of fluids and electrolytes and stimulating adaption of the intestinal remnant.[4,8] Antibiotic therapy to reduce colonic flora reduces these beneficial effects suggesting that they are related to bacterial fermentation of nutrients to short-chain fatty acids.[13] Following intestinal resection, excretion of carbohydrates is proportional to the amount of carbohydrates ingested if the colon is absent.[8] Furthermore, the colon has the capacity to increase its ability to ferment unabsorbed carbohydrates after intestinal resection.[4]

We found that nitrogen absorption was maintained at 80% to 90% of ingested intake after 75% proximal resection. This is consistent with the 10% to 15% fecal loss of nitrogen reported by Stahlgren et al.[14] after 80% proximal resection. Hylander et al.[15] found that very extensive resection (>150 cm) was required to reduce nitrogen absorption in patients with short-bowel syndrome and that preservation of the colon was not an important factor. Nordgaard et al.[8] also found that protein excretion was not markedly influenced by colectomy. Curtis et al.[16] also found that protein absorption was maintained in rats after 70% proximal small intestinal resection, even with bypass of the ileocecal valve. Thus bacterial flora may play a less important role in nitrogen absorption.

Fat absorption was impaired transiently after resection. Fat absorption is generally affected to a greater extent by resection than are carbohydrate and protein absorption.[16] However, an ileal remnant, as was maintained in the present study, is more efficient at fat absorption than a jejunal remnant after 80% to 85% intestinal resection.[14] Bypass of the ileocecal junction in conjunction with resection increases fat malabsorption but not nitrogen malabsorption.[3,14] Although bypass of the ileocecal junction increases bacterial content in the small intestine, it also shortens transit time. In the present study the presence of significant bacteria in the small intestine did not appear to influence fat absorption.[14]

In summary, 75% resection of the proximal intestine in canines resulted in significantly more aerobic and anaerobic bacteria in the ileal remnant. This extent of resection has minimal effect on absorption of carbohydrates and nitrogen and only transiently affected fat absorption. However, nutrient absorption did not correlate with bacterial overgrowth in the small intestine. These findings, taken with the results of other studies demonstrating a role for the colon and colonic bacteria in carbohydrate absorption, suggest that colonic bacteria may have a more important role in nutrient absorption than luminal bacteria in the small intestine after resection.

REFERENCES

1. King CE, Toskes PP. Small intestine bacterial overgrowth. Gastroenterology 1979;76:1035-1055.
2. Quigley EMM, Thompson JS. The motor response to intestinal resection: A study of motor activity in the canine small intestine following distal resection. Gastroenterology 1993; 105:791-798.
3. Thompson JS, Quigley EMM, Palmer JM, et al. Luminal short chain fatty acids and post-resection intestinal adaptation. JPEN 1996;20:338-343.
4. Briet F, Flourie B, Achour L, et al. Bacterial adaptation in patients with short bowel and colon in continuity. Gastroenterology 1995;109:1446-1453.
5. Myrvold H, Tindel MS, Isenberg HD. The nipple valve as a sphincter substitute for the ileocecal valve: Prevention of bacterial overgrowth in the small bowel. Surgery 1984:96:42-47.
6. Roe JH, Rice EW. A photometric method for the measurement of free pentoses in animal tissue. J Biol Chem 1954; 173:507-512.
7. VandeKamer JH, Huinink TB, Weyers HA. Rapid method for determination of fat in feces. J Biol Chem 1949;177:347-355.
8. Nordgaard I, Hansen BS, Mortensen PB. Colon as a digestive organ in patients with short bowel. Lancet 1994;343:373-376.

9. Bergmeyer HV, Bent E, Schmidt F, et al. In Methods of Enzymatic Analysis. Bergmeyer HV, ed. New York: Verlag Chemie, Weinheim/Academic Press, 1974, pp 1196-1201.
10. Corazza OR, Menozzi MG, Strocchi A, et al. The diagnosis of small bowel bacterial overgrowth: Reliability of jejunal culture and inadequacy of breath hydrogen testing. Gastroenterology 1990;98:302-307.
11. Cherbut C, Meivieu O, Ruckebusch Y. Effect of diet on intestinal xylose absorption in dogs. Dig Dis Sci 1996;31:385-391.
12. Ameen VZ, Powell GK, Jones JA. Quantitation of fecal carbohydrate excretion in patients with short bowel syndrome. Gastroenterology 1987;92:493-500.
13. Aghadassi E, Plapler H, Kurau R, et al. Colonic fermentation and nutritional recovery in rats with massive small bowel resection. Gastroenterology 1994;107:637-642.
14. Stahlgren LH, Umana G, Roy R, et al. A study of intestinal absorption in dogs following massive small intestinal resection and insertion of an antiperistaltic segment. Ann Surg 1962;156:483-492.
15. Hylander E, Ladefoged K, Jarnum S. Nitrogen absorption following small intestinal resection. Scand J Gastroenterol 1980;15:853-858.
16. Curtis KJ, Sleisenger MH, Kim YS. Protein digestion and absorption after massive bowel resection. Dig Dis Sci 1984;19:834-840.

Peptide YY Selectively Stimulates Expression of the Colonocytic Phenotype

Susan A. Sgambati, M.D., Gregory A. Turowski, M.D., Ph.D., Marc D. Basson, M.D., Ph.D.

Peptide YY (PYY) is produced by colonic mucosal endocrine cells and modulates gastrointestinal endocrine activity through specific Y-receptors. The direct effects of PYY on intestinal mucosal growth and differentiation remain uncharacterized. The abundance of PYY in colonic mucosa suggests that PYY acts locally to maintain colonocytic differentiation. We tested this hypothesis in human Caco-2 intestinal epithelial cells, which express alkaline phosphatase (AP) and dipeptidyl dipeptidase (DP), brush-border enzymes differentially concentrated in large and small intestinal mucosa, respectively. The effects of PYY on enzyme specific activity were compared with those of pancreatic polypeptide, neuropeptide-Y, vasoactive intestinal peptide, pentagastrin, bombesin, and selective Y1- and Y2-receptor agonists. Brush-border enzyme activity was assessed by AP and DP specific activity in cell lysates quantitated spectrophotometrically following synthetic substrate digestion. PYY, neuropeptide-Y, pancreatic polypeptide, and vasoactive intestinal peptide (10^{-7} mol/L) stimulated AP activity. PYY brought about the greatest increase (38.0% ± 11.0%, n = 48). Only PYY decreased DP specific activity (7.9% ± 2.2%, n = 48). The Y2-agonist but not the Y1-agonist mimicked these PYY effects (increasing AP 28.3% ± 3.5% and decreasing DP 10.4% ± 3.6%). These data suggest that PYY promotes differentiation toward a colonocytic phenotype in Caco-2 intestinal epithelial cells and that this effect may be mediated through the Y2-receptor subtype. (J GASTROINTEST SURG 1997;1:561-568.)

Peptide YY (PYY) is a 36-amino acid regulatory peptide specifically concentrated in the mammalian terminal ileum and colon.[1,2] PYY shares significant amino acid homology with the structurally related members of the pancreatic polypeptide (PP) family of brain-gut peptides, PP, and neuropeptide-Y (NPY).[3,4] PYY is localized and produced by L-type (open) endocrine cells of the ileal and colonic epithelium and released into the circulation in response to luminal nutrients[2,5,6] and certain neural stimuli.[7] A subpopulation of L-cells possesses basal processes characteristic of paracrine cells, which permit the direct release of PYY for local activity.[1,8]

Despite its mucosal localization, PYY is principally recognized as an endocrine modulator of distant target tissues, particularly within the gastrointestinal smooth muscle, where PYY powerfully inhibits gastric and intestinal motility.[9] In the colon, PYY acts through specific mucosal Y-receptor subtypes[10,11] to promote epithelial absorptive function by inhibiting colonic motility and secretagogue-induced secretion while increasing basal net chloride absorption.[8,12,13] The high mucosal concentration and local activity of PYY in the colon suggest the possibility that PYY may act in a paracrine fashion on the mucosa itself.[4] PYY has been reported to stimulate intestinal mucosal proliferation in septic,[14] cachectic,[15] and parenterally fed rats[16] and following massive intestinal resection.[17,18] Furthermore, plasma PYY concentrations are significantly elevated within 24 hours following intestinal resection in rats and have been demonstrated in humans with short gut syndrome.[19] The effects of PYY on colonic mucosal healing, specifically epithelial restitution, is thus a topic of considerable interest.

Given this indirect evidence for trophic effects of PYY on the intestinal mucosa, we theorized that PYY might modulate colonocytic differentiation and stimulate colonic epithelial motility (restitution). We evaluated this hypothesis using Caco-2 human intestinal

From the Departments of Surgery, Yale University School of Medicine (G.A.T. and M.D.B.) and the VA Connecticut Health Care System, West Haven, Conn. (M.D.B.); and the University of Connecticut School of Medicine (S.A.S.), Farmington, Conn.
Supported in part by Developmental Funds from the Yale Cancer Center Support Grant 5 P30 CA16359.
Reprint requests: Marc D. Basson, M.D., Ph.D., Department of Surgery, Yale University School of Medicine, 333 Cedar St., Box 208062, New Haven, CT 06520-8062.

epithelial cells as a model. Although originally derived from a human colonic adenocarcinoma, Caco-2 cells are uniquely well differentiated.[20,21] Caco-2 cells possess morphologic and biochemical characteristics of normal small and large intestinal epithelium[22-24] and exhibit the epithelial sheet migration characteristic of intestinal mucosal wound healing.[25-27] Caco-2 cells can express alkaline phosphatase (AP) and dipeptidyl dipeptidase (DP) brush-border enzymes localized mainly to the large and small intestines, respectively.[28-31] Although detectable throughout the gut, AP is highly concentrated in the colon, where mucosal levels are approximately one order of magnitude greater than those of the small intestine. DP, in contrast, represents a predominantly enterocytic brush-border enzyme, with small intestinal mucosal levels approximately two orders of magnitude greater than those found in the colon.[28-32] Furthermore, DP has been shown to exhibit differentiation-dependent expression in the rat jejunal crypt-villus axis[33] and in Caco-2 intestinal epithelial cells.[34] Because Caco-2 cells express brush-border enzymes characteristic of both small and large intestinal epithelial cells, they resemble fetal colonocytes and may serve as a model of the pluripotent human intestinal epithelial stem cell.[20,23,25,34-36] We, along with other investigators, have previously demonstrated Caco-2 AP and DP levels to be responsive to a variety of in vitro stimuli including amino acids,[37] short-chain fatty acids,[38] phorbol esters,[39] protein kinase C inhibitors,[40] and cyclic nucleotides.[41] These studies have demonstrated statistically significant changes in enzyme specific activity on the order of 20% to 50% for AP and 7% to 10% for DP.

We compared the effects of PYY on Caco-2 differentiation and migration to the effects on these parameters on other gut regulatory peptides. On the basis of preliminary dose-response studies performed for each of the eight peptides tested (range 10^{-12} to 10^{5} mol/L, data not shown), 10^{-7} mol/L concentrations of PYY, NPY, PP, vasoactive intestinal peptide (VIP), and Y1- and Y2-agonists and 10^{-5} mol/L pentagastrin and bombesin were used for our experiments. These concentrations represent the maximal peptide doses that produced a significant response. In addition to PYY, we examined the effects of NPY and PP, which share 70% and 40% structural homology with PYY, respectively,[1,10] which is found throughout the intestinal submucosa,[42] and pentagastrin and bombesin, both of which have been reported to stimulate growth in specific colon cancer models.[43-46] To evaluate the Y-receptor specificity mediating the observed effects of PYY on brush-border enzyme activity, we assessed the effects of the substituted homologue and specific Y1-agonist, leucine^{31} proline^{34} (neuropeptide-Y),[47,48] and the selective Y2-agonist long carboxyl-terminal fragment of PYY, PYY(13-36)[49,50] on Caco-2 AP and DP specific activity.

METHODS

Cells

The Caco-2 cells used in these studies represent a subclonal population specifically selected for its highly differentiated state.[35] Cells were maintained at 37° C in 5% carbon dioxide in Dulbecco's minimal essential medium supplemented with 10% fetal bovine serum (Gibco Laboratories, Grand Island, N.Y.), 10 g/ml transferrin (Boehringer Mannheim Corp., Indianapolis, Ind.), 2 mmol/L glutamine, 1 mmol/L pyruvate, 10 mmol/L HEPES, 100 U/ml penicillin G, and 0.1 mg/ml streptomycin. Cells were cultured to confluence as described previously[25] and subsequently treated with the peptides. All experiments used for this study were performed on cells within 10 to 20 passages.

Matrix Proteins

Type I collagen was purchased from Sigma Chemical (St. Louis, Mo.) and repurified by chloroform phenol extraction. Bacteriologic plastic dishes (Falcon Labware, Becton Dickinson Labware, Franklin Lakes, N.J.) were precoated with saturating quantities of matrix substrate as previously described.[25]

Chemicals and Reagents

PYY, NPY, PP, VIP, pentagastrin, bombesin, leucine^{31}proline^{34} (neuropeptide-Y), and PYY(13-36) were purchased from Peninsula Laboratories, Inc. (Belmont, Calif.). The following concentrations were used: 10^{-7} mol/L for PYY, NPY, PP, VIP, L^{31}P^{34} (NPY), and PYY(13-36), and 10^{-5} mol/L for pentagastrin and bombesin. These concentrations were based on preliminary dose-response curves previously constructed by our laboratory, as well as concentrations used by other investigators.[43-46,51,52] All other chemicals and reagents were of the highest purity available and were purchased from commercial sources.

Brush-Border Enzyme Activity

The specific activity of the brush-border enzymes AP and DP was quantitated spectrophotometrically following synthetic substrate digestion. Cells were treated for 72 hours with peptide-supplemented medium, then lysed on ice in phosphate-buffered saline solution with 10^{-3} mol/L calcium and 10^{-4} mol/L magnesium and containing 0.5% Triton X-100 and 0.35 mol/L NaCl. Total protein in the cell lysates

was quantitated (BCA assay, Pierce Chemical Co., Rockford, Ill.) and aliquots of these cell lysates were diluted to equal protein concentration. AP specific activity was assayed by the digestion of *p*-nitrophenyl phosphate disodium hexahydrate (Sigma) in 100 mmol/L glycine buffer (pH 10.0), and the resulting reaction product was quantitated at 410 nmol/L. DP specific activity was assayed following alanyl-*p*-nitroanilide (Sigma) digestion in 114 mmol/L Tris buffer (pH 8.0), with the resulting reaction product quantitated at 380 nmol/L. Standard dilution curves for AP and DP were performed simultaneously for each assay using synthetic enzyme standards of known activity (Sigma). All experimental assays were performed within the linear range of the assay. Results were standardized in each experiment against known standards and expressed as enzyme activity in international units per gram of cellular protein.[49]

Epithelial Sheet Migration

Epithelial sheet migration was calculated by expansion of a circumscribed and confluent Caco-2 monolayer across an acellular matrix.[26,27] Cells were plated within stainless steel "fences" placed on matrix-precoated 35 mm bacteriologic plastic dishes. After the cells reached confluence, the fences were removed, thereby permitting cells to migrate radially. Cells were fed daily with control medium or peptide-supplemented medium (10^{-7} mol/L of PYY, NPY, PP, and VIP or 10^{-5} mol/L of pentagastrin and bombesin). After 6 days, the monolayers were fixed with 10% formalin in situ. The monolayers were then stained with hematoxylin. The area of each monolayer was measured using a Microtek II-XE flatbed scanner and PC-based morphometric software (SigmaScan/Image, Jandel Scientific, Anaheim, Calif.). The area of the original confining fence was then subtracted from the area covered by the cell monolayers to calculate the area of migration.

Statistics

Statistical analysis was carried out using analysis of variance with a 95% confidence interval.

RESULTS

PYY Effect on AP and DP Specific Activity

Of the peptides tested, the greatest stimulation of AP was seen with PYY (10^{-7} mol/L, shaded bars), which increased AP activity by 38.0% ± 11.0% (n = 48, $P < 0.001$). By contrast, treatment with PYY actually decreased the specific activity of DP by 7.9% ± 2.2% (n = 48, $P < 0.05$) over control values (open bars, n = 48; Fig. 1).

PP and NPY Effect on AP and DP Specific Activity

PP and NPY (10^{-7} mol/L each) stimulated AP specific activity (open bars) by 23.0% ± 4.1% (n = 14, $P < 0.01$) and 12.1% ± 7.8% (n = 12, $P < 0.05$). PP and NPY did not significantly affect DP specific activity (shaded bars, n = 14; Fig. 2).

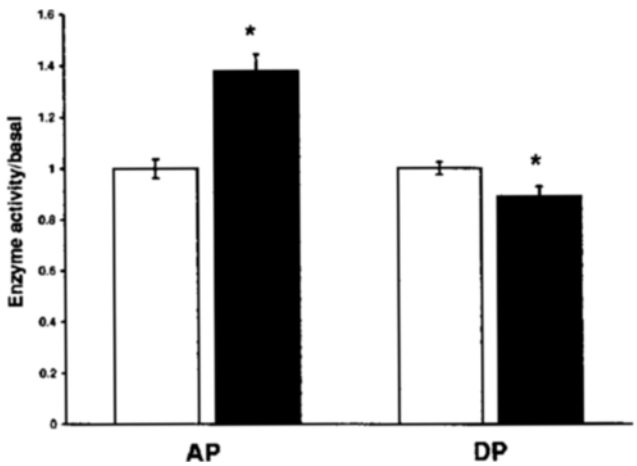


Fig. 1. Effect of PYY on Caco-2 alkaline phosphatase *(AP)* and dipeptidyl dipeptidase *(DP)* specific activity. PYY concentrations of 10^{-7} mol/L (shaded bars) stimulated the specific activity of AP (n = 48, $P < 0.001$) and decreased the specific activity of DP (n = 48, $P < 0.05$) as compared to control values (open bars, n = 48).

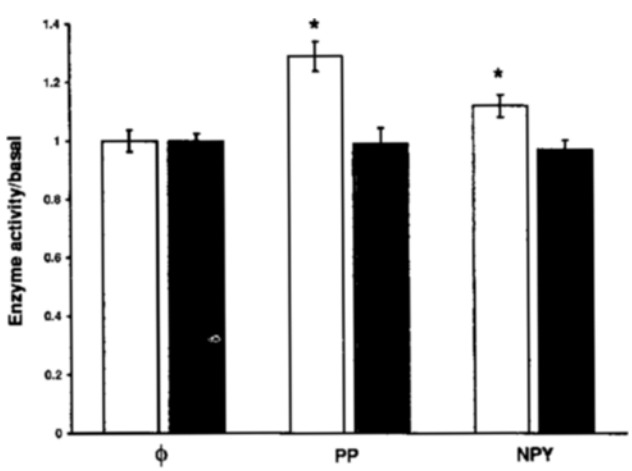


Fig. 2. Effect of pancreatic polypeptide *(PP)* and neuropeptide-Y *(NPY)* on Caco-2 AP and DP specific activity. PP and NPY concentrations of 10^{-7} mol/L increased AP activity (open bars) by 23.0% ± 4.1% (n = 14, $P < 0.01$) and 12.1% ± 7.8% (n = 12, $P < 0.05$). PP and NPY did not significantly affect DP specific activity, however (shaded bars, n = 14).

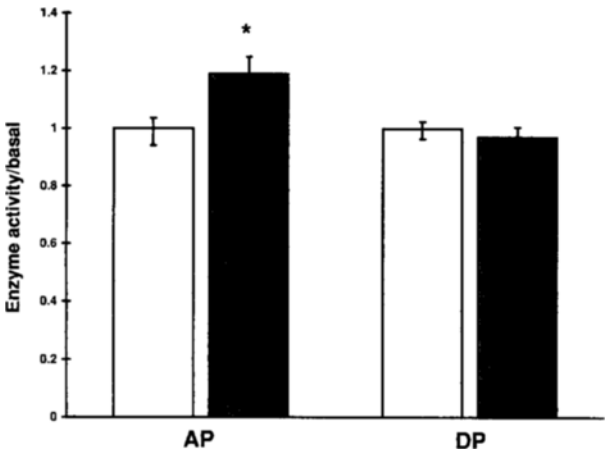


Fig. 3. Effect of vasoactive intestinal peptide (VIP) on Caco-2 AP and DP specific activity. VIP concentrations of 10^{-7} mol/L (shaded bars) stimulated AP (n = 14, $P < 0.02$) but did not significantly affect DP specific activity (n = 14) as compared to control values (open bars, n = 14).

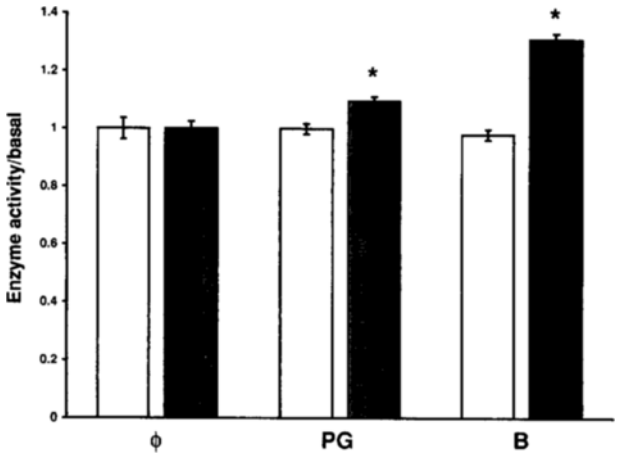


Fig. 4. Effect of pentagastrin *(PG)* and bombesin *(B)* on Caco-2 AP and DP specific activity. PG and B concentrations of 10^{-5} mol/L did not affect AP activity (open bars, n = 14) but significantly stimulated DP (shaded bars, n = 14, $P < 0.05$).

VIP Effect on AP and DP Activity

A VIP concentration of 10^{-7} mol/L (shaded bars) increased AP specific activity by 23.5% ± 7.0% (n = 14, $P < 0.02$) and did not significantly affect DP specific activity (n = 14) compared to control values (open bars, n = 14; Fig. 3).

Pentagastrin and Bombesin Effect on AP and DP Specific Activity

Pentagastrin and bombesin (n = 14, 10^{-5} mol/L each) did not significantly affect AP specific activity (open bars, n = 14). Pentagastrin and bombesin stimulated DP specific activity (shaded bars), however, with a substantially greater effect produced by pentagastrin. Pentagastrin increased DP specific activity by 31.1% ± 1.2% (n = 14, $P < 0.05$), whereas bombesin increased DP activity by 9.6% ± 1.9% (n = 14, $P < 0.05$; Fig. 4).

Receptor Mediation Studies

In studies aimed at characterizing the Y-receptor subtype mediating the observed PYY effects on brush-border enzyme activity, only the selective Y2-receptor agonist PYY(13-36) significantly affected Caco-2 brush-border enzyme activity. PYY(13-36) (10^{-7} mol/L) mimicked the effects of PYY on enzyme specific activity, increasing AP specific activity 28.3% ± 3.5% (open bars, n = 20; $P < 0.02$) and decreasing DP specific activity 10.4% ± 3.6% (shaded bars, n = 20; $P < 0.05$). In contrast, the specific Y1-agonist $L^{31}P^{34}$(NPY) (10^{-7} mol/L, n = 20) did not affect either AP or DP specific activity (Fig. 5).

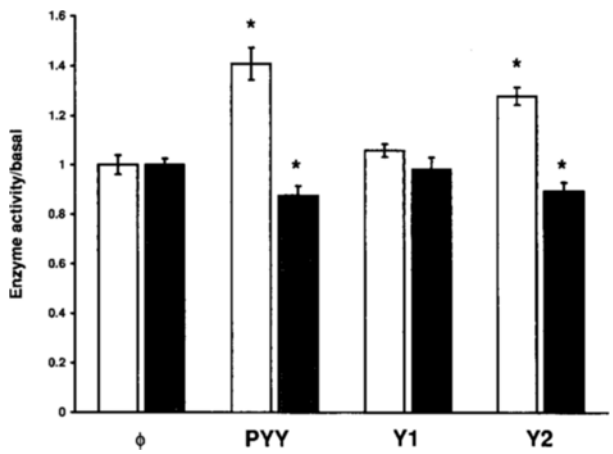


Fig. 5. Effect of Y1- and Y2-agonists on Caco-2 AP and DP specific activity. The Y2-selective agonist PYY(13-36) (10^{-7} mol/L) increased AP activity (open bars, n = 20; $P < 0.02$) and decreased DP activity (shaded bars, n = 20; $P < 0.05$). The specific Y1-agonist $L^{31}P^{34}$(NPY) (10^{-7} mol/L, n = 20) did not significantly affect AP or DP specific activity.

Epithelial Sheet Migration

None of the peptides tested significantly affected Caco-2 epithelial sheet migration across a type I col-

lagen matrix. PYY (10^{-7} mol/L) slightly decreased Caco-2 migration by 1.4% ± 3.0% (n = 12). Similarly, NPY, VIP (10^{-7} mol/L each), and bombesin (10^{-5} mol/L) decreased Caco-2 migration by 1.2% ± 3.0% (n = 12), 6.2% ± 2.9% (n = 12), and 6.1% ± 2.9% (n = 10), respectively. PP (10^{-7} mol/L and pentagastrin (10^{-5} mol/L) slightly stimulated migration by 1.0% ± 3.2% (n = 12) and 4.9% ± 1.9% (n = 10), respectively. The observed increases and decreases in Caco-2 migration were not statistically significant (data not shown).

Table I. Binding specificities of the structurally characterized receptors

	Y1	Y2	Y3
PP	++	++	+
PYY	++	++	++
NPY	++	++	+++
$L^{31}P^{34}$(NPY)	+++	—	?
PYY(13-36)	+	+++	?

DISCUSSION

This study demonstrates that equimolar (10^{-7} mol/L) concentrations of PYY, NPY, PP, and VIP stimulate the specific activity of the predominantly colonocytic brush-border enzyme AP. PYY had the greatest stimulatory effect, increasing AP activity by 38%. Only PYY decreased the specific activity of the enterocytic marker DP, whereas pentagastrin and bombesin increased DP activity. Furthermore, these data suggest that the effects of PYY on Caco-2 brush-border enzyme specific activity may, in part, be mediated through a Y2-receptor since the Y2-agonist PYY(13-36) but not the specific Y1-agonist L^{31} P^{34}(NPY) reproduced the brush-border enzyme effects of PYY, that is, equivalently increased AP specific activity and decreased DP specific activity. The peptide effects on brush-border enzyme activity occurred independent of changes in Caco-2 epithelial cell motility, suggesting specificity of the enzyme effect.

Caco-2 intestinal epithelial cells morphologically and biochemically resemble fetal colonocytes and may express brush-border enzymes characteristic of either the small or large intestinal mucosa on differentiation. They therefore represent an excellent model for the study of human intestinal epithelial growth and differentiation.[20,23,25,35,36] The most widely used marker of mature colonocytes is AP.[53] Although AP is present throughout the bowel, it is particularly concentrated in the colon. In rat colonic mucosa, for example, AP specific activity is quantitatively an order of magnitude higher than small intestinal AP specific activity. In rabbit intestinal mucosa, the differential distribution of the two enzymes is more pronounced, with colonocytic mucosal AP specific activity ranging from one to two orders of magnitude higher than small intestinal AP activity.[28]

DP represents a class of glycoproteins that constitutes the majority of the human villus enterocyte brush-border membrane.[31] DP is generally accepted as an enterocytic brush-border marker enzyme because it is abundant in small intestinal mucosa but expressed at very low levels in colonic mucosa.[29-34] In human small intestinal mucosa, DP specific activity ranges from 75 to 110 mU/mg of protein/min, whereas in colonic mucosa DP specific activity is less than 5 mU/mg of protein/min.[29,30] Certain differentiated colonic tumors and colonic cell lines such as the Caco-2 cell also express DP.[32,35,54] Although neither AP nor DP is exclusively localized to the small or large intestinal mucosa, their differential distribution permits their use as marker enzymes for the colonic and enterocytic phenotypes, respectively.[29,30,53,55-60]

Although intestinal differentiation is an extremely complex process, these data raise the possibility that PYY may function to maintain gut mucosal differentiation, specifically colonocytic differentiation, in Caco-2 human intestinal epithelial cells. The increase in AP demonstrated with NPY and PP may also be related to mucosal Y-receptor activation, as the three peptides possess the requisite structural configuration for Y-receptor binding.[10] The increase in AP activity observed with VIP cannot be explained on the basis of Y-receptor binding, however. The inability to demonstrate a significant decrease in DP specific activity in response to NPY, PP, and VIP may reflect a combination of the relatively lower potency of these compounds compared to PYY and the relatively smaller apparent effect of PYY receptor activation on Caco-2 DP as compared to AP specific activity. Although pentagastrin and bombesin did not affect AP specific activity, both peptides significantly increased Caco-2 DP activity, indicating that Caco-2 cells are capable of responding to appropriate gut peptides by increasing DP specific activity.

PYY and NPY, members of the pancreatic peptide (PP) family of brain-gut peptides, share a common amino acid sequence, the "PP fold," with their parent peptide. The PP fold constitutes the essential structural requirement for the binding of PYY and NPY to Y-receptors.[61] On the basis of the pharmacologic and binding activities of the PP family and related peptide fragments and homologues, Y-receptors have been classified into several structurally distinct subtypes[10,11,62,63] (Table I). Our data raise the possibility

that the PYY effect on Caco-2 AP and DP specific activity may, in part, be mediated by the Y2-receptor, since only the selective Y2-agonist PYY(13-36) stimulated AP and decreased DP specific activity equivalently to PYY. On the basis of these data, we cannot exclude the possibility of other less well-characterized receptors, for example, Y3, Y4, or Y5, contributing to the observed effects as well.[1,5]

In vivo, mucosal levels of PYY in the human colon range from 10^{-8} to 10^{-7} mol/L. Thus the 10^{-7} mol/L PYY concentration used for these in vitro experiments is consistent with in vivo physiology. Although the observed changes in brush-border enzyme activity are, in some instances, relatively small, the magnitude of change in enzyme activity observed in these experiments is consistent with those described previously.[37-41] Specifically, the aforementioned studies establish that the statistically significant changes in AP activity were generally greater (20% to 50% of basal activity) than the changes observed for DP activity (7% to 20% of basal activity). Furthermore, these changes represent differences in the activity of enzyme catalysts. Thus even relatively small changes in enzyme activity may be associated with substantial changes in the proteolytic ability of the intestinal brush border.

The concept that PYY may modulate intestinal epithelial cell biology is consistent with previous observations that PYY may also stimulate intestinal mucosal growth. Changes in plasma PYY levels are seen in all models of altered intestinal epithelial proliferation.[14-19] Although we studied isolated cells in culture, the possibility exists that an autocrine effect of growth factors or inflammatory mediators secreted by the Caco-2 cells in theory may have contributed to the observed results in addition to any direct effect of the gut peptides tested.

None of the peptides tested affected Caco-2 migration across a type I collagen matrix, further supporting the specificity of the observed PYY effect on brush-border enzymes. We have previously demonstrated that changes in brush-border enzyme activity are independent of changes in proliferative activity.[64] The present study further suggests the possibility that changes in brush-border enzyme activity may be regulated independent of changes in epithelial motility.

Although Caco-2 cells in culture represent an established, widely used model of human intestinal epithelial cells, differences in Y-receptor subtype localization, density, and expression, as well as the activity of local growth factors and bioactive peptides, may vary in the in vivo setting. To the extent that we can extrapolate to in vivo conditions from in vitro phenomena, and to the extent to which AP and DP represent markers of colonocytic and enterocytic differentiation, respectively, these data strongly suggest that PYY selectively promotes the colonocytic phenotype in the Caco-2 human intestinal epithelial cell line. These data further suggest that the observed changes in Caco-2 brush-border enzyme activity are mediated through the Y2-receptor subtype.

REFERENCES

1. Adrian TE, Ferri GI, Bacarese-Hamilton AJ, Fuessl HS, Polak JM, Bloom SR. Human distribution of a putative new gut hormone, peptide YY. Gastroenterology 1985;89:1070-1077.
2. Roddy DR, Koch TR, Reilly WM, Carney JA, Go VLW. Identification and distribution of immunoreactive peptide YY in human, canine and murine gastrointestinal tracts: Species-related antibody recognition differences. Regul Pept 1987; 18:201-212.
3. Tatemoto K, Nakano I, Makk G, Angwin P, Mann M, Schilling J, Go VLW. Isolation and primary structure of human peptide YY. Biochem Biophys Res Commun 1988;157:713-717.
4. Mannon PJ, Hernandez SJ, Mervin SJ, Vigna SR, Taylor IL. Characterization of peptide YY receptors in rabbit colonic mucosa. Peptides 1993;14:567-572.
5. Ballantyne GH, Longo WE, Savoca PE, Adrian TE, Vukasin AP, Bilchik AJ, Sussman J, Modlin IM. Deoxycholate-stimulated release of peptide YY from the isolated perfused rabbit left colon. Am J Physiol 1989;257:G715-724.
6. Aponte GW, Fink AS, Meyer JH, Tatemoto K, Taylor IL. Regional distribution and release of peptide YY with fatty acids of different chain length. Am J Physiol 1985;249:G745-750.
7. Sheikh SP, Holst JJ, Orskov C, Ekman R, Schwartz TW. Release of PYY from pig intestinal mucosa: Luminal and neural regulation. Regul Pept 1989;26:253-266.
8. Lundberg JM, Tatemoto K, Terenius L, Hellerstrom PM, Mutt V, Hokfelt T, Hemberger B. Localization of peptide YY (PYY) in gastroenterological endocrine cells and effects on intestinal blood flow and motility. Proc Natl Acad Sci USA 1982;79:4471-4475.
9. Savage AP, Adrian TE, Carolan G, Chattergy VK, Bloom SR. Effects of peptide YY (PYY) on mouth to caecum transit time and on the rate of gastric emptying in healthy volunteers. Gut 1987;17:166-170.
10. Wahlestedt C, Grundemar L, Hakanson R, Heilig M, Shen GH, Zukowska-Grojec Z, Reis DJ. Neuropeptide Y receptor subtypes, Y1 and Y2. Ann NY Acad Sci 1990:611:7-25.
11. Sheikh SP, Hakanson R, Schwartz TW. Y1 and Y2 receptors for neuropeptide Y. FEBS Lett 1989;245:209-214.
12. Cox HM, Cuthbert AW, Hakanson R, Wahlstedt C. The effect of neuropeptide Y and peptide YY on electrogenic ion transport in rat intestinal epithelia. J Physiol Lond 1988;398:65-80.
13. Quin JA, Sgambati SA, Goldenring JR, Basson MD, Fielding LP, Modlin IM, Ballantyne GH. PYY inhibition of VIP-stimulated ion transport in the rabbit distal ileum. J Surg Res 1995;58:111-115.
14. Higashiguchi T, Noguchi Y, Noffsinger A, Fischer JE, Hasselgren PO. Sepsis increases production of total secreted proteins, vasoactive intestinal peptide, and peptide YY in isolated rat enterocytes. Am J Surg 1994;168:251-256.
15. Chance WT, Balasubramaniam A, Thompson H, Zuo L, Fischer JE. Peptide YY and clenbuterol reduce gut atrophy and cachexia during parenteral nutrition of tumor-bearing rats. Abstract presented at the Annual Meeting of the American Institute for Cancer Research, Washington, D.C., 1995.

16. Goodlad RA, Ghatei MA, Domin J, Bloom SR, Gregory H, Wright NA. Plasma enteroglucagon, peptide YY and gastrin in rats deprived of luminal nutrition, and after urogastrone-EGF administration. A proliferative role for PYY in the intestinal epithelium? Experientia 1989;45:168-169.
17. Bilchik AJ, Hines OJ, Adrian TE, Skotzko MJ, McFadden DW, Zinner MJ, Ashley SW. Early regional expression and secretion of peptide YY and enteroglucagon after massive resection of small bowel. J Am Coll Surg 1995;180:417-426.
18. Vukasin AP, Ballantyne GH, Nilsson O, Bilchik AJ, Adrian TE, Modlin IM. Plasma and tissue alterations of peptide YY and enteroglucagon in rats after colectomy. Yale J Biol Med 1992;65:1-15.
19. Andrews NJ, Irving MH. Human gut profiles in patients with short bowel syndrome. Dig Dis Sci 1992;37:729-732.
20. Chantret I, Barbat A, Dussaulx E, Brattain MG, Zweibaum A. Epithelial polarity, villin expression, and enterocytic differentiation of cultured human colon carcinoma cells: A survey of twenty cell lines. Cancer Res 1988;48:1936-1942.
21. Hidalgo IJ, Raub TJ, Borchardt RT. Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. Gastroenterology 1989;96:736-749.
22. LeBivic A, Quaroni A, Nichols B, Rodriguez-Boulan E. Biogenetic pathways of plasma membrane proteins in Caco-2, a human intestinal epithelial cell line. J Cell Biol 1990;111: 1351-1361.
23. Rousset M. The human colon carcinoma cell lines HT-29 and Caco-2: Two in vitro models for the study of intestinal differentiation. Biochemie 1986;68:1035-1040.
24. Eilers U, Klumperman J, Hauri H-P. Nocadazole, a microtubule-active drug, interferes with apical protein delivery in cultured intestinal epithelial cells (Caco-2). J Cell Biol 1989;108:13-22.
25. Basson MD, Modlin IM, Madri JA. Human enterocyte (Caco-2) migration is modulated in vitro by extracellular matrix composition and epidermal growth factor. J Clin Invest 1992;90:15-23.
26. Basson MD, Modlin IM, Flynn SD, Jena BP, Madri JA. Independent modulation of enterocyte migration and proliferation by growth factors, matrix proteins and pharmacologic agents in an in vitro model of mucosal healing. Surgery 1992;112: 299-308.
27. Basson MD, Beidler DR, Turowski GA, Zarif A, Modlin IM, Jena BP, Madri JA. Effect of tyrosine kinase inhibition on basal and epidermal growth factor-stimulated human Caco-2 enterocyte sheet migration. J Clin Invest 1994;160:491-501.
28. Stieger B, Marxer A, Hauri H-P. Isolation of brush border membranes from rat and rabbit colonocytes: Is AP a marker enzyme? J Membrane Biol 1986;91:19-31.
29. Darmoul D, Voisin T, Couvineau A, Rouyer-Fessard C, Salomon R, Wang Y, Swallow DM, Laburthe M. Regional expression of epithelial dipeptidyl peptidase IV in the human intestines. Biochem Biophys Res Commun 1994;203:1224-1229.
30. Gorvel JP, Ferrero A, Chambraud L, Rigal A, Bonicel J, Maroux S. Expression of sucrase-isomaltase and dipeptidylpeptidase IV in human small intestine and colon. Gastroenterology 1991;101:618-625.
31. Kenny JA, Maroux S. Topology of microvillar membrane hydrolases of kidney and intestine, Physiol Rev 1982;62:91-128.
32. Zweibaum A, Triadou N, Kedinger M, Augeron Ch, Robine-Leone S, Pinto M, Rousset M, Haffen K. Sucrase-isomaltase: A marker of foetal and malignant epithelial cells of the human colon. Int J Cancer 1983;32:407-412.
33. Darmoul D, Rouyer-Fessard C, Blais A, Voisin T, Sapin C, Baricault L, Cibert C, Geraud G, Couvineau A, Laburthe M, Trugnan G. Dipeptidyl peptidase IV expression in rat jejunal crypt-villus axis is controlled at mRNA level. Am J Physiol 1991;261:G763-G769.
34. Darmoul D, Lacasa M, Baricault L, Marguet D, Sapin C, Trotot P, Barbat A, Trugnan G. Dipeptidyl IV (CD 26) gene expression in enterocyte-like colon cancer cell lines HT-29 and Caco-2. J Biol Chem 1992;267:4824-4833.
35. Peterson MD, Mooseker MS. Characterization of the enterocyte-like brush border cytoskeleton of the Caco-2_{BBE} clones of the human intestinal cell line, Caco-2. J Cell Sci 1992; 102:581-600.
36. Jumarie C, Malo C. Caco-2 cells cultured in serum-free medium as a model for the study of enterocytic differentiation in vitro. J Cell Physiol 1991;149:24-33.
37. Turowski GA, Rashid Z, Hong F, Madri JA, Basson MD. Glutamine modulates phenotype and stimulates proliferation in human colon cancer cell lines. Cancer Res 1994;54:5974-5980.
38. Basson MD, Hong F. The metabolites of different dietary fibers exert distinct effects on human (Caco-2) colonocytes. Surg Forum 1994;45:192-194.
39. Basson MD, Hong F, Emenaker NJ. Specific modulation of intestinal epithelial brush border enzyme expression by a phorbol ester. J Surg Res 1995;59:121-127.
40. Basson MD, Hong F. Modulation of human Caco-2 intestinal epithelial cell phenotype by protein kinase C inhibitors. Cell Biol Int 1995;119:51-55.
41. Basson MD, Hong F. Regulation of human Caco-2 intestinal epithelial brush border enzyme activity by cyclic nucleotides. Cancer Lett 1996;99:155-160.
42. Ferri GL, Adrian TE, Ghatei MA, O'Shaugnessey DJ, Probert L, Lee YC, Buchan AMJ, Polak JM, Bloom SR. Tissue localization and relative distribution of regulatory peptides in separated layers from the human bowel. Gastroenterology 1983;84:777-786.
43. Lamote J, Williams G. Stimulating effect of pentagastrin on cancer cell proliferation kinetics in chemically induced colon cancer RTAs. Regul Pept 1988;20:1-9.
44. McGregor DB, Morriss LL, Manalo PB, Bomberger RA, Pardini RS. Pentagastrin stimulation of human colon carcinoma. Arch Surg 1989;124:470-472.
45. Winsett OE, Townsend CM Jr, Glass EJ, Thompson JC. Gastrin stimulates growth of a colon cancer. Surgery 1986;90:302-307.
46. Chu KU, Evers BM, Ishizuka J, Townsend CM Jr. Role of bombesin on gut mucosal growth. Ann Surg 1995;222:94-100.
47. Fuhlendorff J, Gether U, Aakerlund L, Langeland-Johansen N, Thogersen H, Melberg SG, Olsen UB, Thastrup O. [Leu^{31},Pro^{34}]Neuropeptide Y: A specific Y1 receptor agonist. Proc Natl Acad Sci USA 1990;87:182-186.
48. Grandt D, Feth F, Schimiczek M, Michel MC, Schlicker E, Rascher W, Goebell H, Eysselein VE, Reeve JR Jr. [Pro^{34}]PYY, a new PYY analogue selectively acts on Y1 receptors [abstr]. Regul Pept 1992;40:159.
49. Grandt D, Schimiczek M, Belinger Ch, Layer P, Goebell H, Eysselein VE, Reeve JR. Two molecular forms of peptide YY (PYY) are abundant in human blood: Characterization of a radioimmunoassay recognizing PYY 1-36 and PYY 3-36. Regul Pept 1994;51:151-159.
50. Mentlein R, Dahms P, Grandt D, Kruger R. Proteolytic processing of neuropeptide Y and peptide YY by dipeptidyl peptidase IV. Regul Pept 1993;49:133-144.
51. Kusyk CJ, McNeil NO, Johnson LR. Stimulation of a colon cancer cell line by gastrin. Am J Physiol 1986;251:G597-G601.

52. Watson SA, Durrant LG, Morris DL. Growth-promoting action of gastrin on human colonic and gastric tumor cells cultured in vitro. Br J Cancer 1988;75:342-345.
53. Gustin MC, Goodman DB. Isolation of brush-border membrane from the rabbit descending colon epithelium. J Biol Chem 1981;256:10651-10656.
54. Zweibaum A, Hauri HP, Sterchi E, Chantret I, Haffen K, Bamat J, Sordat B. Immunohistological evidence, obtained with monoclonal antibodies, of small intestinal brush border hydrolases in human colon cancers and foetal colons. Int J Cancer 1984;34:591-598.
55. Bell I, Williams L. Histochemical demonstration of AP in human large intestine, normal and diseased. Histochemistry 1979;60:85-89.
56. Menard D, Pothier P. Differential distribution of digestive enzymes in isolated epithelial cells from developing human fetal small intestinal and colon. J Pediatr Gastroenterol Nutr 1987;6:509-516.
57. Weiser M. Intestinal epithelial cell surface membrane glycoprotein synthesis: I. An indicator of differentiation. J Biol Chem 1973;7:2536-2541.
58. Ahnen DJ, Reed TA, Bozdech JM. Isolation and characterization of populations of mature and immature rat colonocytes. Am J Physiol 1988;254:G610-G621.
59. Vengesa PB, Hopfer U. Cytochemical localization of AP and Na^+-pump sites in adult rat colon. J Histochem Cytochem 1979;27:1231-1235.
60. Nordstrom C, Dahlqvist A, Josefsson L. Quantitative determination of enzymes in different parts of the villi and crypts of rat small intestine: Comparison of AP, disaccharidases and dipeptidases. J Histochem Cytochem 1968;15:713-721.
61. Yoshinaga K, Mochizuki T, Yanahara N, Oshima K, Izukura M, Kogire M, Sumi S, Gomez G, Uchida T, Thompson JC, Greeley GH Jr. Structural requirements of peptide YY for biological activity at enteric sites. Am J Physiol 1992;263:G695-G701.
62. Sheikh SP, Williams JA. Structural characterization of Y1 and Y2 receptors for neuropeptide Y and peptide YY by affinity cross-linking. J Biol Chem 1990;265:8304-8310.
63. Wahlestedt C, Regunathan S, Reis DJ. Identification of cultured cells selectively expressing Y1, Y2 or Y3-type receptors for neuropeptide Y/peptide YY. Life Sci 1992;50:PL7-PL12.
64. Sgambati SA, Basson MD. Octreotide differentially modulates human Caco-2 intestinal epithelial cell proliferation and differentiation by decreasing intracellular cAMP. Regul Pept 1996;61:219-227.

Effects of Hyaluronic Acid/Carboxymethylcellulose Gel on Bowel Anastomoses in the New Zealand White Rabbit

Anoushirvan Hadaegh, M.D., James Burns, Ph.D., Laurette Burgess, B.M., Rosalind Rose, B.S., Eric Rowe, B.S., Wayne W. LaMorte, M.D., Ph.D., M.P.H., James M. Becker, M.D., F.A.C.S.

Intra-abdominal adhesions form in more than 90% of patients undergoing major abdominal surgery and can lead to significant complications. Application of a bioresorbable gel consisting of chemically modified hyaluronic acid (HA) and carboxymethylcellulose (CMC) has shown promise as a means of preventing intra-abdominal adhesions, but there have been concerns that the presence of the gel might interfere with the integrity and healing of bowel anastomoses. We tested the effects of HA/CMC gel on adhesion formation and anastomotic healing in 60 New Zealand white rabbits after transection and complete (100%) or incomplete (90%) anastomosis of the ileum. Half of the animals underwent application of HA/CMC gel and half served as control subjects. Animals were killed at 4, 7, or 14 days after surgery. Anastomotic adhesions were scored in a blinded fashion. Integrity of the anastomosis was tested by measuring bursting pressure at the anastomotic site and in an adjacent section of intact bowel. With complete anastomosis, HA/CMC gel significantly reduced adhesion formation at 7 and 14 days after surgery ($P < 0.05$), but gel application did not inhibit adhesion formation when the anastomosis was incomplete. Anastomosed segments of bowel burst at a lower pressure than intact bowel 4 days after surgery, but bursting pressures were normal at 7 and 14 days. Burst pressures of anastomoses receiving an application of HA/CMC gel were nearly identical to control anastomoses at all three time points. HA/CMC gel did not interfere with the normal healing process of bowel anastomoses. Furthermore, HA/CMC gel decreased adhesion formation after complete anastomoses, yet it did not affect adhesion formation in the presence of anastomotic disruption. (J GASTROINTEST SURG 1997;1:569-575.)

Postsurgical adhesions are a significant cause of morbidity following abdominal, pelvic, and cardiothoracic operations. In a recent multicenter clinical trial, adhesions were present in more than 90% of patients who underwent major abdominal surgery.[1] The 1991 Operative Laparoscopy Study Group reported that the incidence of intraperitoneal adhesions following open gynecologic pelvic procedures was as high as 97%.[2] Postsurgical adhesions are the most common cause of small bowel obstruction and secondary female infertility in the United States and can present a major difficulty during subsequent operative procedures.[3,4]

Adhesions are caused by a series of cellular and endogenous chemical inflammatory responses secondary to injury to serosal tissues. Various therapeutic modalities have been designed to prevent or reduce adhesions either by reducing the magnitude of the inflammatory response of damaged peritoneal surfaces or by separating the injured surfaces long enough to allow mesothelial repair without adhesion formation.[5]

A bioresorbable membrane of chemically modified hyaluronic acid (HA) and carboxymethylcellulose (CMC) has been shown to reduce postsurgical adhesions in two recent clinical trials.[1,6] A similarly formulated bioresorbable gel, HA/CMC gel, reduced adhesions in three animal models.[7]

Because of uncertainty regarding the impact of adhesion prevention on the healing of intestinal anastomoses, the aims of this study were twofold: (1) to evaluate the integrity of bowel anastomoses after application of HA/CMC gel, and (2) to determine the effect

From the Division of Surgery, Boston University School of Medicine, Boston, Mass., and Genzyme (J.B., L.B., R.R., and E.R.), Cambridge, Mass.

Reprint requests: James M. Becker, M.D., F.A.C.S., Chairman, Division of Surgery, Boston Medical Center, 88 E. Newton St., Boston, MA 02118.

of HA/CMC gel in the presence of anastomotic disruption, a condition during which adhesions might be of benefit.

METHODS

Surgical Preparation

All animal studies were reviewed and approved by the Institutional Animal Care and Use Committees at Boston University Medical Center and Genzyme Corporation (Cambridge, Mass.). Sixty adult female New Zealand white rabbits weighing approximately 4 kg underwent celiotomy, transection of the ileum, and anastomosis. All animals were fasted for approximately 24 hours prior to surgery. Each animal received preoperative antibiotic treatment with cephalothin (20 mg/kg intramuscularly). Animals were preanesthetized with xylazine (10 mg/kg), acepromazine (3 mg/kg), and ketamine (30 mg/kg) intramuscularly. After endotracheal intubation, surgical anesthesia was maintained with isoflurane gas. The abdomen was prepared and draped in a sterile fashion. A 3 to 4 cm midline incision was made below the umbilicus. A segment of ileum approximately 10 cm proximal to the appendix was transected sharply between two atraumatic bowel clamps. The bowel was then anastomosed using a continuous single layer of 5-0 polypropylene sutures (Prolene). One half of the animals underwent a complete (100%) anastomosis. The other one half underwent an incomplete anastomosis with 10% of the anastomosis on the antimesenteric border left open.

One half of the animals in each anastomotic group (complete and incomplete) then received 5 ml of HA/CMC gel, applied as a homogeneous film directly over the anastomosis, 2 cm proximal and 2 cm distal to the anastomosis. An additional 5 ml of gel was instilled into the peritoneal cavity. The midline fascia was closed using a continuous 3-0 polyglycolic acid suture (Dexon) reinforced with interrupted sutures, and the skin was closed with a continuous 4-0 polyglycolic acid suture (Dexon).

Evaluation of Postoperative Adhesions

Five animals from each group were sacrificed at 4, 7, or 14 days postoperatively. At the time of death, their intra-abdominal adhesions were evaluated by a blinded observer who scored the adhesions in three categories: extent, tenacity, and total number of adhesions. The extent of adhesion formation at the anastomotic site was scored on the basis of the percentage of the bowel circumference involved with adhesions, and the tenacity of adhesions was scored based on the firmness of the adhesion's attachment (Table I). The total number of adhesions was the number of individually identifiable and dividable adhesive bands between the anastomosis and the abdominal wall and adjacent bowel segments.

Evaluation of Anastomotic Integrity and Healing

After evaluation of adhesions, a 10 cm segment of bowel, 3 cm proximal to and 7 cm distal to the anastomosis, was resected for evaluation of bursting pressure. In addition, a 10 cm proximal segment of intact bowel from each animal was resected. The intraluminal content of each segment was emptied and irrigated with normal saline solution. The bowel segments were then tested for hydrostatic bursting pressure using a modification of the method of Holzman et al.[8] The distal end of each bowel segment was cannulated with a 14 Fr Foley urinary catheter secured with 0-silk ligature, and the proximal end was occluded with an atraumatic bowel clamp. Normal saline solution was then infused into the bowel lumen through the Foley catheter at a rate of 5 ml/min with a Harvard infusion pump (Harvard Apparatus, Inc., S. Natick, Mass.). One limb of the Foley catheter was connected to a Hewlett-Packard pressure transducer (Hewlett-Packard Medical Products Group, Andover, Mass.) and strip-chart recorder for continuous monitoring of intraluminal pressure. Hydrostatic bursting pressure was defined as the pressure at which the bowel segment leaked.

Table I. Criteria for grading the extent and tenacity of adhesions

Extent		Tenacity	
% of anastomotic circumference involved	Score	Description	Score
None	0	Filmy	1
25	1	Lysis by traction	2
50	2	Lysis by sharp dissection	3
75	3		
100	4		

Statistical Analysis

Results for each group are reported as the mean ± standard error of the mean. Differences in bursting pressures and adhesion scores were tested for significance using a three-factor analysis of variance (completeness of anastomosis, days after surgery, and application HA/CMC gel). Post hoc testing was done using the Tukey-Kramer test. In addition, bursting pressure was also measured in a segment of intact bowel from each animal. The bursting pressures for intact bowel were similar in all experimental groups. Consequently the bursting pressures for intact bowel segments were pooled and used as a single reference standard for both complete anastomoses and incomplete anastomoses.

A chi-square test was used to compare differences in mortality rates.

RESULTS

In the original 60 animals studied there was an overall mortality rate of 16%. Postoperative mortality was similar in animals receiving HA/CMC gel (18%) and controls (14%; $P > 0.05$). Causes of death are summarized in Table II. Animals that were withdrawn from the study because of death or illness were replaced with additional animals.

Effect of HA/CMC Gel on Complete Anastomoses

Fig. 1 summarizes adhesion scores among animals with complete anastomosis of the ileal segment. In general, adhesions tended to increase somewhat from day 4 to day 7, but results obtained after 7 and 14 days were nearly identical, and these groups were combined for simplicity. The extent of adhesions, that is, the percentage of the anastomotic circumference involved with adhesions, was similar at day 4 after surgery, but after 7 days the extent of adhesion formation was significantly lower in anastomoses to which gel was applied ($P < 0.05$). The number of adhesions and the tenacity of adhesions were both significantly lower with HA/CMC gel application at all time points ($P < 0.05$).

Fig. 2 shows the mean bursting pressures for complete ileal anastomoses and compares them with bursting pressures observed in intact segments of ileum. On postoperative day 4, all anastomotic segments burst at the anastomosis, but after 7 or 14 days bursting occurred adjacent to the anastomosis. Anastomosed segments of ileum burst at lower pressures than intact bowel on day 4 ($P < 0.05$), but bursting pressures were similar in anastomoses to which gel was applied and control (non-gel) anastomoses. At

Table II. Causes of postoperative death

Cause of death	No gel	Gel applied
With complete (100%) anastomosis		
Respiratory infection	2	2
Bowel obstruction	0	2
Anastomotic dehiscence	0	1
Other	1	1
With incomplete (90%) anastomosis		
Respiratory infection	1	0
Bowel obstruction	1	0
Abdominal wound dehiscence	0	1

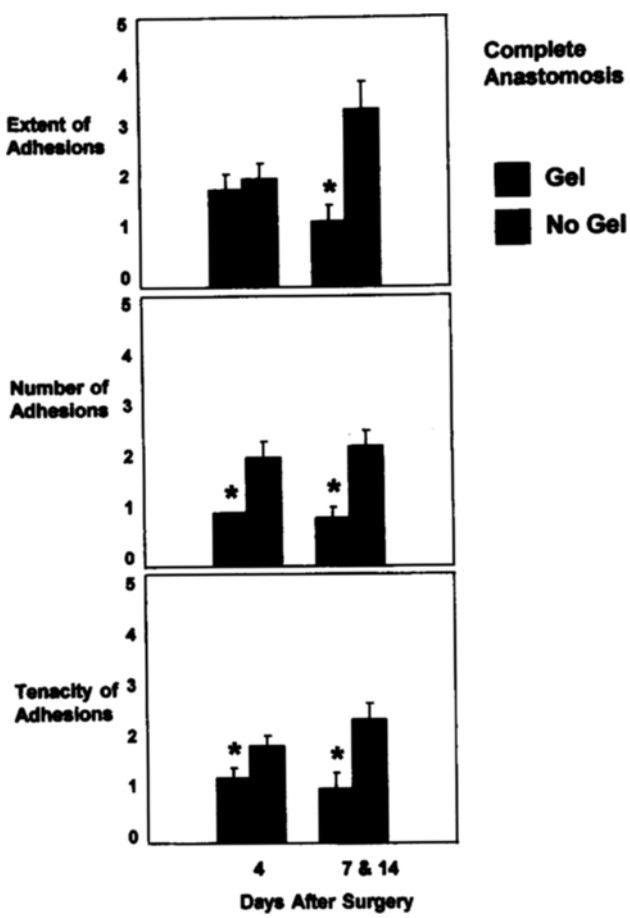


Fig. 1. Effects of HA/CMC gel on adhesion formation in animals undergoing complete anastomosis of the ileal segment. Criteria used for scoring the extent and tenacity of adhesions are listed in Table I. (* = $P < 0.05$ compared to non-gel controls at the same time point.)

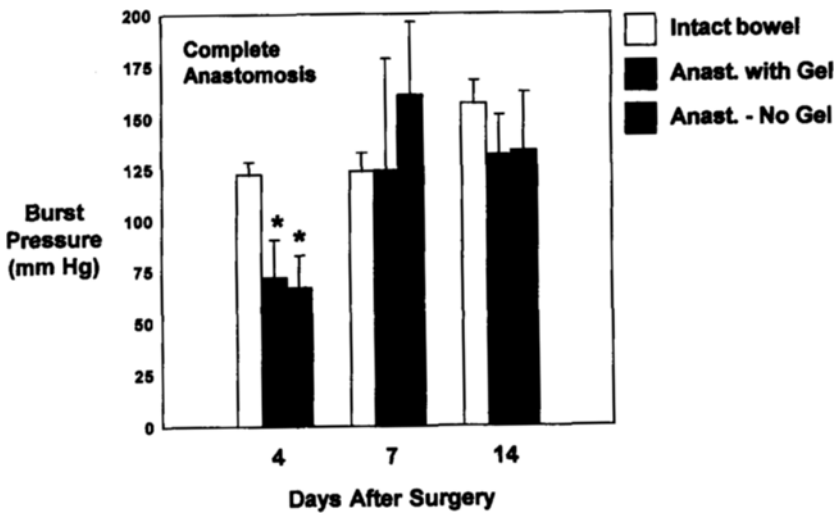


Fig. 2. Ileal bursting pressures in animals with complete anastomoses. (* = $P < 0.05$ compared to intact segments of bowel from the same animals.)

days 7 and 14, bursting pressures of the anastomosed segments were similar to those observed in the intact bowel segment. Moreover, bursting pressures were similar in anastomoses receiving HA/CMC gel and non-gel control anastomoses. Finally, there was no apparent association between anastomotic bursting pressure and the extent of adhesion formation in either animals to which gel was applied or in non-gel controls.

Effect of HA/CMC Gel on Incomplete Anastomoses

Fig. 3 shows the effects of HA/CMC gel on adhesion formation in animals in which the anastomosis was incomplete (90% of bowel circumference). In control animals with anastomotic disruption, the extent of adhesion formation was significantly greater at 4 days than it had been with complete anastomosis (compare Figs. 1 and 3), although the number of adhesions formed and the tenacity scores were similar to those observed in animals with complete anastomoses. However, in contrast to what was observed with complete anastomoses, HA/CMC gel application had no significant effect on adhesion formation in either the early or late postoperative period. Specifically, the greater extent of adhesion formation seen with anastomotic disruption at 4 days was not diminished by the application of gel.

Fig. 4 illustrates mean bursting pressures observed in animals with incomplete ileal anastomoses and again compares them with bursting pressures in intact segments of ileum. Similar to what was seen with

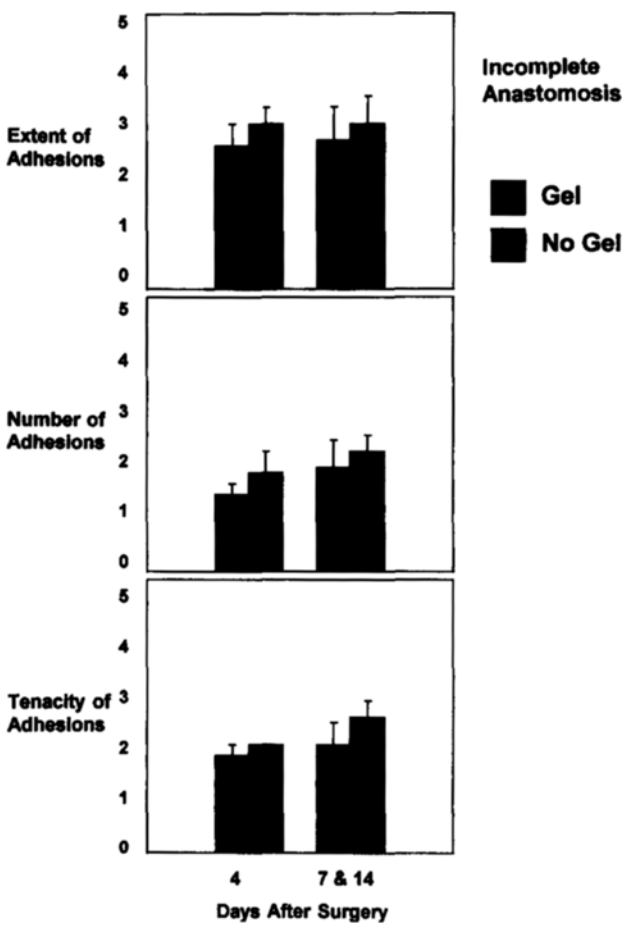


Fig. 3. Effects of HA/CMC gel on adhesion formation in animals undergoing incomplete anastomosis (90% of bowel circumference). (None of the differences were statistically significant; $P > 0.05$.)

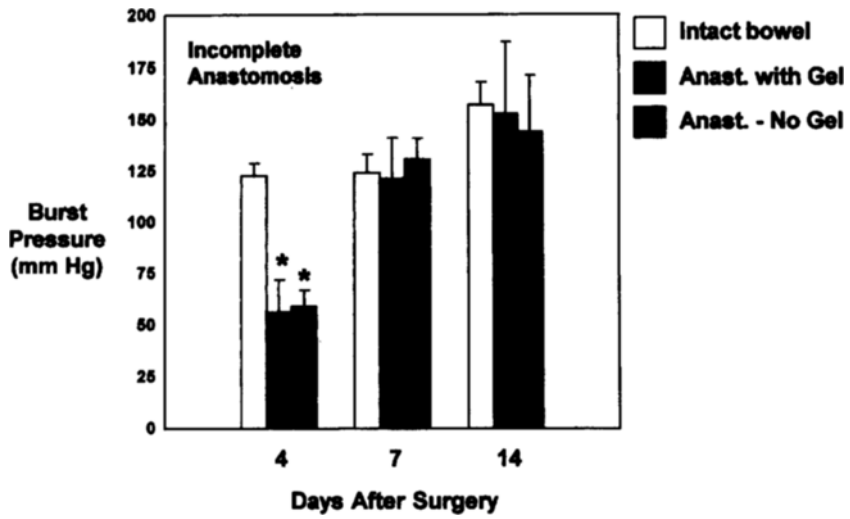


Fig. 4. Ileal bursting pressures in animals with incomplete anastomoses (90% of bowel circumference). (* = $P < 0.05$ compared to intact segments of bowel from the same animals.)

complete anastomoses, all anastomotic segments burst at the site of the anastomosis on day 4, but after 7 or 14 days bursting occurred adjacent to the anastomosis. Once again, anastomosed segments of ileum burst at lower pressures than intact bowel on day 4 ($P < 0.05$), but bursting pressures were similar for anastomoses to which gel was applied and non-gel controls. At days 7 and 14, bursting pressures of the anastomosed segments were similar to those observed in intact bowel segments, and bursting pressures again were similar in anastomoses receiving HA/CMC gel and non-gel control anastomoses.

DISCUSSION

Adhesions are a significant cause of postsurgical complications, resulting in bowel obstruction, infertility, and pain. In addition to their undesirable clinical impact, adhesions are associated with substantial increases in health care costs.[8] According to the 1993 National Inpatient Profile, more than 400,000 operations were performed in the United States for the lysis of adhesions in 1992. The total costs related to adhesions have been estimated to be $1.2 billion per year.[9]

Adhesions are defined as healing fibrosis between two damaged tissue surfaces that normally are separated.[10] Peritoneal tissue trauma activates a series of cellular and endogenous chemical responses involving cytokines, complement, and the clotting system. With the use of electron microscopy, Raftery[11] demonstrated that adhesion formation begins with fibrin deposition within an inflammatory exudate formed over an injured serosal layer. The fibrin matrix is used as a scaffolding for new vascular granulation tissue containing macrophages, fibroblasts, and giant cells,[12] which later organize into a distinct network of histamine-releasing cells, fibroblasts, and collagen,[5] resulting in adhesion formation. Mesothelial cells exhibit fibrinolytic activity, which is influenced by a variety of factors including inflammation and infection. If the net fibrinolytic activity following injury is sufficient, the fibrin matrix resolves and mesothelial repair occurs without adhesion formation.[13,14]

Current strategies regarding adhesion reduction or prevention are threefold. The first is reduction of tissue trauma by careful tissue handling and surgical technique. Studies have shown that tissue ischemia, desiccation, unnecessary tissue damage due to retraction, clamping, extensive electrocautery, and foreign bodies such as suture material, talc, or starch powder from surgical gloves are associated with an increased incidence of adhesions.[15] The second strategy is to interfere with the initial inflammatory response or fibrin matrix formation by use of agents such as corticosteroids,[16] nonsteroidal anti-inflammatory drugs (NSAIDs),[17] or heparin.[18] This approach has had variable effects on adhesion formation.[5] The third strategy focuses on separating damaged peritoneal surfaces for a long enough period to permit mesothelial repair without adhesion formation using barrier materials such as dextran 70 (Hyskon, Pharmacia Laboratories, Princeton, N.J.),[19,20] oxidized regenerated cellulose (Interceed[TC7], Johnson & Johnson Medical, Inc., Arlington, Tex.),[21-23] polytetrafluoroethylene membrane (Gore-Tex Surgical Membrane, W.L. Gore & Associates, Inc., Flagstaff,

Ariz.),[24,25] or sodium hyaluronate/carboxymethylcellulose membrane (Seprafilm Bioresorbable Membrane, Genzyme, Cambridge, Mass.).[1,6,26]

HA is a naturally occurring glycosaminoglycan that is found in the basement membrane of cells and in soft tissues. HA has unique physical properties such as tissue protection and joint lubrication, and it enhances tissue elasticity.[27] CMC is a bioresorbable synthetic polysaccharide polymer that has been shown to reduce adhesions in its liquid form.[28] A lyophilized, dehydrated CMC sponge has been shown to reduce postoperative adhesions more effectively than oxidized regenerated cellulose (Interceed).[29] The HA/CMC gel has been chemically altered to make it less water soluble and less susceptible to in vivo degradation. This preparation forms a hydrophilic viscous gel that coats tissue surfaces and remains in the peritoneal cavity long enough to provide a mechanical barrier during the phase of peritoneal repair when adhesions form. The ability of HA/CMC gel to prevent adhesions was recently demonstrated in two animal species employing standardized surgical trauma.[6] A similar membrane formulation (Seprafilm Bioresorbable Membrane) has been shown to reduce the extent and severity of postsurgical adhesions in two multicenter clinical trials following abdominal and pelvic operations.[1,5]

The goal of this study was to examine the effects of HA/CMC gel on intestinal anastomotic healing, with or without leakage, by evaluating hydrostatic bursting pressure. According to Laplace's law, bowel wall tension is directly proportional to the radius of the bowel and intraluminal pressure.[30] Intraluminal hydrostatic bursting pressure determines the multiaxial force or energy required to disrupt the bowel at its weakest point. Christensen et al.[31] showed that determination of hydrostatic bursting pressure is a valid technique for evaluating anastomotic integrity in the early stages of bowel healing. In this study, bursting in the early phase of healing occurred at the site of anastomosis and occurred at a lower pressure than that of intact bowel. This was true regardless of whether the anastomosis was complete or incomplete. These findings are consistent with earlier reports that the integrity of a bowel anastomosis during the first 5 days after surgery depends on the holding strength of the sutures.[31] Furthermore, intra-abdominal infection interferes with the early stages of anastomotic healing.[32] Ahrendt et al.[33] demonstrated that the hydrostatic bursting pressure and collagen content of colonic anastomoses were lower in the presence of intra-abdominal sepsis. The results of our study show that bursting occurred adjacent to the anastomosis on the seventh and fourteenth postoperative days, when bursting pressures were similar to those of intact bowel, regardless of whether the anastomosis was complete or incomplete. The radiologic and mechanical studies of Christensen et al.[31] found that by the sixth postoperative day 60% of the bowel segments ruptured adjacent to the anastomosis rather than at the anastomosis, per se. They postulated that disruption tended to occur in the adjacent bowel segments as a consequence of Laplace's law, which predicts that at a given intraluminal pressure, wall tension will be higher in the adjacent segments where luminal diameter is greater than at the healing anastomotic site.[31] Regardless of the mechanism involved, HA/CMC gel did not influence bursting pressure with complete or incomplete anastomoses.

Studies by Ryan et al.[34] and Diamond et al.[35] showed that there are no changes in adhesion formation after 7 days. We observed a similar time course for adhesion formation. Adhesion formation as measured by the extent, total number, and tenacity of adhesions was reduced in the presence of HA/CMC gel in animals with complete intestinal anastomoses. These results are consistent with earlier findings that HA/CMC film or gel decreased adhesion formation following an intra-abdominal procedure.[1,6,7,26] It is perhaps noteworthy that the application of HA/CMC gel to complete anastomoses resulted in fewer adhesions, yet healing of these anastomoses was comparable to that observed in the non-gel controls. This would suggest that adhesions to the anastomosis are not a prerequisite for normal healing. However, in the presence of an incomplete anastomosis, HA/CMC gel did not influence adhesion formation. We speculate that the greater inflammatory response elicited by anastomotic disruption overwhelms the barrier function of HA/CMC gel.

CONCLUSION

HA/CMC gel did not interfere with the normal process of bowel anastomotic healing as measured by hydrostatic bursting pressure, even in the presence of anastomotic disruption. Further, HA/CMC gel decreased adhesion formation in complete anastomoses but did not affect adhesion formation in the presence of an anastomotic leak.

We gratefully acknowledge the assistance of Jude Cult in study design and manuscript preparation and the assistance of Roberto Rodriguez in animal care.

REFERENCES

1. Becker JM, Dayton MT, Fazio VW, et al. Sodium hyaluronate-based bioresorbable membrane (HAL-F) in the prevention of postoperative abdominal adhesions: A prospective, randomized, double-blinded multicenter study. J Am Coll Surg 1996;183:297-307.
2. The Operative Laparoscopy Study Group. Postoperative adhesion development after operative laparoscopy: Evaluation at early second-look procedures. Fertil Steril 1991;55:700-704.
3. Monk BJ, Berman ML, Montz FJ. Adhesions after extensive gynecological surgery: Clinical significance, etiology, and prevention. Am J Obstet Gynecol 1994;170:1396-1403.
4. Stewart RM, Page CP, Brender J, et al. The incidence and risk of early postoperative small bowel obstruction: A cohort study. Am J Surg 1987;154:643-647.
5. di Zerega GS. Contemporary adhesion prevention. Fertil Steril 1994;61:219-235.
6. Diamond M. The Seprafilm Adhesion Group. Seprafilm (HAL-F) reduces postoperative adhesions: Initial results of a multicenter gynecologic clinical study. Presented at the Fourth Congress of the European Society for Gynecological Endoscopy. Brussels: December, 1995.
7. Burgess LS, Rose RL, Colt MJ, et al. The evaluation of an injectable bioresorbable gel on adhesion reduction in two animal models. Presented at the Third International Congress on Pelvic Surgery and Adhesion Prevention. San Diego: March, 1996.
8. Holzman S, Connolly RJ, Schwaitzberg SD. The effects of hyaluronic solution on healing of bowel anastomoses. J Invest Surg 1994;7:431-437.
9. Peritoneal adhesiolysis. National Inpatient Profile 1993: Baltimore, HICA Inc., 1994;427:635-655.
10. Hertzler AE. The Peritoneum. St. Louis: CV Mosby, 1919, pp 20-22.
11. Raftery AT. Regeneration of parietal and visceral peritoneum: An electron microscopical study. J Anat 1973;115:375-392.
12. Raftery AT. Regeneration of peritoneum: A fibrinolytic study. J Anat 1979;129:659-664.
13. Thompson JN, Whawell SA. Pathogenesis and prevention of adhesion formation. Br J Surg 1995;82:3-5.
14. Whawell SA, Thompson JN. Cytokine induced release of plasminogen activator inhibitor-1 by mesothelial cells. Eur J Surg 1995;161:315-317.
15. Stone K. Adhesions in gynecologic surgery. Curr Opin Obstet Gynecol 1993;5:322-327.
16. Stangel JJ, Nisbett JD, Settles H. Formation and prevention of postoperative abdominal adhesions. J Reprod Med 1984; 29:143-156.
17. Nishimura K, Shimanuki T, di Zerega GS. Ibuprofen in the prevention of experimentally induced postoperative adhesion. Am J Med 1984;77:102-106.
18. Jansen RPS. Failure of peritoneal irrigation with heparin during pelvic operations upon young women to reduce adhesions. Surg Gynecol Obstet 1988;166:154-160.
19. Adhesion Study Group. Reduction of postoperative pelvic adhesions with intraperitoneal 32% dextran 70: A prospective, randomized clinical trial. Fertil Steril 1983;40:612-619.
20. Rosenberg SM, Board JA. High molecular weight dextran in human infertility surgery. Am J Obstet Gynecol 1984;148: 380-385.
21. Interceed (TC7) Adhesion Barrier Study Group. Prevention of postsurgical adhesions by Interceed (TC7), an absorbable adhesion barrier: A prospective, randomized multicenter clinical study. Fertil Steril 1989;51:933-938.
22. Interceed (TC7) Adhesion Barrier Study Group 2. Pelvic sidewall adhesion reformation: Microsurgery alone or with Interceed absorbable adhesion barrier. Surg Gynecol Obstet 1993;177:135-139.
23. Sekiba K. The Obstetrics and Gynecology Adhesion Prevention Committee. Use of Interceed (TC7) absorbable adhesion barrier to reduce postoperative adhesion reformation in infertility and endometriosis surgery. Obstet Gynecol 1992;79:518-522.
24. The Surgical Membrane Study Group. Prophylaxis of pelvic sidewall adehsions with Gore-Tex Surgical Membrane: A multicenter clinical investigation. Fertil Steril 1991;57:921-923.
25. McCabe-Fowler JD, Lacy SM, Montz FJ. The inability of Gore-Tex Surgical Membrane to inhibit post-radical pelvic surgery adhesions in the dog model. Gynecol Oncol 1991; 43:141-144.
26. Skinner KC, Colt MJ, Kirk JF, et al. HAL-F film: A new hyaluronic acid based bioresorbable barrier for the prevention of abdominal adhesion [abstr]. J Invest Surg 1991;4:381.
27. Comper WD, Laurent TC. Physiologic function of connective tissue polysaccharides. Physiol Rev 1978;58:255-315.
28. Fredericks CM, Kotry I, Holz G, et al. Adhesion prevention in the rabbit with sodium carboxymethylcellulose solutions. Am J Obstet Gynecol 1986;155:667-670.
29. Ryan CK, Sax HC. Evaluation of a carboxymethylcellulose sponge for prevention of postoperative adhesions. Am J Surg 1995;169:154-160.
30. Ganong WF. Review of Medical Physiology, 13th ed. New York: Appleton & Lange, 1987, pp 483-484.
31. Christensen H, Langfert S. Laurberg S. Bursting strength of experimental colonic anastomosis. Eur Surg Res 1993;25: 38-45.
32. Hesp FL, Hendriks T, Lubbers EJ, de Boer HH. Wound healing in the intestinal wall. Effects of infection on experimental ileal and colonic anastomoses. Dis Colon Rectum 1984; 27:462-467.
33. Ahrendt GM, Gardner K, Barbul A. Loss of colonic structural collagen impairs healing during intra-abdominal sepsis. Arch Surg 1994;129:1179-1183.
34. Ryan GB, Gruberty J, Majno G. Postoperative peritoneal adhesions. Am J Pathol 1971;65:117-148.
35. Diamond MP, Daniell JF, Feste J, et al. Adhesion reformation and de nova reformation after pelvic reproductive surgery. Fertil Steril 1987;47:864-866.

Management of Hepatic Metastases From Colorectal Cancer: Systemic Chemotherapy

Brian Leyland-Jones, M.D., Susan Burdette-Radoux, M.D.

The current phase III studies of chemotherapy in advanced colorectal cancer include 60% to 85% of patients with the liver as a site of metastatic disease. Within the past 10 years, various modulatory combinations of 5-fluorouracil (5-FU) with agents such as leucovorin, interferon, N-(phosphonacetyl)-L-aspartate (PALA), and methotrexate have produced higher response rates than 5-FU alone. A major seven-arm study, conducted by the Southwestern Oncology Group and reported in 1995, suggested that single-agent, continuous-infusion 5-FU demonstrated the most encouraging results. Nine of 12 reported randomized studies comparing the combination of 5-FU and leucovorin with 5-FU alone report significant increases in response rates; two studies reported significant increases in survival. The meta-analysis project involving 1381 patients confirmed the increase in response rate with the combination (23%) vs. 5-FU alone (11%) but did not demonstrate any significant difference in median survival. The current issues involving 5-FU administration largely concentrate on the best approach (modulation vs. scheduling) and comprehensive evaluation of end points (quality of life, survival, and pharmacoeconomics). The current literature examining quality-of-life issues suggests that 5-FU and low-dose leucovorin produce the best overall improvement in symptoms. Others argue that continuous-infusion scheduling is also associated with a very good quality of life (although the increased cost and morbidity of continuous-infusion administration has to be factored into this consideration). An important phase III study is currently being conducted by the National Cancer Institute of Canada comparing immediate vs. delayed (until symptomatic) chemotherapy in patients with advanced colorectal cancer. Of the new approaches to therapy, perhaps the most immediately applicable are the new thymidylate synthase inhibitors (in particular, Tomudex, which produces a response rate equivalent to that of 5-FU plus leucovorin with less toxicity and a more convenient schedule). (J GASTROINTEST SURG 1997;1:576-582.)

This article attempts to summarize the current state of the art of chemotherapy for metastatic colon cancer. Whereas the current phase III chemotherapy studies are usually conducted in patients with advanced colorectal cancer, regardless of the site of metastatic disease, they include 60% to 85% of patients with the liver as the site of metastatic disease.[1] Hence these studies have been used as the prime source of references for this report.

Within the past 10 years, various modulatory combinations of 5-fluorouracil (5-FU) with agents such as leucovorin (LV), interferon, N-(phosphonacetyl)-L-aspartate (PALA), and methotrexate have produced higher response rates than 5-FU alone. It is interesting, however, to compare review articles from the late 1980s[2,3] with more recent editorials.[4,5] Whereas the former articles were more hopeful, the latter editorials confirm the results of major phase III studies demonstrating a significant increase in response rates compared with bolus 5-FU; however, the benefit of these 5-FU modulatory combinations on overall survival is questionable.

It is useful to review the history of 5-FU in metastatic colon cancer. Its efficacy was first demonstrated in the 1970s, with most studies confirming an overall response rate of approximately 10% to 20%. The majority of these responses are partial, with complete responses being rare. The median survival for all metastatic colorectal cancer patients treated with 5-FU is 6 to 8 months, compared with a median survival

From the Department of Oncology, McGill University, Montreal, Quebec, Canada.
Presented as part of the SSAT Consensus Conference on the Treatment of Hepatic Metastases From Colorectal Cancer, San Francisco, Calif., May 19-22, 1996.
Reprint requests: Brian Leyland-Jones, M.D., Department of Oncology, McGill University, McIntyre Medical Sciences Building, 3655 Drummond, Suite 701, Montreal, Quebec, Canada H3G 1Y6.

of 12 to 18 months for the responding patients.[6] Toxicity of 5-FU increases in parallel with the dosage used, the age of the patient, and poor performance status. Other agents have not performed much better. Nitrosoureas, chlorozotocin, methyl-CCNU (cyclohexyl-2-chloroethyl-nitrosourea), and mitomycin C are all reported to have a 10% to 15% *partial response* rate.[1,7] Interestingly, the fluorouracil/cisplatin (FU/cis-DDP) combinations developed for use in head and neck malignancies have been extended to colorectal cancer to obtain a 15% to 30% response rate in phase II studies. Regrettably, some of our most active cancer chemotherapeutic agents—Vinca alkaloids, anthracyclines, taxol, and most alkylating agents—show either minimal or no activity in advanced colorectal cancer. The past 10 years have shown us that combining 5-FU with other agents tends to produce more toxicity with little gain in efficacy. The problems with 5-FU resistance include (1) a high expression of MDR-1 (multidrug-resistant gene), (2) intracellular inactivation of 5-FU, and (3) the ability of the cell to use alternative pathways.[1] The characteristic slow doubling time of colon tumor cells also makes them less susceptible to phase-specific agents. Current 5-FU variations include continuous infusion, combinations with other agents, and modulation with leucovorin (10-formyl tetrahydrofolate, 10-CHO-FH_4), methotrexate, PALA, and interferon-α. Modulation of 5-FU with leucovorin has become the most widely accepted variation combining an improved response rate with ease of administration and the smallest increase in toxicity. Controversial issues include (1) the best way to administer 5-FU (i.e., modulation vs. scheduling), (2) the applicability of the results to adjuvant therapy, and (3) the impact on quality of life of these patients who have advanced disease.

The Southwestern Oncology Group (SWOG) conducted one of the most important studies on 5-FU modulation in advanced colorectal cancer; the results were presented in 1995.[8] Six hundred twenty patients were entered into this trial within a 3½-year period and were entered into one of seven FU-based regimens to assess the efficacy and toxicity afforded by biochemical modulation or schedule variations. The results of this trial are summarized in Table I.

In this "study" no regimen achieved a higher response rate than single-agent bolus 5-FU. The median survival time was 14 months for the entire cohort. High-grade toxicities occurred more frequently in the 5-FU bolus arms (predominately granulocytopenia and diarrhea). Survival hazards ratios showed a positive trend in favor of the unmodulated infusion regimens. In this study the 5-FU continuous infusion was administered at a dosage of 300 mg/m^2 daily for 28 days with a rest period between days 29 and 35 before recycling; the 24-hour infusion of 5-FU was administered at a rate of 2000 mg/m^2. Overall response rates varied from 15% to 29% according to the regimen.

In contrast, the meta-analysis of 1381 patients reported in 1992 showed an overall response rate of 11% for 5-FU and an overall response rate of 23% for 5-FU plus LV administered either as weekly or monthly regimens.[9] However, this increase in response did not result in a discernible improvement in overall survival (approximating 11 months in both arms). The authors carefully emphasized that in future trials, tumor response should not be considered a

Table I. Pivotal 5-FU modulation clinical trials: Summary of results

	Response rate (%)	Stable disease (%)	Progression (%)	Median survival (mo)
A. Southwestern Oncology Group Trial, 1995				
Treatment				
5-FU IVP	29	35	32	14
5-FU IVP/LDLV	27	36	27	14
5-FU IVP/HDLV	21	28	37	13
5-FU CI	29	25	31	15
5-FU CI/LDLV	28	28	34	14
5-FU 24 hr	15	32	35	15
5-FU 24 hr/PALA	25	40	25	11
B. Advanced Colorectal Cancer Meta-analysis Project, 1992				
Meta-analysis				
5-FU	11			11
5-FU/LV	23			11.5

5-FU = 5-fluorouracil; IVP = intravenous push; LDLV = lose-dose leucovorin; HDLV = high-dose leucovorin; CI = continuous infusion; PALA = N-(phosphonacetyl)-L-aspartate; LV = leucovorin.

Table II. 5-FU plus leucovorin: Results of seven prospective randomized studies (summarized from Cohen et al.[1])*

Study	No. of patients	LV dose	Response with LV	Survival advantage
Roswell Park Cancer Institute[11]	74	High	44% PR 4% CR	No
GITSG[12]	343	Low, high	30% RR (high)	Yes (slight)
NCCTG/Mayo[13]	429	Low, high	Statistically significant difference	Yes (low + high)
NORDIC[14]	249	Low	Statistically significant difference	Yes (slight)
NCOG[15]	249	Low, high	No difference	No
City of Hope National Medical Center[16]	79	High	Statistically significant difference	No
Princess Margaret Hospital[17]	130	High	33% vs. 7%	Yes

GITSG = Gastrointestinal Tumor Study Group; NCCTG = North Central Cancer Treatment Group; NORDIC = Nordic Gastrointestinal Tumor Adjuvant Therapy Group; NCOG = Northern California Oncology Group; PR = partial response; CR = complete response; RR = response rate.

*Toxicity of the combination was greater than with 5-FU alone; once weekly schedule was less toxic than the daily times five schedule.

valid surrogate end point for survival in this patient population. A further meta-analysis by this group reported a doubling of the response rate to 5-FU by modulation with LV, yielding a small improvement in survival.[10]

Table II (summarized from Cohen et al.[1]) presents the data from seven prospective randomized studies of various 5-FU modulatory combinations with either high- or low-dose LV; response rates and survival advantages are indicated. In all, 9 of 12 reported randomized studies comparing the combination of 5-FU and LV with 5-FU alone report significant increases in response rates, whereas only two report significant increases in survival. Overall, for these 12 studies the toxicity of the combination was greater than that with 5-FU alone; furthermore, the once weekly schedule was less toxic than the daily times five schedules. It must be emphasized that the majority of these trials were of good design with excellent randomization strategies in appropriately selected patient populations. Furthermore, the meta-analysis conducted in 1992 (Tables III and IV) also offers an excellent statistical presentation of the odds ratios for both response and survival, both in the individual trials and overall; they report a median survival of 11.5 months for the 5-FU plus LV studies and 11 months for 5-FU alone.[9]

Two important studies that concentrated on quality-of-life issues were reported in 1989 and 1994. Poon et al.,[18] in 1989, conducted a comparison between 5-FU alone and 5-FU plus cisplatin, 5-FU/methotrexate/LV rescue, 5-FU/methotrexate, 5-FU/high-dose LV, and 5-FU/low-dose LV. Four hundred twenty-nine patients showed a survival of 12 months for the 5-FU/low-dose LV arm vs. 7.7 months for 5-FU alone. Symptomatic improvement measured in terms of performance status, weight gain, and symptomatic relief was greatest in the 5-FU/low-dose LV arm (5-FU, 370 mg/m^2, together with LV, 20 mg/m^2 daily times five every 4 weeks).[18]

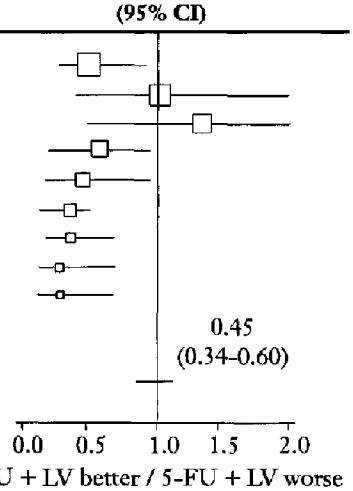
In 1994 Buroker et al.[19] compared 5-FU and low-dose LV on a daily times five schedule vs. 5-FU and high-dose LV on a weekly times six schedule. A total of 372 ambulatory patients were evaluated; no significant differences were observed in either the response rate (35% vs. 31%) or survival (9.3 months vs. 10.7 months). Symptomatic improvement as measured in terms of performance status, symptomatic relief, and weight gain again favored the low-dose arm (44% vs. 31%). Furthermore, the high-dose LV regimen caused more diarrhea and an increased requirement for hospitalization (to manage the toxicity) accompanied by a concomitant increase in financial cost.

As mentioned previously, the authors of the SWOG study concluded that single-agent, continuous-infusion 5-FU demonstrated the most encouraging results, producing less hematologic toxicity and, at low doses, less stomatitis.[4,9] However, it does have its own associated toxicity of palmar plantar erythrodysesthesia.[20]

Current new approaches to the management of colorectal metastatic disease include thymidylate synthase inhibitors such as Tomudex and LY231514. Tomudex (Zeneca Pharma, Macclesfield, U.K.) produced a 27% response rate in a European phase II trial[21]; a recent randomized study comparing To-

Table III. Meta-analysis, 1992[9]: Response odds ratios in individual trials and overall*

Trial	No. of events/No. entered		O-E	Variances	Odds ratio (95% CI)
	5-FU + LV	5-FU			
GITSG	58/269	13/113	−8.00	12.07	
NCOG	19/107	10/55	0.15	5.38	
GOIRC	12/91	14/90	1.07	5.59	
GISCAD	19/92	9/90	−4.85	5.95	
Genova	16/75	6/73	−4.85	4.72	
Toronto	21/66	4/64	−8.31	5.08	
City of Hope National Medical Center	15/39	5/40	−5.13	3.79	
RPCI	12/30	2/23	−4.07	2.58	
Bologna	9/34	1/30	−3.69	2.14	
					0.45 (0.34-0.60)
OVERALL	181/803	64/578	−37.68	47.30	



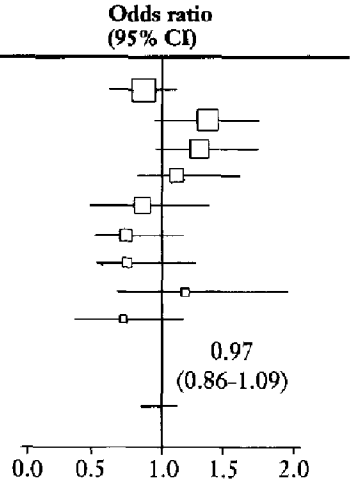
O = number of patients who do not respond to therapy in the treatment group; E = hypergeometric expectation; CI = confidence interval;

Odds ratio = $\frac{\text{Probability of failing to treatment/probability of responding to treatment}}{\text{Probability of failing to control/probability of responding to control}}$; GITSG = Gastrointestinal Tumor Study Group; RPCI = Roswell Park Cancer Institute; GISCAD = Italian Group for the Study of Digestive Tract Cancer; NCOG = Northern California Oncology Group; GOIRC = Italian Oncology Group for Clinical Research.

*Test for treatment effect: $\chi^2 = 30.02$ (1 df): $P < 10^{-7}$. Test for heterogeneity: $\chi^2 = 17.77$ (8 df); $P = 0.023$.

Table IV. Meta-analysis, 1992[9]: Survival odds ratios in individual trials and overall*

Trial	No. of events/No. entered		O-E	Variances	Odds ratio (95% CI)
	5-FU + LV	5-FU			
GITSG	231/269	101/113	−8.45	66.19	
NCOG	102/107	50/55	6.68	34.67	
GOIRC	74/91	66/90	7.17	34.50	
GISCAD	62/92	62/90	2.60	28.58	
Genova	71/75	73/73	−3.72	35.10	
Toronto	59/66	58/64	−6.67	28.40	
City of Hope National Medical Center	39/39	40/40	−4.66	18.60	
RPCI	30/30	23/23	1.54	12.48	
Bologna	28/34	28/30	−3.81	12.73	
					0.97 (0.86-1.09)
OVERALL	696/803	501/578	−9.33	271.25	



O = number of deaths in the treatment group; E = log-rank expectation; CI = confidence interval;

Odds ratio = $\frac{\text{Death rate on treatment/survival rate on treatment}}{\text{Death rate on control/survival rate on control}}$; GITSG = Gastrointestinal Tumor Study Group; RPCI = Roswell Park Cancer Institute; GISCAD = Italian Group for the Study of Digestive Tract Cancer; NCOG = Northern California Oncology Group; GOIRC = Italian Oncology Group for Clinical Research.

*Test for treatment effect: $\chi^2 = 0.32$ (1 df); $P = 0.57$. Test for heterogeneity: $\chi^2 = 8.33$ (8 df); $P = 0.41$.

mudex with 5-FU plus LV reported an equivalent response rate with less toxicity and a more convenient schedule.[22] A shorter time to progression was also reported in this study. Similarly, LY231514 is a pyrrolopyrimidine analogue of folic acid with thymidylate synthase inhibition as its primary mechanism of action. However, when given on a schedule of every 3 weeks, it produced more myelotoxicity. Phase III studies are ongoing.[23]

Other new approaches are also being studied including various uracil derivatives. Investigators in Milan have recently reported their results with a combination of the uracil derivative doxifluridine and LV; they achieved a 32% response rate in chemotherapy-naive patients compared with a 13% response rate in previously teated patients.[24] In addition, investigators at M.D. Anderson Cancer Center have recently reported results from combination trials of uracil and tegafur, which produced a 42% response rate in chemotherapy-naive patients.[25] This regimen has the advantage of being totally orally administered and produces low toxicity. Similar results have also been reported by Saltz et al.[26]

Among the camptothecins, irinotecan produced a 27% overall response rate in phase II trials (25% response rate in previously treated patients) with reasonable toxicity.[27,28] This drug has already been licensed for the treatment of advanced colorectal cancer in France and is undergoing clinical testing in North America.

Also on the horizon are new trials of MDR reversal agents including liposome encapsulation,[29] various calcium channel blockers, and anti-P glycoprotein antibodies.[30]

In the field of immunotherapy, aggressive studies are proceeding on more specific monoclonal antibodies[31-33] and tumor vaccines.[34-37]

In the field of gene therapy, work continues with both "suicide" and cytokine genes; results of in vivo preclinical studies were reported last year.[38,39]

An important phase III study is currently being conducted by the National Cancer Institute of Canada; this study compares survival of asymptomatic patients with advanced (metastatic) colorectal cancer treated immediately with fluorouracil and folinic acid with survival of patients in whom treatment is delayed until symptoms arise. The end points of this study—in addition to survival—include a direct comparison of the quality of life in these two populations.[40]

SUMMARY

The following conclusions have been drawn from the current phase III chemotherapy trials:

1. 5-FU with modulation is the best agent.
2. The best response rates are in the 25% to 45% range; occasional complete responses are reported.
3. Meta-analysis did *not* demonstrate an overall survival advantage in metastatic disease with 5-FU/LV treatment over 5-FU alone. However, two randomized studies showed an advantage.
4. The therapeutic effect can be maximized with variations in schedule and modulation, but toxicity increases as well.
5. The best quality of life is obtained with 5-FU/low-dose LV or 5-FU continuous-infusion scheduling.
6. New agents currently undergoing clinical testing may prove advantageous because of their more convenient (often oral) schedules of administration and reduced toxicity compared to current regimes, leading to an improved quality of life.

REFERENCES

1. Cohen AM, Minsky BD, Schilsky RL. Colon cancer. In DeVita VT Jr, Hellman S, Rosenberg SA, eds. Cancer: Principles and Practice of Oncology, 4th ed. Philadelphia: JB Lippincott, 1993, pp 929-977.
2. Pinedo HM, Peters GFJ. Fluorouracil: Biochemistry and pharmacology. J Clin Oncol 1988;6:1653-1664.
3. Einhorn LH. Improvements in fluorouracil chemotherapy? J Clin Oncol 1989;7:1377-1379.
4. Kemeny N. Chemotherapy for colorectal carcinoma: One small step forward, one step backward. J Clin Oncol 1995;13:1287-1290.
5. Borner MM. More is not always better: A case for low-dose leucovorin. J Clin Oncol 1993;11:382-383.
6. Moertel CG. Large bowel. In Holland JF, Frei E, eds. Cancer Medicine. Philadelphia: Lea & Febiger, 1973, pp 1497-1626.
7. Conti JA, Kemeny NE, Saltz LB, Andre AM, Grossano DD, Bertino JR. Continuous infusion fluorouracil/leucovorin and bolus mitomycin-C as a salvage regimen for patients with advanced colorectal cancer. Cancer 1995;75:769-773.
8. Leichman CG, Fleming TR, Muggia FM, Tangen CM, Ardalan B, Doroshow JH, Meyers FJ, Holcombe RF, Weiss GR, Mangalik A, MacDonald JS. Phase II study of fluorouracil and its modulation in advanced colorectal cancer: A Southwestern Oncology Group study. J Clin Oncol 1995; 13:1303-1311.
9. Advanced Colorectal Cancer Meta-Analysis Project. Modulation of fluorouracil by leucovorin in patients with advanced colorectal cancer: Evidence in terms of response rate. J Clin Oncol 1992;10:896-903.
10. Advanced Colorectal Cancer Meta-Analysis Project. Meta-analysis of randomized trials testing the biochemical modulation of fluorouracil by methotrexate in metastatic colorectal cancer. J Clin Oncol 1994;12:960-969.
11. Petrelli N, Herrera L, Rustum Y, Burke P, Creaven P, Stulc J, Emrich L, Mittleman A. A prospective randomized trial of 5-fluorouracil versus 5-fluorouracil and high-dose leucovorin versus 5-fluorouracil and methotrexate in previously untreated patients with advanced colorectal carcinoma. J Clin Oncol 1987;5:1559-1565.

12. Petrelli N, Douglass H Jr, Herrera L, Russell D, Stablein D, Bruckner H, Mayer R, Schinella R, Green M, Muggia F, Megibow A, Greenwald E, Bukawski R, Harris J, Levin B, Gaynor E, Loutifi A, Kalser M, Barkin J, Benedetto P, Woolley P, Nauta R, Weaver D, Leichman L. The modulation of fluorouracil with leucovorin in metastatic colorectal carcinoma: A prospective randomized phase III trial. J Clin Oncol 1989; 7:1419-1426.
13. Poon M, O'Connell M, Wieand H, Krook J, Gerstner J, Tschetter L, Levitt R, Kardinal C, Mailliard J. Biochemical modulation of fluorouracil with leucovorin: Confirmatory evidence of improved therapeutic efficacy in advanced colorectal cancer. J Clin Oncol 1991;9:1967-1972.
14. Nordic Gastrointestinal Tumor Adjuvant Therapy Group. Superiority of sequential methotrexate, fluorouracil, and leucovorin to fluorouracil alone in advanced symptomatic colorectal carcinoma: A randomized trial. J Clin Oncol 1989;7:1437-1446.
15. Valone F, Friedman M, Wittlinger P, Drakes T, Eisenberg P, Malec M, Hanningan J, Brown B Jr. Treatment of patients with advanced colorectal carcinomas with fluorouracil alone, high-dose leucovorin plus fluorouracil, or sequential methotrexate, fluorouracil, and leucovorin: A randomized trial of the Northern California Oncology Group. J Clin Oncol 1989;7:1427-1436.
16. Doroshow J, Multhauf P, Leong L, Margolin K, Litchfield T, Akman S, Carr B, Bertrand M, Goldberg D, Blayney D, Odujinrin O, DeLap R, Shuster J, Newman E. Prospective randomized comparison of fluorouracil versus fluorouracil and high-dose continuous infusion leucovorin calcium for the treatment of advanced measurable colorectal cancer in patients previously unexposed to chemotherapy. J Clin Oncol 1990;8:491-501.
17. Erlichman C, Fine S, Wang A, Elhakim T. A randomized trial of fluorouracil and folinic acid in patients with metastatic colorectal carcinoma. J Clin Oncol 1988;6:469-475.
18. Poon MA, O'Connell MJ, Moertel CG, Wieand HS, Cullinan SA, Everson LK, Drook JE, Mailliard JA, Laurie JA, Tschetter LK, Wiesenfeld M. Biochemical modulation of fluorouracil: Evidence of significant improvement of survival and quality of life in patients with advanced colorectal carcinoma. J Clin Oncol 1989;7:1407-1418.
19. Buroker TR, O'Connell MJ, Wieand HS, Krook JE, Gerstner JB, Mailliard JA, Schaefer PL, Levitt R, Dardinal CG, Gesme DH Jr. Randomized comparison of two schedules of fluorouracil and leucovorin in the treatment of advanced colorectal cancer. J Clin Oncol 1994;1:14-20.
20. Lokich JJ, Ahlgren JD, Gullo JJ, Philips JA, Fryer JG. A prospective randomized comparison of continuous infusion fluorouracil with a conventional bolus schedule in metastatic colorectal cancer: A Mid-Atlantic Oncology Program study. J Clin Oncol 1989;7:425-432.
21. Cunningham D, Zalcberg JR, Rath U, Olver I, Van Cutsem E, Svensson C, Geitz JF, Harper P, Kerr D, Perez-Manja G, et al. Tomudex (ZD1694): Results of a randomised trial in advanced colorectal cancer demonstrate efficacy and reduced mucositis and leucopenia. The Tomudex Colorectal Cancer Study Group. Eur J Cancer 1995;31A:1945-1954.
22. Zalcberg JR, Cunningham D, Van Cutsem E, Francois E, Schornagel J, Adenis A, Green M, Iveson A, Azab M, Seymour I. ZD1694: A novel thymidylate synthase inhibitor with substantial activity in the treatment of patients with advanced colorectal cancer. Tomudex Colorectal Study Group. J Clin Oncol 1996;14:716-721.
23. Rinaidi DA, Burris HA, Dorr FA, Woodworth JR, Kuhn JG, Eckardt JR, Rodriguez G, Corso SW, Fields SM, Langley C, Clark G, Faries D, Lu P, Von Hoff DD. Initial phase I evaluation of the novel thymidylate synthase inhibitor, LY231514, using the modified continual reassessment method for dose escalation. J Clin Oncol 1995;13:2842-2850.
24. Bajetta E, Colleoni M, DiBartolomeo M, Buzzoni R, Bozzetti F, Doci R, Somma L, Cappuzzo F, Stampion GC, Guenzi A, Balant LP, Zilembo N, DiLeo A. Doxifluridine and leucovorin: An oral treatment combination in advanced colorectal cancer. J Clin Oncol 1995;13:2613-2619.
25. Pazdur R, Lassere Y, Rhodes V, Ajani JA, Sugarman SM, Patt YZ, Jones DV Jr, Markowitz AB, Abbruzzese JL, Bready B, Levin B. Phase II trial of uracil and tegafur plus oral leucovorin: An effective oral regimen in the treatment of metastatic colorectal carcinoma. J Clin Oncol 1994;12:2296-2300.
26. Saltz LB, Leichman CG, Young CW, Muggia FM, Conti JA, Spiess T, Jeffers S, Leichman LP. A fixed-ratio combination of ruacil and ftorafur (UFT) with low dose leucovorin: An active oral regimen for advanced colorectal cancer. Cancer 1995;75:782-785.
27. Shimada Y, Yoshino M, Wakui A, Nakoo I, Futatsuki K, Sakata Y, Kambe M, Taguchi T, Ogawa N. CPT-11 Gastrointestinal Cancer Study Group. Phase II study of CPT-11, a new camptothecin derivative, in metastatic colorectal cancer. J Clin Oncol 1993;11:909-913.
28. Rothenberg ML, Eckardt JR, Kuhn JG, Burris HA III, Nelson J, Hilsenbeck SG, Rodriguez GI, Thurman AM, Smith LS, Eckhardt SG, Weiss GR, Elfring GL, Rinaldi DA, Schaaf LF, VanHoff DD. Phase II trial of irinotecan in patients with progressive or rapidly recurrent colorectal cancer. J Clin Oncol 1996;14:1128-1135.
29. Sela S, Husain SR, Pearson JW, Longo DL, Rahman A. Reversal of multidrug resistance in human colon cancer cells expressing the human MDR-1 gene by liposomes in combination with monoclonal antibody or verapamil. J Natl Cancer Inst 1995;87:123-127.
30. Beck WT. Circumvention of multidrug resistance with anti-P-glycoprotein antibodies: Clinical potential or experimental artifact? J Natl Cancer Inst 1995;87:73-75.
31. Fagerberg J, Steinitz M, Wigzell H, Askelof P, Mellstedt H. Human anti-idiotypic antibodies induced a humoral and cellular immune response against a colorectal carcinoma-associated antigen in patients. Proc Natl Acad Sci USA 1995; 92:4773-4777.
32. Fagerberg J, Hjelm AL, Ragnhammar P, Frodin JE, Wigzell H, Mellstedt H. Tumor regression in monoclonal antibody-treated patients correlates with the presence of anti-idiotype-reactive T lymphocytes. Cancer Res 1995;55:1824-1827.
33. Buckley DT, Robins AR, Durrant LG. Clinical evidence that the human monoclonal anti-idiotypic antibody, 105AD7, delays tumor growth by stimulating anti-tumor T-cell responses. Hum Antibodies Hybridomas 1995;6:68-72.
34. Moertel CG. Vaccine adjuvant therapy for colorectal cancer: "Very dramatic" or ho-hum? J Clin Oncol 1993;11:385-386.
35. Hoover HC Jr, Brandhorst JS, Peters LC, Surdyke MG, Takeshita Y, Madariaga J, Muenz LR, Hanna MG Jr. Adjuvant active specific immunotherapy for human colorectal cancer: 6.5-year median follow-up of a phase III prospectively randomized trial. J Clin Oncol 1993;11:390-399.
36. Conry RM, LoBuglio AF, Loechel F, Moore SE, Sumerel LA, Barlow DL, Pike J, Curiel DT. A carcinoembryonic antigen polynucleotide vaccine for human clinical use. Cancer Gene Ther 1995;2:33-38.

37. Adluri S. Helling F, Ogata S, Zhang S, Itzkowitz SH, Lloyd KO, Livingston PO. Immunogenicity of synthetic TF-KLH (keyhole limpet hemocyanin) and sTn-KLH conjugates in colorectal carcinoma patients. Cancer Immunol Immunother 1995;41:185-192.
38. Chen SH, Chen XH, Wang Y, Kosai K, Finegold MJ, Rich SS, Woo SL. Combination gene therapy for liver metastasis of colon carcinoma in vivo. Proc Natl Acad Sci USA 1995; 92:2577-2581.
39. Trinh QT, Austin EA, Murray DM, Knick VC, Huber BE. Enzyme/prodrug gene therapy: Comparison of cytosine deaminase/5-fluorocytosine versus thymidine kinase/ganciclovir enzyme/prodrug systems in a human colorectal carcinoma cell line. Cancer Res 1995;55:4808-4812.
40. Moore M, Cripps C, DeGara C, Langer B, Pedersen J, Wong A, James K, Myles J, Iscoe N, Sargeant A. A phase III study of immediate versus delayed chemotherapy for asymptomatic advanced colorectal cancer, National Cancer Institute of Canada CTG trial CO.10, June 20, 1994.