Special Problems in Minimally Invasive Surgery of the Foregut. Part III: Special Problems With Antireflux Procedures

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The number of laparoscopic antireflux procedures continues to rise yearly. This increased volume has added to the accumulated experience of surgeons interested in gastroesophageal reflux disease. Out of this experience has come the reconfirmation that certain key abnormalities greatly increase the complexity of surgical therapy for this disease. These abnormalities include (1) acquired esophageal shortening, (2) enlargement of the esophageal hiatus, (3) coexisting esophageal motility disorders, (4) delayed gastric emptying, and (5) previous esophageal surgery.

Three of these issues were addressed at the SSAT/SAGES Joint Symposium. Dr. Donald Low defined the short esophagus and identified it as a major factor contributing to a recurrent hiatal hernia after surgical repair. He discussed how to recognize the presence of a shortened esophagus and how to manage it when encountered. He concludes that esophageal shortening is real, has a frequency of 15%, and is one of the most important determiners of surgical outcome. In my assessment, it is one of the best narratives on this highly debated subject.

Dr. John Hunter provided a delightful discussion concerning the patient with recurrent gastroesopha-

geal reflux disease. He states that the taxonomy of failed repairs can be symptomatic or anatomically based—that is, how much the postoperative anatomy deviates from the ideal repair. The clarity with which the subject is discussed makes it a "must read" for all esophageal surgeons.

Dr. Lee Swanstrom gave a short, concise presentation on the management of patients with gastroesophageal reflux disease complicated by esophageal and/or gastric dysmotility. He states that mild delays in gastric emptying are of little concern in patients undergoing fundoplication, and serious symptomatic delays are rather easily corrected by near-total gastric resection. The surgical principles to follow when encountering an esophageal motility abnormality in patients with gastroesophageal reflux disease are clearly outlined by Dr. Swanstrom and are an excellent guide for all esophageal surgeons.

Together these reports summarize the current strategies in the management of complex problems associated with surgical therapy of gastroesophageal reflux disease and are well worth reading.

Management of Patients With Gastroesophageal Reflux Disease and Esophageal or Gastric Dysmotility

Lee L. Swanstrom, M.D.

Dysfunction of the normal peristalsis of the upper gastrointestinal tract can exacerbate and sometimes cause symptoms of gastroesophageal reflux disease (GERD), and certainly complicates the surgical treatment of this disease. The antegrade movement of food, prevention of acid reflux, and clearance of the refluxed gastric contents depends on the complex relationship of swallowed alkaline saliva, consistent antegrade peristalsis of the esophagus, a functioning lower esophageal sphincter or "valve," and a gastric reservoir that produces normal volumes and empties normally.

GASTRIC DYSMOTILITY

Delayed gastric emptying (DGE) can have multiple etiologies (Table I), all of which can contribute to a scenario where an overly full or slowly emptying gastric reservoir may "overcome" the normal protective function of the lower esophageal sphincter and esophagus, thereby causing GERD. A common analogy is that of a plugged sink drain, which will lead to overflow of the sink with normal use. Symptoms of DGE can range from severe and disabling to none whatsoever (Table II). It is always indicated, however, to ask about such symptoms during the preoperative evaluation of all patients with GERD. DGE should be suspected in patients with reflux whose primary presenting complaint is one of the symptoms of DGE or when preoperative pH testing documents severe reflux but motility tests show normal esophageal function and a normal lower esophageal sphincter. Diagnosis of DGE is best made by imaging studies, although endoscopy is also a mandatory part of the evaluation in order to rule out mechanical or acute causes of obstruction (see Table I).

Plain abdominal x-ray films or a barium upper gastrointestinal series are relatively nonspecific tests that can sometimes document gastromegaly, obstructing lesions, or a bezoar. They may also confirm total obstruction or show peptic ulcer disease. Various tests have been devised to both diagnose and quantify the degree of DGE. These include carbon 14–labeled meals with spirometry breath tests (the amount of tracer exhaled decreases as the food moves into the duodenum) and radionuclide scintigraphy. The quantitative radionuclide gastric emptying study has become the diagnostic "gold standard" because of its ease and validity. This examination involves the ingestion of a standardized meal that has been labeled with a radionuclide tracer (^{99}Tc or sulfur colloid), which can then be scanned in real-time with a scintigraphic gamma camera. The resulting time for 50% gastric emptying can then be compared to established normal values. The test meal can be either solid or liquid, or even a dual study with different tracers for the solid and liquid components. This dual-labeled study can be the most sensitive in differentiating fine motor disorders from mechanical blockage.

When reflux is due to DGE, its treatment depends on the determined cause of the DGE disorder plus or minus the correction of LES problems. Mechanical obstruction of the stomach will obviously require a mechanical correction, which could involve endoscopic dilatation of strictures or the dysfunctional pylorus, stent placement for malignant obstruction, surgical drainage procedures (pyloroplasty, pyloromyotomy, gastrojejunostomy, or even gastric resection). DGE due to motility disorders can usually be helped with medical therapy, and this should always be attempted before resorting to surgery. Pharmacologic treatments work either by decreasing gastric secretions (lowering the "reservoir") or by stimulating gastric peristalsis (Table III). If the patient's GERD symptoms fail to improve on medical therapy or the patient shows evidence of progressive failure (uncontrollable esophagitis, weight loss, pulmonary complications, etc.), surgical correction can and should be considered.

Table I. Causes of delayed gastric emptying

Mechanical
  Tumor infiltration
  Chronic scarring of the pylorus
Gastric volvulus
  Continued to inflammation of the pylorus
Intrinsic
  Diabetic gastropathy
  Scleroderma
  Gastrointestinal ileus
  Idiopathic

Table II. Symptoms related to delayed gastric emptying

None
Bloating
Chronic vomiting
Weight loss
Gastric bezoar

Table III. Pharmacologic treatment of delayed gastric emptying

Medication	Mechanism of action
Histamine antagonists	Decreased gastric reservoir
Proton pump inhibitors	volume or treatment of acute inflammatory conditions
Cisapride	
Metaclopramide	
Erythromycin	Increased gastric peristalsis

SURGICAL INTERVENTIONS FOR GASTROESOPHAGEAL REFLUX DISEASE RELATED TO DELAYED GASTRIC EMPTYING

As mentioned, surgery is ideal for mechanical causes of DGE. If the cause of the obstruction can be identified, it can usually be corrected with a surgical repair or bypass, which should improve the patient's reflux symptoms. The exception is obstruction due to malignancy, which typically has a poor prognosis and should be treated with the least morbid palliative intervention possible. Surgery for DGE related to motility disorders has a lower overall success rate. These patients often have complex motor disorders of the entire gastrointestinal tract, which can compromise surgical attempts to improve gastric emptying.

It has long been demonstrated that the addition of a fundoplication will increase gastric emptying to some degree. The risk of relying on this phenomenon is that one may create a scenario of chronic "gas bloat

syndrome" if the increase is insufficient to overcome the emptying problem. We would recommend that patients with reflux who are determined to have a mild degree of DGE by objective studies (i.e., less than one standard deviation over the norm) simply undergo a fundoplication and patients with a more severe degree of DGE undergo a gastric emptying procedure. These patients often respond symptomatically to the addition of a pyloroplasty (or pyloromyotomy) without vagotomy. The mechanism behind this success is not well known as postoperative emptying studies in the past have shown only moderate improvement in the $t_{1/2}$ emptying times. The most logical explanation is that the emptying procedure combined with a fundoplication both stimulates antegrade gastric motility and provides a preferred path of least resistance to improve emptying time overall.

TREATMENT OF ESOPHAGEAL MOTILITY DISORDERS

There are three cardinal principles to be followed for the treatment of esophageal motility disorders: (1) relief of any existing outlet obstruction; (2) avoiding the creation of an outflow restriction; and (3) establishing a competent antireflux mechanism. To a certain degree these principles are contradictory. Treatment of outlet obstructions by dilatation, stenting, or myotomy can cause or exacerbate gastroesophageal reflux, which can then lead to peptic strictures—perhaps the most difficult of the outflow restrictions to treat. Likewise the most competent and effective antireflux surgery, the Nissen fundoplication, is thought to achieve success by the creation of a hypercompetent outflow (and reverse flow) mechanism. This resistance can be roughly calculated by multiplying the length by the mean resting pressure of the high-pressure zone, although preservation of receptive relaxation during swallowing also plays a large role in ease of esophageal emptying. Because of this dilemma it has become almost dogma that patients with esophageal pump failure, as defined by poor motility (Table IV), should undergo a partial fundoplication. Partial wraps are known to create a lower mean resting pressure, which gives them less outflow resistance. This also yields decreased *reverse* flow characteristics. This accounts for their perception as a "more physiologic" (patient friendly) antireflux mechanism. Many reports describe not only low rates of postoperative dysphagia but also minimal gas bloat and inability to belch and vomit following partial wraps.

Although laparoscopic partial wraps may have a low side effect profile, this comes at the price of less reflux control because of the relative weakness of the reflux barrier. This phenomenon has been docu-

Table IV. Defining characteristics of poor esophageal motility

Failed peristalsis
>50% simultaneous contractions
>50% dropped peristalsis
Ineffective peristalsis
Contraction amplitudes <30 mm Hg in two or more
segments
Painful peristalsis
Diffuse esophageal spasm: Disordered contractions of high
amplitude (>150 mm Hg)
Nutcracker esophagus: Normal but hypertensive
peristalsis (>150 mm Hg)

mented by Bell et al., who showed adequate early reflux control when comparing the Rosetti and Toupet repairs, but significantly less postoperative augmentation of the lower esophageal sphincter following the partial wrap. In their report, the Rosetti repair increased the lower esophageal sphincter pressure from 7.8 to 26.9 mm Hg, but the Toupet fundoplication only showed an increase from 8.6 to 18.6 mm Hg. Horvath et al. have further noted that as many as 48% of patients at 2-year follow-up had objective evidence of reflux in spite of endoscopically intact fundoplications. This was a matter of particular concern in that many of these patients (50%) were totally asymptomatic in spite of abnormal 24-hour pH studies. It was also noted that the failure rate was highest in patients with more severe or complicated disease.

The one exception to the apparent compromise between reflux prevention and esophageal outflow restriction is the use of partial wraps (Dor or Toupet)

following Heller myotomy for achalasia. Many studies have shown dramatic reductions in dysphagia with this procedure and the reported incidence of reflux, by pH testing or symptoms, is very low.

CONCLUSION

Knowledge of the presence of gastric or esophageal motility disorders is important to the surgeon treating GERD for a variety of reasons. Appropriate treatment of either condition remains controversial but almost certainly affects the patient's long-term success and risk of side effects or morbidity. The best esophageal surgeons will take all aspects of the patient's physiology into account when counseling them preoperatively and while performing antireflux procedures.

SUGGESTED READINGS

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Approach and Management of Patients With Recurrent Gastroesophageal Reflux Disease

John G. Hunter, M.D.

Gastroesophageal reflux disease (GERD) is the most common gastrointestinal disease for which individuals take medication. Omeprazole is the most frequently prescribed prescription drug in the United States, yet this is the tip of the iceberg. Most patients suffering from heartburn treat themselves with over-the-counter products such as antacids and H_2-receptor blockers. Nonetheless, it is estimated that one in ten Americans have frequent symptoms of GERD.

Since the development of laparoscopic fundoplication, more individuals with severe GERD have chosen to undergo surgical correction of GERD rather than continuing on lifetime medical therapy. As with open surgery, the most popular laparoscopic operations are total fundoplication (Nissen) and partial (270-degree) esophagogastric fundoplication. Whether performed as an open procedure or laparoscopically, these operations have an initial success rate of greater than 95% in stopping reflux symptoms. When performed through a laparotomy or thoracotomy, somewhere between 9% and 30% of patients develop recurrent symptoms or new troublesome symptoms that result as a side effect of fundoplication.[1,3] Reported failure rates with laparoscopic fundoplication range from 2% to 17% depending on how failure is defined.[4,8] That these failure rates are indeed lower than those reported for open fundoplication probably reflects the fact that laparoscopic fundoplication is a relatively new technique, not that laparoscopic fundoplication is intrinsically better.

The taxonomy of failure can be symptom based (e.g., heartburn, dysphagia, gas bloat) or it may be anatomically based, using a description of how the current anatomy deviates from the ideal. From a mechanistic and therapeutic standpoint, the latter definition is preferable. The anatomy of failure includes four common types of fundoplication failure previously described with open surgery. These are (1) slipped or misplaced fundoplication, (2) disrupted fundoplication, (3) herniated fundoplication, and (4) fundoplication that is too tight or too long.[9] To this list of four can be added two new anatomic problems—the "two-compartment stomach" and the twisted fundoplication.[10]

EVALUATION OF PATIENTS WITH RECURRENT OR NEW SYMPTOMS OF GASTROESOPHAGEAL REFLUX DISEASE
Early Postoperative Symptoms

The management of patients with new or recurrent symptoms after an operation for GERD is dependent on the time of presentation. In the early postoperative period (less than 3 months), the presence of certain symptoms is extremely common and no treatment other than reassurance is necessary.

The most common postoperative symptom is solid food dysphagia. Because distal esophageal edema (with or without hematoma) and transient esophageal dysmotility are common sequelae of fundoplication, it is no wonder that most patients have difficulty swallowing solid foods after a loose, floppy fundoplication is performed. We generally recommend that the patient stay on a full liquid diet for a week after surgery and then maintain a soft diet for the next 3 weeks following the operation. This protocol has dramatically reduced the incidence of postoperative food impaction, nausea, and vomiting seen when a regular diet is begun too soon after the operation. When patients complain of postoperative dysphagia, we urge them to return to a liquid diet until swallowing is easy, and then advance to a soft diet. If a patient has difficulty tolerating a full liquid diet, early intervention may be necessary. Options for early intervention include esophageal dilation and/or placement of a gastrointestinal feeding tube. We have used nasal enteric tubes and gastrostomy tubes when early postoperative dysphagia or nausea becomes so severe as to cause weight loss and dehydration (Fig. 1).

A.   Early Dysphagia (First 3 Months)

Solid Food   Liquids and Solids

**Diet Modification,   Dilation
Dilation   Feeding Tube Placement or TPN**

**B.   Late Dysphagia (> 3 Months) ⟶⟶⟶⟶⟶⟶ Barium Swallow
                                              (with 12.5 mm
                                               Barium Pill)**

Normal   Abnormal

**Severity of
Dysphagia**

Mild (to Meat, Bread)   Severe (to All Solids ± Liquids)   **EMS**

                                          Ineffective   Normal
                                          Peristalsis
**Reassurance   Redo
± Dilation   EMS   Floppy
                                                           Nissen**
                        Ineffective
                        Peristalsis   **Convert Nissen
                                            to Toupet (with
                        Normal   Heller Myotomy
                                             for Aperistalsis)**

                        EGD

                Normal   Slipped Nissen

**Nutritional Support ± Feeding Tube;
± Esophageal Dilation, Reassurance**
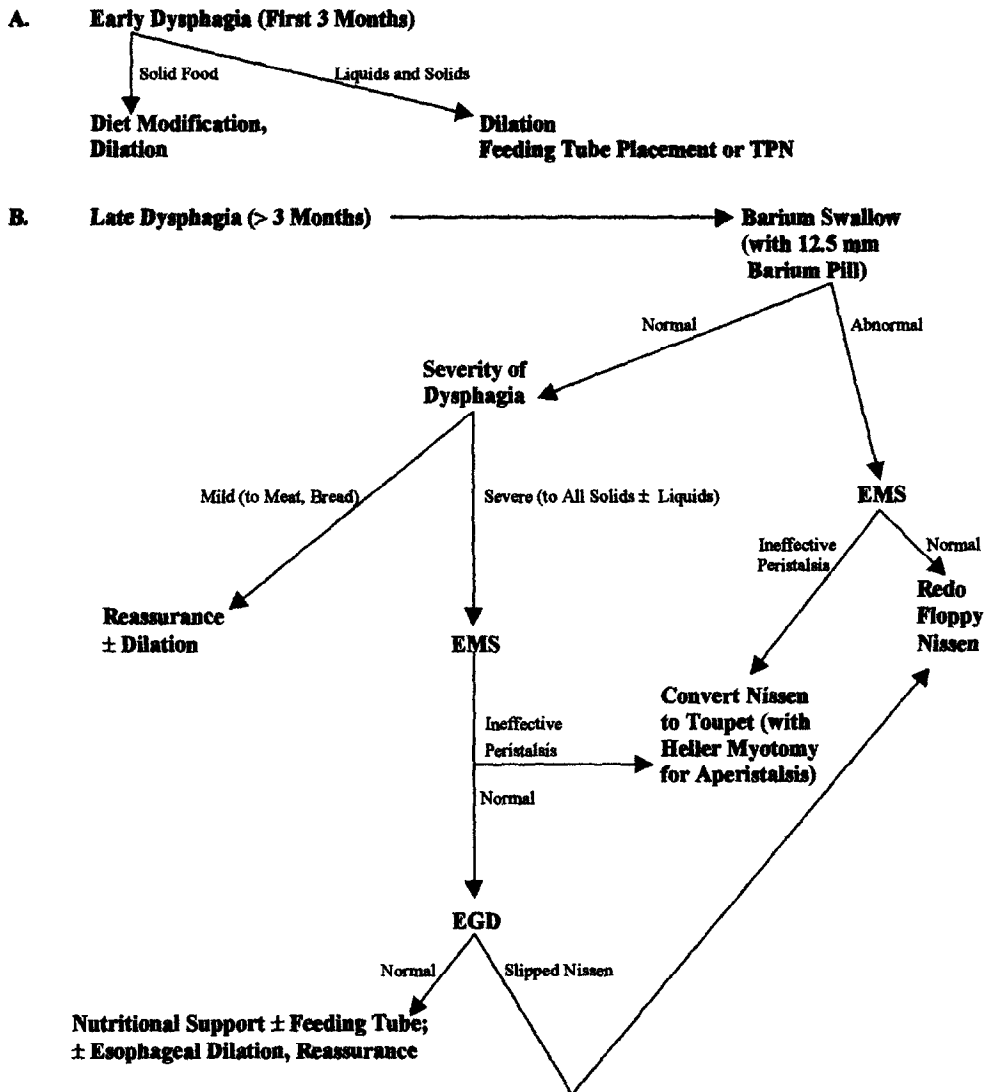
Fig. 1. Evaluation of the patient with new dysphagia following laparoscopic Nissen fundoplication.

The second early postoperative symptom of no great consequence (usually) is the development of chest pain or recurrent reflux symptoms. During the first 3 months after surgery, the patient must be reassured that it is unlikely that he or she is experiencing reflux if no postoperative events such as retching have occurred. A "boatload" of reassurance may be necessary, and occasionally a barium swallow may be needed to reassure the patient that the fundoplication is intact. A trial of proton pump inhibitors is often begun but is rarely effective for these early postoperative difficulties. The best management of these early postoperative complaints is patience, not reoperation.

Recurrent Symptoms

When recurrent or new symptoms of GERD develop in the late postoperative period (greater than 3

months), investigation is warranted. For individuals who return with symptoms identical to those for which they underwent surgery, a diagnostic trial of antireflux medication is appropriate. In addition, we generally order a barium swallow, as we have determined that at least 90% of all fundoplication abnormalities can be seen with this study.[10] If the barium swallow is normal, it is unusual for patients to respond to proton pump inhibitor therapy. The most frequent explanation for the recurrence of symptoms is that the symptom reported is the result of a problem distinct from gastroesophageal reflux. Because respiratory symptoms and atypical gastroesophageal reflux symptoms are so often intertwined, it may take the performance of a fundoplication to determine once and for all which supraesophageal symptoms are related to reflux and which are not. Frequently the typical symptoms of reflux (heartburn, dysphagia, regurgitation)

A.   Early (first 3 months) ──────► Reassurance
                                    ± Barium Swallow

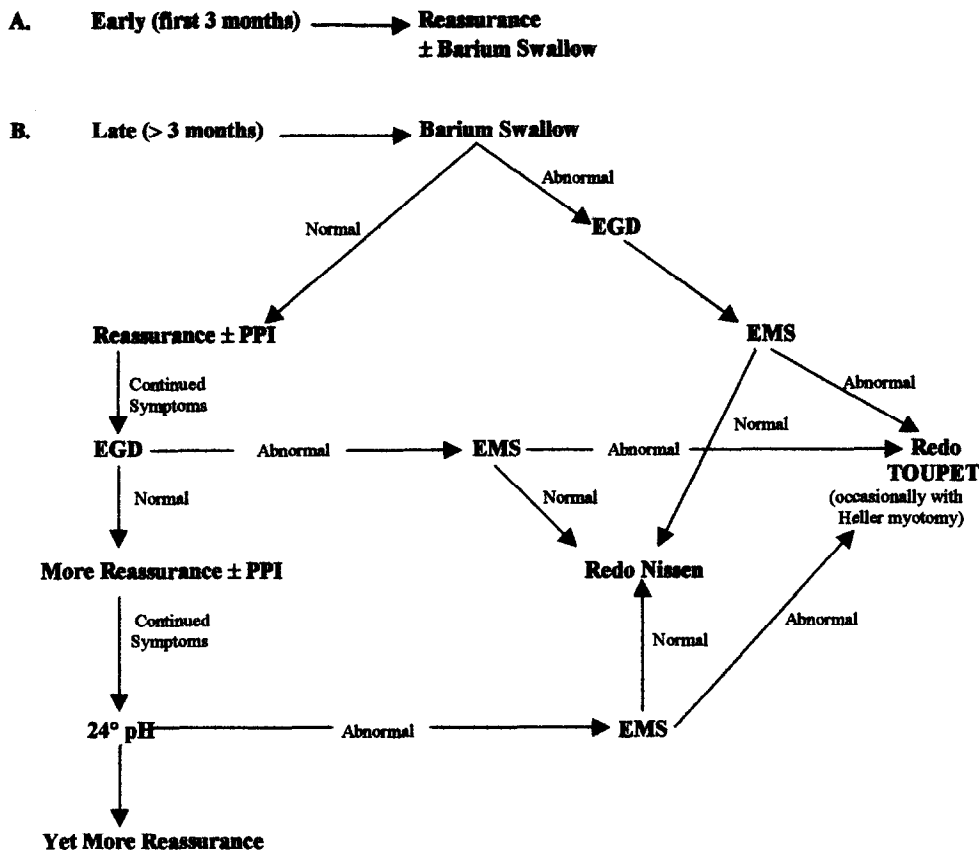B.   Late (> 3 months) ──────► Barium Swallow

Fig. 2. Evaluation of the patient with recurrent reflux symptoms following laparoscopic Nissen fundoplication.

will be eliminated by fundoplication, but the supraesophageal symptoms in the same patient (globus, cough, hoarseness, wheezing) will not be eliminated by antireflux surgery. The best preoperative predictors of supraesophageal symptom relief after a fundoplication are the response of the symptom to proton pump inhibition and/or the correlation of supraesophageal symptoms with reflux events on a 24-hour pH study.

If preliminary evaluation with a postoperative barium swallow does not reveal any abnormalities, and a trial of medical therapy fails, further investigation is unlikely to bear fruit but should be done nevertheless. In 10% of our patients referred for postoperative symptoms, esophagogastroduodenoscopy (EGD) revealed an anatomic problem missed by barium swallow.[10] The most common anatomic problem discovered on EGD, when the barium swallow looked normal, was a slipped or misplaced Nissen fundoplication. Although the gastroesophageal junction may be difficult to define on barium swallow, an EGD demonstrating the presence of gastric folds above a fundoplication indicates a Nissen valve that has been misplaced or slipped onto the stomach. Also, a partially disrupted fundoplication will be visible on EGD in the retro-

flexed position, demonstrated by a gastroesophageal junction that is patulous (does not hug the scope), but this finding may be missed on barium swallow. When the results of EGD are normal and the barium swallow is normal, the 24-hour pH study (the fourth test) is almost always normal, as well (Fig. 2).

Persistent Postoperative Dysphagia

In contradistinction to the patient with recurrent reflux symptoms, the patient with new-onset dysphagia represents a different problem. The management of the patient with early dysphagia is discussed above. In the patient with dysphagia that persists past 3 months, we confirm that an anatomic abnormality exists by performing a video barium swallow with a 12.5 mm barium pill. If the pill passes the gastroesophageal junction readily, one must suspect that the dysphagia is a result of an esophageal motility disturbance or is of psychogenic nature. Thus the normal barium swallow is followed by an esophageal motility study in patients with significant dysphagia. If the barium swallow demonstrates an anatomic abnormality near the gastroesophageal junction, a motility study is necessary, but only in preparation for a "redo" operation. The

decision to reoperate at this point must be individualized based on the patient's nutritional status and the severity of the dysphagia. A patient who is still confined to liquids 3 months postoperatively, or a patient who is losing weight because of dysphagia, should be offered early elective reoperation. If the solid food dysphagia is mild or moderate, dietary restrictions are few, and weight loss is not present, we prefer a conservative course of management for the first year postoperatively. If, at that time, the barium tablet still hangs in the distal esophagus and the patient is bothered by the dietary restrictions that are necessary, a second operation is offered. The last scenario is one in which the postoperative barium swallow demonstrates an obvious anatomic problem, such as a slipped or herniated fundoplication. Most of these problems will require reoperation. Although esophageal dilation may be beneficial for early postoperative dysphagia, it is rarely helpful in late dysphagia (see Fig. 1).

ANATOMIC FAILURE OF NISSEN FUNDOPLICATION
Fundoplication Herniation

The most frequent anatomic problem we have encountered in our patients following laparoscopic fundoplication has been herniation of the fundoplication across the diaphragm.[10] This has occurred in four settings. The first is the patient who strains or retches in the early postoperative period. Occasionally the patient will feel something pop, and usually patients have severe chest pain after herniation of the fundoplication. This is a surgical emergency and should be confirmed with water-soluble contrast radiography, followed by rapid return to the operating room for a laparoscopic or open reduction of the herniated stomach.

The second scenario is the patient who has a similar event, remote from the time of surgery. Although these patients may develop severe acute pain after herniation of the fundoplication, it is more usual for the event to be followed by heartburn, new onset of dysphagia, or development of postoperative chest pain resulting from gas or food distending the mediastinal portion of the herniated fundoplication. These patients should be evaluated by means of a barium swallow test and esophagogastroscopy. Depending on the length of time between the first operation and the development of the recurrence, we will perform esophageal motility and/or gastric emptying studies to further define the foregut physiology before reoperation.

The third scenario is more insidious yet. It involves the patient who has a slow onset of recurrent or new symptoms (chest pain, dysphagia, heartburn) in the absence of a precipitating event. Reflection on the preoperative status of these patients usually demonstrates the presence of a paraesophageal hernia, esophageal stricture, or Barrett's esophagus before the first operation. In these patients the herniation has occurred because esophageal foreshortening was not detected and adequately treated at the first operation or because the hiatus has stretched to allow cephalad migration of the stomach. Elective re-repair should include a Collis gastroplasty when esophageal foreshortening is discovered intraoperatively, along with re-repair of the esophageal hiatus.

The fourth group of patients who have herniation of their fundoplications and the vast majority of patients with small herniations are asymptomatic. Nearly half of the patients who develop fundoplication herniation will be truly asymptomatic. This group most commonly includes patients whose first operation was for a paraesophageal hiatal hernia.[11] If the patient is asymptomatic, is not anemic, and has no evidence of ulceration in the herniated fundoplication, we will usually not recommend a reoperation.

In summary, patients with acute herniation need an emergency operation, those with an "event-induced" recurrence should undergo elective reoperation, those with a recurrence secondary to esophageal foreshortening should undergo Collis gastroplasty and repeat fundoplication, and those with asymptomatic occurrence need not undergo reoperation.

Slipped Nissen Fundoplication

Patients with a "slipped" Nissen fundoplication represent a different challenge. Those with a gastric pouch above the fundoplication that is either long or bulbous will suffer the most severe symptoms of reflux and regurgitation. Not only is food trapped in this pouch during swallowing, it serves as a trap for acid-rich refluxate immediately below an incompetent sphincter. These patients are extremely grateful when the fundoplication is placed in the correct location on the esophagus. The challenge with this deformity is to determine whether the fundoplication slip is related to a repair under tension around a short esophagus or represents a gastroesophageal junction that was never reduced into the abdominal cavity. Reoperation in patients with a misplaced fundoplication often reveals a virgin segment of mediastinal esophagus just above the gastroesophageal junction, evidence that the esophagus was never adequately mobilized during the first operation. The operative principles for management of this problem will be discussed later.

Disrupted Fundoplication, Twisted Fundoplication, and Two-Compartment Stomach

The disrupted fundoplication is perhaps the easiest to diagnose and repair. The operative evaluation of these patients will usually include a 24-hour pH study as well as esophageal motility testing, barium swallow, and EGD. In the absence of erosive esophagitis or Barrett's esophagus, it is important to document gastroesophageal reflux with a pH study before reoperating on a patient with a disrupted fundoplication.

The new defects, which are unique to laparoscopic surgery, are the twisted fundoplication and the two-compartment stomach. The twisted fundoplication results from failure to mobilize the greater curvature of the stomach from the spleen and diaphragm (Rosetti-Nissen). A portion of the anterior wall of the stomach is pulled from the left around the esophagus to the right and sutured in this position. This creates tension at the gastroesophageal junction, which can result in rotation of the distal esophagus and a spiral-type deformity seen in retroflection of the endoscope. This deformity is usually associated with symptoms of dysphagia and severe postoperative gas bloat. This defect is very resistant to esophageal dilation and requires reoperation to correct it. Occasionally individuals who undergo this Rosetti modification of the Nissen fundoplication will have an additional problem—the two-compartment stomach. This occurs when a point on the greater curvature too far from the fundus is pulled up to the gastroesophageal junction to form the fundoplication. The result is a two-compartment stomach; the fundic compartment resides against the posterior left hemidiaphragm and the distal compartment (the antrum) lies below the septation. The proximal compartment is filled preferentially and will create early satiety, upper gastric distress, nausea, and retching. The hypercompetent valve will usually prevent vomiting in these patients. They are extremely uncomfortable and require urgent reoperation once the diagnosis is made. Barium swallow and upper endoscopy usually reveal the septated nature of the stomach, and the diagnosis is not difficult.

Bloating, Nausea, and Epigastric Pain

A small subset of patients who undergo laparoscopic Nissen fundoplication will be plagued by persistent bloating, nausea, and epigastric pain postoperatively. These patients fall into two groups: those with functional problems and those with delays in gastric emptying, which may be a result of inadver-

tent vagal injury. In the early postoperative period, treatment with antiemetics is the best therapy. When nausea persists beyond the usual 3-month postoperative period, however, investigation is warranted. Initially we believed that these symptoms might represent gastritis, but found little benefit from proton pump inhibition and little EGD evidence of gastritis in these patients. If the EGD is normal, it is usually sufficient to treat these patients with antiemetic medications including ondansetron, Phenergan, and the prokinetic agent metaclopromide. In contrast, when the EGD demonstrates food in the stomach after a 12-hour fast, one must postulate that gastroparesis has occurred postoperatively or was overlooked on the preoperative evaluation. Significant amounts of food in the stomach is strong evidence of gastroparesis. There is probably little need for a gastric emptying study in these patients, but we generally perform this study to quantify the amount of gastric retention. If gastric emptying cannot be normalized on prokinetic agents (and it rarely is), we recommend that pyloroplasty be performed. If the patient is losing weight, a feeding jejunostomy is added. Following these two procedures, we prefer to wait for a year to determine whether gastric emptying will return. If there is no appreciable improvement in gastric emptying after a 12-month follow-up period, subtotal gastrectomy with Roux-en-Y gastrojejunostomy is appropriate (Fig. 3).

Reoperation for Failed Fundoplication

In addition to our study mentioned earlier, there have been a number of others that have addressed redo laparoscopic fundoplication.[12-15] Some surgeons attempt all redo fundoplications laparoscopically, some will perform all redo fundoplications through a thoracotomy, and some perform all redo fundoplications through a laparotomy. We generally tailor our redo operations according to the method used for the previous operation(s). That is, when the first operation was performed through a thoracotomy or with laparoscopy, our preferred approach is a laparoscopic approach. When the first operation was performed through a laparotomy, our preferred operative approach is through a laparotomy. When we have approached this latter group through a thoracotomy, the intra-abdominal adhesions make redo surgery difficult. When we have performed the redo operation in this latter group with laparoscopy, we have found, as did the Austrian investigators,[14] that intra-abdominal adhesions made the laparoscopic procedure quite lengthy. Whether the redo opera-
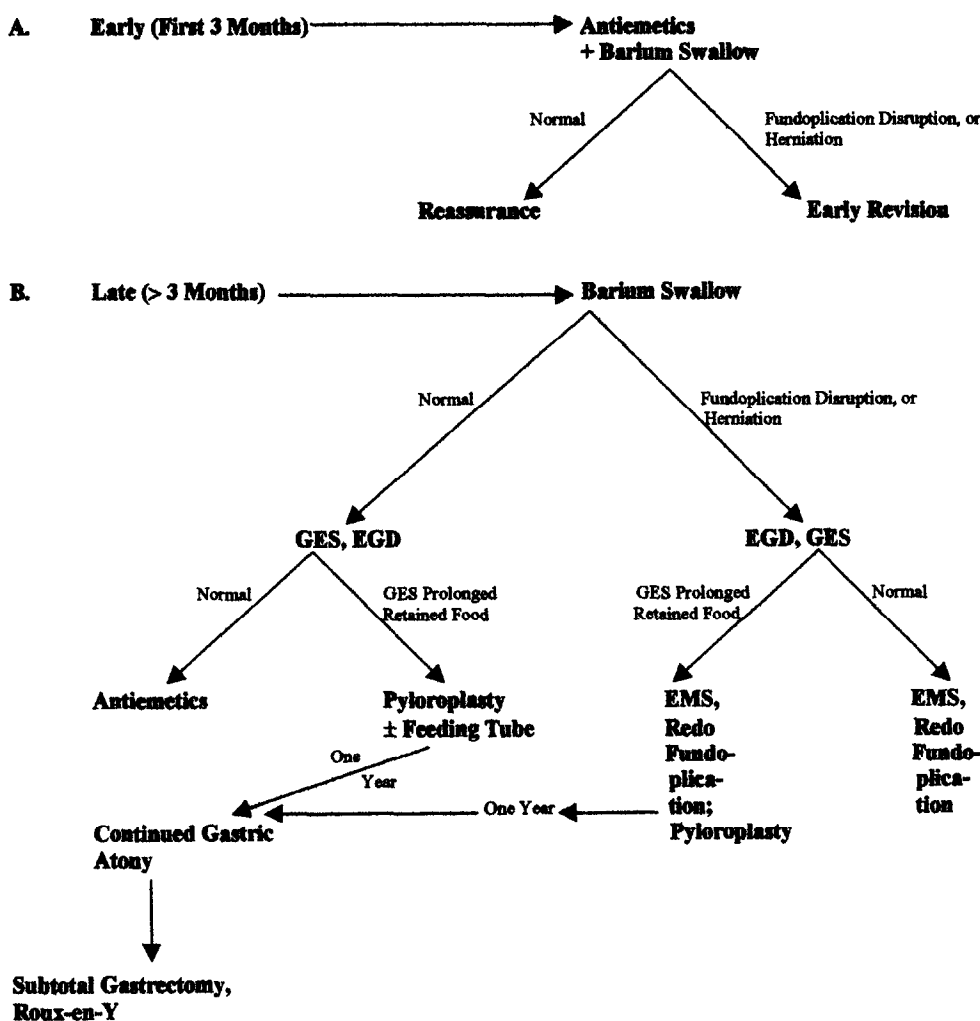
A. **Early (First 3 Months)** ⟶ **Antiemetics + Barium Swallow**

Normal ↙ ↘ Fundoplication Disruption, or Herniation

Reassurance **Early Revision**

B. **Late (> 3 Months)** ⟶ **Barium Swallow**

Normal ↙ ↘ Fundoplication Disruption, or Herniation

GES, EGD **EGD, GES**

Normal ↙ ↘ GES Prolonged Retained Food — GES Prolonged Retained Food ↙ ↘ Normal

Antiemetics **Pyloroplasty ± Feeding Tube** **EMS, Redo Fundoplication; Pyloroplasty** **EMS, Redo Fundoplication**

One Year — One Year ⟵

Continued Gastric Atony

↓

Subtotal Gastrectomy, Roux-en-Y

Fig. 3. Evaluation of the patient with severe bloating, nausea, and retching following laparoscopic Nissen fundoplication.

tion is performed laparoscopically or through a laparotomy, the principles are the same.

Exposure for Laparoscopic Redo Fundoplication

We use the same five-trocar technique that was used for the primary operation. Because there will frequently be adhesions between the fundoplication and the liver, it may be necessary to adjust the liver retractor several times as the adhesions are taken down. Adhesiolysis is best performed with electrosurgical scissors or ultrasonic shears. Dissection then proceeds by identifying the diaphragmatic crura. This is generally easiest on the left side of the fundoplication. If the short gastric vessels have been previously mobilized, it is easy to follow the left hemidiaphragm down to its base. The right diaphragm is best approached

by identifying the caudate lobe of the liver and proceeding superiorly and to the left until the right crus is encountered. If the hepatic branch of the vagus has not been divided during the first operation, it is usually necessary to do so at the second operation to facilitate dissection and repair. If the short gastric vessels were not taken down during the first operation, this is performed. A 360-degree dissection of the diaphragmatic crura allows a Penrose drain to be placed behind the esophagus. This drain is held in place with clips or with an endoloop. The surgeon should note that if the fundoplication has become herniated, the Penrose drain will be around the stomach and not around the esophagus. Inferior traction on the drain allows the surgeon to reduce the herniated fundoplication back into the abdomen. Again, the dissection is kept close to the stomach to avoid causing a pneumothorax. If a pneumothorax does occur, generally

the intra-abdominal pressure is decreased to 8 to 10 mm Hg, and dissection proceeds without physiologic difficulty. Once the fundoplication has been reduced from the chest, the next step is to take the old fundoplication apart. This is performed with sharp dissection by identifying the stitches on the anterior portion of the fundoplication and dividing them sharply. The fundus of the stomach is then peeled to the left and to the right from the midposition to take down the fundoplication circumferentially. Again sharp dissection without the use of electrosurgery or a harmonic scalpel is preferred to ensure that the vagal nerves are not damaged in the course of this dissection. Generally, the vagal trunks will be found within the prior fundoplication. The posterior vagus can usually be preserved if it was previously left inside the fundoplication, but it is more difficult to preserve if it was left outside the fundoplication. The anterior vagus nerve should and can be preserved if it has not been encased in scar tissue at the level of the diaphragm. Once the fundoplication has been entirely taken down, an assessment of intra-abdominal length is performed by pulling the crura together with a grasper and letting go of the drain. If 2 cm of tension-free esophagus exists in the abdomen, the esophagus is not foreshortened and a Collis procedure need not be done. If the gastroesophageal junction lies within 2 cm of the closed hiatus, a Collis gastroplasty is performed as previously described.[16] Occasionally patients appear to have adequate intra-abdominal length but will have had a herniated fundoplication twice previously without known diaphragmatic stressors. Under these circumstances we advocate performing a Collis gastroplasty regardless.

I am often asked whether a pyloroplasty is indicated when neither vagal trunk can be identified because of one or two previous operations. We generally do not recommend this, as many vagotomized stomachs will empty reasonably normally, and pyloroplasty can then be used for those patients who develop difficulties with poor gastric emptying postoperatively. It has been extremely rare that we have found the need to return at a later date for pyloroplasty. Last, the topic of second and third revisional operations frequently arises. We have reported that the results of redo fundoplications deteriorate with each successive operation.[10] Whereas success for the first operation ranges between 90% and 95%, second operations are successful between 80% and 90% of the time, and third operations are successful between 50% and 66% of the time. Because fourth operations are rarely successful at all, many individuals suggest that esophageal resection be performed after three failed fundoplications. This is generally our policy when all other conservative measures have failed.

In conclusion, the revolution in laparoscopic antireflux surgery has created a huge new area for thought and investigation—the failed laparoscopic Nissen fundoplication. With careful and thorough preoperative evaluation, most of these patients may undergo re-repair using meticulous laparoscopic technique with good to excellent outcome.

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The Short Esophagus—Recognition and Management

Donald E. Low, M.D.

Before one can adequately confront the question of whether the short esophagus exists, a generally accepted definition of the entity must be established. I believe the short esophagus to be "a relative shortening of the expected length of the esophagus associated with intramural and periesophageal scarring and fibrosis, which inhibits the easy reestablishment of normal length during esophageal surgical procedures." In reality, although other medical practitioners may acknowledge its existence, it is clinically important predominantly to surgeons who must routinely recognize the presence of esophageal shortening and potentially modify their treatment approach in acknowledgment of its potential effect on standard antireflux operations.

With the establishment of a working definition, it is certainly safe to say that if the short esophagus does not exist as a true clinical entity, it is undoubtedly one of the most cited myths in the surgical literature. What is apparent from a perusal of publications on the subject of esophageal surgery is that most surgeons who have significant experience with esophageal operations have acknowledged its existence and importance in antireflux procedures for more than 50 years. This recognition has taken on renewed emphasis within the past decade because of the increased number of surgeons involved in antireflux surgery and the acceptance of the laparoscopic approach as the standard in the vast majority of antireflux operations.

In the middle part of this century, new technical approaches were suggested for acquired short esophagus. These approaches included esophageal resection, repositioning of the esophagogastric junction through the dome of the left diaphragm, and stricturoplasty of areas involved in esophageal stricture and fibrosis (Thal procedure). These efforts culminated in Collis' 1957 publication of the description of the gastroplasty procedure to create a gastric tube extension or neoesophagus in patients with esophageal shortening to facilitate locating the new esophagogastric junction

within the abdominal cavity. This procedure saw fairly wide acceptance within the surgical antireflux community and experienced a wide application in conjunction with the most commonly applied antireflux procedures of the time, specifically the Belsey Mark IV operation[1] and the Nissen fundoplication.[2]

Gozzetti et al.[3] reviewed a series of patients thought to have a short esophagus and hypothesized that acid gastroesophageal reflux produced significant mural damage, specifically esophagitis and stricture formation affecting the submucosal layers and resulting in shortening of the esophagus. Interestingly, they suggested that pancreaticobiliary reflux could produce the same degree of submucosal fibrosis (possibly by increasing the penetration of H^+ ions) without evidence of mucosal injury.[3]

Possibly the best demonstration of the existence of the short esophagus is expressed in a careful examination of the most common reasons for failure of antireflux procedures reported in collective series. Between 59% and 90% of patients with failed antireflux operations will demonstrate a recurrent hiatal hernia involving displacement of the esophagogastric junction, or in fact the entire antireflux procedure, into the chest (Table I). This pattern of failure demonstrates that there are forces in certain patients that increase the likelihood of hiatal hernia recurrence. One component of these failures is the inability to achieve a tension-free reduction of the esophagogastric junction into the abdomen at the time of the initial operation. This common pattern of failure is additional testimony to the existence of the short esophagus and its ability to affect outcome when unrecognized in patients undergoing antireflux surgery.

PREDICTING THE PRESENCE OF ESOPHAGEAL SHORTENING

Following the acceptance of the short esophagus as a real clinical entity, the next important issue would

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Table I. Etiology of failure in surgical procedures for GERD

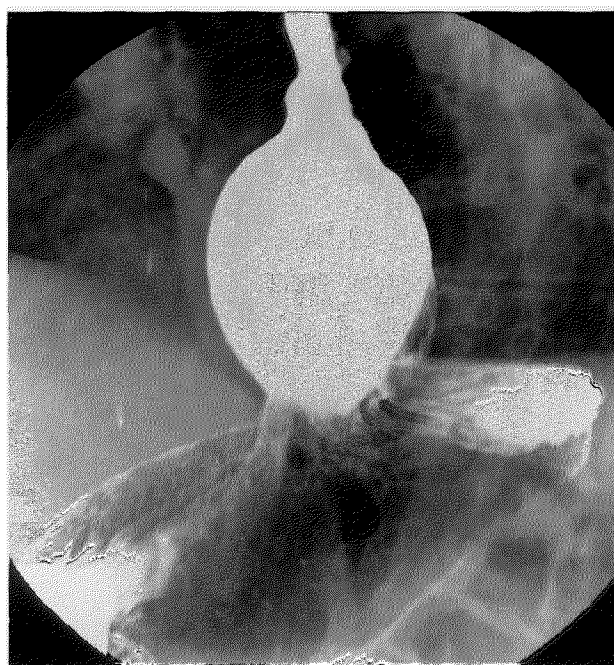| Reference	No. of patients	Recurrent hiatal hernia (%)
Low et al.[4]	116	77
Soper and Dunnegan[5]	20	90
Kimber et al.[6]	66	87
Horgan et al.[7]	48	59
Hunter et al.[8]	100	84

Table II. Factors associated with the presence of clinically important short esophagus in patients undergoing antireflux surgery

Factor	Predictability
Esophageal stricture and hiatal hernia (>5 cm)	+ + +
Esophageal stricture	+ +
Hiatal hernia (>5 cm)	+/−
Barrett's esophagus	+/−
Grade IV esophagitis	+/−

Fig. 1. Large hiatal hernia with proximal stricture. During barium swallow, this hernia did not move or reduce, indicating it is "fixed" within the chest.

be the inability to identify these patients preoperatively. The clinical findings most commonly seen as precursors to esophageal shortening are esophageal stricture and the presence of large "fixed" hiatal or paraesophageal hernias. Of lesser importance, but still thought to play a role is a history of severe (type III or IV) esophagitis or Barrett's esophagus (Table II).

The incidence of revisional surgery has been shown to be significantly increased in patients with esophageal stricture following standard Belsey and Nissen repairs.[9,10] Gastal et al.[11] have shown that the combination of a large hiatal hernia and an esophageal stricture is particularly significant and as a result have recommended routinely operating on these patients through an open transthoracic approach when these factors coincide. These same investigators were able to document a manometrically measurable decrease in esophageal length in these patients, although they could not identify a threshold value below which esophageal shortening could be reliably identified. Pearson[12] not only noted a significant decrease in esophageal length but also documented decreases in esophageal peristaltic amplitude in the distal esophagus, which he interpreted to be consistent with intramural fibrosis.

In our experience, the patients in whom the greatest caution should be exercised are those who demonstrate strictures and large hiatal hernias (i.e., ≥5 cm), which

remain "fixed" or nonreducing during repositioning of the patient from the supine to the upright position at the time of the barium swallow tests (Fig. 1).

Previous publications have predicted the need for esophageal lengthening procedures in 14% to 28% of all patients with GERD. However, at the time of surgery only 4% to 16% required modifications of the standard technique (i.e., gastroplasties) indicating that the actual findings during the procedure and surgical experience will be the ultimate judge of which patients will require esophageal lengthening procedures or alternative techniques to maintain good results.[11,13,14]

MANAGEMENT OF PATIENTS WITH SHORT ESOPHAGUS

The management of patients with short esophagus seems to settle into two distinct groups, specifically, those who undergo a gastroplasty and those who are managed without esophageal lengthening. The basic premise of the management of all these patients involves extensive mobilization of the esophagus, meticulous closure of the esophageal hiatus, and anchoring the repair securely within the abdominal cavity with careful avoidance of any physiologic issues, which will increase intra-abdominal pressure postoperatively such as retching, vomiting, or excessive coughing.

Although most surgeons agree that 6 to 8 cm of esophagus can be routinely mobilized laparoscopically, some would recommend a thoracic approach to allow mobilization to be completed up to the level of the aortic arch.[11,12] However, the decision on the approach and type of repair should be based on the ultimate ability to provide enough esophageal mobilization to allow reduction of the esophagogastric junction into the abdomen for at least 1 to 2 cm and for it to remain in position without tension. If this cannot be accomplished, a gastroplasty or a more firmly anchored repair should be contemplated. In addition, notwithstanding the technical approach, a very conservative approach to postoperative management, specifically, routine use of antiemetics, prolonging the use of nasogastric suction, and more judicious reintroduction of normal foods, should be considered in patients with documented esophageal shortening. This is due to the tendency of these patients to demonstrate early anatomic failure with any significant degree of postoperative retching or vomiting.

If tension-free reduction of the esophagogastric junction into the abdominal cavity cannot be achieved, management should be a choice between a gastroplasty and a Hill antireflux operation. There are currently three technical descriptions for the application of gastroplasty with laparoscopic antireflux operations.[13,15,16] One technique described by Swanstrom et al.[16] provides for the passage of an endoscopic stapler through the right side of the chest to perform a gastroplasty while the antireflux procedure is carried out using a standard laparoscopic approach. Another method advocated by Johnson et al.[13] involves a completely laparoscopic approach, but one involving a minilaparotomy incision to facilitate the insertion of a circular stapling device, which along with a linear endoscopic stapler makes the gastroplasty easier to perform. These early reports obviously include learning curves, but also entail significantly longer operative times and lengths of hospital stay (in the report by Johnson et al., a mean procedure length of 4 hours and 54 minutes and a mean length of hospital stay of 3 days).

Just as important as understanding the technical aspects of laparoscopic gastroplasty is understanding all of the potential ramifications of gastroplasty procedures and the effect the procedure itself can have on clinical outcome. This was specifically addressed in a supplementary paper by Jobe et al.[14] in which they reported on the follow-up of 15 patients undergoing thoracoscopic/laparoscopic Collis gastroplasties. Of the 14 patients available for long-term follow-up, standard parameters suggested excellent results (i.e., mean length of stay 2 days, return to work 8 days, and 100% of patients considering their operations successful at a mean long-term follow-up of 14 months). However,

within this same group, two patients (14%) reported significant postoperative heartburn, and two (14%) reported prolonged postoperative dysphagia. Endoscopic follow-up of this entire group demonstrated intact repairs and good flap valve formation in 100%. However, five patients (36%) has documented postoperative esophagitis and seven (50%) had abnormal 24-hour pH tests postoperatively. The presence of esophagitis and an abnormal 24-hour pH test is explained by the postoperative documentation of gastric mucosa proximal to the fundoplication in 100% of patients. Seven of these patients demonstrated positive Congo red staining proximal to the repair and six (43%) showed loss of distal esophageal peristalsis within the neoesophagus segment decreasing esophageal clearance and increasing the tendency for postoperative dysphagia. Jobe et al.[14] concluded that "Collis gastroplasty allows a tension-free fundoplication to be performed to correct a shortened esophagus. It results in an effective antireflux mechanism, but can be complicated by the presence of acid secreting gastric mucosa proximal to the intact fundoplication and a loss of distal esophageal motility. These patients require close objective follow-up and maintenance of acid suppression therapy." Although Collis gastroplasty remains a recognized and important aspect of the management of short esophagus, surgeons applying this technical adaptation to the standard Belsey, Nissen, and Toupet repairs need to keep these important factors in mind postoperatively.

Conversely, our practice in managing patients who are suspected preoperatively of having a short esophagus involves the routine application of an open Hill procedure. From 1960 to the present, a total of 3170 antireflux operations have been performed at Virginia Mason Medical Center. Twenty percent of these patients had esophageal strictures or grade IV esophagitis, and 18% were redo procedures. In this entire operative experience of more than 3000 operations, we have never performed a gastroplasty. This has been possible due to the fact that the Hill antireflux operation remains the only procedure to be based on not only reconstruction of the gastroesophageal flap valve but also firmly and reliably anchoring the repair within the abdominal cavity. The vast majority of routine procedures performed at Virginia Mason Medical Center are carried out laparoscopically. For the small subset of patients who are still undergoing an open repair, which over the past 4 years has encompassed 80 operations, the mean operative time is 92 minutes and the mean hospital length of stay is 3.9 days. Even though this subgroup contains all patients with large hiatal hernias and a history of esophageal strictures, and in many cases patients who are obese, this patient population has demonstrated a failure rate of less than 5% (i.e., requiring medications postoper-

atively) and no documented incidences of anatomic failure.

With the regular application of regional anesthetic techniques facilitating early mobilization, we have seen dramatic increases in hospital lengths of stay and times to return to work with the open Hill procedure applied in these highly selected patients. We have previously reported the largest long-term follow-up of the open Hill procedure with 88% of patients demonstrating satisfaction at a mean of 17.8 years' follow-up.[17] We have also reported on one of the most extensive experiences of antireflux surgery in patients with esophageal strictures.[18] We believe that this experience is more than sufficient to justify the continued application of an open procedure to maintain the anatomic integrity of the esophageal gastric junction and distal esophagus and to avoid some of the issues clearly demonstrated by Jobe et al.[14] This philosophy is supported in a qualified manner by Johnson et al.[13] in their discussion of laparoscopic Collis gastroplasty[13] in which they point out in the discussion: "Another very acceptable approach is to convert to an open Collis-Nissen procedure when esophageal shortening is discovered intraoperatively. It is more important that the operation be performed correctly than a laparoscopic access be maintained." We would agree with this statement wholeheartedly, but advocate consideration of the Hill procedure over a gastroplasty.

CONCLUSION

Esophageal shortening is a real and clinically important issue for surgeons performing antireflux surgery. The ability to identify these patients preoperatively is based predominantly on experience; however, particular awareness and appropriate preparations should be made for patients demonstrating esophageal strictures and large fixed hiatal hernias. Management should include extensive mobilization of the esophagus to facilitate a tension-free reduction of the esophagogastric junction into the abdominal cavity with subsequent meticulous closure of the esophageal hiatus and anchoring of the repair within the abdomen. If tension-free reduction cannot be achieved, surgeons should be prepared to proceed with a procedure involving Collis gastroplasty or an operation such as the Hill procedure, which involves reliable anchoring within the abdominal cavity.

It is currently estimated that approximately 15% of patients presenting for antireflux surgery will have some degree of esophageal shortening, and various estimates indicate that 20% to 70% of this population will require specialized surgery because of the inability to establish a tension-free reduction of esophago-

gastric junction. Based on projections that 20,000 to 40,000 antireflux procedures will be performed in the United States in the year 2000, calculations would show that between 1200 and 2500 patients will require specialized operations for esophageal shortening. Surgeons should be making every attempt to identify these patients preoperatively and have the flexibility to manage them appropriately at the time of antireflux repair.

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Laparoscopic Fundoplication for Symptomatic but Physiologic Gastroesophageal Reflux

Reginald C.W. Bell, M.D., Philip Hanna, M.D., Sandy Brubaker, B.S.

Esophageal pH monitoring identifies some patients who have physiologic amounts of esophageal acid exposure but have a strong correlation between symptoms of esophageal reflux events. These patients with symptomatic physiologic reflux probably have enhanced sensory perception of reflux events and may be difficult to control with acid-suppressive therapy. Little is known about the role of fundoplication in such patients. Patients with no endoscopic evidence of gastroesophageal reflux disease and a normal 24-hour pH composite score (<22.4 in our laboratory), but a symptom index (SI = number of symptoms with pH <4/total number of symptoms) greater than 50% were offered laparoscopic fundoplication if acid-suppressive therapy was unsatisfactory. This group comprised 18 (4%) of 459 patients undergoing fundoplication at our institution. Heartburn, dysphagia, and reflux symptoms were scored on a scale of 0 to 10 with patients on and off medicine preoperatively, and at a mean of 7.2 months (range 1 to 32 months) postoperatively. The 18 patients with symptomatic physiologic reflux (6 males and 12 females) had heartburn as a major complaint. Preoperative response to proton pump inhibitors for heartburn was 72% and for all symptoms was 60%. The group had a mean pH composite score of 14 (range 4 to 22). The symptom used to calculate the symptom index was heartburn in 12 patients, regurgitation in three, chest pain in two, and cough in one. An average of 18 symptoms (range 2 to 56) were recorded. The mean symptom index was 82% (range 50% to 100%). A Nissen fundoplication was performed in nine patients and a Toupet fundoplication in nine. Surgery was successful (>90%) in alleviating reflux symptoms in 14 patients and partially successful (>75%) in three of the remaining four patients. Gas bloat and dysphagia were seen in one patient each. Fundoplication is effective at relieving reflux symptoms in carefully selected patients with symptomatic physiologic reflux, with minimal side effects. (J GASTROINTEST SURG 2001;5:462-467.)

KEY WORDS: GERD, physiologic reflux, fundoplication, symptom index

Gastric fundoplication controls symptoms and reflux in patients who have excessive esophageal exposure to gastric juice. Twenty-four-hour pH monitoring is the accepted standard for documenting both the presence of acid gastroesophageal reflux and determining how well a patient's symptoms correlate with acid reflux events. Symptom correlation may be quantitated as a symptom index, which is the number of times a patient's symptom coincides with acid reflux divided by the number of times that symptom is reported. A symptom index above 50% has been shown to correlate with excessive acid exposure on 24-hour pH monitoring.[1,2]

Although the majority of patients with acid-induced heartburn have excessive amounts of esophageal acid exposure, occasional patients will exhibit an excess sensitivity to acid despite having normal amounts of acid exposure on 24-hour pH testing, the so-called "acid-hypersensitive esophagus."[3] These patients with symptomatic but not excessive gastroesophageal reflux may be diagnosed when a positive symptom index is found despite physiologic levels of esophageal acid exposure and absence of visible esophagitis on endoscopy.

Patients with symptomatic physiologic reflux (SPR) exhibit the typical symptoms of gastroesopha-

geal reflux disease (GERD)—heartburn, reflux, and dysphagia—but may exhibit bloating and dyspeptic symptoms more frequently. Medical therapy for patients with symptomatic physiologic reflux has been demonstrated to be successful in controlling typical symptoms.

Surgery has traditionally been reserved for patients with esophagitis or abnormal amounts of esophageal acid exposure on 24-hour pH testing. It has not been clear what role if any gastric fundoplication would have for patients with SPR. This study evaluates the functional outcome of a group of patients with SPR undergoing laparoscopic fundoplication.

MATERIAL AND METHODS

Data for patients undergoing laparoscopic fundoplication by a single surgeon from 1992 to 2000 were prospectively entered into a computerized database. Retrospective analysis of this database revealed 18 patients who met the criteria listed below for symptomatic physiologic reflux, and these patients are the basis of this study.

Twenty-Four-Hour pH Testing

All patients underwent 24-hour pH testing preoperatively. Proton pump inhibitors were discontinued for a minimum of 5 days, and typically 7 to 10 days, before pH testing. Ambulatory outpatient testing was performed with an antimomy catheter for a minimum of 20 hours (Sandhill Scientific, Inc., Highlands Ranch, Colo.). Computer analysis of pH recordings was performed according to the scoring system of Johnson and DeMeester.[5] The normal value determined according to the Sandhill Scientific computer algorithm for the Johnson-DeMeester scoring system is a composite score less than 22.4. (This normal value is different from frequently published reports of less than 14.7; these reports use a slightly different computer scoring system incorporated by the Synectics/Medtronics devices.)

The symptom index was determined by taking the number of times the major symptom coincided with a pH of less than four and dividing it by the number of times the major symptom was recorded.[2] All patients in this study had a composite score of less than 22.4 and a symptom index of greater than or equal to 50%.

Esophageal Manometry

Complete esophageal body and lower esophageal sphincter (LES) studies were performed with a miniature solid-state transducer manometry catheter (Sandhill Scientific). Stationary techniques were used to de-

termine LES pressure and LES length. A defective LES was present with any one of the following manometric findings: LES pressure less than 10 mm Hg measured in midrespiration at the point of maximal pressure, LES length less than 2 cm, or LES intra-abdominal length less than 1 cm. This follows the definition of Zaninoto et al.,[6] recognizing that the lower normal limit for LES pressure (ninety-fifth percentile) measured at midrespiration at the respiratory inversion point is 6 mm Hg and at midrespiration in the zone of maximal pressure is 10 mm Hg.[7]

Upper Endoscopy

Complete upper endoscopy was performed by the referring gastroenterologist with attention to the following: (1) grade of esophagitis (following the criteria of Ollyo et al.[8]); (2) presence of hiatal hernia; (3) presence of Barrett's esophagus; (4) presence of a stricture or need for dilation; and (5) presence of gastric or duodenal acid–peptic disease (ulceration, erosions, gastritis). All patients in this study were free of visible esophagitis, stricture, Barrett's esophagus, or a hiatal hernia larger than 2 cm on preoperative upper endoscopy.

Symptom Assessment

Heartburn, dysphagia, and reflux symptoms were scored on a scale of 0 to 10 by an impartial nurse interviewer (S.B.). Patients were asked about the severity of these symptoms preoperatively both on medication and off medication (prior to the 24-hour pH test), and at postoperative follow-up. Patients rated, as a percentage, the best ever and the current relief of heartburn provided by proton pump inhibitors. A similar percentage rating was given for the response of global reflux symptoms to medication.

Surgical Treatment

Laparoscopic fundoplication was performed according to techniques previously described by us and others.[9] Both techniques of a Nissen 360-degree fundoplication and a Toupet 270-degree fundoplication were used in these patients. The choice of surgical technique reflected evolution of our surgical technique and was not based on preoperative motility findings.

Statistical Analysis

Data were collected prospectively on a computerized database (Microsoft Access, Microsoft, Seattle, Wash.). Statistical analysis was performed on SYSTAT 7.01 (SPSS Inc., Chicago, Ill.). Specific tests

used are reported with the results; Pearson's chi-square, t test, and Kruskal-Wallis nonparameteric analysis were used as appropriate.

RESULTS
Preoperative

Eighteen (4%) of 459 patients undergoing surgical fundoplication met the above-mentioned criteria for SPR. The group had a mean pH composite score of 14 (range 4 to 22). Had we used the original criteria for the acid-hypersensitive esophagus—less than 4.2% total time with a pH <4 with a positive symptom index[3]—we would have identified 24 such patients. Because our decision regarding surgery had been based on the composite score, and this criterion proved more restrictive, we stayed with the composite score in the selection of patients for this study. Three of the study patients had more than 4.2% but less than 7% of the time with a pH of 4. An average of 18 symptoms (range 2 to 56) were recorded during the 24-hour period. The mean symptom index was 82% (range 50% to 100%). The specific symptoms with their indexes are listed in Table I.

The study group included twelve women and six men. All patients had heartburn as a major complaint, and all had been tried on either single-dose[12] or double-dose[6] proton pump inhibitor therapy to control their symptoms. The mean *best* percentage relief of heartburn provided by proton pump inhibitors was 72% (range 20% to 100% relief of heartburn), and for all symptoms was 60% (range 20% to 100%). Four patients had a defective LES as defined above. One patient had defective peristalsis (mean amplitude in the lower esophageal body during 10 wet swallows of less than 30 mm Hg).

Postoperative

All operations were completed laparoscopically, and there were no operative or postoperative complications. Seventy-two percent of patients were discharged within 24 hours of surgery. Clinical follow-up was accomplished in all patients at a mean of 7.2 months (range 1 to 32 months) after surgery.

Table II and Figs. 1 and 2 show the effects of surgery symptoms. Heartburn (see Fig. 1) postoperatively was better than it had been with no medication in 17 patients (94%) and resolved completely in 14 patients (78%). In addition, heartburn was better controlled by surgery than by medication in 15 patients (83%).

Regurgitation (see Fig. 2) was eliminated in 15 (89%) of 17 patients who experienced this symptom preoperatively. Additionally, surgery controlled regurgitation significantly better than medication alone.

Dysphagia, which was not a major preoperative symptom, did not change significantly with surgery.

Table I. Patients with component score, symptoms, and symptom index in 18 patients undergoing fundoplication

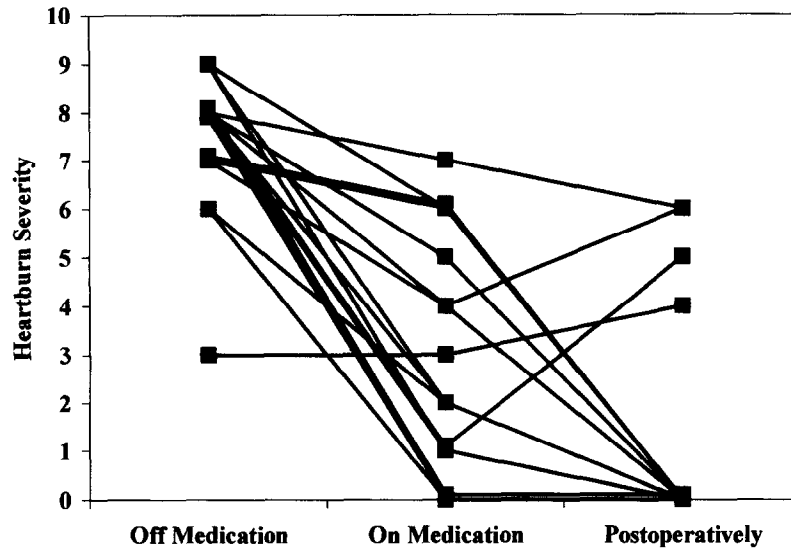| Patient	Johnson-DeMeester component score (normal <22)	% of total time pH < 4	Symptom recorded for symptom index	No. of symptoms reported (S)	No. of reflux episodes associated with symptoms (R)	Symptom index $\frac{(R)}{(S)} \times 100$
1	13	4	Chest pain	18	12	67
2	7	1	Chest pain	12	7	58
3	15	4	Cough	9	7	77
4	12	3	Heartburn	22	21	94
5	16	3	Heartburn	9	9	100
6	13	4	Heartburn	19	16	88
7	13	3	Heartburn	6	4	66
8	10	3	Heartburn	18	16	94
9	20	4	Heartburn	24	21	87
10	15	2	Heartburn	6	6	100
11	10	4	Heartburn	2	2	100
12	21	6	Heartburn	2	1	50
13	8	2	Heartburn	56	27	48
14	20	5	Heartburn	7	6	85
15	4	Unknown	Heartburn	39	39	100
16	14	Unknown	Regurgitation	10	10	100
17	18	3	Regurgitation	9	8	88
18	22	5	Regurgitation	55	34	62

Fig. 1. Changes in heartburn severity with medication and with fundoplication.
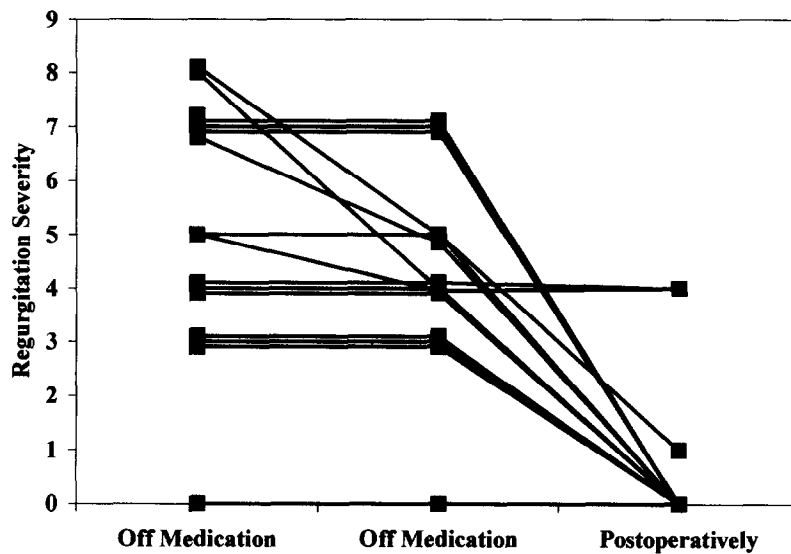

Fig. 2. Changes in severity of regurgitation with medication and with fundoplication.

Table II. Mean symptom scores before and after surgery

| | Preoperative | | |
Symptom score	Off medication	On medication	Postoperative (no medication)
Heartburn	7.5 (6.8-8.2)	2.8 (1.6-4.0)*	1.2 (0.03-2.3)*
Regurgitation	4.5 (3.2-5.9)	1.2 (0.03-2.3)*†	0 (0)*†
Dysphagia	1.7 (0.9-2.5)	2.7 (1.4-4.0)*	1.7 (0.7-2.8)*

Values are mean (95% confidence interval); paired t test.
*P <0.01 vs. preoperative off medication.
†P <0.05 vs. preoperative on protein pump inhibitor medication.

No patient developed significant de novo dysphagia postoperatively. Patients rated the effectiveness of surgery relative to the best results of medication at relieving their reflux symptoms. Surgery worked an average of 87% better than medication at relieving reflux. (One patient reported control equal to that achieved with medication, and one patient reported that medication had been more effective than surgery.)

All patients reported an ability to belch after surgery. Side effects of surgery included excess bloating in one patient and excess gas/meteorism in one patient. All (100%) of the patients stated that they were satisfied or very satisfied with the results of surgery. All but two said that their symptom control was better after surgery than it had ever been on medication.

Three patients continued to take proton pump inhibitors but report very little improvement in their residual symptoms on proton pump inhibitors. Four patients, three with some persistent symptoms after fundoplication, agreed to postoperative 24-hour pH testing. The composite scores were normal (0, 1, 4, and 16, respectively) in all four patients; however, only the patient with a score of 16 had a positive symptom index for heartburn.

DISCUSSION

Surgical fundoplication in GERD has typically been reserved for patients with either erosive or complicated esophagitis, or excess amounts of gastroesophageal reflux defined by 24-hour pH testing. The latter criterion, initially thought to be a marker for increased risk of esophageal injury,[10,11] has proved to be more important as esophagitis has become a less frequent finding on upper endoscopy because of patient pretreatment. With improved medical therapy, patients are more commonly considering surgery for persistence of symptoms, even though their esophagitis may have healed. Surgery for GERD is shifting from disease control to symptom control. That shift from disease control to symptom control has come about partly because of minimally invasive (laparoscopic) techniques. Milder forms of GERD, including patients with upright reflux and patients with physiologically normal sphincter, are now considered potential surgical candidates.[12]

Patients with symptomatic physiologic reflux represent perhaps the mildest form of GERD as an injurious disease process. By definition there is no esophageal injury and there is only a physiologic amount of acid exposure in the esophagus. These patients exhibit clinical characteristics, chronicity of disease, and long-term requirement for medical therapy similar to patients with abnormal esophageal acid exposure.[13]

There is probably a spectrum of symptomatology extending to functional dyspepsia, but patients with SPR are distinguishable by the presence of a strong symptom association on 24-hour pH testing.[3,14,15] Traditional criteria for recognizing reflux may not detect all reflux,[16] but we stayed with traditional criteria for this analysis.

Patients with SPR frequently respond well to acid-suppressive medication. A double-blind crossover trial between omeprazole and placebo showed a significant decrease in the frequency and severity of reflux symptoms only in a subgroup of patients with GERD-type symptoms who had a positive symptom index.[4] Multivariate analysis of factors predicting outcome after fundoplication has shown that typical symptoms, a good response to acid-suppressive medication, and an abnormal 24-hour pH test are strong predictors of success.[17] It was therefore with some trepidation that we offered fundoplication to patients with symptomatic physiologic reflux when their symptom control was inadequate on medication. We did not know whether reflux surgery would work in a situation where cumulative acid exposure was normal. We suspected that the major mechanism of reflux in these patients was during transient LES relaxations, and reports on the effect of fundoplication on transient LES relaxations were promising.[18,19] We therefore limited our selection to patients who had typical symptoms and at least initially a good response to medical therapy. From a clinical perspective, the results have been very encouraging.

The limitations of this study are the relatively small size of the cohort, the short follow-up (mean of 8 months), and the lack of postoperative 24-hour pH testing in most patients. The limited number of patients reflects the rarity of this process, as it accounted for only 4% of almost 500 patients undergoing laparoscopic fundoplication. The utility of a short follow-up is justified because the long-term durability of the Nissen and Toupet fundoplications in patients with only modest amounts of reflux has been well demonstrated. We do not believe the results reflect a placebo effect, but longer term follow-up will be needed to confirm this. We did ask all patients to undergo postoperative 24-hour pH testing. Unfortunately, as has been reported in other studies of patients undergoing antireflux surgery, postoperative testing is difficult to accomplish in asymptomatic patients, especially in a managed care environment.

CONCLUSION

Patients with symptomatic but physiologic gastroesophageal reflux comprise a small portion of patients

with symptomatic GERD. They are diagnosed on the basis of a symptom index of greater than 50% in the absence of an excess amount of acid exposure and in the absence of any abnormality on upper endoscopy. If they also have typical symptoms of GERD and have had a reasonable (>50%) response of their symptoms to medication, our results indicate that these patients respond well to laparoscopic fundoplication.

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Hepatitis B or C Virus Serology as a Prognostic Factor in Patients With Hepatocellular Carcinoma

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It is not clear whether chronic hepatitis B or C virus (HBV or HCV) infection is a prognostic factor for hepatocellular carcinoma. We performed this study to determine if chronic HBV or HCV infection had any impact on postresection survival or affected patterns of failure. The records of 77 patients undergoing surgical resection for hepatocellular carcinoma between January 1990 and December 1998 were reviewed. Forty-four patients (57%) had HCV infection, 18 patients (23%) had HBV infection, and 15 patients (20%) had negative serology. There were no differences in age, sex, or tumor size among the groups, and all patients had margin-negative resections. There was a significantly higher incidence of satellitosis and vascular invasion in patients with HCV infection (32% and 41%, respectively; P <0.05 vs. other groups). With a median follow-up of 30 months, a significantly decreased local disease–free survival (LDFS) was seen in HBV-positive (5-year LDFS 26%) or HCV-positive (5-year LDFS 38%) patients compared to those with negative serology (5-year LDFS 79%; P <0.05). There was also a trend toward a decreased overall survival in patients with positive hepatitis serology compared to patients with negative serology (37% vs. 79%; P = 0.12). Univariate analysis revealed that only satellitosis was related to local recurrence and overall survival. Patients with positive serology for hepatitis B or C undergoing resection for hepatocellular carcinoma have a trend toward worse overall prognosis and a significantly decreased LDFS when compared to patients with negative serology. (J GASTROINTEST SURG 2001;5: 468-476.)

KEY WORDS: Hepatitis B, hepatitis C, hepatocellular carcinoma, prognostic factors

Hepatocellular carcinoma (HCC) is among the most common solid human malignancies. It is one of the 10 most common cancers in the world and is one of the most lethal malignancies with a mortality index of 0.94.[1] Chronic hepatitis B virus (HBV) and C virus (HCV) infections have been implicated as important etiologic factors in the development of HCC.[2-5] A review of the literature reveals that of all patients diagnosed with HCC, the range of coexistent chronic HBV or HCV infection is 13% to 73% and 11% to 88%, respectively (Table I).

Previous studies have shown that clinical, surgical, and pathologic findings such as tumor size, tumor-free resection margin, tumor stage, vascular invasion, and satellitosis serve as important prognostic factors for HCC.[14] The probability of long-term survival is higher in patients whose tumors are smaller than 5 cm and who have a tumor-free resection margin greater than 1 cm, absence of vascular invasion, and a solitary tumor.[15-17] As yet, the prognostic significance of coexistent HBV or HCV infection in HCC patients in Western countries has not been clearly established. The purpose of this study was to compare the following in patients undergoing resection for HCC: (1) differences in the incidence of negative prognostic factors in patients with chronic HBV or HCV infection to the incidence of these factors in hepatitis virus–negative patients and (2) survival of hepatitis-positive HCC patients with hepatitis-negative HCC patients.

Table I. Incidence of chronic hepatitis B virus (HBV) and C virus (HCV) infections in patients with hepatocellular carcinoma

Reference	Year	No. of patients	Country	% HBV-positive	% HCV-positive
Villa et al.[6]	1988	166	Italy	26	—
Yuki et al.[7]	1992	89	Japan	73	88
Tomimatsu et al.[8]	1994	121	Japan	13	70
Vauthey et al.[9]	1995	106	USA	48	—
Yu et al.[10]	1997	359	China	18	66
Olubuyide et al.[11]	1997	—	Nigeria	19	59
Zhang et al.[12]	1998	152	China	63	11
Lee et al.[13]	1999	284	Taiwan	55	4

MATERIAL AND METHODS

Patients

A retrospective review of patients undergoing hepatic resection for HCC at The University of Texas, M.D. Anderson Cancer Center and the G. Pascale National Cancer Institute between January 1990 and December 1998 was undertaken. In all patients, hepatitis B surface antigen (HbsAg) and antihepatitis C antibody (HCVAb) concentrations were measured by radioimmunoassay and enzyme-linked immunosorbent assay techniques, respectively. Patients were divided into three groups based on results of their hepatitis virus serology tests: group I (HbsAg positive), group II (HCVAb positive), and group III (negative serology).

Standard demographic data were collected on all patients. Tumor stage, vascular invasion, presence of satellitosis, local disease–free survival (LDFS), distant disease–free survival (DDFS), and overall survival were analyzed. All patients were followed every 3 months after surgical treatment with chest radiography, abdominal helical computerized tomographic (CT) scans or magnetic resonance imaging (MRI), and serum alpha-fetoprotein levels.

Operations

Preoperative evaluation included imaging studies (ultrasonography, CT scans, MRI), routine blood work (complete blood count, coagulation profile, electrolytes, liver function tests, alpha-fetoprotein, hepatitis serology), and assessment of comorbid factors. Operative procedures were performed based on the size and intrahepatic location of the tumor and hepatic functional reserve. Hepatic function was assessed preoperatively by indocyanine green retention studies, aminopyrine breath tests, and the Child-Pugh classification of severity of cirrhosis.[18] The extent of hepatectomy was defined according to the functional anatomy of the liver. Lobectomy was defined as re-

section of the functional right lobe or left lobe, segmentectomy as resection of one of the four portal sectors individualized by three hepatic veins, and wedge resection as a nonanatomic resection.[19] Operations were planned with the aid of intraoperative ultrasonography to achieve at least a 1 cm tumor-negative resection margin. In addition, intraoperative ultrasonography was also performed during all operations to determine the presence of additional hepatic tumors not visualized on preoperative imaging studies. An operation was considered to be an R0 resection if the entire tumor was removed with microscopic negative surgical margins, an R1 resection if microscopic surgical margins were positive, and an R2 resection if gross tumor was left behind.

Statistical Analysis

Summary statistics were obtained using established methods. Differences in proportions were assessed by means of Fisher's exact test. Overall survival was defined as the time from the date of initiation of therapy at M.D. Anderson Cancer Center to death from any cause. Differences between the entire cohort and the subset of patients with localized disease were assessed by either the chi-square or Wilcoxon rank-sum test, depending on the type of variable (i.e., categorical, dichotomous, or continuous). Fisher's exact test was used in place of the chi-square test if the sample size was too small in a subgroup. Overall survival and disease-free (local and distant) survival curves were estimated by the Kaplan-Meier method. Log-rank testing was used to evaluate differences in overall survival or disease-free survival rates between subgroups of patients. Univariate models were fitted for potential predictive variables. Continuous variables are presented as the mean. Comparison of frequencies was performed with the Pearson chi-square test. Overall survival, LDFS, and DDFS were calculated using Kaplan-Meier life-table methods. Log-rank testing

was used to compare survival in different groups. Differences were considered significant at $P <0.05$.

RESULTS

A total of 77 HCC patients who underwent margin-negative (R0) hepatic resection were identified from our databases. Patients who were unresectable or those who underwent R1 or R2 resections were excluded from the study. Forty-five of the 77 patients were identified from the G. Pascale National Cancer Institute database; 32 patients were identified from the M.D. Anderson Cancer Center database. There were 17 HBV-infected patients (22%), 44 HCV-infected patients (57%), and 16 patients (21%) with negative serology for HBV or HCV infection. No patients with dual HBV and HCV infections were identified. All of these patients had evidence of cirrhosis based on pathologic examination. Child-Pugh classification, background data, and tumor characteristics of these patients are presented in Table II. There was a higher male-to-female ratio in patients with positive hepatitis serology. Based on the TNM staging system, 95% of the patients had stage II or stage III disease. Regarding tumor characteristics, there were no significant differences among the three groups with respect to the presence of solitary or multiple tumors. There was a significantly higher incidence of satellitosis and tumor vascular thrombus formation (32% and 41%, respectively) for patients with HCV infec-

tion when compared to patients with HBV infection (6% and 12%, respectively) and negative serology (19% and 6%, respectively) ($P <0.05$). There were no differences among the three groups with respect to the degree of cirrhosis or the Child-Pugh classification. All patients underwent either an R0 segmental resection or a lobectomy. No wedge resections were performed. There were no differences among patients with negative serology (segmental resection, n = 4; lobectomy, n = 11), HBV (segmental resection, n = 12; lobectomy, n = 6), or HCV (segmental resection, n = 40; lobectomy, n = 4) with regard to lobectomies or segmental resections performed.

The median follow-up for the entire cohort of patients was 30 months. The 5-year actuarial disease-free survival for the HBV, HCV, and negative serology groups was 28%, 36%, and 79%, respectively (Fig. 1). Patients with HBV or HCV infection had a worse overall disease-free survival when compared to patients with negative hepatitis virus serology ($P <0.05$). This was also seen with respect to LDFS. Patients with chronic HBV or HCV infection had LDFS rates of 28% and 38%, respectively. This was significantly different when compared to patients with negative hepatitis virus serology (LDFS 79%; $P <0.05$) (Fig. 2).

Worse overall survival was seen in the patients with either type of hepatitis infection compared to the non–hepatitis-infected patients; however, this trend did not reach statistical significance ($P = 0.12$). Me-

Table II. Patient demographics and tumor characteristics in 77 patients undergoing liver resection for hepatocellular carcinoma

|	HBV-positive	HCV-positive	Neither	Total
No. of patients	18 (23%)	44 (57%)	15 (20%)	77
Age (yr)	60	61	63	61
Male:Female ratio	2.4:1	3.4:1	0.6:1	2:1
TNM stage				
I	0	1	1	2
II	12	34	8	54
III	5	8	6	19
IV	0	1	1	2
No. of tumors				
Solitary	15	36	13	64
Multiple*	2 (12%)	8 (18%)	3 (19%)	13 (17%)
Satellitosis*	1 (6%)	14 (32%)	3 (19%)	18 (23%)
Tumor thrombus	2 (12%)	18 (41%)	1 (6%)	21 (27%)
Median follow-up (mo)	30	27	33	30
Child-Pugh classification				
A	18	43	13	74
B	0	1	2	3

HBV = hepatitis B virus; HCV = hepatitis C virus.
*Satellitosis is defined as separate foci of the primary tumor; multiple tumors are synchronous primary lesions.
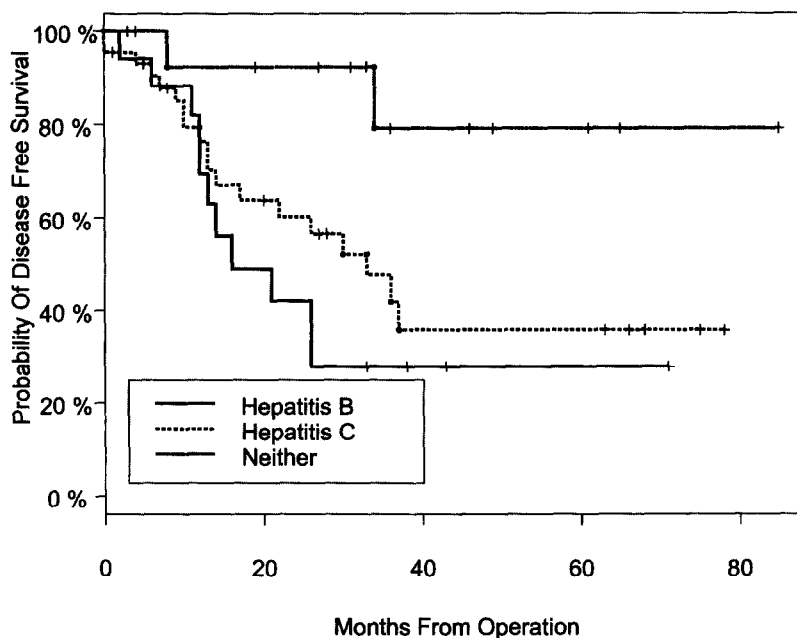
Fig. 1. Overall disease-free survival hepatitis status. There was a significant difference in disease-free survival between patients who were HBV or HCV positive vs. patients with negative hepatitis virus serology. The 5-year disease-free survival after resection of HCC in the HBV, HCV, and negative serology groups was 28%, 36%, and 79%, respectively (P <0.05 HBV and HCV groups vs. negative serology group).
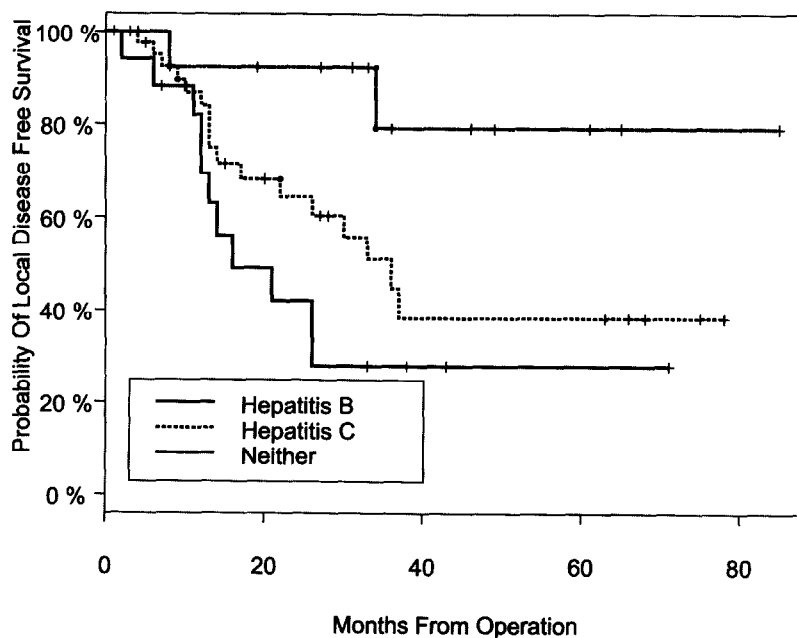
Fig. 2. Local disease-free survival by hepatitis status. Patients with chronic hepatitis B or C virus infections and HCC had LDFS rates of 28% and 38%, respectively, following hepatic tumor resection. This was a significant difference when compared to patients with negative serology (LDFS 79%; P <0.05).
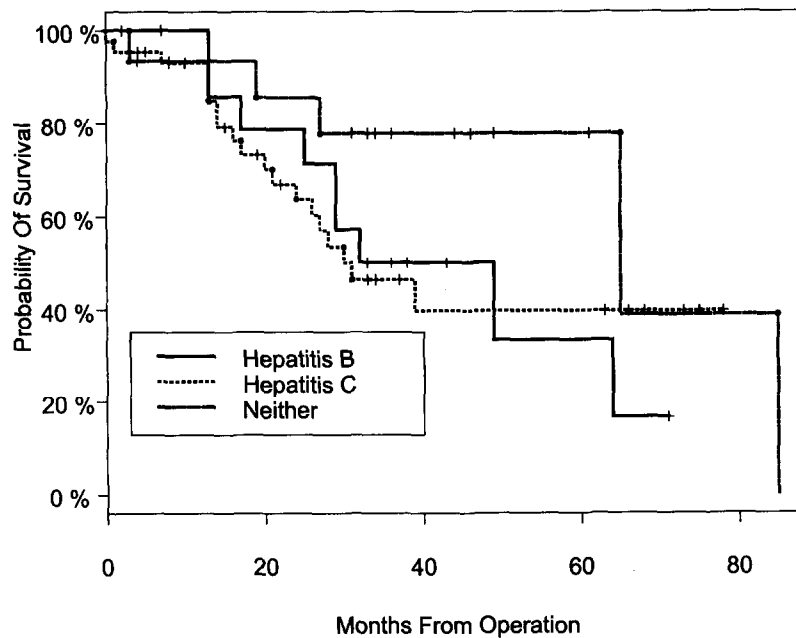
Fig. 3. Overall survival by hepatitis status. A trend toward a difference in overall survival rates was seen in the hepatitis virus–positive groups compared to the hepatitis virus–negative patients; however, this did not reach significance. The overall 5-year survival rates for the HBV, HCV, and negative serology groups were 34%, 40%, and 78%, respectively ($P = 0.12$, HBV- and HCV- infected vs. negative serology group).
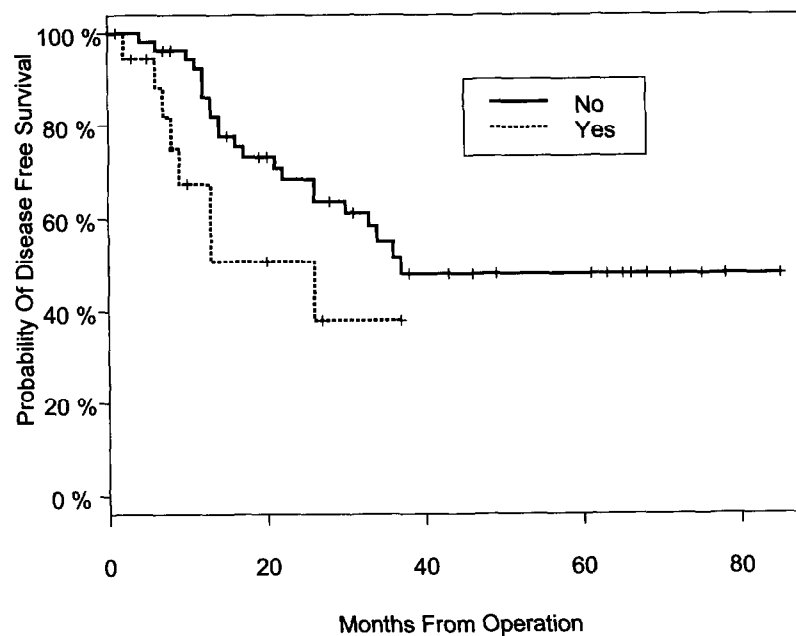
Fig. 4. Local disease-free survival by satellitosis. Univariate analysis determined satellitosis to be a significant predictor of local recurrence after resection of HCC ($P < 0.05$).
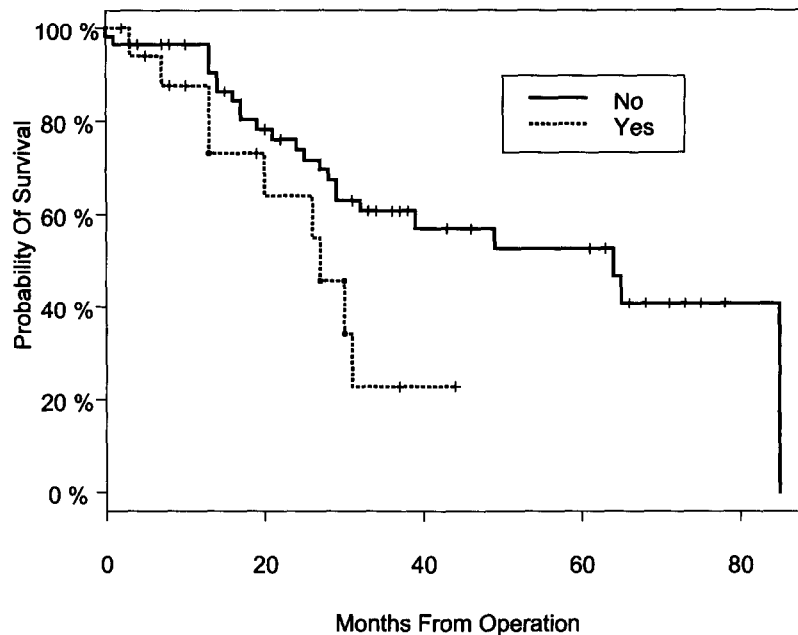
Fig. 5. Overall survival by satellitosis. Univariate analysis determined satellitosis to be a significant predictor of overall survival after resection of HCC (P <0.05).

Table III. Univariate analysis of local recurrence and overall survival after resection of hepatocellular carcinoma

Risk factor	Local recurrence (P value)	Overall survival (P value)
Age	0.50	0.75
Sex	0.77	0.77
TNM stage	0.23	0.78
Vascular invasion	0.21	0.29
Satellitosis	0.04	0.04

dian survival for patients with HBV, HCV, and negative serology was 29 months (range 2 to 71 months), 21 months (range 1 to 78 months), and 34 months (range 3 to 85 months), respectively. The overall 5-year survival rates for the HBV, HCV, and negative serology groups were 34%, 40%, and 78%, respectively (Fig. 3). There was no difference in DDFS among the three groups.

Univariate analysis was performed for local recurrence and overall survival (Table III). Factors including age, sex, TNM stage, vascular invasion, and satellitosis were analyzed. Only satellitosis was significantly related as a negative prognostic factor for local recurrence and overall survival (Figs. 4 and 5).

DISCUSSION

The tumorigenic mechanism of HBV infection leading to HCC has been proposed to be directly related to the chronic inflammatory process causing an increased rate of random mutations in hepatocytes.[20] Another possible mechanism is integration of double-stranded DNA proviral proto-oncogenes into the host genomic DNA resulting in alterations in cell cycle control, signal pathways, and apoptosis.[21] The oncogenic mechanisms of chronic HCV infection leading to HCC has not been clearly elucidated.

Traditionally, factors associated with prognosis in patients with HCC have included age, tumor size, tumor number, presence of vascular invasion, satellitosis, stage of disease, presence of cirrhosis, and positive margin of resection.[22-24] Ozawa et al.[17] showed increased patient survival rates when margins of resection were greater than 1 cm compared to margins less than 1 cm (77% vs. 21% 3-year survival, respectively). Yamanaka et al.[15] reported no 2- and 3-year survivors after surgical resection when portal or hepatic veins were involved with tumor invasion, compared to a 32% 5-year survival rate in patients with no vascular invasion. Finally, the Liver Cancer Study Group in Japan demonstrated improved survival rates in patients undergoing resection for solitary HCC and tumors less than 5 cm in diameter.[16]

Recently, a history of chronic HBV or HCV infection as a prognostic factor in HCC patients has been investigated. Unfortunately, long-term results after liver resection in patients with HCC and chronic hepatitis virus infection are inconsistent.[24-28] Yamanaka et al.[26] reported that patients with HBV-associated HCC have improved survival rates compared to patients with HCV-associated HCC (54% vs. 42% 5-year survival). Haratake et al.[24] found the opposite to be true. In their study, patients with HCV-associated HCC had improved 1-, 2-, and 3-year survival rates when compared to patients with HBV-associated HCC. Further confounding these results were claims by other investigators that long-term prognosis after resection of HCC is not influenced by hepatitis viral status.[25,27,28]

Our study represents the first Western report of hepatitis serology as a prognostic determinant for patients with HCC (Table IV). The significant finding in our study was that hepatitis status negatively influenced LDFS. Patients with HBV or HCV infection had a 28% and 38% LDFS, respectively; this was a significantly lower rate than was the case for patients with negative viral hepatitis serology, who demonstrated a 79% LDFS rate. Investigators from Asian centers have reported similar findings. Takenaka et al.[28] reported 5-year disease-free survival rates of 23% and 46% for HCV and HBV patients, respectively. Wu et al.[29] reported 5-year disease-free survival rate of 22% for HCV-associated HCC, 29% for HBV patients, and 66% for patients with negative serology.

One possible explanation for this phenomenon of decreased LDFS following resection of HCC in hepatitis virus-positive patients is the presence of residual tumor (i.e., positive margin); however, in our cohort, all patients had pathologically confirmed margin-negative resections with at least 1 cm tumor-free margin. Nagao et al.[30] proposed intrahepatic metastasis via the portal venous system to be the cause of intrahepatic recurrence. Others have proposed that the persistent inflammation related to chronic hepatitis virus infection and subsequent focal hepatic regeneration is the cause of carcinogenesis.[31] Finally, Ko et al.[32] proposed multicentric hepatocarcinogenesis as the primary etiology of hepatic recurrence after curative resection for HCC in hepatitis virus-positive patients. In this model, activated proto-oncogenes, such as c-jun or fos, cause multicentric disease that becomes manifest in the face of active hepatitis. In our series, the majority of recurrences were found in the segments near the surgical resection. This suggests that microscopic or subclinical satellitosis is likely a frequent occurrence in hepatitis virus-associated HCC, and thus pathologically tumor-negative margins alone are not sufficient to prevent recurrence near the area of hepatic resection.

In our series there was a trend toward worse overall survival in hepatitis virus-infected patients, although this trend did not attain statistical significance. Five-year survival rates for patients with HBV, HCV, and negative viral serology were 34%, 40%, and 78%, respectively. Because the median follow-up was only 30 months, the failure to demonstrate a significant difference was likely due to the fact that median survival has not yet been reached in this group of patients. Once again, our data are consistent with series reported from Eastern countries. Takenaka et al.[28] and Wu et al.[29] have reported 5-year survival rates of 64%

Table IV. Comparison of surgical outcomes in patients with hepatitis B- or C-virus-related hepatocellular carcinoma

Reference	Year	No. of patients	Hepatitis	5-year survival (%)	Disease-free survival (%)
Takenaka et al.[28]	1995	126	B	62	46
			C	53	23
Yamanaka et al.[26]	1997	202	Negative	64	34
			B	52	38
			C	42	10
Wu et al.[29]	1999	261	Negative	83	66
			B	57	29
			C	65	22
			B and C	40	36
Present study	2000	77	Negative	78	79
			B	34	28
			C	40	36

and 83%, respectively, for patients with negative hepatitis viral serology following hepatic resection for HCC. These investigators have also reported 5-year survival rates of 57% to 62% for HBV-associated HCC and 53% to 65% for HCV-associated HCC.

CONCLUSION

Our series demonstrates that patients with positive hepatitis B or C viral serology have an increased incidence of intrahepatic tumor recurrence after resection of HCC. Because of this increased likelihood of hepatic tumor recurrence, these patients also have a trend toward worse overall survival. Patients with chronic HBV or HCV infection undergoing surgical resection for HCC need to be carefully selected with regard to perioperative risk, functional hepatic reserve, and surgical indications. Clearly effective adjuvant therapy is needed for hepatitis virus–positive patients with HCC in order to improve outcome.

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Ultrasound-Guided Radiofrequency Thermal Ablation of Liver Tumors: Percutaneous, Laparoscopic, and Open Surgical Approaches

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Only 10% to 20% of patients with primary and colorectal metastatic liver tumors are candidates for curative surgical resection. Even after curative treatment, tumors recur commonly in the liver. As a less invasive therapy, radiofrequency thermal ablation (RFA) of primary, metastatic, and recurrent liver tumors was performed under percutaneous, laparoscopic, or open intraoperative ultrasound guidance. The safety and local control efficacy of RFA were investigated. RFA was performed mostly in patients with unresectable hepatomas or metastatic liver tumors. Patients with large tumors, major vessel or bile duct invasion, limited extrahepatic metastases, or liver dysfunction were not excluded. An RFA system with a 15-gauge electrode-cannula with four-pronged retractable needles was used. All patients were followed for more than 8 months to assess morbidity and mortality, and to determine tumor recurrence. Sixty RFA operations were performed in 46 patients: 11 patients underwent repeat RFA once or twice. A total of 204 tumors were treated: 70 hepatomas and 134 metastatic tumors. Tumor size ranged from 5 mm to 180 mm (mean 36 mm). RFA was performed in 29 operations for 81 tumors percutaneously, in seven operations for 14 tumors laparoscopically, and in 24 operations for 109 tumors by open surgery. Combined colorectal resection was carried out in five operations and combined hepatic resection was carried out in three operations. There was one death (1.7%) from liver failure, and there were three major complications (5%): one case of bile leakage and two biliary strictures due to thermal injury. There were no intra-abdominal infectious or bleeding complications. The length of hospital stay ranged from 0 to 2, 1 to 3, and 4 to 7 days for percutaneous, laparoscopic, and open surgical RFA, respectively. During a mean follow-up period of 20.5 months, local tumor recurrence at the RFA site was diagnosed in 18 (8.8%) of 204 tumors. The risk factors for local recurrence included large tumor size and major vessel invasion: recurrence rates for tumors less than 4 cm, 4 to 10 cm, and greater than 10 cm, and for those with vessel invasion were 3.3%, 14.7%, 50%, and 47.8%, respectively. Ten of 18 tumors recurring locally were retreated by RFA, and eight of them showed no further recurrence. Ultrasound-guided RFA is a relatively safe, well-tolerated, and versatile treatment option that offers excellent local control of primary and metastatic liver tumors. The appropriate use of percutaneous, laparoscopic, and open surgical RFA is beneficial in the management of patients with liver tumors in a variety of situations. (J GASTROINTEST SURG 2001;5:477-489.)

Surgical resection is the established curative treatment method for both primary and secondary malignant liver tumors. However, only 10% to 20% of patients with hepatocellular carcinoma or metastasis from colorectal cancer are candidates for a potentially curative resection. Resection is often not indicated, even for patients with liver-only malignant diseases, for various reasons including the presence of multiple bilobar tumors, invasion or close proximity of tumor to major vessels or bile ducts, limited liver func-

tion, or high surgical risk due to comorbidity. Moreover, tumor recurrence in the liver is common even after curative resection.

Nonresectional ablation therapy was initially used in hopes of achieving local control of unresectable liver tumors, and more recently its use was considered with curative intent. Chemical ablation, mainly ethanol injection, is effective for small hepatomas, but is not effective in large tumors or metastatic tumors. Cryoablation has been most extensively investigated as treatment for unresectable primary and secondary liver tumors in the United States.[1-5] Although less invasive than resection, cryoablation is associated with various complications and usually requires laparotomy. Local recurrence rates after cryoablation have been reported to range from 10% to 30%.[2-5]

Thermal ablation is a new local ablation modality, which has been increasingly investigated lately with advances in technology and development of special radiofrequency ablation devices.[6-9] In this study we report our initial experience with radiofrequency thermal ablation (RFA) of primary and metastatic liver tumors treated percutaneously, laparoscopically, or as open surgery in 60 operations. The safety in terms of complications of RFA and the efficacy in terms of local control or local recurrence after RFA are described. The advantages, disadvantages, and indications for percutaneous, laparoscopic, and open surgical approaches for RFA are summarized.

PATIENTS AND METHODS

Forty-six patients with histologically proved malignant primary and metastatic liver tumors who underwent a total of 60 operations for RFA from August 1997 to July 1999 were prospectively studied. Patients with extensive extrahepatic metastases were excluded, although some patients with limited extrahepatic metastases were included for treatment with RFA. Also excluded were patients with extensive liver tumors, that is, 15 tumors or more, or tumor involvement of more than 50% of the entire liver. Large tumor size, invasion, or proximity of tumor to major blood vessels or bile ducts were not considered contraindications for RFA in this study. Patients with liver dysfunction (e.g., Child class C cirrhosis) or with significant comorbidity were included as long as patients were preoperatively judged to be able to tolerate RFA treatment and anesthesia.

All patients had preoperative laboratory tests including liver function tests and tumor markers such as alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA), and imaging studies including computed tomography (CT) and percutaneous ultrasonography. Ultrasound examination was performed

in all patients by one of us (J.M.) and compared with CT scans to determine the optimal approach for RFA. Percutaneous, laparoscopic, or an open surgical approach was selected, depending on the number, size, and location of liver tumors, the accessibility (visualization) of tumors by percutaneous ultrasound, the need for precise staging by laparoscopy or open surgery, the presence or absence of synchronous primary cancer (e.g., colorectal cancer), newly diagnosed tumor or recurrent tumor, and the patient's condition with regard to surgical and anesthesia risks.

General anesthesia was usually used. Local anesthesia was used in selected patients undergoing percutaneous RFA who were at risk for general anesthesia or who required a short RFA period. The radiofrequency system (RITA Medical Systems, Mountain View, Calif.) used in this study consisted of a generator providing 460 kHz alternating current up to 50 watts, a 15-gauge cannula with four-pronged retractable curved electrode-needles, and a dispersive electrode pad. Under ultrasound guidance, a cannula was inserted into the tumor and four needles were deployed. The RFA process was monitored by continuous measurement of temperature by thermocouples located in the tips of four needles. The tissue impedance and the delivered energy were also recorded. The target temperatures at the needle tips were set at 100° C. When open surgical RFA was performed, hepatic inflow occlusion by the Pringle maneuver was used to facilitate temperature elevation when necessary. Once the temperatures approached 100° C, the ablation was continued for 5 to 10 minutes, depending on the size of the desired ablated lesion of 3 to 4 cm in diameter. For tumors smaller than 2 cm, usually one ablation session was used. Larger tumors required multiple overlapping ablation sessions at 2 to 2.5 cm intervals. Generally, two to eight ablation sessions were performed for tumors of 2 to 5 cm, and more than eight sessions were performed for tumors larger than 5 cm (Fig. 1). The RFA process was also monitored by intraoperative ultrasound imaging; the ablated lesion became hyperechoic because of outgassing from heated tissues (Fig. 2). If intraoperative color or power Doppler imaging demonstrated intratumoral blood flow prior to RFA, Doppler imaging was repeated after ablation to confirm loss of blood flow within the tumor. When the cannula was withdrawn at the completion of each ablation session, the cannula tract within the liver parenchyma was ablated to prevent bleeding and possible tumor seeding. For percutaneous and laparoscopic RFA, the cannula tract within the abdominal wall was also ablated.

Routine postoperative care was provided. A 1-week postoperative CT scan was obtained as a baseline to document the ablated lesions. CT scans were subse-
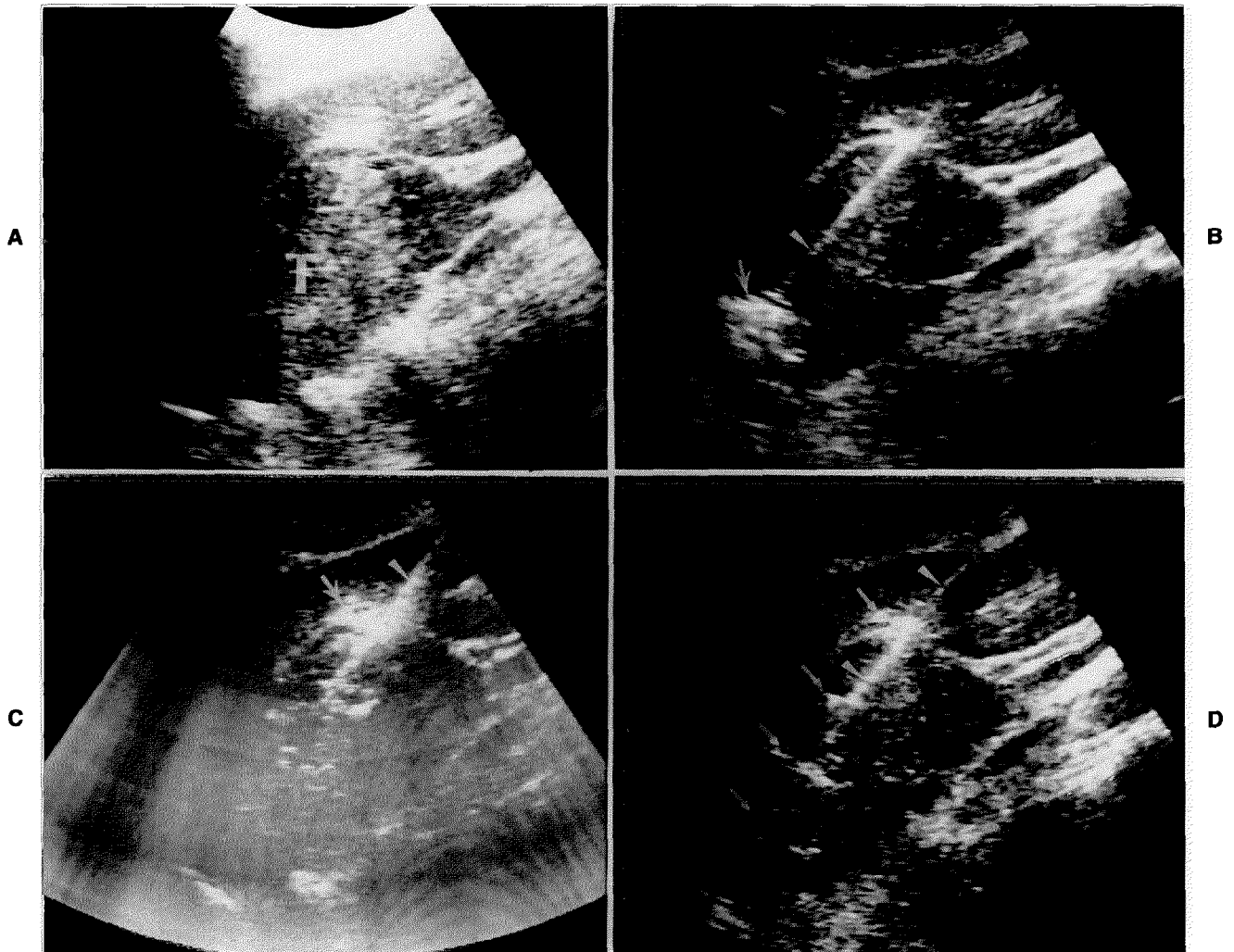
Fig. 1. Large hepatoma successfully treated by multiple sessions of percutaneous RFA. **A,** A 102 × 75 mm tumor *(T)* was present in segments 1 and 4, extending to segment 2, and invading the left hepatic vein. **B,** The most posterior-superior portion of the tumor was ablated first. Image shown is immediately after the first ablation session. The ablated lesion became hyperechoic (arrow). Arrowheads point to the electrode-cannula. **C,** On the same cannula tract, four overlapping ablation sessions were performed. This image was obtained during the fourth ablation session. **D,** Ablated areas after the fourth session. Four ablated lesions are indicated by arrows. This tumor was treated with a total of 22 sessions. At 24 months postoperatively, there was no local recurrence.
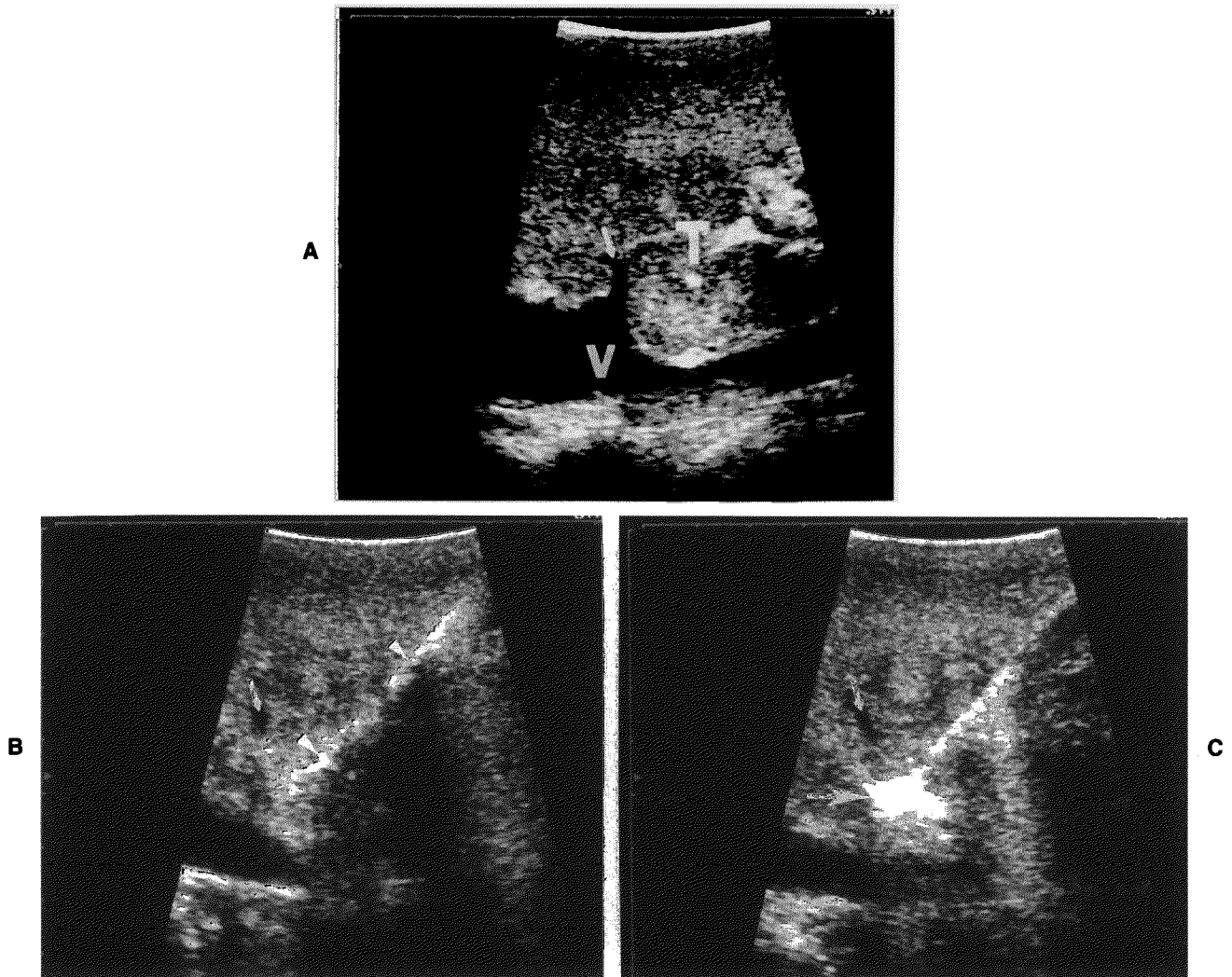
Fig. 2. Metastatic tumor from a sigmoid colon cancer treated by open surgical RFA. A, A 63 × 56 mm tumor (T) was present in segments 1 and 4 extending to segment 5. The tumor was involving the middle hepatic vein (arrow) and was likely invading the right hepatic vein and the vena cava (V). B, The electrode-cannula (arrowheads) was inserted into the posterior portion of the tumor. C, After ablation of this portion, the ablated lesion became hyperechoic (large arrow). Small arrow points to middle hepatic vein. Despite multiple sessions of ablation treatment, this tumor showed signs of local recurrence at 6.5 months postoperatively.
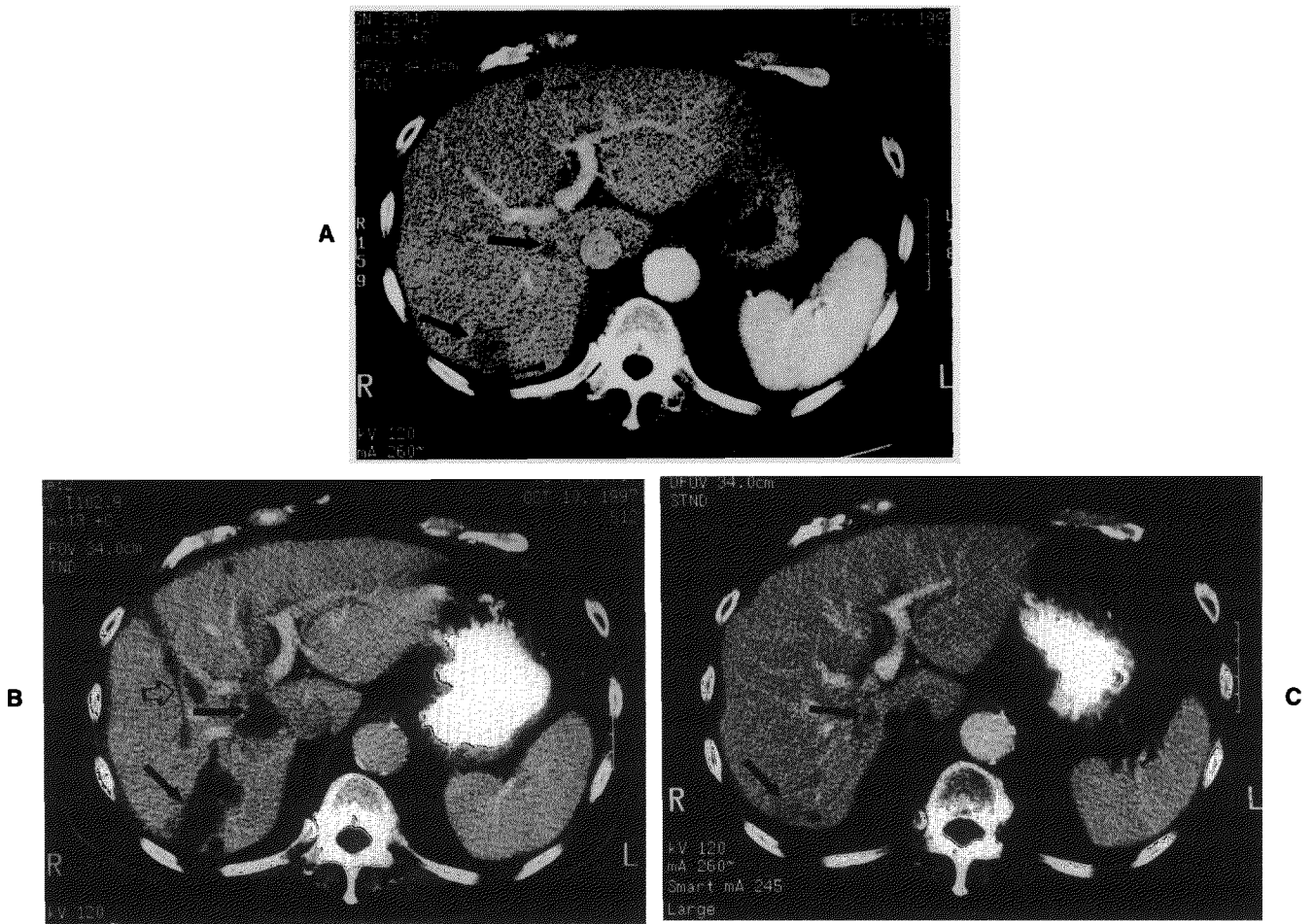
Fig. 3. A, Two metachronous metastatic tumors from an ascending colon cancer. Preoperative CT shows two tumors (arrows) and two cysts (arrowheads). One tumor was 2 × 3 cm on CT and located near the posterior surface of segment 7, and one tumor was 1 cm and located deep in segment 1 near the vena cava. **B,** CT scan 1 week after RFA. Ablated lesions (arrows) were larger than the original tumors. This shows complete ablation of the deep tumor in segment 1, but only shows an inferior part of ablation of the tumor in segment 7. Ablations of cannula tracts (open arrow) were also seen. **C,** CT scan 9 months after RFA. Both ablated lesions (arrows) became small with no local tumor recurrence.

quently performed at 3-month intervals for at least 1 year and at 6-month intervals thereafter. If preoperative serum tumor markers were elevated, they were also measured postoperatively. Whenever the tumor marker that decreased soon after RFA was reelevated during a follow-up period, metastatic workup studies including CT of the liver were conducted. To evaluate the local recurrence of tumor (i.e., recurrence at the site of ablated tumor due to RFA treatment failure), all patients were followed for at least 8 months or until death. Therefore all surviving patients had at least three (1-week, 3-month, and 6-month) postoperative CT scans. At 1 week postoperatively, the successfully ablated lesions appeared larger than the original tumors. During follow-up, the successfully ablated lesions progressively decreased in size on CT

scans (Fig. 3). Local recurrence was diagnosed when CT scans demonstrated an increase in size or change in CT contrast-enhanced appearance characteristic of original malignant tumors. When local recurrence could not be determined by CT alone or there was a discrepancy between CT findings and tumor markers, gallium scan (for hepatomas) or positron emission tomography (PET) (for colorectal metastatic tumors) was used to evaluate local recurrence or new recurrence.

RESULTS

A total of 60 RFA operations were performed in 46 patients: eight patients underwent repeat operations once and three patients underwent repeat operations twice for recurrent liver tumors during the study pe-

Table I. Liver tumors treated by radiofrequency thermal ablation

Pathology	No. of patients	No. of operations	No. of liver tumors
Hepatoma	18	24	70
Colorectal cancer metastasis	25	32	130
Gastric cancer metastasis	1	2	2
Ovarian cancer metastasis	1	1	1
Leiomyosarcoma metastasis	1	1	1
TOTAL	46	60	204

Table II. Indications for radiofrequency thermal ablation

Indications	No. of operations for hepatoma*	No. of operations for metastatic tumors*
Not candidates for resection		
Not resectable (multiple, vessel invasion)	13	18
Extrahepatic metastasis	2	6
High risk for resection	8	6
Combined major colorectal resection	0	4
Patient preference	1	2

*Only the main reason was noted for each operation, although many patients had multiple conditions precluding hepatic resection.

riod. There were 26 men and 20 women. Ages ranged from 40 to 83 years, with a mean age of 66.3 years. Twenty-four operations were performed in 18 patients with hepatomas (total 70 tumors) and 36 operations were performed in 28 patients with metastatic tumors (total 134 tumors), as summarized in Table I. The number of tumors treated by RFA per operation ranged from 1 to 14: 2.9 tumors per operation for hepatomas and 3.7 tumors per operation for metastases. The size (the longest axis) of the tumors ranged from 5 mm to 180 mm, with a mean of 36 mm (40 mm for hepatomas and 34 mm for metastases): 123 tumors were smaller than 4 cm, 75 tumors were 4 to 10 cm, and six tumors were larger than 10 cm. Twenty-three of a total of 204 tumors exhibited invasion to or were abutting major vessels (the first and second branches of the portal vein or major branches of the hepatic veins). Among patients with hepatomas, eight patients (12 operations) were in Child class A, seven patients (7 operations) were in Child class B, and three patients (5 operations) were in Child class C. All patients with metastases were in Child class A.

Indications for RFA in the 60 operations are summarized in Table II. In the majority of the operations, patients were considered not to be candidates for surgical resection for a variety of reasons. Approximately one half of the patients were unresectable because they had multiple (more than 4) bilobar tumors or

major vessel invasion of tumors. Three patients had potentially curative resectable tumors; however, they refused hepatic resection and opted for percutaneous or laparoscopic RFA after the benefits and risks of each operation had been fully explained to them and they were informed of the unavailability of long-term results of RFA.

Twenty-nine RFA operations as treatment for 81 tumors were performed percutaneously (2.8 tumors per operation), seven operations for 14 tumors were performed laparoscopically (2 tumors per operation), and 24 operations for 109 tumors were performed as an open procedure (4.5 tumors per operation). During open surgical RFA, other major organ resections were performed in 11 operations. In five operations, colorectal resections for the primary cancer were performed together with RFA for synchronous liver metastases. In three operations, major hepatic resections were combined with RFA for remaining tumors. In two operations, metastasectomies for lung metastatic tumors were performed with RFA for liver tumors. In one operation, combined partial gastrectomy was performed with RFA for liver and splenic metastatic tumors. Six percutaneous operations were performed under local anesthesia, and 54 operations were performed under general anesthesia.

Transient fever and leukocytosis were seen in many patients, usually in proportion to the extent of ablated

Table III. Complications of radiofrequency thermal ablation of liver tumors

Complications	Hepatoma	Metastasis	Total
Death			
Liver failure	1	0	1
Major complications			
Bile leak	0	1	1
Bile duct thermal injury (biliary stricture)	0	2	2
Minor complications			
Skin burn	1	1	2
Wound infection	0	1	1
Thrombocytopenia	1	2	3
Myoglobinuria	2	2	4
Pacemaker malfunction	1	0	1
Congestive heart failure	1	0	1
Total	7	9	16

No bleeding or abdominal abscess-infection.

lesions and ablation time. Similarly, liver function tests including bilirubin were elevated for a few days to several weeks, depending on the patient's baseline liver function and the extent of ablation. Six patients with hepatomas had new onset of ascites; however, it resolved with recovery of liver function except in one patient who developed progressive liver failure. Asymptomatic pleural effusions were observed in seven patients, associated with ascites or after RFA of subdiaphragmatic tumors.

Table III summarizes the complications of RFA in this study. There was one death (1.7%). This patient had a 180 × 115 mm tumor in the right lobe, and a poorly differentiated carcinoma was suspected based on preoperative biopsy. Because he responded to initial chemotherapy with decreasing tumor marker (AFP) levels, percutaneous RFA was performed for the purpose of cytoreduction. Although his AFP levels further decreased, he had progressive liver failure and died on postoperative day 36. An autopsy demonstrated additional multiple small bilobar tumors along with extrahepatic metastases, and the final histologic diagnosis was hepatoma. There were three major complications (5.0%), which required further interventions or caused long-term sequelae. A bile leak due to direct injury of the left hepatic duct by a cannula occurred in one operation, and required prolonged percutaneous drainage and placement of an endoscopic biliary stent. Segmental and lobar biliary stricture due to thermal injury of the intrahepatic bile duct occurred in two operations (Fig. 4). One patient needed stent placement at 4 months postoperatively. In nine operations, there were 12 minor complications, which did not result in major or long-term problems. Two 2 × 1 cm second-degree skin burns occurred at the edge of an electrode pad placed on the
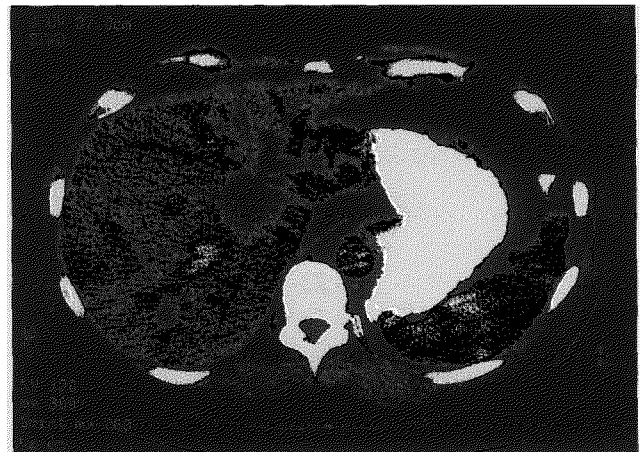
Fig. 4. Original colon cancer metastatic tumor was located in segment 4, extending to segments 5 and 3, and was invading the left portal vein. CT scan 3 months after RFA shows segmental bile duct dilatations (arrowheads) of the left lobe (due to biliary stricture likely caused by bile duct thermal injury) in addition to the ablated lesion (arrow). Ablations of another small lesion and a cannula tract (open arrows) are also seen in the right lobe.

thigh and on the back. They healed with nonoperative management. Thrombocytopenia (platelets <50,000, excluding patients with preexisting thrombocytopenia due to cirrhosis and hypersplenism) and/or gross myoglobinuria (grossly red-colored urine and myoglobin positive by urine analysis) were encountered in five operations. In these five operations, RFA was performed for more than 240 minutes: two operations in which the ablation time was longer than 580 minutes had both complications. With appropriate manage-

ment, myoglobinuria did not result in renal failure. In the present study, there were six other operations with an RFA time exceeding 240 minutes, which were not associated with thrombocytopenia or gross myoglobinuria. Three patients in this study had a pacemaker, and in one operation, RFA caused a pacemaker to malfunction, requiring reprogramming postoperatively. There were no cases of intrahepatic or intraperitoneal bleeding or infectious complications such as abscesses.

The length of hospital stay was 0 to 2 days for percutaneous RFA, 1 to 3 days for laparoscopic RFA, and 4 to 7 days for open surgical RFA, with the exception of the following: one thoracotomy for lung metastasectomy (9 days), one rectosigmoid colon resection (10 days), one bile leak (15 days), and one death (36 days).

Tumor markers (AFP or CEA) were preoperatively elevated in 43 operations. After RFA, there was a reduction from preoperative levels in 40 operations (93%). Tumor markers did not decrease in two operations for patients with extrahepatic metastases and in one operation for a patient who had extensive early intrahepatic recurrence.

The postoperative follow-up period was 8 to 32 months with a mean of 20.5 months. Local tumor recurrence (due to RFA failure) was noted in 14 operations (23.3% of 60 operations). A total of 18 local recurrent tumors were diagnosed, representing 8.8% of 204 tumors treated by RFA. Twelve recurrent tumors were diagnosed by CT and 6 tumors were diagnosed by PET. The characteristics of these tumors are shown in Table IV. Tumor size and major vessel invasion appeared to be the main risk factors for local recurrence due to treatment failure. Whereas small tumors (<4 cm) had a low recurrence rate (3.3%; 4 of 123), larger tumors were associated with higher recurrence rates: 14.7% (11 of 75) for tumors 4 to 10 cm in size and 50% (3 of 6) for tumors larger than 10 cm. The average size (the mean of the longest axis) of the tumors with local recurrence was 68.4 mm. Among 23 tumors with major vessel invasion in this study, 11 (47.8%) showed local recurrence (see Figs. 1 and 2). Metastatic tumors seemed to be associated with high local recurrence compared to hepatomas. The method of approach for RFA did not affect the local recurrence rate. Most local tumor recurrence was noted within 6 months postoperatively; however, some recurrent tumors were diagnosed late during the follow-up period. The time interval from RFA treatment to diagnosis of recurrence ranged from 1 week (obvious incomplete ablation on a 1-week postoperative CT scan in 1 patient) to 15 months with a mean of 5.1 months. Ten of 18 recurrent tumors were retreated by repeat RFA. Eight reablated tumors had no evidence of further recurrence, whereas two reablated

Table IV. Local tumor recurrence after radiofrequency thermal ablation (18 tumors in 14 patients)

	No. of liver tumors with recurrence/total	(% of total)
Pathology		
Hepatoma	3/70	(4.3)
Colorectal metastasis	12/130	(9.2)
Other metastasis	3/4	(75)
Location		
Right lobe*	2	
S_7	5	
S_4	3	
S_5, S_6, S_8	2 each	
S_1, S_3	1 each	
Size		
<4 cm	4/123	(3.3)
4-10 cm	11/75	(14.7)
>10 cm	3/6	(50)
(mean size of tumors with recurrence 68.4 mm)		
Major vessel invasion	11/23	(47.8)
RFA approach		
Percutaneous	8/81	(9.9)
Laparoscopic	0/14	(0)
Open surgical	10/109	(9.2)
Recurrence interval		
≤3 mo	8	
3-6 mo	4	
6-12 mo	4	
>12 mo	2	
Repeat RFA		
Performed	10	
(8 no further recurrence)		
Not performed	8	

S = segment; RFA = radiofrequency thermal ablation.
*Tumors extending into more than two segments of the right lobe were included.

tumors (both with major vessel invasion) recurred again. There was no recurrence due to tumor seeding in the abdominal wall where the cannula was introduced during percutaneous and laparoscopic RFA.

Among 46 patients, 17 patients (37.0%) were alive during the follow-up period without any cancer recurrence after the first or a single RFA operation (Table V). The remaining 29 patients (63.0%) had a new recurrence (not at the site of RFA treatment), local liver tumor recurrence (at the RFA site), or both. New recurrence or metastasis was noted in the liver and/or at extrahepatic organs (e.g., lung, bone, brain, intraperitoneum) in 21 patients (45.7%): six of these patients also had local liver tumor recurrence. After repeat RFA for new recurrence or local tumor recurrence (performed in 11 patients), eight patients (17.4%) were alive without further recurrence (see

Table V. Outcome of patients after first and repeat radiofrequency thermal ablation

	No. of patients	(%)
Alive without recurrence after first RFA	17	(37.0)
Alive without recurrence after repeat RFA	8	(17.4)
Alive with recurrence after first or repeat RFA	11	(23.9)
Died of cancer	9	(19.5)
Died of cirrhosis (no recurrence after repeat RFA)	1	(2.2)
TOTAL	46	(100)

Table V). Eleven patients (23.9%) were alive but with new or local recurrence. Ten patients (21.7%) died during the follow-up period, including one whose death was attributed to the operation itself: three patients with hepatomas and seven patients with metastases. The cause of death was liver failure due to progression of liver tumors in four patients, cirrhosis in one patient (without tumor recurrence), and failure of other organs due to extrahepatic spread of cancer in five patients. Among the three patients who preferred and underwent RFA for resectable tumors, two were alive without recurrence at 26 months and 28 months postoperatively, and one patient was alive with a new recurrence (lung metastases) at 12 months postoperatively.

DISCUSSION

Thermal ablation of liver tumors can be achieved by a variety of methods such as microwave coagulation, laser hyperthermia, high-intensity focused ultrasound, and RFA. Radiofrequency energy has long been used to ablate or coagulate small areas as electrocautery. Recently, advancement in radiofrequency technology and the introduction of newly designed devices have allowed large areas of the liver to be ablated. The initial large clinical experience with RFA was reported by Rossi et al.[10,11] in Italy. They used a percutaneous RFA technique mainly for treatment of hepatomas including resectable tumors. Their results in 39 patients with small hepatomas were encouraging: only one minor complication, 5% local recurrence, and 40% 5-year survival. Over the past few years, in the United States and Europe, several investigators have reported their preliminary results with RFA, with yet relatively short-term follow-up, mostly for unresectable primary and secondary liver tumors (Table VI).[12-20] Overall, the complication rates were less than 5% to 10%: major complications were rare

Table VI. Literature on radiofrequency thermal ablation of liver tumors

Reference	Year	Patients (operations)	Tumors	Pathology	Approach	Complications	Local recurrence (incomplete ablation)*	Mean or median follow-up
Rossi et al.[10]	1996	39	41	H	P	None	2/41 of tumors (4.9%)	22.6 mo
Siperstein et al.[12]	1997	6	13	M	L	None	0%	(1 wk-15 mo)
Solbiati et al.[13]	1997	29	44	M	P	1/29 (3.4%)	15/44 tumors (34.1%)	10.3 mo
Scudamore et al.[14]	1999	10	12	M	P	None	1/9 tumors (11.1%)	(resection at 6 wk)
Cuschieri et al.[15]	1999	10	34	M, H	L	None	2/34 tumors (5.8%)	(6-20 mo)
Allgaier et al.[16]	1999	12	13	H	P	None	2/13 patients	143 days
Jiao et al.[17]	1999	35	—	M, H	O, P	6/35 (17%)	11/35 patients	8.5 mo
Curley et al.[18]	1999	123	169	M, H	O, P	3/123 (2.4%)	3/169 tumors (1.8%)	15 mo
Bilchik et al.[19]	1999	50	132	M, H	P, L, O	2/50 (4%)	3/132 tumors (2.3%)	6 mo
Siperstein et al.[20]	2000	43	181	M, H	L	—	22/181 tumors (12.2%)	13.9 mo
Machi et al. (present study)		60	204	M, H	P, O, L	4/60 (6.7%)	18/204 tumors (8.8%)	20.5 mo

H = hepatoma; M = metastasis; P = percutaneous; L = laparoscopic; O = open surgical.
*Local recurrences include incomplete ablation diagnosed at the time of ablation, by immediate postoperative CT, or by histologic examination in addition to local tumor recurrence diagnosed during the follow-up period.

and included bleeding, infection (abscess), and visceral injury. There were no previously reported deaths directly related to the RFA treatment itself. The rates of local tumor recurrence or incomplete ablation were generally reported to be less than 10% on tumor-by-tumor (not patient-by-patient) analysis. In one study by Solbiati et al.,[13] the local recurrence rate was 34% for tumors treated by RFA. However, in recent studies in a large number of patients and tumors by Curley et al.[18] and Bilchik et al.,[19] the recurrence rates were 1.8% and 2.3%, with median follow-up periods of 15 months and 6 months, respectively.

In the present study, patients with varying conditions including more extensive tumors and high surgical risk underwent RFA treatment. The average tumor size and the average number of tumors ablated in each operation were greater than in previous studies. The main objective of this study was to determine the safety and the local control efficacy of RFA using various ultrasound-guided approaches. Although intra-abdominal bleeding or infectious complications were absent, there were four major complications. One death from liver failure was the result of underestimation of the extent of the hepatoma (overestimation of the remaining liver function) and too extensive RFA treatment. One bile leak was caused by direct intrahepatic duct injury by a cannula, which was recognized during open surgical RFA. Two bile duct thermal injuries resulting in biliary stricture, which were recognized during the follow-up period, were caused by RFA of centrally located tumors (in segments 1, 4, and 5). Thrombocytopenia and myoglobinuria, well-known complications of cryoablation but not previously documented in RFA, were encountered in some patients who underwent extensive RFA in this study. Pacemaker malfunction occurred during one operation soon after the onset of RFA.

The local tumor recurrence rate in this study during a mean follow-up period of 20.5 months was 8.8%. Large tumor size and major vessel invasion were the greatest risk factors for local recurrence due to RFA treatment failure. Larger tumors required more overlapping RFA sessions, thereby increasing the likelihood of incomplete ablation. Although the recurrence rate was only 3.3% for tumors smaller than 4 cm, it was 50% for tumors larger than 10 cm. Complete ablation of tumors larger than 10 cm was deemed difficult to achieve with the current RFA devices. Higher local recurrence rates were also expected for tumors adjacent to or invading major vessels because of the heat-sink effect. Although half of such tumors recurred, it is noteworthy that the remaining one half of these tumors did not show signs of recurrence. In addition, aggressive RFA treatment of these tumors in which electrode-needles were positioned close to major vessels did not cause vascular complications such as bleeding or thrombosis. The method of approach for RFA was unlikely to be a causative factor in local recurrence as long as tumors were visualized and the electrode-cannula was introduced appropriately under ultrasound guidance. The lower incidence of local recurrence with the use of laparoscopic RFA in this study is due to the ablated tumor selection (i.e., smaller tumor size and fewer tumors per operation). In addition to tumor markers and CT, PET was helpful in diagnosing recurrence in this study.

It was not the intent of the present study to determine long-term survival or mortality in patients treated by RFA. Several patients already had extrahepatic metastases at the time of RFA. During follow-up, 21 (45.7%) of 46 patients were diagnosed with new recurrence in the liver or other organs, much higher than the rate of local liver tumor recurrence. However, 11 selected patients with new or local recurrent tumors in the liver were able to be treated by repeat RFA. Eight of these patients, who underwent repeat RFA once or twice, were alive without further recurrence. With longer follow-up, the incidence of such new recurrent tumors is anticipated to rise. Therefore the long-term outcome in patients who have undergone RFA depends more likely on the patient inclusion criteria and the tumor response to multimodality treatment. Patients with resectable liver tumors would have a better prognosis after RFA, as seen in three patients in this study.

RFA has several advantages over cryoablation. The complication rate for RFA (less than 10%) appears to be less than that for cryoablation, which has ranged from 10% to 25%.[1-5, 21-23] Cryoablation is known to be associated with a variety of complications including myoglobinuria with renal failure, hypothermia, coagulopathy, thrombocytopenia, freezing injury to abdominal viscera, liver parenchymal fracture, abscess, and direct impalement of major vessels or bile ducts leading to significant bleeding, bile leakage, or fistula. Serious morbidity, referred to as cryoshock, has been observed in 1% of operations.[23] Mortality rates have ranged from 0% to 4%.[1-5,21,23] On the other hand, infectious and bleeding complications are possible but rare with RFA. Thrombocytopenia, myoglobinuria, and liver failure can occur after extensive RFA treatment, as was seen in our patients, but are uncommon. The ability of local tumor control using RFA is at least similar or perhaps even superior to that of cryoablation. The rate of local tumor recurrence for cryoablation has been 10% to 30%,[2-5] whereas that for RFA in most of the recent studies has ranged from a few percent to 15%.[10-20] Even with longer follow-up, the local recurrence rate for RFA will be expected

to be less than 10% to 20%, although it will vary depending on the size and location of tumors subjected to RFA treatment. The important advantage of RFA is the availability of different approaches applicable for RFA. Cryoablation still requires open surgery in general, although laparoscopic and percutaneous cryoablation devices have been investigated.[24-26] On the other hand, percutaneous and laparoscopic RFA is possible and plays an important role in selected patients as a less invasive approach with faster recovery. RFA does not require more than one ablation cycle, such as freeze–thaw cycles of cryoablation, to create one ablation lesion at one location. Therefore particularly small tumors that do not require multiple overlapping ablation sessions can be ablated more rapidly by RFA.

Current RFA has certain limitations or disadvantages. The device used in this study creates an RFA lesion of up to approximately 4 cm in diameter, which is smaller than cryoablation lesions currently in use. Therefore, for treatment of large tumors, more multiple overlapping RFA sessions are required, which increases the risk of incomplete ablation and local recurrence. Multiple overlapping RFA is time consuming. Newer devices such as longer and more retractable needles or cooled-tip electrodes have been under investigation to achieve larger ablation areas.[7,13] Although the ablated lesion becomes hyperechoic during the RFA process, the margin of ablation is not always distinct on real-time ultrasound imaging. In addition, when a part of a large tumor is ablated, the ultrasound appearance of the tumor becomes obscured, making subsequent ultrasound imaging and monitoring increasingly difficult. Although the RFA procedure is best guided by real-time ultrasound, immediate intraoperative assessment of the completeness of RFA can be difficult, unlike surgical resection in which the margin of resection can be examined intraoperatively. As with all ultrasound procedures, ultrasound-guided RFA techniques are highly operator dependent. There is a definite learning curve for mastering the RFA procedure, and this is especially true for surgeons unfamiliar with ultrasound imaging and guidance. Although RFA is safer than surgical resection or cryoablation, RFA can be associated with serious complications such as bile duct injuries. RFA of tumors adjacent to the major intrahepatic bile ducts must be avoided or performed with caution.

There are advantages and disadvantages to each ultrasound-guided approach. The advantages of percutaneous RFA include the fact that it is the least invasive, it can be performed as an outpatient procedure, the hospital stay is shorter, and the ultrasound guide system is readily available, which allows precise RFA cannula placement. Patient satisfaction and acceptability are high with quick recovery and minimal post-operative pain. The disadvantages of percutaneous RFA are as follows: less accuracy in cancer staging with the possibility of missing small or occult tumors, the presence of ultrasonically inaccessible areas in certain patients, and possible burn injury in adjacent organs. Small tumors in the superior areas of the liver (usually segments 7 or 8) may not be delineated by percutaneous ultrasound. CT or magnetic resonance imaging (MRI)–guided percutaneous RFA has been described[27] but is often time consuming. The advantages of laparoscopic RFA include less invasiveness with a shorter hospital stay, better cancer staging with laparoscopy and laparoscopic ultrasound, and avoidance of adjacent organ injury by laparoscopically separating organs. Preoperatively unrecognized liver tumors or cancer spread can be diagnosed, and surgical management may be altered by laparoscopic approach. The disadvantages of laparoscopic RFA include technical difficulty in treating deeply situated tumors and possible conversion to open surgery. Currently, no laparoscopic ultrasound guide system for cannula insertion is available. Therefore accurate placement of the RFA cannula under laparoscopic ultrasound guidance into tumors, especially deep tumors in segments 6, 7, or 8, is technically demanding and at times impossible; successful laparoscopic RFA is highly surgeon dependent. As with all laparoscopic procedures, conversion to an open surgical approach is sometimes necessary because of significant adhesions from previous operations or inability to place the cannula appropriately. The advantages of open surgical RFA include better cancer staging with open intraoperative ultrasound, availability of an intraoperative ultrasound guide system, accessibility to tumors in all areas of the liver, avoidance of adjacent organ injury, the ability to perform hepatic inflow occlusion, which reduces the heat-sink effect, and the possibility of combining RFA with hepatic resection or other organ (e.g., colorectal cancer) resection. As is the case with laparoscopic ultrasound, occult liver tumors are often diagnosed with open intraoperative ultrasound. No matter where tumors are located, all detected tumors can be ablated with the use of intraoperative ultrasound guidance. The main disadvantages of open surgical RFA are its invasiveness with increased surgical risks and the longer hospital stay and recovery time.

There are indications with each ultrasound-guided approach for RFA that are based on these advantages and disadvantages. Percutaneous RFA is indicated for patients at high risk for laparoscopic or open surgery. It is also indicated when RFA is performed for palliation (e.g., pain control), to prevent rapidly growing tumors from leading to liver failure, or to prolong life, for example, in patients with associated extrahepatic metastases. For patients with new or local liver tumor

recurrence after previous liver resection or ablation, percutaneous RFA is preferable because of the higher likelihood of future recurrence. In this situation, repeat percutaneous RFA is minimally invasive, well tolerated, and accepted by patients. Last, patients who refuse hepatic resection can be candidates for percutaneous RFA after they are made fully aware of the benefits and risks of surgical resection and ultrasound-guided RFA. Percutaneous RFA is contraindicated when tumors are situated in close proximity or in contact with other organs, mainly the gastrointestinal tract. Laparoscopic or open surgical RFA is indicated more for potentially curative intent. Laparoscopic or open intraoperative ultrasound examination is indispensable when surgical treatment of liver tumors is performed with curative intent. When tumors are relatively small, when the number of tumors is limited, and when tumors are not deep or inaccessible, laparoscopic RFA is indicated. Because laparoscopic ultrasound guidance of RFA is highly operator dependent, the indications for laparoscopic RFA would expand with increased experience and skill of the laparoscopic surgeon.[12,15,20] Open surgical RFA is indicated for patients who are suitable for open surgery with large, numerous, or deeply located tumors that cannot be accurately accessed using a laparoscopic approach. Furthermore, when patients have synchronous liver metastases, open surgical RFA can be performed in conjunction with resection of the primary cancer. For example, during operations for colorectal cancer, although major hepatic resection is usually not performed, RFA can be performed even when tumors are large or there are multiple tumors.

Although the goal of treatment in patients with primary and secondary liver tumors is to achieve long-term disease-free survival, many patients cannot be cured by local tumor control alone. A multimodality approach including systemic and regional treatment is necessary. Surgical resection with regional chemotherapy such as hepatic infusion chemotherapy has provided certain survival benefits. Similarly, RFA should be investigated in conjunction with other modalities.[17,19,28] Because of minimal morbidity, less invasiveness, availability of different approaches, and encouraging local control capability, as shown in the present study and other recent studies, RFA will have wider indications than surgical resection or cryoablation. RFA used as a primary or adjunctive treatment method will allow more options for treating liver tumors that have been previously considered unresectable. RFA may increase the number of patients who undergo potentially curative operations by itself or by combining with surgical resection; for example, a resection of a large tumor with RFA of remaining

multiple smaller tumors, or pulmonary metastasectomy with RFA of multiple liver tumors. Because of minimal invasiveness and morbidity, it will be possible to perform RFA more frequently than other local treatment methods for the purpose of palliation or cytoreduction.

CONCLUSION

Ultrasound-guided RFA is a relatively safe, novel modality that achieves excellent local control of liver tumors. Percutaneous, laparoscopic, and open surgical RFA approaches have advantages and disadvantages, and the appropriate use of each is beneficial for patients in different situations of primary, secondary, or recurrent liver tumors. Tumor recurrence in the liver is common for both primary and secondary tumors, even after potentially curative treatment. For management of such recurrent liver tumors, easily repeatable and minimally invasive local control therapy is desired, and RFA can be such a therapy. RFA alone or in combination with other locoregional or systemic treatment has the potential to improve local liver tumor control and to increase surgical resectability, and may result in a survival benefit, although this has not yet been proved. The encouraging results of the present study as well as other recent studies justify further clinical use and investigation of RFA.

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Alterations in Intrahepatic Hemodynamics of the Harvested Porcine Liver

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Hemodynamic properties of a donor liver, during initial reperfusion, are associated with the degree of graft preservation injury and have been proposed to correlate with subsequent markers of liver function. In the present study, hepatic hemodynamics, that is, portal venous pressure, hepatic vascular resistance, and compliance (vascular distensibility), were characterized (1) in situ before porcine livers were manipulated, (2) after these same livers were isolated and perfused within a bypass circuit, and (3) on reperfusion after 2 hours of cold ischemia. Hepatic vascular resistance was determined in each of these three states from the portal vein pressure response to differing hepatic blood flows. In addition, the response of the same livers to norepinephrine and nitroprusside was evaluated in each condition. In the in situ and isolated perfused liver, portal venous pressure increased only modestly despite doubling of hepatic flows. After cold ischemia, the pressure response to higher flows was significantly greater and much less of a reduction in hepatic vascular resistance was noted than in studies prior to cold ischemia. Unlike livers prior to cold ischemia, the pressure response to norepinephrine was attenuated following cold ischemia. The response to nitroprusside, however, remained intact reducing the portal pressure to that of in situ livers. Therefore the portal hypertension that follows cold ischemia appears to be largely provoked by the preservation injury and not by surgical manipulation or the bypass circuit. This increment in portal pressure is responsive to a nitric oxide donor. (J GASTROINTEST SURG 2001;5:490-498.)

KEY WORDS: Ischemia-reperfusion, compliance, resistance, nitric oxide, norepinephrine, hemodynamics

The harvested organ undergoes several types of injuries that produce pathologic damage that can result in universal early postoperative graft malfunction or loss.[1] These events, termed "preservation injury," begin prior to the actual harvest and include damage from loss of neurologic input, surgical manipulation, warm ischemia, cold ischemia, and reperfusion. Although each injury exerts differing effects on the organ, together they act to severely alter the gross and microscopic appearances of the organ as well as impair organ function.[2]

Most functional alterations that occur after liver harvest for transplantation or ex vivo support are reversible. The alterations can affect bile formation,[3] response to insulin,[2] lipoprotein metabolism,[4] and protein synthesis.[5] With severe injury to the liver, these functional and pathologic alterations are irreversible, rendering the harvested liver unusable for either transplantation or ex vivo support perfusion.

Several methods have been used to quantitate organ damage and predict donor organ success. Endothelial damage has been estimated through hyaluronate, creatine kinase, or thrombomodulin[6-9] levels in plasma. Hepatocellular injury can be determined by biopsy,[10] marker enzymes,[11] or clearance of lidocaine, indocyanine green, or other compounds.[7,12] Some of these measures correlate directly with organ dysfunction, but none are immediately available on a "real-time" basis during surgery. A rapid, real-time accurate estimation of subsequent organ function would be a valuable addition to hepatic transplantation.[1]

There is no current intraoperative measure of the eventual function of the transplanted liver. Recent evidence indicates that the eventual function of a har-

vested liver, as measured by bile flow, correlates with its hemodynamic properties on initial reperfusion.[13] Hepatic grafts with the most abnormal blood flows manifested the poorest bile secretion. Considering these findings, the present study attempts to evaluate differences in hemodynamic properties of the isolated and perfused liver before and after cold ischemia. A more comprehensive understanding of hepatic hemodynamics may provide the surgeon with a useful real-time measure of eventual hepatic graft function.

METHODS

Protocols were approved by the University of Massachusetts Medical School animal review board. Hepatic hemodynamic measurements were obtained from the same livers under the following three conditions: (1) in situ (vascular attachments intact), (2) isolated (after placement on a venovenous circuit), and (3) harvested (following procurement and cold ischemia). Donor Yorkshire pigs weighing 30 to 40 kg were used for the experiments. The animals were sedated with a mixture (1.0 ml/kg) of telazol, 150 mg/ml (Ft. Dodge Animal Health, Ft. Dodge, Iowa); ketamine, 50 mg/ml (Ft. Dodge Animal Health); and xylazine, 10 mg/ml (Phoenix Pharmaceuticals, Inc., St. Joseph, Mo.). Animals were intubated and anesthesia was induced by means of 1.5% inhaled isoflurane. The femoral artery and vein were cannulated for blood pressure monitoring and intravenous infusions.

In Situ Liver

Through a midline laparotomy, the cystic duct was ligated without removing the gallbladder, and a 14 F cannula (Sherwood Medical, St. Louis, Mo.) was inserted into the common bile duct. The portal vein and hepatic artery were then carefully dissected in the porta hepatis. Electromagnetic flow probes (Transonic Systems, Inc., Ithaca, N.Y.) were used to record flow within these vessels. A 5 F cannula (Daig Co., Minnetonka, Minn.) was placed into the splenic vein and directed toward the portal vein for continuous recordings of portal venous pressure (PVP). A mesenteric vein was also cannulated with a 5 F catheter for direct injections into the portal system.

Isolated Liver

The same organ that was used in the in situ model was converted to an isolated state. To achieve isolation, 15,000 units of heparin was administered intravenously. To ensure adequate heparinization, activated clotting time values were maintained above 200. A 21 F cannula (Sherwood Medical, St. Louis, Mo.) was placed into the external jugular vein and guided toward the suprahepatic inferior vena cava. A median sternotomy allowed direct visualization of the cannula into the vena cava. Blood (1000 ml) was drained from the animal and into the circuit system (Fig. 1). The circuit was placed on bypass around the liver while an 8 F cannula (Medtronics Biomedicus, Eden Prairie, Minn.) was directed into the hepatic artery and a 17 F
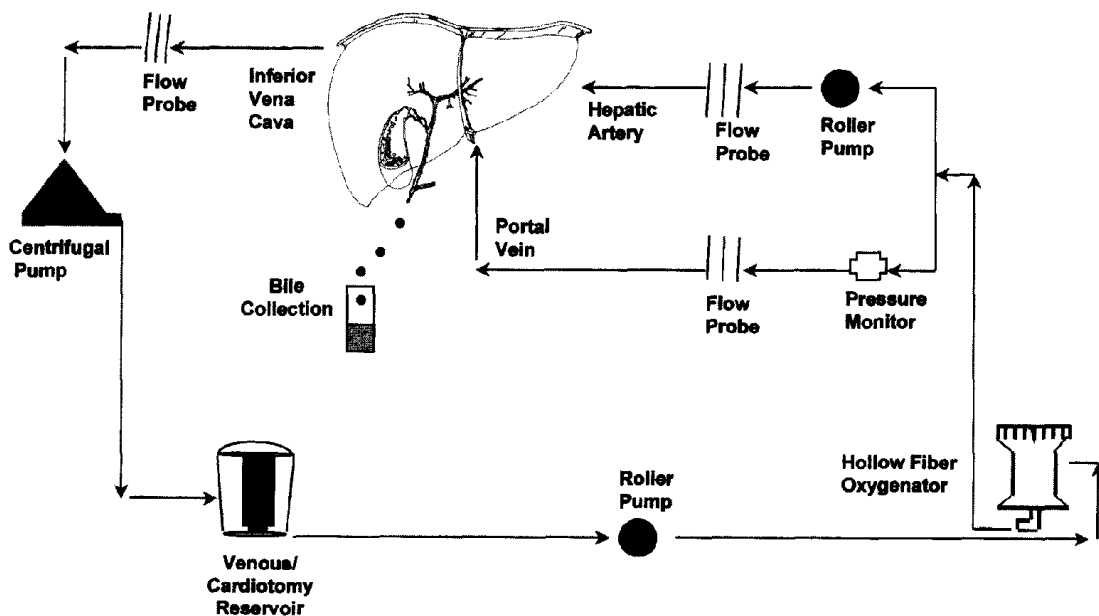
Fig. 1. Venovenous circuit for perfusion of isolated and harvested livers.

cannula was inserted into the portal vein. Cannulation was completed within 5 minutes to minimize ischemia.

After cannulation, blood flow to the liver was gradually increased. Portal vein perfusion reached 550 to 750 ml/min (0.50 to 0.75 ml/g of liver/min) and the hepatic artery, perfused with additional nonpulsatile flow, reached a level of 100 to 200 ml/min (0.16 to 0.2 ml/g of liver/min). Both portal vein and hepatic artery blood flows were controlled with mechanical pumps as previously described.[11] The infrahepatic vena cava was ligated, as well as the hepatoduodenal ligament, to ensure complete isolation of the liver. Organ denervation occurs following this step, as all attachments were severed.[3] The animals were sacrificed with pentobarbital. A small 16-gauge catheter (Becton Dickenson, Sandy, Utah) was directed into the portal vein cannula to continuously monitor PVP. Flow probes (Transonic Systems, Inc.) were used for recordings in the portal vein, hepatic artery, and inferior vena cava. Vasoactive agonists were injected through a three-way stopcock on the portal vein cannula.

Harvested Liver

The isolated liver from the previous stage was converted to the harvested model through the following steps. The circuit was again placed on bypass around the liver, whereas 3 liters of Euro-Collins solution (Fresenius USA, Ogden, Utah) were infused at 4° C (2 L into the portal vein; 1 L into the hepatic artery). It is estimated that the conditions produced by preserving an organ in Euro-Collins solution for 6 hours are equal to 24 hours in Wisconsin solution.[14] The liver was cooled to 4° C with iced saline solution and the inferior vena cava drainage discarded. The liver remained on ice for 2 hours without flow as the venovenous circuit continued on bypass. After cold ischemia, 2 liters of normal saline were infused into the portal vein and hepatic artery (1 L into the portal vein; 1 L into the hepatic artery) while the liver was slowly rewarmed. Perfusate was allowed to enter the liver as the circuit was reconnected. Initial inferior vena cava outflow, approximately 500 ml, was discarded to prevent Euro-Collins contamination in the system. The liver was allowed 1 hour to warm and regain function prior to experimental manipulation. PVP and flow recordings were monitored as in the isolated liver.

Experimental Protocol

Hepatic vascular resistance (HVR) was calculated with the equation:

$$\frac{[PVP \ (mm \ Hg) - Inferior \ vena \ cava \ pressure \ (mm \ Hg)]}{Portal \ blood \ flow \ (ml/min)}$$

PVP and HVR were evaluated at differing flow rates in the in situ, isolated, and harvested livers at approximately 1 hour following experimental manipulation. In situ liver portal blood flows were manipulated by restricting venous return from the splanchnic circulation. Both isolated and harvested portal blood flows were altered through manual adjustment of pump speed. Vasomotor responses to norepinephrine, infused at 2.5 μg/kg/min, and sodium nitroprusside, infused at 3.0 μg/kg/min, were evaluated in each liver stage over a 10-minute infusion period.

Statistical Analysis

Data were analyzed by Student's t tests and analysis of variance for multiple analyses. A P value less than 0.05 was considered significant.

RESULTS
In Situ Liver

All hemodynamic measurements of isolated, perfused, and harvested livers were compared to the same liver while in situ. As described earlier, the in situ liver was characterized by minimal manipulation, intact innervation, and a splanchnic source of portal flow. Hepatic artery flow ranged from 12% to 25% of total hepatic blood flow. When in situ portal vein flow increased from 400 to 1100 ml/min, the liver responded with a minimal rise in PVP from 6.8 ± 0.7 to 9.6 ± 0.24 mm Hg (Fig. 2, A) with a slope of 0.005. HVR fell from 0.016 ± 0.0018 to 0.0086 ± 0.0004 mm Hg min/ml (n = 9) (Fig. 2, B) at the corresponding flows. Hepatic artery flows decreased from 133 ± 36 ml/min at portal flow of 400 ml/min to 64 ± 1 ml/min at portal flow of 1100 ml/min. Previous evaluations of in situ feline livers also demonstrated minimal increments in pressure with large increases in flow, indicating considerable vascular distensibility or compliance.[15]

PVP of the in situ liver averaged 8.2 ± 0.66 mm Hg, whereas HVR averaged 0.014 ± 0.0018 mm Hg min/ml. In four animals, a 10-minute infusion of norepinephrine resulted in a significant rise in PVP from 9.0 ± 1.0 to 16.5 ± 0.87 mm Hg (P = 0.001) (Fig. 3, A), which is similar to the observed effect in feline livers.[16] HVR also increased from 0.010 ± 0.0015 to 0.016 ± 0.002 mm Hg min/ml (P = 0.04) (Fig. 3, B) without a significant change in portal vein flow. Sodium nitroprusside significantly decreased PVP of four in situ livers from 8.8 ± 1.1 to 5.5 ± 0.65 mm Hg (P = 0.03) (Fig. 4, A) with a minimal effect on HVR (P = NS) (Fig. 4, B). Sodium nitroprusside did produce a dramatic decrease in systolic pressure, which resulted in decreased splanchnic flow.
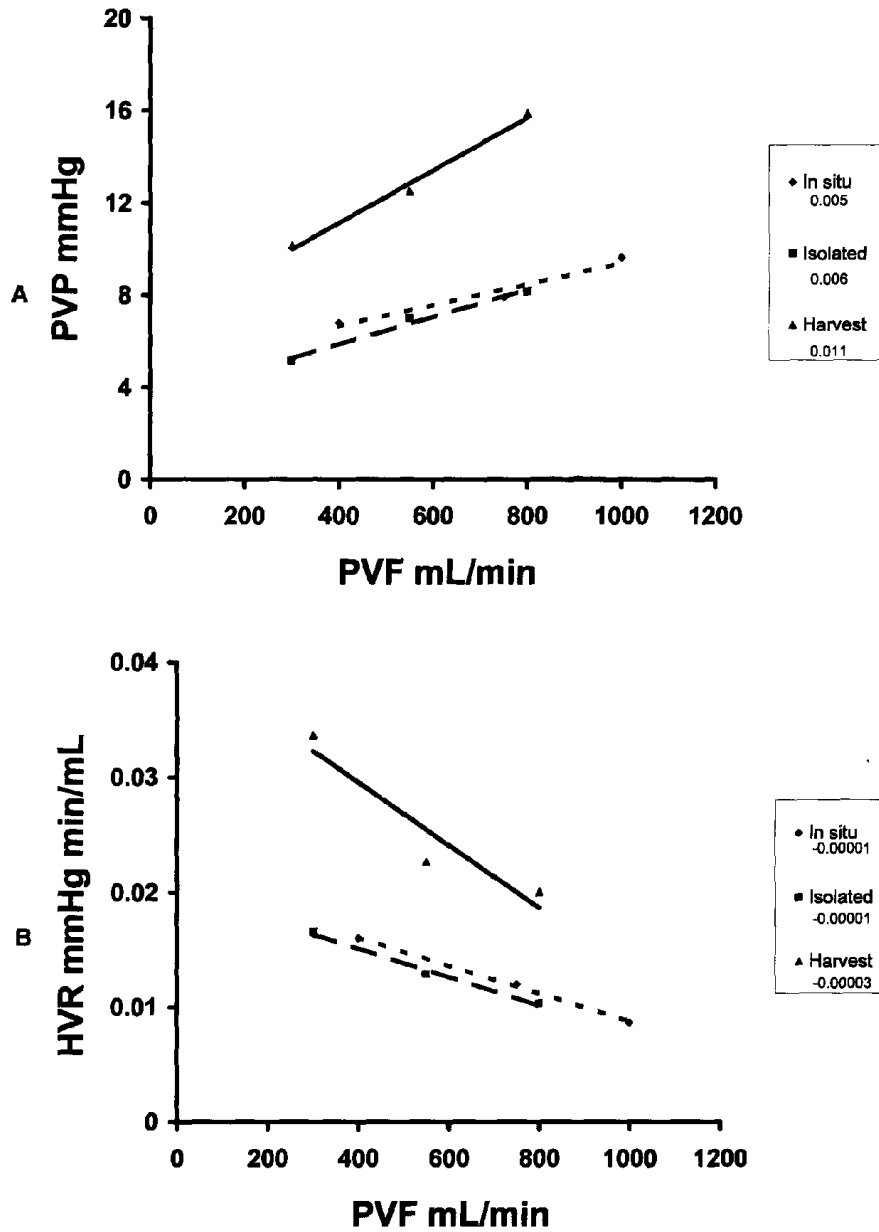
Fig. 2. A, Effect of alterations in portal vein flow *(PVF)* on portal vein pressure *(PVP)* in in situ (◆ — ◆),
isolated (■ — ■), and harvested (▲—▲) livers. The data represent the mean PVP for three organs in
the three models. The slope of each line is listed in the legend beneath the model. B, Effect of alterations
in portal vein flow *(PVF)* on hepatic vascular resistance *(HVR)* in in situ (◆ — ◆), isolated (■ — ■),
and harvested (▲—▲) livers. The data represent the mean HVR for three organs in the three models.
The slope of each line is listed in the legend beneath the model.
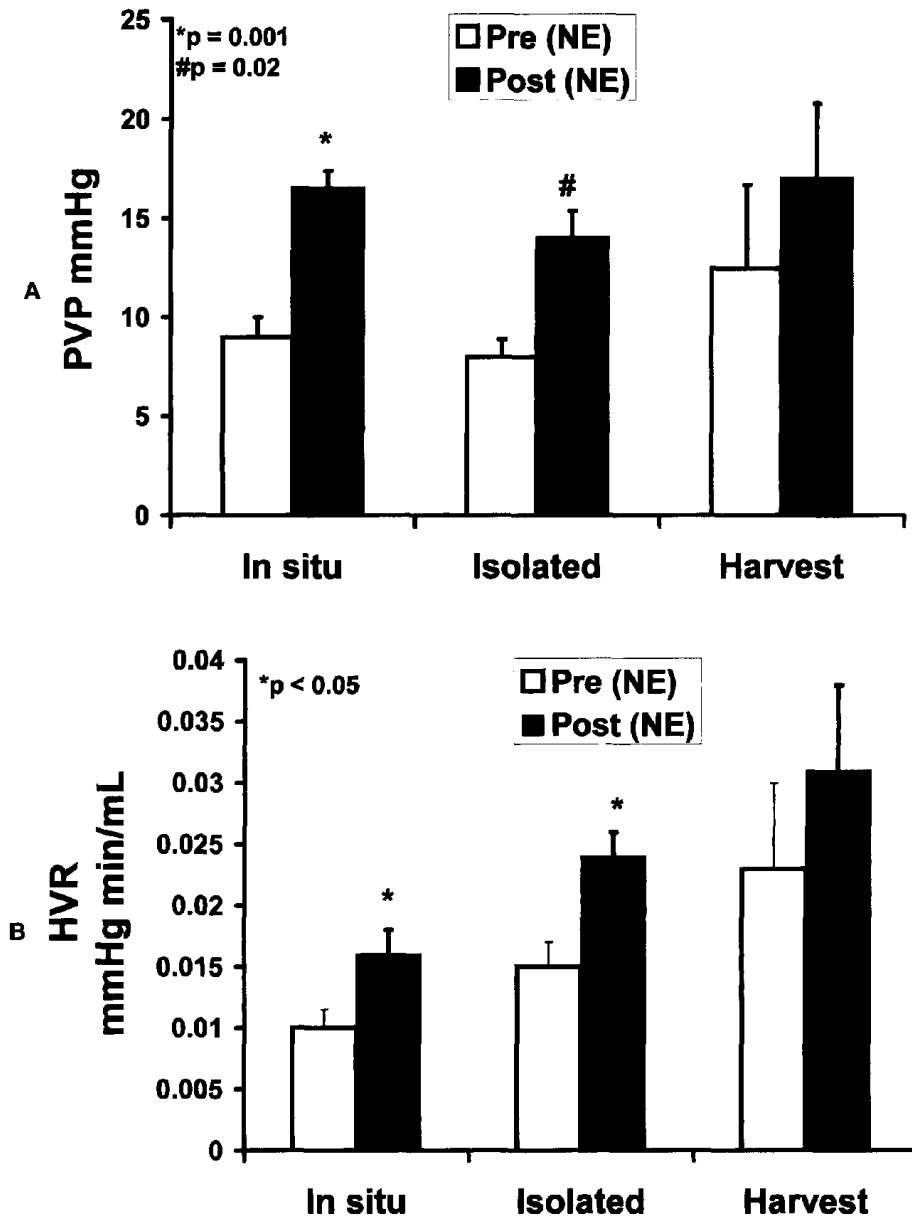
Fig. 3. A, Effect of norepinephrine *(NE)* infusion on portal vein pressure *(PVP)* in the in situ liver, the isolated liver, and the harvested liver. The data represent the mean PVP, with standard errors of the mean, before and after NE infusion. **B,** Effect of norepinephrine *(NE)* infusion on hepatic vascular resistance *(HVR)* in the in situ liver, the isolated liver, and the harvested liver. The data represent the mean HVR, with standard errors of the mean, before and after NE infusion.
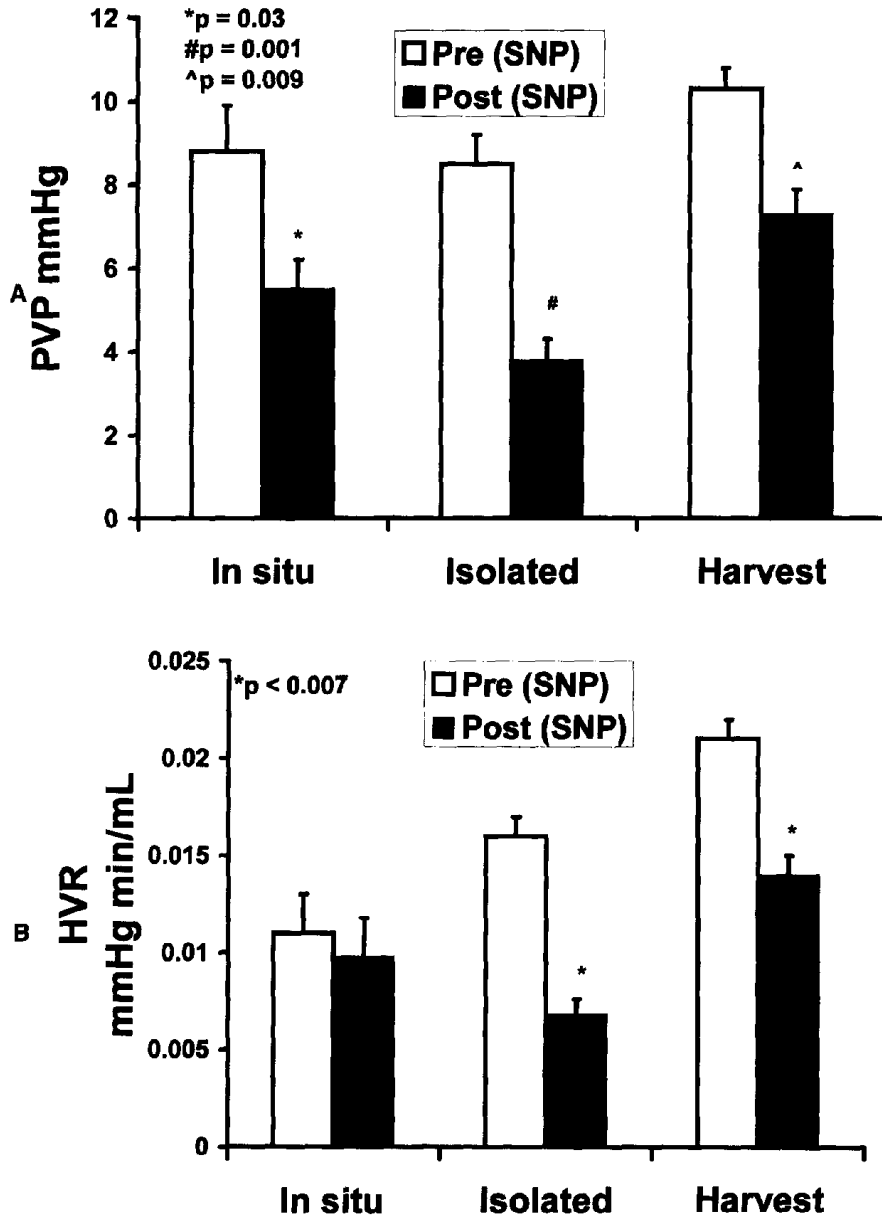
Fig. 4. A, Effect of sodium nitroprusside *(SNP)* infusion on portal vein pressure *(PVP)* in the in situ liver, the isolated liver, and the harvested liver. The data represent the mean PVP, with standard errors of the mean, before and after SNP infusion. **B,** Effect of sodium nitroprusside *(SNP)* infusion on hepatic vascular resistance *(HVR)* in the in situ liver, the isolated liver, and the harvested liver. The data represent the mean HVR, with standard errors of the mean, before and after SNP infusion in four livers.

Isolated Liver

As mentioned earlier, placement of the liver into the perfusion circuit denervates the organ and, by virtue of its polymer surface, the venovenous circuit activates the complement cascade stimulating inflammatory cells.[17] A change in portal vein flow from 300 to 800 ml/min produced a relatively small rise in PVP with a slope of 0.006, demonstrating a vascular compliance similar to that of the in situ liver. PVP increased from 5.14 ± 0.3 to 8.14 ± 0.46 mm Hg (see Fig. 2, A), whereas HVR decreased from 0.017 ± 0.001 to 0.01 ± 0.0006 mm Hg min/ml (see Fig. 2, B) in seven livers. Hepatic artery flow was maintained constant at 100 to 150 ml/min at these differing portal flows. The flow pressure response of the isolated liver was not significantly different from that of the in situ liver, even with the complement and coagulation activation of the polymer surface and the loss of neurologic input.

At baseline portal vein flow, approximately 550 ml/min, the isolated liver PVP and HVR remained relatively constant throughout a 2-hour perfusion, 7.8 ± 0.7 to 8.0 ± 1.3 mm Hg and 0.015 ± 0.002 to 0.015 ± 0.0025 mm Hg min/ml, respectively (n = 5; P = NS). Thus length of time on the perfusion circuit did not alter the hepatic hemodynamic data, although neutrophils were consumed and platelets activated.[17] Flow pressure functions at the end of the 2-hour isolated perfusion were no different from those obtained earlier in the perfusion.

During a 10-minute infusion of norepinephrine, a significant rise in isolated liver PVP was noted in four animals from 8.0 ± 0.9 to 14.0 ± 1.4 mm Hg (P = 0.02) (see Fig. 3, A), similar to the effects of norepinephrine in situ. Isolated liver HVR rose from 0.015 ± 0.002 to 0.024 ± 0.002 mm Hg min/ml (P = 0.03) (see Fig. 3, B), analogous to the in situ response. During a 10-minute infusion of sodium nitroprusside, PVP declined from 8.5 ± 0.7 to 3.8 ± 0.5 mm Hg (P = 0.001) (see Fig. 4, A), comparable to the in situ data. However, with stable hepatic artery and portal vein flows during sodium nitroprusside infusion, HVR significantly decreased from 0.016 ± 0.001 to 0.007 ± 0.0008 mm Hg min/ml (P = 0.0007) (see Fig. 4, B).

Harvested Liver

When the portal vein flow of six harvested livers was altered from 300 to 800 ml/min, PVP increased from 10.2 ± 1.2 to 15.8 ± 1.3 mm Hg (see Fig. 2, A) and HVR decreased from 0.034 ± 0.0039 to 0.02 ± 0.0016 mm Hg min/ml (see Fig. 2, B). The rate of change in portal pressure with flow alterations in the liver after cold ischemia was twice (0.011) that of the

in situ (0.005) or isolated livers (0.006), indicating an appreciable reduction in vascular distensibility or compliance. The y intercept of the flow pressure function was greater in the harvested liver than in either the in situ or isolated liver. The liver after cold ischemia demonstrated higher vascular resistance values than both isolated and in situ livers at comparable flows. The similarity of the in situ and isolated flow pressure functions and the alterations after harvest suggest that the decrease in compliance is explained primarily by the effect of cold ischemia.

At baseline portal venous (550 ml/min) and hepatic arterial (140 ml/min) flows, the PVP of the harvested liver was significantly higher (12.6 ± 1.0 mm Hg) than the values in the isolated or in situ liver (P = 0.008). HVR of the harvested liver was also significantly higher (0.022 ± 0.0016 mm Hg min/ml) than that in the isolated or in situ control liver (P = 0.01). PVP and HVR remained relatively constant during the time course of the perfusion. During a 3-hour perfusion, PVP of the harvested liver was 12.3 ± 1.7 mm Hg at the start and 11.5 ± 1.2 mm Hg at the close of the experiment (P = NS). Similarly, HVR was 0.022 ± 0.0016 mm Hg min/ml immediately following cold ischemia and 0.025 ± 0.002 mm Hg min/ml at the end of perfusion (P = NS). These data indicate that although PVP and HVR were significantly elevated after cold ischemia, hepatic resistance did not change appreciably with time.

During a 10-minute infusion, sodium nitroprusside profoundly reduced PVP in four harvested livers from 10.25 ± 0.5 to 7.25 ± 0.6 mm Hg (P = 0.009) (see Fig. 4, A), as well as HVR from 0.021 ± 0.001 to 0.014 ± 0.0012 mm Hg min/ml (P = 0.008) (see Fig. 4, B). These reductions in resistance parameters were similar to the reductions observed when these same livers were treated in isolated perfusions prior to cold ischemia. PVP of the harvested liver rose during norepinephrine infusion from 12.5 ± 4.2 to 17.0 ± 3.8 mm Hg (P = NS), although the variances were large (see Fig. 3, A). HVR also rose slightly but not significantly during norepinephrine infusion from 0.023 ± 0.007 to 0.030 ± 0.007 mm Hg min/ml (P = NS) (see Fig. 3, B). Compared to the isolated and in situ livers, the vasomotor responses to norepinephrine seemed more variable and blunted after cold ischemia.

DISCUSSION

These data demonstrate that the vasomotor characteristics of the in situ and isolated livers differ significantly from those of the harvested liver. Vascular distensibility in response to flow, or vascular compliance, in the in situ and isolated livers and vasomotor

responses to norepinephrine or sodium nitroprusside were comparable in both preparations. Thus loss of neurologic input or placement of the organ onto a venovenous circuit does not significantly alter hepatic hemodynamics compared to the in situ state. Significant changes in hepatic resistance and compliance were noted after cold ischemia. The slope of the harvested liver flow pressure function, an index of vascular compliance, was twice that of the in situ or isolated livers and the effect of norepinephrine infusion was blunted. Therefore increased vascular resistance and decreased compliance after harvest are due to cold ischemia and not loss of innervation.

The polymer surface in a venovenous circuit or simple denervation of the organ may potentially produce altered hepatic hemodynamics. Venovenous bypass induces complement activation, endotoxin release, leukocyte activation, expression of adhesion molecules, activation of clotting factors, and the release of inflammatory mediators.[17] Polymer-activated leukocytes or platelets plug the sinusoids and may also alter hepatic blood flow. Activation of complement, which also occurs on these surfaces, has the potential to initiate a broad inflammatory response.[18] However, the relatively similar hemodynamics of the in situ and isolated liver, after prolonged exposure to the venovenous circuit, indicate that neither surface activation, denervation, nor inflammatory cell activation play a major role in the hepatic circulatory alterations after cold ischemia. In these studies, oxygenated blood is provided by both the portal vein and hepatic artery in the isolated and harvested models. This difference is in contrast to the in situ model in which only the hepatic artery provides oxygenated blood. Yet this difference in oxygen supply does not explain the differing hemodynamic results obtained between the isolated and harvested livers as these models both received elevated oxygen levels.

The observed alterations in hepatic hemodynamics after cold ischemia may be explained by a number of pathologic processes induced by this injury. Cold ischemia injures and activates endothelial cells,[1] promoting intravascular coagulation and platelet adhesion.[19,20] Endothelial cell activation leads to cellular death from apoptosis, after protracted cold preservation.[21] The pathologic damage or death of the endothelium may subsequently lead to changes in endothelial or stellate cell contractility resulting in significant alterations in hepatic hemodynamics. The harvest also induces hepatocellular damage through the effects of warm ischemia. Hepatocytes become ballooned with focal zonal necrosis and a variable degree of microvesicular steatosis after ischemia, particularly in zone 3.[22] Hepatocyte damage may induce interstitial edema expanding the space of Disse and

compressing the sinusoids causing altered hepatic hemodynamics after exposure to cold ischemia. Agents that attenuate both endothelial and hepatocyte injury or reduce hepatic edema may improve vascular compliance following cold ischemia.

Many agents are influential in modulating hepatic blood flow. Vasoactive mediators are involved in the pathophysiology of hepatic microcirculatory disturbances after cold ischemia and reperfusion.[23-25] Modulation of nitric oxide[25,26] and carbon monoxide[24,27] as well as the release of endothelin[23,28] are important determinants of hepatic vasomotion. Cold ischemia alters the production and release of these compounds during the harvest. Release of endothelin is more pronounced with more significant damage to the organ,[29] resulting in greater vascular resistance. Given the observed vasodilatation in response to nitroprusside after cold ischemia, this may be an approach to improve both blood flow and hepatocellular function.

Altered hepatic hemodynamics may also be related to "no reflow," a phenomenon that has puzzled transplant surgeons and researchers for many years. This phenomenon is characterized by areas of unperfused, underperfused, or relative stasis of liver sinusoids after cold ischemia and reperfusion.[30] Etiologic factors producing this phenomenon include oxygen-derived free radicals, capillary leukostasis, and platelet adhesion.[30,31] It is possible that these areas of "no reflow" produce a shunting effect on the liver, channeling more blood flow through open vessels, thereby raising hepatic vascular resistance. The functional status of the liver has been correlated with the degree of the perfusion failure after cold ischemia. Methods to reduce "no reflow" or improve distribution of blood flow in the liver after cold ischemia would theoretically reduce resistance and minimize ischemia and reperfusion injury.

The etiology of the hepatic hemodynamic alterations following cold ischemia reperfusion may be due to one or more of the factors described. Although the exact mechanisms responsible for the hemodynamic alterations are not known, the predictive value of early reperfusion hemodynamic measurements is beginning to be understood. As described earlier, the harvested organ with significant perturbations in hepatic hemodynamics is associated with depressed hepatocellular function as measured by bile flow.[13] Consequently it may be possible to predict the functional outcome of the donor liver at the operating table, through these simple hemodynamic measurements. These hemodynamic data can be obtained immediately, whereas other predictive measures require hours of analysis. More important, if an accurate functional prediction can be developed from these data, interventions can be employed to optimize subsequent function. Fail-

ing any improvement, a different support or transplant organ may be required. Ultimately the ability to predict and possibly alter the function of the harvested liver would enhance the available donor pool.

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Diminished Morbidity and Mortality in Portal Hypertension Surgery: Relocation in the Therapeutic Armamentarium

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Although several effective therapeutic options are available for bleeding from portal hypertension, surgery has a well-defined role in the management of patients with good liver function who are electively operated. The aim of this investigation was to evaluate the operative mortality and morbidity of portal blood flow–preserving procedures in a highly select patient population. The records of 148 patients operated on between 1996 and 2000 using one of two techniques (selective shunts or a Sugiura-Futagawa operation [complete portoazygos disconnection]) were analyzed with particular attention to operative mortality, postoperative rebleeding, and encephalopathy. Survival was calculated according to the Kaplan-Meier method. Sixty-one patients had distal splenorenal shunts placed, and 87 patients had a devascularization procedure. Operative mortality for the group as a whole was 1.2%. In the group with selective shunts, the rebleeding rate was 4.9%, the encephalopathy rate was 9.8%, and the shunt obstruction rate was 1.6%. Survival at 24 months was 94% and at 48 months was 92%. In those undergoing devascularization, the encephalopathy rate was 5% and the rebleeding rate was 14%. Survival at 24 months was 90% and at 48 months was 86%. Portal blood flow–preserving procedures have very low morbidity and mortality rates at specialized centers. In addition, a low rebleeding rate is associated with a good quality of life. Low-risk patients with bleeding portal hypertension should be considered for surgical treatment. (J GASTROINTEST SURG 2001;5:499-502.)

KEY WORDS: Portal hypertension surgery, shunt surgery, devascularization procedures

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Treatment of bleeding portal hypertension has expanded widely over the past few years. Several treatment modalities are now available, each with its own particular indications and results in certain subsets of patients. In emergency situations, pharmacotherapy and endoscopic therapy are the treatments of choice.[1] Pharmacotherapy can also be used for primary prophylaxis,[2] and transjugular intrahepatic portosystemic shunts (TIPS) are indicated in refractory cases in the acute setting, as well as in patients with poor liver function who are awaiting a liver transplant.[3] Liver transplantation is considered the treatment of choice at many centers, because not only does portal pressure return to normal but liver function is also restored.[4]

Surgery may be considered in a subset of patients with good liver function whose principal problem is bleeding esophageal varices from portal hypertension.[5] The relative complexity of these operations as well as their long-term results in terms of survival, which differ little from other treatment modalities, has restricted their utilization at many centers.

At our institution, surgery for portal hypertension has been maintained as a therapeutic option for several reasons. Selection of patients has broadened as technical expertise has become more highly developed. In this report we analyze the results of portal hypertension surgery (blood flow–preserving procedures) over the past few years, with special emphasis on mortality, and current indications for surgery, and status of patients.

METHODS

Patients evaluated for management of bleeding esophageal varices from portal hypertension at our institution, either as an elective procedure or in the

Table I. Criteria for patients undergoing portal hypertension surgery

History of bleeding portal hypertension
Good liver function
   Albumin >3 g/dl
   Prothrombin time <2 seconds
   Direct bilirubin <2 mg/dl
   No encephalopathy
   No ascites
Good cardiopulmonary function
Good renal function
No hepatocarcinoma

emergency setting, are treated by a multidisciplinary team approach. Patients with acute bleeding are managed with endoscopic treatment and/or pharmacotherapy. Once the bleeding is controlled, liver function is evaluated. Patients in Child-Pugh class C remain on endoscopic and/or pharmacologic therapy. Some may be placed on the liver transplant waiting list according to whether or not they meet the criteria of our institution. Patients fulfilling the criteria listed in Table I are evaluated for portal hypertension surgery. These criteria reflect good liver function, good cardiac function, and good lung function. A prothrombin time of less than 2 seconds is indicative of an international normalization ratio of less than 1.16. This is a quick and easy means of evaluating liver function in addition to a serum albumin level above 3.5 g/dl. Celiac angiography is performed for complete evaluation of the complete splanchnic vessels in each patient. If adequate vessels are found (splenic vein and left renal vein), the patient is scheduled for a distal splenorenal shunt.[6] If no adequate vessels are found, the patient is scheduled for a Sugiura-Futagawa operation[7] or complete portoazygos disconnection.[8] During the postoperative period, routine angiography is performed (between 2 and 4 weeks postoperatively) to evaluate shunt patency and portal blood flow. Within the past 5 years, a total of 193 operations have been performed in 148 patients. The records of these patients were evaluated with a focus on operative mortality and morbidity. Survival curves were constructed according to the Kaplan-Meier method.

The following definitions were used in our evaluations: Rebleeding was defined as hematemesis and/or melena with hemodynamic decompensation (i.e., heart rate >100 beats/min and hypotension) and decreasing hemoglobin levels. Encephalopathy was determined by means of clinical evaluation and, when deemed necessary, motor function tests. Patients with categoric clinical signs of encephalopathy (i.e., som-

nolence, asterixis, etc.) were considered positive, and no further tests were conducted. Three patients with no categoric signs were subjected to motor function tests. Quality of life was assessed according to our established criteria.[5] Patients with no need for hospitalization, no rebleeding, and no encephalopathy who were able to carry out their daily activities were considered to have a good quality of life. The opposite was true for those judged to have a poor quality of life.

RESULTS

Sixty-one patients had a distal splenorenal shunt placed and 87 patients had a Sugiura-Futagawa operation or complete portoazygos disconnection (Table II). Operative mortality was 1.2% for the group as a whole. Nine patients in the distal splenorenal shunt group and five patients in the devascularization group were lost to follow-up. These patients were included in the Kaplan-Meier survival analyses.

Distal Splenorenal Shunt

All of the patients in this group were cirrhotic, with a slight predominance of viral over alcoholic cirrhosis. There were five cases of primary biliary cirrhosis. Operative mortality was 1.6% (1/61). The rebleeding rate was 4.9% (3/61). The encephalopathy rate was 9.8% (6/61) and the shunt obstruction rate was 1.6% (1/61). Survival was 98% at 1 month, 94% at 24 months, and 92% at 48 months. Patients with encephalopathy were treated medically (protein-restricted diet, lactulose).Three of these patients met the criteria for liver transplantation and were placed on the waiting list.

Sugiura-Futagawa Operation and Complete Portoazygos Disconnection

In 45 patients both stages of the operation (thoracic and abdominal) were performed, and in 42 patients only the abdominal stage was necessary. No operative deaths were recorded in this group. Forty-eight percent of the patients in this group had no cirrhosis on liver biopsy, whereas the remaining 52% had alcoholic or posthepatitis cirrhosis. These patients were selected for this type of operation because they were judged unsuitable for shunt surgery because of the presence of vascular abnormalities and/or thrombosis. No cases of esophageal dehiscence or fistulization of the modified esophageal transection were recorded. Five patients were lost to follow-up. At 5 years, the encephalopathy rate was 5% and the rebleeding rate was 14%. Survival was 100% at 1 month, 94% at 24 months, and 86% at 48 months.

Table II. Patient characteristics

	DSRS	Devascularization
Total patients in each group	61	87
Cirrhosis	61	45 (52%)
Alcoholic	26	21
Viral	30	22
Primary biliary	5	2
No cirrhosis	0	42 (48%)
Prehepatic and/or idiopathic	0	42
Splenomesoportal patency	61	43
Operative mortality	1/61 (1.6%)	0
Rebleeding	3/61 (4.9%)	12/87 (14%)
Encephalopathy	6/61 (9.8%)	4/87 (5%)
Shunt obstruction	1/61 (1.6%)	
Survival at 60 months	90%	82%
Postoperative angiography	55/60	21/43
Portal vein		
Unaltered	45/55 (81%)	20/21 (95%)
Diminished	7/55 (13%)	0
Thrombosed	3/55 (5%)	1/21 (5%)

DSRS = distal splenorenal shunt.

DISCUSSION

Surgery for portal hypertension has evolved over the past 50 years and its location in the therapeutic armamentarium has varied widely. In the 1960s and 1970s it was the treatment of choice for all subsets of patients, but it soon became evident that surgery had only a minor impact on survival, although it did have a favorable outcome with regard to the rebleeding rate.[9] As other treatment modalities emerged, its role was redefined. Endoscopic treatment (sclerotherapy) emerged as a good alternate choice in emergency situations, replacing surgery at most centers around the world (including our own); one exception was Orloff et al.[9] from San Diego, who achieved superb results with total shunts in emergency situations. Pharmacotherapy also appears to be a good choice for patients in the acute setting.[10] The evolution of endoscopic therapy has made band ligation the treatment of choice for patients with acute bleeding episodes as well as those with poor liver function.[11] The TIPS procedure has a role in patients with acute bleeding refractory to endoscopic and pharmacologic therapy (as a short-term bridge to liver transplantation), and liver transplantation is indicated primarily for patients who have poor liver function concomitant with bleeding portal hypertension.

Patients with good liver function and a history of variceal bleeding all share the following characteristics:

1. They have a high probability of rebleeding (although they are better able to tolerate these episodes than Child-Pugh C patients).

2. Those treated with pharmacotherapy and/or endoscopic therapy have a high rate of rebleeding (30% to 50%). This rebleeding can cause patients with good liver function to develop posthemorrhagic liver failure, with a high probability of being reclassified as Child-Pugh C.

3. In patients treated with the TIPS procedure, high rates of encephalopathy and shunt obstruction are to be expected, which necessitate reexploration and replacement of stents. The advantages of portal blood flow–preserving procedures are improved shunt patency (if a shunt is used) and a lower incidence of encephalopathy.

4. The disadvantages of liver transplantation are the shortage of donor livers and the well-known complications of immunosuppression. Although this treatment modality has had an extraordinary evolution, the morbidity and mortality of this procedure must be considered. Liver transplantation is indicated for patients with end-stage liver cirrhosis with or without bleeding portal hypertension. Portal hypertension surgery is indicated in patients whose principal problem is bleeding but who have an adequate liver reserve. Some patients with bleeding portal hypertension are not candidates for a liver transplant (e.g., alcoholic patients who continue to drink). Others, even though they are suitable candidates, will never receive a transplant because of the shortage of donor organs. Thus the most promising alternative may not always be

the one most readily available, and most of these patients will have recurrent bleeding episodes while they are on the waiting list.

5. Portal blood flow–preserving procedures performed by a highly skilled surgical team in a well-selected patient population offer excellent results. Over the past 5 years at our institution, we have achieved a very low operative mortality rate (1%) with very good 5-year survival, along with a low encephalopathy rate and a low rebleeding rate. No other option can offer such promising results. Nevertheless, for patients in Child-Pugh class C, replacement of the cirrhotic liver restores functional mass and relieves portal hypertension. Surgery for portal hypertension alone is not appropriate for these patients because a high morbidity and mortality can be anticipated.

Selecting the most suitable type of operation is crucial. When no adequate anatomy can be found, devascularization is a better option. In our surgically treated patients, extensive devascularization is used more frequently than shunts. In patients with a thrombosed portal vein (even one that has been recanalized) and a patent splenic vein, a shunt cannot be used. We have previously found that these patients have a high rate of shunt thrombosis. Patients with small splenic veins (<1 cm) were also excluded. It is possible that some of these patients would be considered for a shunt at other centers. Because we have achieved good (comparable) results with devascularization, we prefer to avoid the risk posed by an inadequate anatomy.

When the choice of operation is based on the individual characteristics of each patient, the results achieved with portal blood flow–preserving procedures in low-risk (Child-Pugh A and B) patients are excellent. Nevertheless, no studies (prospective, controlled, randomized) have been done comparing the five treatment options in low-risk patients. Our group has conducted a prospective, controlled, randomized study comparing beta blockers, endoscopic therapy, and portal blood flow–preserving procedures in low-risk patients, which showed better results for surgery when rebleeding rates were analyzed.[12] These findings confirm the role of surgery as a long-term bridge for patients awaiting liver transplantation when needed,[13] because many of these patients do well after the operation, maintaining good liver function and a

good quality of life. Liver transplant centers have the opportunity to reassess the role of portal hypertension surgery in the therapeutic armamentarium, because these centers usually have the technologically advanced facilities and highly skilled surgeons needed to ensure the success of types of operations.

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Preoperative Intraluminal Application of Capsaicin Increases Postoperative Gastric and Colonic Motility in Rats

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In a model to investigate postoperative gastrointestinal motility with strain gauge transducers in awake rats, we tested the effects of intraluminal capsaicin infusion into the cecum 2 days or 14 days prior to abdominal surgery. Acute infusion of capsaicin into the cecum for 30 minutes increased the gastric, small intestinal, and colonic motility index by up to 115%, 34%, and 59%, respectively, compared to vehicle infusion. Intraluminal capsaicin infusion 2 days prior to abdominal surgery significantly increased the intraoperative gastric and colonic motility index by 166% and 100%, respectively, compared to vehicle, but had no effect on small intestinal motility. The postoperative decrease in gastric or colonic motility was completely prevented by capsaicin pretreatment, representing a 73% and a 72% increase in the motility index during the first postoperative hour and a 40% and a 29% increase in the motility index during the second postoperative hour compared to vehicle, whereas the postoperative decrease in small intestinal motility was not altered by capsaicin pretreatment. In contrast, intraluminal capsaicin infusion 14 days prior to abdominal surgery had no effect on postoperative inhibition of gastrointestinal motility. Our results suggest that capsaicin-sensitive visceral afferent C-fibers, presumably of the submucosa, play an important role in mediating postoperative ileus. Intraluminal capsaicin does probably ablate these nerve fibers temporarily, with no systemic side effects observed with the use of the tail flick test as a measure of skin nociception. (J GASTROINTEST SURG 2001;5:503-513.)

KEY WORDS: Postoperative ileus, visceral afferent nerve fibers, capsaicin, gastric motility, small intestinal motility, colonic motility

Postoperative inhibition of gastrointestinal motility, so-called postoperative ileus, is induced by laparotomy and intra-abdominal procedures. The transient inhibition of gatrointestinal motility, occurring in humans mainly in the stomach and the colon may last for several days and can considerably contribute to a patient's postoperative discomfort. Oral food intake may be delayed until postoperative ileus has resolved, and prolonged nasogastric suction or, in rare cases, even relaparotomy becomes necessary.[1,2] The expenses incurred by postoperative ileus in the United States have been estimated at $1500 per patient, or $750,000,000 annually.[3]

There is evidence that postoperative ileus is a multifactorial event, since several neurotransmitters or neuropeptides have been shown to contribute to postoperative ileus.[4-8] Recent studies suggest that surgical manipulation of the gut elicits an inflammatory response in the gut wall, which leads to dysfunction of gastrointestinal smooth muscle cells.[9]

We have previously shown that visceral afferent nerve fibers and the neuropeptides contained within them contribute to postoperative inhibition of gastrointestinal motility. Ablation of afferent sympathetic nerve fibers in the celiac/superior mesenteric ganglia by topical capsaicin treatment hastened recovery from postoperative gastric and colonic ileus in these studies.[10,11] Intraoperative topical treatment of sympathetic nerve fibers is possible but has not yet been investigated in humans as a means of treating postop-

erative ileus. However, if postoperative ileus develops once the abdomen is closed, topical treatment of sympathetic nerve fibers is no longer feasible. As we have shown that enteroenteric inhibitory reflexes can be ablated by intraluminal capsaicin and recover in days,[12] intraluminal capsaicin treatment might be an alternative. A specific capsaicin receptor has been identified,[13] and orally effective capsaicin analogues have already been described.[14] We therefore investigated the effects of intraluminal capsaicin treatment on postoperative contractile activity utilizing a recently developed strain gauge transducer model to record gastrointestinal motility in awake rats. With this model, the postoperative changes in contractile activity of the stomach, the small intestine, or the colon can be used as a measure of postoperative ileus.[15]

METHODS
Animals

Experiments were performed on male Sprague-Dawley rats (Charles River, Kisslegg, Germany) weighing 280 to 320 g. Rats were housed under conditions of controlled temperature (22° C) and 12-hour light-dark cycles. The institutional guidelines for the care and use of laboratory animals were followed throughout the study, and the research protocol was approved by the local animal research committee. Rats were fasted for 16 hours before the experiments but were allowed free access to water.

Strain Gauge Transducers

The strain gauge transducer technique has been described by us previously.[15] In brief, two miniature strain gauge transducers (type EA-06-062 DN-350 E; Measurements Group, Raleigh, N.C., final size 3 × 4 mm) were glued together with tetrahydrofuran (M-Bond 610-E, Measurements Group). Insulated copper wires (0.127 mm diameter, Measurements Group) were soldered to the transducers and coated with epoxy resin (M-Bond 43 B-E, Measurements Group). The strain gauge transducers were immersed in silicone paste (Wacker, München, Germany), placed between two reinforced silicone sheets (0.178 mm; Silastic, Dow Corning, Midland, Mich.), hardened for 24 hours in a mold, and trimmed to their final size of 4 × 6 mm.

Implantation of Strain Gauge Transducers

Rats were anesthetized by intraperitoneal injection of ketamine (Ketanest, 100 mg/kg; Parke-Davis, Berlin, Germany) and xylazine (Rompun, 15 mg/kg;

Bayer, Leverkusen, Germany), and the gastrointestinal tract was exposed by a midline laparotomy. Strain gauge transducers were sutured with 8-0 silk (Braun-Dexon, Melsungen, Germany), in parallel with the circular muscle layer, to one of the following: (1) the gastric corpus, (2) the proximal jejunum, or (3) the right colonic and the left colonic flexure, beginning 10 cm distal to the ligament of Treitz. At the jejunum, the three strain gauges were placed 5 cm apart from each other. The copper wires of the strain gauge transducers were exteriorized between the shoulder blades. In addition, a polyethylene catheter (inner diameter = 0.40 mm, outer diameter = 0.80 mm; Protex Limited, Hythe, U.K.) was implanted into the cecum and exteriorized between the shoulder blades, as described previously for the duodenum.[12] Rats recovered from the procedures within hours as indicated by normal feeding and grooming behavior. No body weight loss was observed until the start of the postoperative study, which was performed 3 days later.

Gastrointestinal Motility Recording and Data Analysis

During the recovery period after implantation of the strain gauge transducers, and prior to the start of experiments, rats were accustomed to light restraint cages (Bollman cages) in training sessions. On the day of the experiments, rats were placed in the Bollman cages and the strain gauge transducer wires were connected to a wheatstone bridge (2100 System, Measurements Group). The signals were simultaneously recorded by a personal computer with an A/D board (AD12-16(PC)E; Contec Microelectronics, San Jose, Calif.) and a multichannel data acquisition recorder (BD 300; Kipp & Zonen, Delft, The Netherlands).

Signal analysis was done with the help of dedicated software (Intestinal Data Acquisition and Analysis, version 3.40.15; Standard Instruments, Karlsruhe, Germany), calculating the area under the curve (motility index), the contraction frequency, the mean contraction amplitude, and the mean area under the contraction amplitude. The appearance of giant contractions in the colon was evaluated visually. Baseline motility was analyzed in 5-minute segments, and the average of six segments (=30 minutes) was set as 100%. Intraoperative and postoperative motility was analyzed in 5-minute segments as well, and the average of three time segments (=15 minutes) was compared to preoperative motility, with each animal serving as its own control. Contraction frequency was analyzed in 5-minute segments as well. For each time segment of 30 minutes, the average of six 5-minute segments was calculated.

Effects of Capsaicin Infusion Into the Cecum on Gastrointestinal Motility

Rats were placed in Bollman cages and baseline motility was recorded for 30 minutes. Then capsaicin (Sigma Chemical, Steinheim, Germany), dissolved in dimethyl sulfoxide (DMSO, 5%; Sigma Chemical), Tween 80 (5%, Sigma Chemical), and NaCl 0.15 mol/L (90%), was infused into the cecum at a concentration of 600 μmol/L and a rate of 0.2 ml/min for 30 minutes (total volume 6 ml), correlating to a total dose of 3.6 μmol (1.099 mg), as described previously for the duodenum.[12] Control experiments consisted of vehicle infusion (DMSO, 5%; Tween 80, 5%; and NaCl 0.15 mol/L, 90%) at a rate of 0.2 ml/min for 30 minutes (total volume 6 ml). Gastrointestinal motility was recorded during capsaicin or vehicle infusion and for 120 minutes thereafter.

Postoperative Gastrointestinal Motility 2 Days After Capsaicin Infusion Into the Cecum

Two days after capsaicin infusion, baseline gastric, small intestinal, or colonic motility was recorded for 30 minutes. Rats were then anesthetized in an enflurane-filled bowl and kept under continuous anesthesia by means of enflurane (5% volume/volume). Abdominal surgery was simulated by a laparotomy with gentle manipulation of the cecum for 5 minutes. The cecum was returned to the abdominal cavity and the midline incision was closed with running sutures; the entire procedure lasted no longer than 15 minutes. Rats were placed in the Bollman cages again, and gastrointestinal motility was recorded for another 120 minutes.

Postoperative Gastric and Colonic Motility 14 Days After Capsaicin Infusion Into the Cecum

Rats were anesthetized by means of intraperitoneal injection of ketamine (Ketanest, 100 mg/kg) and xylazine (Rompun, 15 mg/kg). After laparotomy, capsaicin was infused into the cecum via a cannula at a concentration of 600 μmol/L and a rate of 0.2 ml/min for 30 minutes (total volume 6 ml), corresponding to a total dose of 3.6 μmol (1.099 mg). The abdomen was closed with running sutures and the rats were allowed to recover for 12 days. Three strain gauge transducers were then implanted on the gastric corpus and the right and left colonic flexure as described earlier. Rats were allowed to recover for 2 days, after which they were placed in Bollman cages, and baseline gastric and colonic motility was recorded for 30 minutes. Under enflurane anesthesia, abdominal surgery was simulated by laparotomy and cecal manipu-

lation for 5 minutes as described earlier. Gastrointestinal motility was recorded during abdominal surgery and for 120 minutes thereafter.

Tail Flick Test

To investigate whether capsaicin produced any systemic effects on nociception, the tail flick test as a measure of skin nociception was performed before and 2 days after intraluminal (see above) or intraperitoneal (1 μmol/kg) capsaicin application. Rats were placed in the Bollman cages, and their tails were put into hot water (50° C). The reaction time until the rat withdrew its tail was measured with a stopwatch 10 times, with pauses of 2 minutes between each test. For each animal, the average of all 10 tests was calculated.[16,17]

Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM). Differences between capsaicin and vehicle treatment or between baseline motility and subsequent motility segments were determined by one-way analysis of variance (ANOVA), followed by Dunn's test, which corrects for multiple comparisons. A probability level of $P < 0.05$ was taken as significant.
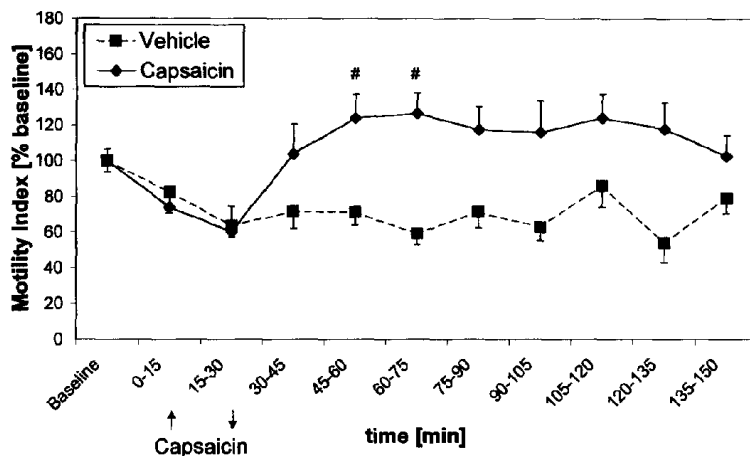
RESULTS
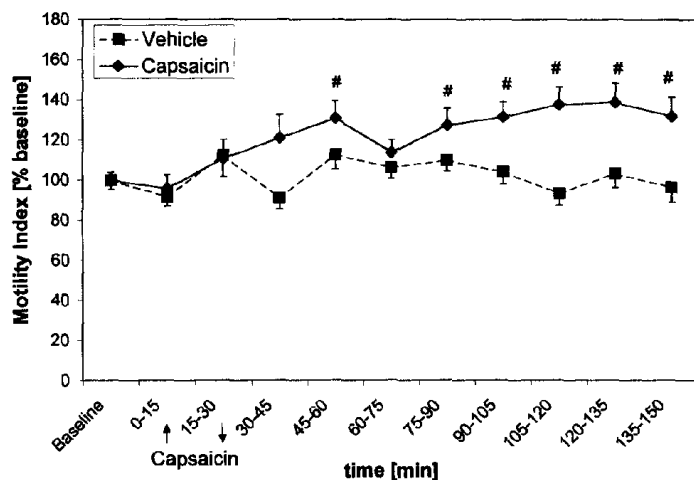### Effects of Capsaicin Infusion Into the Cecum on Gastrointestinal Motility

Capsaicin infusion into the cecum significantly increased gastric, small intestinal, and colonic motility. The maximum increase in the motility index, compared to baseline values and analyzed at 15-minute intervals, was 115% for the stomach, 34% for the small intestine, and 59% for the colon. While colonic motility was increased during capsaicin infusion only, gastric and small intestinal motility showed a delayed but continuous increase for at least 120 min (Figs. 1, 2, and 3). Capsaicin infusion did not cause any noticeable harm to the animals, and food intake, stool pellet output, and body weight on the following days were unchanged compared to vehicle-treated rats (data not shown).

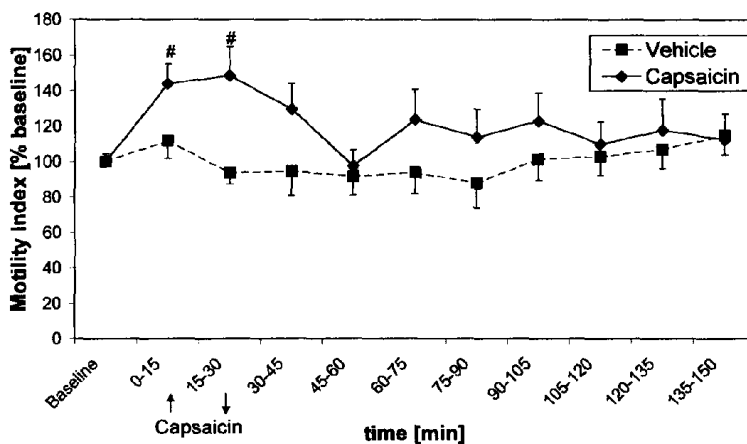## Effects of Capsaicin Infusion Into the Cecum on Gastrointestinal Contraction Frequency

Gastric contraction frequency was significantly increased during capsaicin infusion into the cecum but not thereafter, whereas small intestinal contraction frequency showed a delayed increase 2 hours after capsaicin infusion into the cecum. Colonic contrac-

Fig. 1. Capsaicin infusion into the cecum (n = 5) significantly increased gastric motility approximately 45 minutes after the start of capsaicin infusion compared to baseline values, whereas vehicle infusion (n = 5) had no effect. # = P <0.05 vs. baseline.

Fig. 2. Capsaicin infusion into the cecum (n = 5) significantly increased small intestinal motility approximately 45 minutes after the start of capsaicin infusion compared to baseline values, whereas vehicle infusion (n = 5) had no effect. # = P <0.05 vs. baseline.

Fig. 3. Capsaicin infusion into the cecum (n = 5) significantly increased colonic motility during capsaicin infusion compared to baseline values, but not thereafter. Vehicle infusion (n = 5) had no effect. # = P <0.05 vs. baseline.

tion frequency did not show any changes during or after capsaicin infusion (Table I).

Postoperative Gastrointestinal Motility 2 Days After Capsaicin Infusion Into the Cecum

Capsaicin treatment 2 days prior to abdominal surgery significantly increased intraoperative and postoperative gastric and colonic motility compared to vehicle, but had no effect on small intestinal motility. After capsaicin pretreatment, the gastric motility index increased by 73% during the first postoperative hour and by 40% during the second postoperative hour compared to vehicle pretreatment, whereas the colonic motility index increased by 72% during the first postoperative hour and by

29% during the second postoperative hour compared to vehicle pretreatment. Further, gastric or colonic baseline motility levels were reached in the first postoperative recording segment in capsaicin-treated rats, indicating immediate postoperative recovery of gastric or colonic contractile activity (Figs. 4, 5, 6, and 7).

Postoperative Gastrointestinal Contraction Frequency 2 Days After Capsaicin Infusion Into the Cecum

We observed significant changes in contraction frequency 2 days after capsaicin treatment compared to vehicle treatment. The contraction frequency of the stomach and the small intestine was increased by 9%

Table I. Acute effects of capsaicin infusion into the cecum on gastrointestinal contraction frequency*

Time (min)	Stomach		Small intestine		Colon	
	Vehicle	Capsaicin	Vehicle	Capsaicin	Vehicle	Capsaicin
0-30	30.5 ± 1.0	30.0 ± 0.5	111 ± 2.9	117 ± 2.4	6.0 ± 0.3	7.0 ± 0.2
30-60	31.5 ± 0.7	36.0 ± 1.0*	113 ± 2.6	113 ± 2.6	7.0 ± 0.3	7.0 ± 0.2
60-90	35.5 ± 1.1	29.5 ± 0.8	109 ± 2.3	121 ± 2.2	8.0 ± 0.3	7.0 ± 0.2
90-120	32.0 ± 0.9	30.5 ± 0.9	110 ± 2.8	125 ± 2.1	7.0 ± 0.3	7.0 ± 0.2
120-150	34.5 ± 0.8	30.5 ± 0.7	110 ± 2.1	122 ± 2.1	8.0 ± 0.2	7.0 ± 0.2
150-180	33.0 ± 0.7	30.0 ± 0.5	115 ± 2.1	129 ± 2.0*	7.0 ± 0.3	6.0 ± 0.2

*Motility was recorded under baseline conditions (0 to 30 minutes), followed by capsaicin infusion (30 to 60 minutes). After capsaicin infusion, motility was recorded for another 120 minutes (60 to 180 minutes). Capsaicin infusion significantly increased the frequency of gastric and small intestinal contractions compared to baseline values, whereas it had no effect on the frequency of colonic contractions. Frequency is given as contractions per 5 minutes. For each time segment of 30 minutes, the average of six 5-minute segments was calculated.
*P <0.01 vs. vehicle.
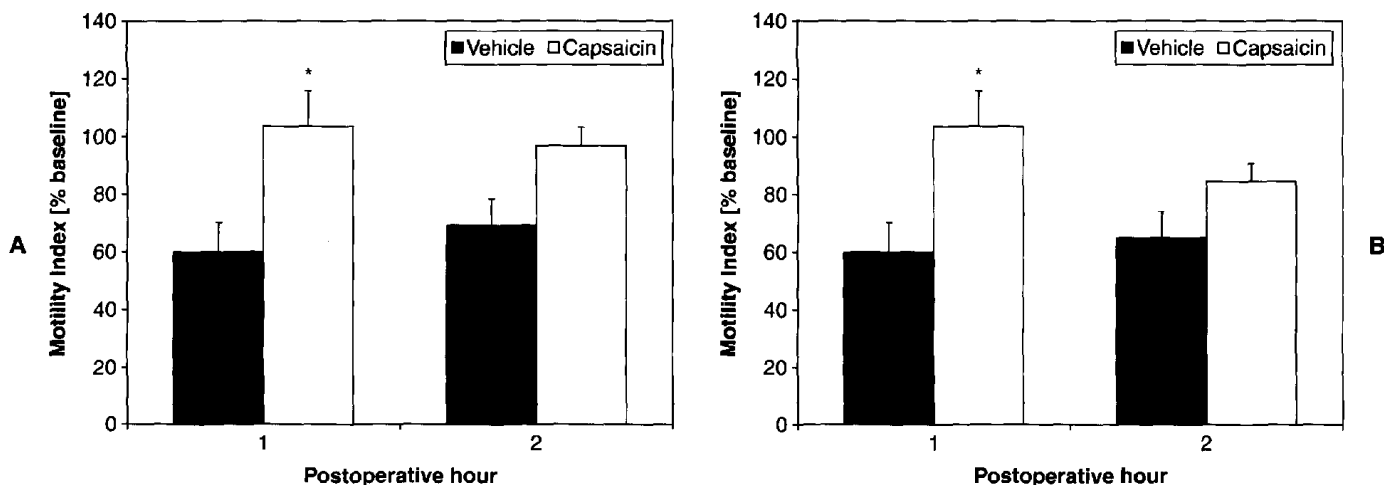
Fig. 4. Capsaicin treatment 2 days prior to abdominal surgery significantly increased the gastric (**A**) and colonic (**B**) motility index during the first postoperative hour compared to vehicle treatment. * = P <0.05 vs. vehicle.
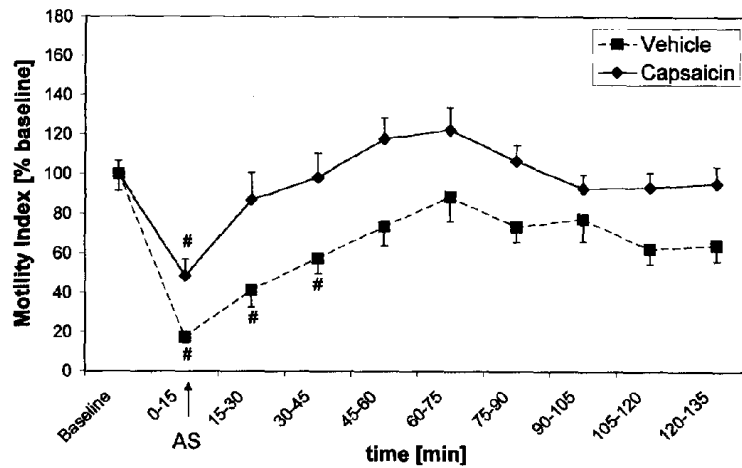
Fig. 5. Capsaicin infusion into the cecum (n = 5) 2 days prior to abdominal surgery *(AS)* increased intraoperative and postoperative gastric motility compared to vehicle infusion (n = 5). In capsaicin-treated rats, no postoperative decrease in gastric motility could be observed compared to baseline motility, indicating immediate recovery of postoperative gastric contractile activity. # = P <0.05 vs. baseline.
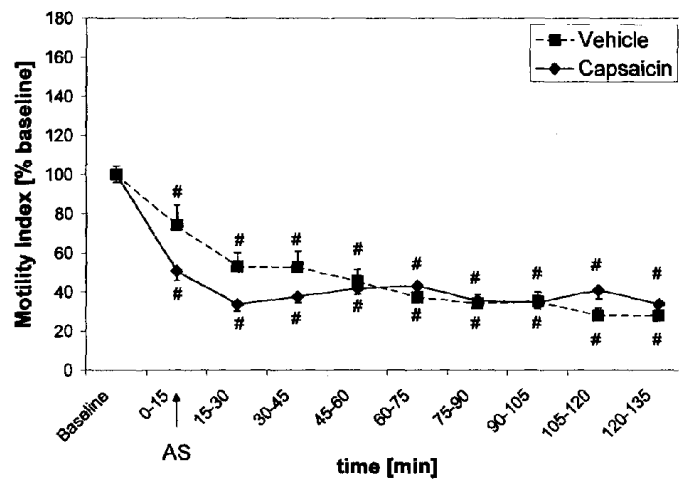
Fig. 6. Capsaicin infusion into the cecum (n = 5) 2 days prior to abdominal surgery *(AS)* had no effect on postoperative small intestinal motility compared to vehicle infusion (n = 5). Postoperative small intestinal motility was decreased throughout the recording period. # = P <0.05 vs. baseline.
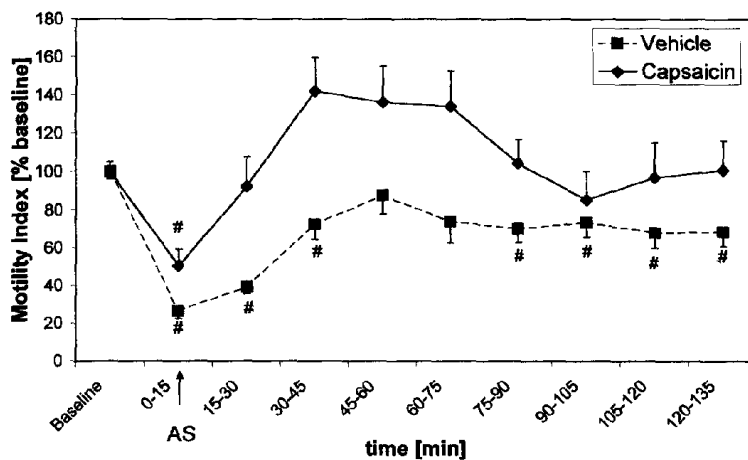
Fig. 7. Capsaicin infusion into the cecum (n = 5) 2 days prior to abdominal surgery *(AS)* increased intraoperative and postoperative colonic motility compared to vehicle infusion (n = 5). In capsaicin-treated rats, no postoperative decrease in colonic motility was observed compared to baseline motility, indicating immediate recovery of postoperative colonic contractile activity. # = P <0.05 vs. baseline.

and by 10%, respectively, whereas no changes were observed in the colon (Table II).

Contraction frequency was difficult to analyze during abdominal surgery because of the very irregular contraction patterns. Quite often it could not be determined, either by computer program analysis or by visual analysis, whether or not a slight increase in the motility curve was representative of a contraction. We therefore waived analysis of contraction frequency during abdominal surgery.

Capsaicin treatment prior to abdominal surgery significantly increased baseline gastric and small intestinal contraction frequency, but only small intestinal contraction frequency was increased postopera-

tively compared to vehicle treatment. Colonic contraction frequency was unchanged by capsaicin treatment (see Table II).

Postoperative Gastric and Colonic Motility 14 Days After Capsaicin Infusion Into the Cecum

Capsaicin infusion into the cecum 14 days prior to abdominal surgery did not affect recovery of postoperative gastric or colonic motility. Motility remained significantly decreased compared to baseline motility throughout the recording period (Fig. 8). Postoperative small intestinal motility 14 days after capsaicin infusion into the cecum was not investigated, since capsaicin in-

Table II. Effects of intraluminal capsaicin infusion into the cecum 2 days prior to abdominal surgery on postoperative gastrointestinal contraction frequency*

Time (min)	Stomach		Small intestine		Colon	
	Vehicle	Capsaicin	Vehicle	Capsaicin	Vehicle	Capsaicin
0-30	31.5 ± 1.5	34.5 ± 0.9†	117 ± 2.0	129 ± 1.8†	9.0 ± 0.3	8.0 ± 0.4
30-45	NA	NA	NA	NA	NA	NA
45-75	28.0 ± 1.5	33.0 ± 1.4	83 ± 2.9	76 ± 2.7	8.0 ± 0.4	8.0 ± 0.3
75-105	26.0 ± 1.2	28.0 ± 0.7	76 ± 3.6	85 ± 2.3	8.0 ± 0.3	8.0 ± 0.3
105-135	27.0 ± 1.2	28.0 ± 0.5	64 ± 3.8	74 ± 2.2‡	7.0 ± 0.4	7.0 ± 0.3
135-165	29.0 ± 1.3	30.0 ± 0.7	54 ± 4.3	79 ± 2.3‡	7.0 ± 0.3	6.0 ± 0.3

NA = not analyzed.

*Motility was recorded under baseline conditions (0 to 30 minutes), followed by abdominal surgery under enflurane anesthesia (30 to 45 minutes). Postoperative motility was recorded for another 120 minutes (45 to 165 minutes). Capsaicin treatment significantly increased the frequency of gastric and small intestinal contractions compared to baseline values but had no effect on the frequency of colonic contractions. Postoperatively capsaicin treatment significantly increased the frequency of small intestinal contractions compared to vehicle treatment, whereas it had no effect on the frequency of gastric or colonic contractions. Frequency is given as contractions per 5 minutes. For each time segment of 30 minutes, the average of six 5-minute segments was calculated. Frequency of intraoperative contractions was not analyzed because of the very irregular contraction patterns.
†$P < 0.01$ vs. baseline contraction frequency prior to capsaicin infusion into the cecum (0 to 30 minutes; Table I).
‡$P < 0.01$ vs. vehicle.
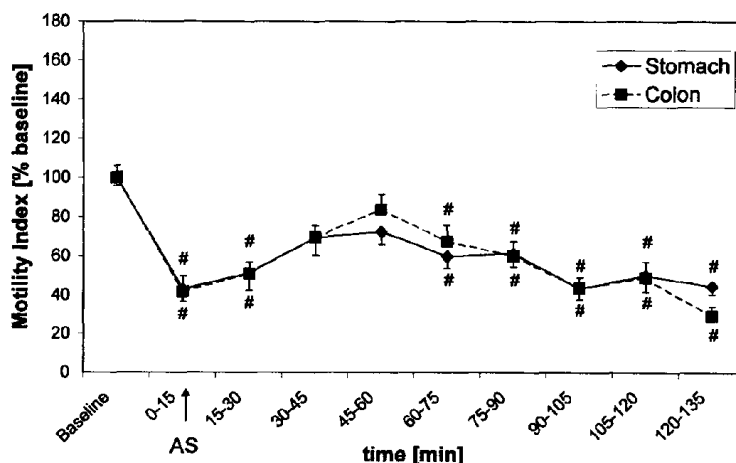
Fig. 8. Capsaicin infusion into the cecum 14 days before abdominal surgery (AS) did not significantly increase postoperative gastric (n = 4) or colonic (n = 4) motility. # = $P < 0.05$ vs. baseline.
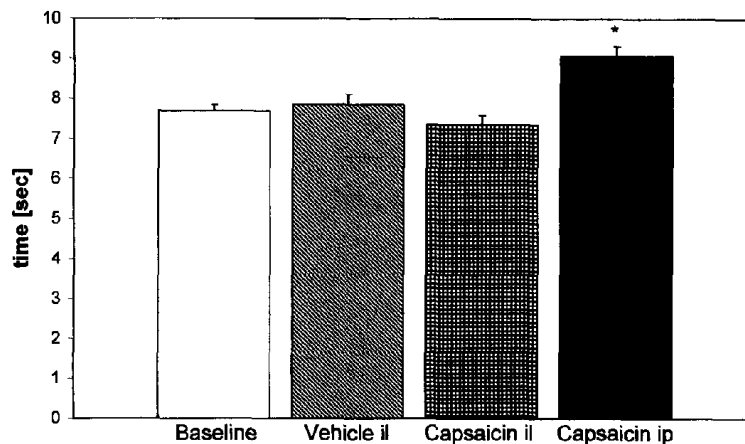
Fig. 9. Intraperitoneal *(ip)* capsaicin application (1 μmol/kg, n = 10) significantly increased the time to withdrawal of the tail in the tail flick test compared to untreated rats (baseline, n = 10), intraluminal *(il)* vehicle infusion into the cecum (n = 10), or intraluminal capsaicin infusion into the cecum (3.6 μmol, ≈12 μmol/kg, n = 10). * = P <0.05 vs. baseline, vehicle il, or capsaicin il.

fusion into the cecum 2 days prior to abdominal surgery had no effect on postoperative small intestinal motility.

Tail Flick Test

Intraperitoneal capsaicin injection at a dosage of 1 μmol/kg (0.305 mg/kg), corresponding to approximately 0.3 μmol (0.102 mg) of capsaicin per rat, significantly increased the tail withdrawal time during the tail flick test. In contrast, intraluminal capsaicin application of 3.6 μmol per rat (≈12 μmol/kg; 1.099 mg) or vehicle did not increase the tail withdrawal time (Fig. 9).

DISCUSSION

Capsaicin is the pungent substance in red peppers of the genus *Capsicum*. When given either systemically or locally, it produces a functional ablation of neurons with primary afferent C-fibers by depleting the terminal fields of these neurons of its neuropeptides, such as substance P or calcitonin gene-related peptide (CGRP).[18,19] When capsaicin is given systemically by intravenous, subcutaneous, or intraperitoneal injection, primary afferent C-fibers are permanently ablated, the threshold dose being estimated between 5 and 15 mg/kg.[18] After systemic capsaicin application, high tissue levels have been observed in the brain, the spinal cord, and intra-abdominal organs.[20] In contrast, after gastric perfusion with capsaicin, only small amounts of capsaicin were detected in the gastric mucosa, and almost nothing in the gastric muscle layer.[21] Although it has been shown that capsaicin can be absorbed from the gastrointestinal tract, it is degraded

immediately in the liver, and less than 5% appears in the circulation.[22]

In immunohistologic studies, intraintestinal capsaicin treatment in adult rats transiently reduced submucosal CGRP and, to a minor extent, submucosal substance P, with rapid restoration of CGRP or substance P content within days. No such changes were observed in the myenteric plexus.[23] Since we have previously shown that CGRP and capsaicin-sensitive visceral afferent nerve fibers play an important role in mediating postoperative gastric and colonic ileus,[10,11] and others have shown an important role for CGRP in postoperative small intestinal ileus,[24] we investigated whether intraluminal capsaicin is able to reduce postoperative ileus.

Acute infusion of capsaicin into the cecum induced a delayed, long-lasting increase in gastric or small intestinal motility. Capsaicin can either stimulate or relax gastric corpus smooth muscle cells, depending on the dosage applied.[25] The stimulatory component can be blocked by tachykinin antagonists, indicating substance P involvement, whereas the inhibitory component might be CGRP mediated, as CGRP has been shown to inhibit gastric smooth muscle cells.[26] However, because capsaicin was infused into the cecum, a direct capsaicin effect is unlikely. Rather, capsaicin stimulated afferent C-fibers in the cecal submucosa, which in turn stimulated gastric and small intestinal motility. Capsaicin infusion into the cecum also induced a temporary increase in colonic motility, which subsided once the capsaicin infusion was stopped. As for the stomach, capsaicin has been shown to induce stimulatory and inhibitory effects on colonic smooth muscle strips.[27] Possibly the increase in colonic motil-

ity observed during capsaicin infusion was the net effect of submucosal substance P and CGRP release, since intraluminal capsaicin would be expected to release submucosal CGRP and substance P.[23]

Laparotomy with gentle manipulation of the cecum is an established model for inducing postoperative ileus in rats,[28-30] possibly because of the rich sensory innervation of the cecum in rats.[31] Since presumably most of the operative trauma in our model occurred in the cecum, we applied capsaicin here in order to ablate submucosal CGRP fibers.[23] Thus we tried to evaluate the role of capsaicin-sensitive submucosal visceral afferent C-fibers in mediating postoperative ileus.

Abdominal surgery decreased gastric and colonic motility by approximately 83% and 75% in vehicle-treated rats, which is no different from what was found in untreated rats, as previously described.[15,32] In contrast, in capsaicin-treated rats, intraoperative gastric or colonic motility was decreased by only about 52% and 50%, representing motility increases of 166% and 100%, respectively, compared to vehicle-treated rats. Intraoperative inhibition of gastric or colon motility could not be prevented completely by capsaicin pretreatment. However, intraluminal capsaicin treatment would not be expected to have any effect on enflurane anesthesia, skin incision, or abdominal muscle layer incision, all of which have been shown to inhibit gastrointestinal motility as well.[15,33,34]

Capsaicin treatment completely prevented postoperative inhibition of gastric and colonic motility, indicating immediate postoperative recovery of contractile activity. For the first time we provide evidence that intraluminal capsaicin treatment is able to abolish postoperative inhibition of gastric and colonic motility. Since little or none of the intraluminal capsaicin applied 2 days prior to abdominal surgery is absorbed beyond the submucosal layer,[21,22] it is likely that capsaicin's effects on postoperative motility were mediated via the ablation of submucosal afferent nerve fibers. Our data suggest that capsaicin-sensitive submucosal visceral afferent nerve fibers play an important role in mediating postoperative ileus. Since capsaicin treatment into the cecum did not prevent postoperative inhibition of small intestinal motility, different pathways for the small intestine must be assumed. Intestino-intestinal inhibitory reflex pathways have been repeatedly shown to be mediated by capsaicin-sensitive afferent nerve fibers.[12,35,36] It is conceivable that these reflex pathways are activated by abdominal surgery and contribute to postoperative ileus.[10,11] Possibly activation of these pathways can be prevented by intraluminal capsaicin treatment.

There is recent evidence that abdominal surgery induces an inflammatory response in the gut wall,

thereby impairing gut smooth muscle cell function.[9] Visceral afferent nerve fibers might participate in this inflammatory response, since immune cells and afferent nerve fibers are known to interact in the gut wall.[37] Since capsaicin treatment has been shown to prevent neurogenic inflammation,[38,39] it is conceivable that intraluminal capsaicin treatment prior to abdominal surgery might inhibit the inflammatory response observed in the gut wall postoperatively, thereby improving postoperative recovery of smooth muscle cell function.

When applying capsaicin, the possibility of permanent nerve damages must be taken into consideration. We therefore tested whether intraluminal capsaicin treatment had long-lasting effects on postoperative gastric or colonic motility. However, the beneficial effects detected 2 days after intraluminal capsaicin treatment were no longer present after 14 days, indicating that afferent nerve fibers in the gut wall had probably recovered. As mentioned earlier, this is in agreement with studies showing recovery of neuropeptide content of submucosal nerve fibers after intraluminal capsaicin treatment within days.[23] Similarly, intestino-intestinal inhibitory reflex pathways ablated by intraluminal capsaicin treatment have been shown to completely recover within 18 days.[12] Obviously it is possible to temporarily ablate visceral afferent C-fibers that play an important role in the mediation of postoperative ileus.

We also investigated whether intraluminal capsaicin treatment caused alterations of nociception by testing skin nociception with the tail flick test. In our study, intraluminal capsaicin treatment did not influence skin nociception, suggesting that no afferent nerve fiber ablation outside the gut wall occurred. In contrast, intraperitoneal injection of a considerably lower capsaicin dose significantly delayed the reaction time in the tail flick test, indicating ablation of afferent skin nerve fibers. Probably capsaicin was absorbed via the peritoneum, and thus was able to act systemically. In contrast, intraluminal capsaicin treatment does not seem to induce any systemic afferent nerve fiber ablation, although more detailed studies would be necessary to definitively exclude this.

In summary, our data suggest a new pathway for the mediation of postoperative ileus, since the involvement of capsaicin-sensitive submucosal visceral afferent nerve fibers has not been shown before. Postoperative nociception and postoperative ileus are probably interrelated, since both are mediated via afferent C-fibers and the neuropeptides contained within them—namely, substance P and CGRP.[10,11,40-42] Capsaicin treatment of nociception in hyperalgesic states has already been tried successfully in humans,[43] and it is conceivable that capsaicin or newly developed

capsaicin analogues might be used to treat postoperative ileus in the future.[14,41,43]

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Myenteric Plexus in Spastic Motility Disorders

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Previous studies have often revealed an absence or reduction of ganglia in Auerbach's plexus in many patients with achalasia, which has been postulated to be related to the elevated lower esophageal sphincter pressure in these patients. We undertook a prospective study to determine whether microscopic changes were present in the myenteric plexus of patients with hypertensive lower esophageal sphincter, nutcracker esophagus, and diffuse esophageal spasm and if there was a correlation with lower esophageal sphincter pressure. Nine patients (3 men and 6 women; ages 49 to 72 years, mean 58 years) underwent a laparoscopic esophagomyotomy with fundoplication for symptomatic spastic motility disorder. A 10 mm × 5 mm segment of esophageal muscle was removed from the border of the myotomy incision, fixed in formalin, and examined under light microscopy for the presence or absence of ganglia and inflammation. Correlation between the presence of ganglia and lower esophageal sphincter pressure was tested by Pearson's bivariant correlation. Manometry revealed three patients with hypertensive lower esophageal sphincter, four patients with nutcracker esophagus, and two patients with diffuse esophageal spasm. All three patients with a hypertensive lower esophageal sphincter revealed an absence of ganglia, whereas the six patients with nutcracker esophagus and diffuse esophageal spasm exhibited ganglia despite an elevated lower esophageal sphincter pressure in four. Hypertensive lower esophageal sphincter resembled achalasia in its absence of ganglia in Auerbach's plexus, whereas nutcracker esophagus and diffuse esophageal spasm exhibited ganglia. There was no significant correlation in our series between the presence of ganglia and an elevated lower esophageal sphincter pressure in spastic motility disorders. (J GASTROINTEST SURG 2001;5:514-516.)

KEY WORDS: Manometry, histology, esophageal motility disorders, Auerbach's plexus, myotomy

The primary esophageal motility disorders of achalasia, nutcracker esophagus, diffuse esophageal spasm (DES), and hypertensive lower esophageal sphincter (LES) are characterized by spastic motor disturbance on manometry, which results in dysphagia or chest pain.[1] Previous histologic studies have focused primarily on achalasia and have often demonstrated an absence or reduction of ganglia in Auerbach's plexus, which may be associated with inflammatory cells.[2-5] The resultant denervation and loss of postganglionic nerve fibers have been postulated to be related to the hypertensive or nonrelaxing LES, which may be present in this disorder.[6] A few studies included histologic examination of DES and noted no alternation in ganglion; however, patients with an elevated LES pressure were excluded.[4,7] Patients with nutcracker esophagus and DES may also demonstrate an elevation in LES pressure or a nonrelaxing LES, making distinct characterization of primary motility disorders difficult.[1]

The purpose of this prospective study was to determine whether ganglia were present or absent in the myenteric plexus in patients with hypertensive LES, nutcracker esophagus, and DES, and if there was a correlation with LES pressure.

MATERIAL AND METHODS

Nine patients (3 men and 6 women; ages 49 to 72 years, mean 58 years) were included in this study. All were symptomatic with dysphagia or chest pain that had been managed unsuccessfully with conservative medical treatment.

Preoperative evaluation included esophagogastroscopy and manometry. Manometric studies were performed with a three-channel solid-state catheter using a 1 cm station pull-through technique to localize and measure the LES. Body motility was assessed with a catheter positioned 3 cm above the LES utilizing 10

wet swallows. Normal LES pressure in our laboratory is 18 to 35 mm Hg. Primary motility disorders were characterized by criteria established by Castell and Castell.[1] Achalasia was required to demonstrate absent esophageal body peristalsis, and some patients may have incomplete LES relaxation or a hypertensive LES. DES was required to demonstrate greater than 20% simultaneous contractions with intermittent normal peristalsis, and some patients may have incomplete relaxation of the LES or elevated LES pressure, with repetitive, prolonged, or high-amplitude contractions. Patients with nutcracker esophagus demonstrated high-amplitude peristaltic waves of at least 180 mm, with normal progression, which may be of prolonged duration. Hypertensive LES is characterized by a resting LES pressure above 45 mm Hg with normal peristalsis, and patients may have abnormal LES relaxation.

All patients underwent a laparoscopic esophagomyotomy and fundoplication. The myotomy was 6 cm in length and extended across the gastroesophageal junction. After completion of the myotomy, a 10 mm × 5 mm segment of longitudinal and circular esophageal muscle was incised from the lower border of the esophageal incision.

The tissue was fixed in formalin, stained with hematoxylin and eosin, and examined under a microscope for ganglia and inflammatory cells. A correlation between the presence of ganglia and elevated LES pressure was tested by Pearson's bivariant correlation.

RESULTS

Preoperative manometry revealed three patients with hypertensive LES (LES pressure 57, 55, and 55 mm Hg, respectively; mean 56 mm Hg), four patients with nutcracker esophagus (LES pressure 65, 45, 47, and 37 mm Hg; mean 46 mm Hg), and two patients with DES (LES pressure 50 and 27; mean 47 mm Hg).

All three patients with hypertensive LES revealed an absence of ganglia, whereas the six patients with nutcracker esophagus and DES exhibited ganglia despite an elevated LES pressure in four. One patient with nutcracker esophagus and one with DES had a normal LES pressure. None of the patients demonstrated inflammatory cells.

There was no correlation ($P = 0.32$) between the presence of ganglia and elevated LES pressure.

DISCUSSION

The etiology of primary motility disorders is unknown. Previous reports of the ultrastructure of the esophageal muscle in achalasia and DES have revealed no alteration in the smooth muscle fibers other than hyperplasia and have excluded a primary myopathy.[4]

The focus in achalasia has been on a neurogenic etiology because of the reduction or absence of ganglia, which may be demonstrated in the myenteric plexus, and fine structural changes in the vagus nerves.[2,3] More recently the number of ganglion cells and degree of inflammation or fibrosis are believed to change with the stage of achalasia.[5] The hypertensive or nonrelaxing LES in achalasia has been attributed to Cannon's law of denervation with a sphincter that is highly sensitive to cholinergic drugs.[3] Relaxation of the LES from its resting tonic state occurs with the activation of postganglionic inhibitory neurons by an unknown neurotransmitter. With the loss of ganglion cells in the myenteric plexus and degeneration of postganglionic nerve fibers, a hypertensive LES should occur. Histologic studies, however, have demonstrated no correlation between LES pressure and absence or decrease of ganglion cells in achalasia.[3]

Our study confirms the observations of others that no histologic abnormalities are demonstrated in DES.[4,7] In addition, we documented the presence of ganglion cells and the absence of inflammation in nutcracker esophagus. The presence of ganglion cells in the four patients with nutcracker esophagus and one DES patient with elevated LES pressure raises additional questions about the etiology of the sphincter dysfunction. The mere presence or absence of ganglion cells is unrelated to LES pressure in any of the spastic primary motility disorders in our study. Other factors must be active in regulating sphincter tone. There may be qualitative changes in the innervation, or regulatory peptides may assume a more important role than is recognized at present. Recent research suggests that nitric oxide and vasoactive intestinal polypeptides may serve as regulators of LES tone.[5]

The association of an elevated LES pressure in patients with nutcracker esophagus or DES is not characteristic of these spastic motility disorders, but it was important to include this subset in our study since it was excluded previously.[4,7] Of 968 patients who presented to our laboratory for evaluation of gastroesophageal reflux disease, 174 (18%) demonstrated a primary motility disorder and 78 (45%) underwent a surgical myotomy for intractable dysphagia or chest pain of 8 years' duration on average. Of the 78 patients who underwent myotomy, 26 had nutcracker esophagus with 42% demonstrating an elevated LES pressure, and 13 had DES with 23% demonstrating an elevated LES pressure. It is not surprising that this group would not respond to medical management and require surgical myotomy, making histologic examinations possible. Of the nine patients in this study, eight (89%) have complete resolution of their dysphagia or chest pain at 36 months. One patient had persistent chest pain for more than 6 weeks and underwent a 24-hour pH study,

which was negative for reflux; the pain resolved with intermittent oral antispasmotic medication (amitriptyline, 10 mg three times a day).

The classification of abnormal manometric patterns into distinct disorders can be difficult. Patterns can be transient and absent on a particular day, and have also been shown to evolve from one pattern to another over time.[1,6,8] The distinct labels we place on spastic motility patterns may only represent different stages in the spectrum of one disorder rather than separate disorders. Histologic biopsies may help to further classify motility disorders. Our patients with nutcracker esophagus and DES exhibited ganglia, however, both nutcracker esophagus and DES have been reported on occasion to evolve to achalasia or to revert back to a normal pattern.[6,8] The presence of ganglion cells on biopsy could indicate an earlier stage in the process, which may still be reversible. Achalasia and hypertensive LES share the histologic feature of absent ganglion cells but patients with a hypertensive LES have peristalsis and body motility is preserved. This raises the question of whether hypertensive LES is also a precursor to achalasia. To date we are not aware of any reports documenting a transition from hypertensive LES to achalasia on manometry. Further long-term follow-up will be required to determine whether a correlation exists. Absence of ganglia, however, signifies a permanent change in the ultrastructure of the esophagus, which is not reversible.

CONCLUSION

Our study demonstrates a histologic change of absence of ganglion cells in the myenteric plexus in patients with a hypertensive LES, which resembles achalasia, whereas patients with nutcracker esophagus or DES did exhibit ganglia. There was no significant correlation in our small series between the presence of ganglia and an elevated LES pressure in the spastic motility disorders of nutcracker esophagus, DES, and hypertensive lower esophageal sphincter, but future studies with larger numbers of patients will be required to further validate our findings.

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Ileal Absorptive Adaptation to Jejunal Resection and Extrinsic Denervation: Implications for Living-Related Small Bowel Transplantation

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Net absorption of water, electrolytes, and simple nutrients decreases early after jejunoileal autotransplantation (extrinsic denervation) in a canine model but recovers toward normal by 8 weeks. However, the ability of the extrinsically denervated ileum to adapt after total jejunectomy, which would be relevant as a model of segmental small bowel transplantation, remains unknown. Two groups of five dogs each were studied before and 2 weeks and 12 weeks after 50% proximal enterectomy. A control group remained neurally intact, whereas the other group underwent extrinsic denervation (Ext Den) of the remaining ileum. Using a perfusion technique, net absorption of water, electrolytes, and five simple nutrients (glucose, arginine, glutamine, and oleic and taurocholic acids) was measured at the three time points. Ileal morphometry was also evaluated. All dogs developed diarrhea, which resolved by 12 weeks in all but two of the Ext Den dogs. Weight in both groups was decreased at 2 weeks ($P < 0.05$), returned to normal at 12 weeks in control dogs, but remained low in Ext Den dogs ($P < 0.05$). Maximal weight loss was greater in the Ext Den group ($P < 0.05$). No consistent or important differences in net absorptive fluxes of water, electrolytes, or simple nutrients were noted either within or between groups at any time point. Villous height, crypt depth, and longitudinal muscle width increased significantly at 12 weeks after jejunectomy in the Ext Den dogs, but not in the control dogs ($P < 0.05$). Extrinsic denervation of the ileum results in persistent weight loss after proximal 50% enterectomy. Despite diarrhea, only minor changes in electrolyte absorption occur, and ileal net absorption of simple nutrients remains unaffected. The ileum of extrinsically denervated dogs undergoes a more prominent morphometric adaptation after jejunectomy. Extrinsic denervation necessitated by small bowel transplantation, independent of immune effects, does not appear to suppress the ileal adaptive response to maintain net absorption of water, electrolytes, and simple nutrients. (J GASTROINTEST SURG 2001;5:517-524.)

KEY WORDS: Small bowel transplantation, extrinsic denervation, intestinal adaptation

Small bowel transplantation has now become a clinical reality,[1] and the new frontier in small bowel transplantation may involve segmental grafts with the use of living-related donors.[2] Several experimental studies in rats have shown the feasibility of segmental small bowel transplantation in maintaining nutrition and supporting growth.[3] The concept of segmental small bowel transplantation, aside from important immunologic considerations, raises the question of the functional "adequacy" of different regions of trans-planted small bowel to support the nutritional requirements of the recipient. However, the regional ability of the transplanted (and thus extrinsically denervated) segment to adapt is not well described and may prove to be a factor that limits the potential of segmental transplants.

Many factors modulate the intestinal adaptive response to resection, including luminal nutrients and fiber, pancreatobiliary secretions, circulating humoral agents such as hormones, growth factors, and puta-

tive regulatory peptides, and the anatomic region of the remaining gut.[4-9] The role of extrinsic innervation to the gut, which modulates absorption, motility, and other functions of the gut, may represent another important factor in this adaptive response that has not been well investigated.

The aim of this study was to determine the role of extrinsic innervation in the adaptive responses of the ileum in a large animal (canine) model of moderate short bowel syndrome. Our goals were of both physiologic interest (role of extrinsic innervation in modulating adaptation) and clinical relevance (does the ileum adapt well to transplantation). Our previous work showed that extrinsic denervation of the entire jejunoileum led to an impressive early diarrhea that eventually resolved 6 to 8 weeks postoperatively,[10,11] suggesting an adaptive response. Moreover, extrinsic denervation decreased net absorption in both the jejunum and the ileum at 2 weeks after denervation.[12,13] Our hypothesis was that the adaptive response to jejunal resection in the extrinsically denervated canine ileum would be blunted, and that early net absorptive function would be less efficient. Our experimental preparation of in situ neural isolation of the jejunoileum is a model of intestinal autotransplantation devoid of immune considerations and permits study of the enteric function of the extrinsically denervated gut.

MATERIAL AND METHODS
Overall Design

Two groups of five dogs each were studied before and after 50% proximal small bowel resection. One group remained neurally intact (control), and the other group underwent extrinsic denervation of the remaining ileum (Ext Den). Both groups underwent baseline in vivo experiments of net ileal absorption of water, electrolytes, and five simple nutrients (glucose, arginine, glutamine, oleic acid, and taurocholate). After completing baseline studies, both groups underwent a 50% proximal jejunoileal resection; in addition, the Ext Den group underwent complete extrinsic denervation of the remaining ileum. Thereafter both groups were restudied 2 weeks and 12 weeks postoperatively with identical experiments of absorption in vivo.

Animal Preparation

Healthy female mongrel dogs weighing 16 to 22 kg were used adhering to the standards set forth by the Institutional Animal Care and Use Committee of the Mayo Foundation in accordance with the guidelines of the National Institutes of Health and the United States Public Health Service policy on the humane care and use of laboratory animals. Anesthesia was induced by means of intravenous sodium methohexital induction (12.5 mg/kg) and maintained with endotracheal halothane. All dogs underwent a midline celiotomy. At a point 115 cm proximal to the ileocecal junction, an infusion catheter (outside diameter [OD] 1.5 mm) was inserted into the ileal lumen, and through the same enterotomy, a larger bore aspiration catheter (OD 2 mm) was positioned with its tip 15 cm distally. These two catheters were sutured in place and embedded in a metal cannula exteriorized through the abdominal wall. A modified Thomas cannula was then inserted 95 cm distal to the proximal ileal catheter (Fig. 1). This configuration allows for a 15 cm mixing segment and an 80 cm study segment similar in principle to our previous study.[14] For the first 3 days, dogs were given intramuscular butorphanol for pain control and maintained on parenteral fluid and electrolytes before being allowed ad libitum feeding. After a 2-week recovery, the dogs underwent baseline absorption studies. At this stage both groups of dogs had a neurally intact ileum.

After completing the baseline studies, all dogs underwent resection of the proximal 50% of the jejunoileum starting 10 cm distal to the ligament of Treitz. Intestinal continuity was restored with an end-to-end jejunoileostomy. This procedure has been shown to establish a moderate short bowel syndrome with weight loss but without mortality related to the extent of resection.[12,15,16] Dogs were then randomly divided into two groups. Control dogs (neurally intact) had no further treatment. Ext Den dogs underwent our model of in situ neural isolation of the remaining jejunoileum as described and validated previously as a model of extrinsic denervation.[11] In brief, the ileum was transected 5 cm proximal to the ileocecal junction, and the mesenteries at the site of jejunal and distal ileal transection were incised in radial fashion back to the superior mesenteric artery and vein just distal to the inferior pancreaticoduodenal vessels. Next, all extrinsic nerves, lymphatics, and connective tissue traveling to the jejunoileum along these vessels were ligated and transected. In addition, the superior mesenteric artery and vein were meticulously skeletonized and stripped of their investing adventitia for 1 to 2 cm under optical magnification. Intestinal continuity was restored by end-to-end ileoileostomy. We purposely did not divide and reanastomose the superior mesenteric artery and vein because this maneuver would introduce a confounding variable of ischemia/reperfusion injury. Similarly, using dogs as their own controls avoided confounding immune phenomena. After the dogs recovered, absorptive studies were performed 2 weeks and 12 weeks postoperatively.
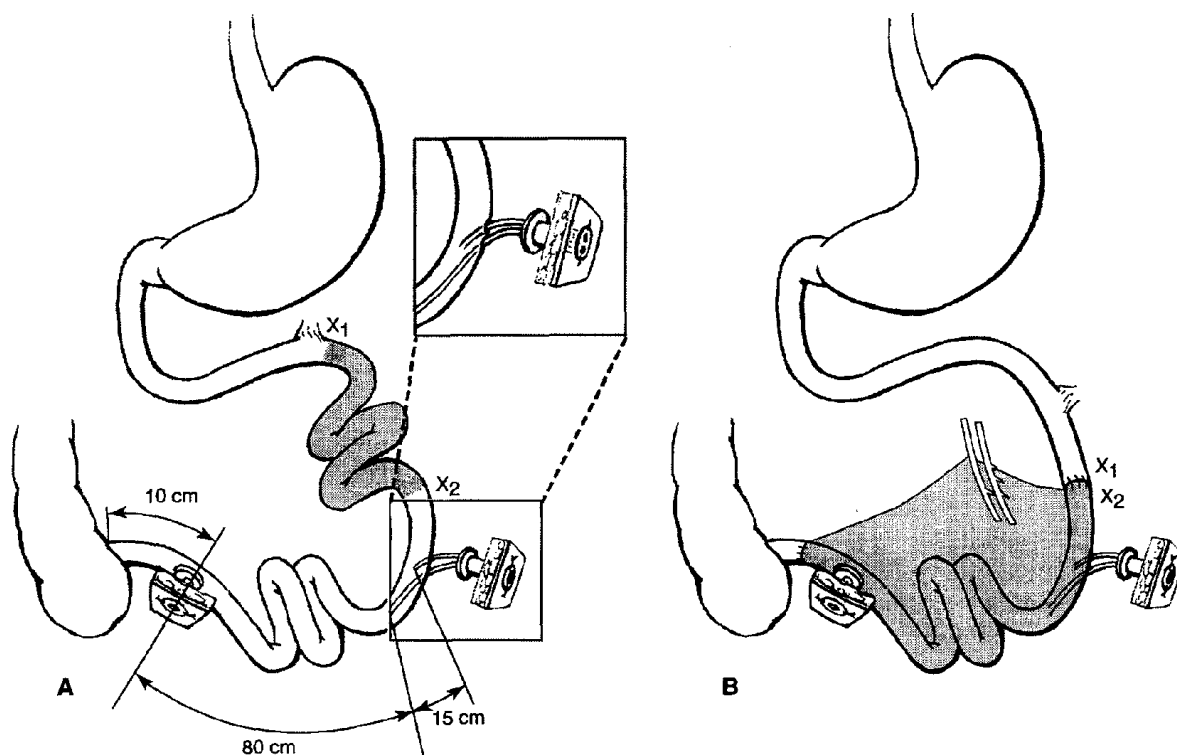
Fig. 1. Experimental model. **A,** Insertion site of perfusion catheter system in the ileum; shaded bowel between X_1 and X_2 is the part that will be resected. *Inset* shows the proximal infusion and proximal aspiration sites. **B,** After resection of the jejunum, the shaded area is the extrinsically denervated ileum in the Ext Den group and remains extrinsically innervated in the control group.

Tissue Sampling

For morphometric studies, a piece (1 × 2 cm) of bowel was excised from the distal end of the resected bowel at the time of the jejunal resection and from the proximal end of the remaining "adapted" bowel just before the dogs were killed after completion of all absorptive experiments. Each sample was carefully pinned out, mucosa side up, on styrofoam and fixed in formalin overnight.

Conduct of Studies

All experiments were conducted after an overnight fast in conscious dogs resting comfortably in a Pavlov sling. The following six test solutions were evaluated: (1) basal solution (water and electrolytes) containing Na^+ (140 mEq/L), K^+ (5 mEq/L), HCO_3^- (35 mEq/L), and Cl^- (110 mEq/L) titrated to pH 7.4 (this solution was designed to reproduce the normal jejunal/proximal ileal electrolyte content); (2) basal solution, but also containing 2.5 mmol/L ^{3}H-glucose (Glc); (3 and 4) basal solution and 2.5 mmol/L ^{3}H-arginine (Arg) or 2.5 mmol/L ^{3}H-glutamine (Glu),

respectively; (5) basal solution and 5 mmol/L ^{3}H-oleic acid delivered as a bile salt emulsion[17] by adding desiccated, unfractionated bovine bile (Sigma Chemical Co., St. Louis, Mo.) containing 11.8 mmol/L bile salts; and (6) basal solution and 5 mmol/L ^{3}H-taurocholate (the primary bile salt in the dog). Via the proximal ileal infusion catheter, the prewarmed (39° C) test solutions containing 5 g/L polyethylene glycol (PEG) labeled with 5 μCi of ^{14}C-PEG as a nonabsorbable volume marker was infused at a rate of 5 ml/min for 4 hours. This protocol was repeated on separate days for each of the six test solutions. Net absorption of each solution was evaluated twice at each time point whenever possible. Samples of enteric fluid were collected from the proximal aspiration catheter at 1 ml/min using a Harvard constant aspiration pump (Harvard Apparatus, Boston, Mass.). Effluent from the 80 cm test segment was collected from the distal cannula at 1-hour intervals.

Analytic Methods

Concentrations of sodium and potassium were determined using flame photometry and chloride using

a chloridometer. Concentrations of the nonabsorbable marker ^{14}C-PEG and the solutes ^{3}H-Glc, ^{3}H-Arg, ^{3}H-Glu, ^{3}H-oleic acid, and ^{3}H-taurocholate were determined by dual-label, liquid scintillation techniques.[12]

Intestinal samples for morphometric studies were fixed, embedded, and cut into multiple strips from each of three separate areas and stained with hematoxylin and eosin. Villous height, crypt depth, and widths of the circular and longitudinal muscle layers were measured with light microscopy using an optical reticule as described previously.[18]

Analysis of Data

Net absorption of water, electrolytes, and simple nutrients was determined using principles of the triple-lumen infusion technique.[13,14] After allowing the first hour for establishment of steady-state conditions (i.e., the amount of nonabsorbable marker entering the study segment equals the amount of marker leaving the distal end of the segment), the change in concentration of nonabsorbable marker and solutes between the infusion and proximal aspiration catheters allowed us to calculate the rate and concentration of each substance delivered to the test segment. Then, by using the change in concentration of PEG to correct for volume absorbed, the difference between amount of solute at the proximal aspiration catheter and the distal ileal cannula yields net absorption; net absorption is then calculated as net absorptive flux according to one of the following: ml/cm/min, mEq/cm/min, or mmol/cm/min.

The means of the individual values for net absorptive flux for each of the 1-hour intervals per experiment were calculated, and the means of these mean values for the two replicate experiments were also obtained. Grand means across dogs were calculated for the basal, 2-week, and 12-week absorptive experiments.

For morphometric studies, three separate measurements of villous height, crypt depth, and circular and longitudinal muscle width were determined on each of three strips from three separate pieces of each sample of bowel wall (n = 27 measurements per parameter/per dog). Samples from control dogs provided baseline values for adapted "neurally intact" bowel and were compared to samples from Ext Den dogs reflecting the adapted "neurally isolated" bowel.

Statistical Analysis

Comparisons within each group were made to baseline values using paired analysis. Comparisons between groups were made at each time point using unpaired analysis. Differences in values were compared using both analysis of variance (ANOVA) and, when differences were seen, individual comparisons using Student's t test for paired data with a Bonferroni correction for the multiple comparisons. Data in the text are presented as mean values ± standard error of the mean (SEM).

RESULTS

All dogs tolerated catheter and cannula placement and subsequent jejunal resection well. The Ext Den dogs also tolerated ileal denervation well. All dogs had liquid stool after jejunectomy, which persisted at 6 weeks postoperatively. Twelve weeks postoperatively, all control dogs had normal, solid, formed stool, whereas only three of the five Ext Den dogs had normal, solid, formed stool; the other two had persistent diarrhea. Preoperative body weights in the Ext Den and control groups were similar (17.1 ± 0.3 kg vs. 17.3 ± 0.8 kg, respectively). All dogs lost weight after jejunectomy, but maximal weight loss was greater in the Ext Den dogs than in the control dogs (3.2 ± 0.4 kg vs. 1.7 ± 0.3 kg; $P = 0.01$). At 12 weeks after jejunectomy, when compared to baseline weight, Ext Den dogs had lost 1.0 ± 0.3 kg, whereas control dogs had gained 0.3 ± 0.4 kg ($P = 0.03$). These differences occurred despite similar appetites, activity levels, and overall subjective health in the two groups.

Absorption Studies

Net water absorption, presented as net absorptive flux (ml/cm/min), is shown in Table I. Net water absorption did not differ at any time point between groups (t test; $P > 0.05$), or within groups over the 12-week study period (ANOVA; $P > 0.05$) in any of the six test solutions. Net absorption of electrolytes and simple nutrients, presented as net absorptive flux (mEq/cm/min or mmol/cm/min), are also shown in Table I. No significant differences in absorption were observed at any time point between groups (t test, $P > 0.05$) or within groups over the 12-week study period (ANOVA; $P > 0.05$).

Morphometric Studies

Villous height, crypt depth, longitudinal muscle width, and circular muscle width in the distal jejunum/proximal ileum were similar (as expected) in the two groups at baseline (Fig. 2). In the control group, none of the histologic parameters were significantly different 12 weeks after jejunectomy. However, in the Ext Den group, villous height ($P = 0.02$), crypt depth ($P = 0.03$), and longitudinal muscle width ($P = 0.009$) were increased at 12 weeks.
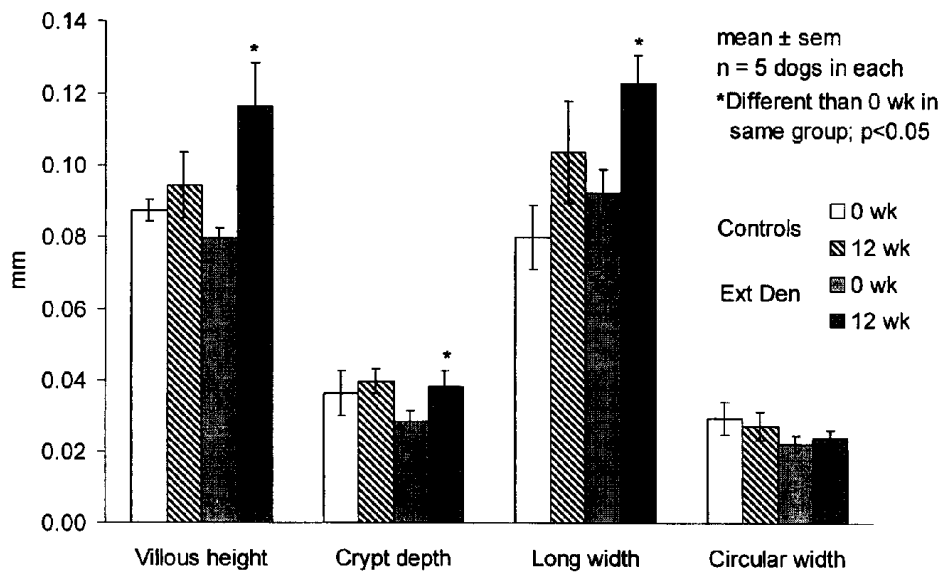
Fig. 2. Morphometric characteristics of proximal ileum before and after jejunectomy.

Table I. Net ileal absorptive fluxes of water (ml/cm/hr), electrolytes (mEq/cm/hr), and simple nutrients (mmol/cm/hr) in dogs before (0 weeks) and after (2 weeks and 12 weeks) 50% proximal enterectomy*

	Control group (neurally intact)			Ext Den group (extrinsically denervated)		
	0 wk	2 wk	12 wk	0 wk	2 wk	12 wk
H_2O	0.8 ± 0.1	1.1 ± 0.3	1.2 ± 0.3	1.1 ± 0.1	0.8 ± 0.1	0.9 ± 0.1
Na	17 ± 3	22 ± 4	20 ± 5	19 ± 2	18 ± 2	14 ± 1
K	0.3 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
Cl	15 ± 3	19 ± 3	19 ± 4	17 ± 2	16 ± 1	14 ± 1
Glucose	0.7 ± 0.1	0.7 ± 0.2	0.6 ± 0.1	0.67 ± 0.1	0.6 ± 0	0.6 ± 0.1
Arginine	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0	0.5 ± 0.1
Glutamine	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0	0.6 ± 0.1
Oleic acid	0.7 ± 0.2	0.7 ± 0.2	0.9 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
Taurocholate	1.3 ± 0.1	1.2 ± 0.2	1.3 ± 0.1	1.2 ± 0.1	1.0 ± 0.2	0.9 ± 0.2

*Mean ± SEM; n = 5 dogs per group; no significant differences between or within groups.

DISCUSSION

Most of the advances and interest in the field of small bowel transplantation have focused on the immunobiology of the transplanted gut, whereas many aspects of enteric physiology after small bowel transplantation remain poorly understood. In particular, segmental small bowel transplantation is an intriguing new frontier in human small bowel transplantation for several important reasons. First, a shorter graft would minimize the antigen burden for the host. Second, this approach would allow use of grafts from living-related donors, further minimizing immunologic complications. Additional possible benefits include increased availability of grafts and the ability to plan the small bowel transplant as an elective proce-dure with many potential benefits including shorter ischemic time for the graft.

This concept of segmental small bowel transplantation, however, raises the question of the functional "adequacy" of the shorter segment to support the nutritional requirements of the recipient. Although it has been demonstrated clinically that a patient can adapt to loss of more than 75% of the small intestine, the length of small bowel necessary to restore gut function in the setting of transplantation is unknown. Furthermore, not only the length but also the anatomic origin of the segment of small intestine used for segmental small bowel transplantation may have an impact on this functional adequacy. Variations in function and adaptation of different regions of the small

intestine become clinically relevant in the setting of segmental small bowel transplantation. Our study was designed to investigate the effect of extrinsic denervation on the adaptation of the ileum after jejunectomy, a model of segmental small bowel transplantation of the ileum; this model was developed specifically to be devoid of the confounding effects of immunosuppression, immune rejection, ischemia/reperfusion injury, or systemic venous drainage of the gut.

In our previous work we developed a canine model of jejunoileal autotransplantation designed specifically to eliminate confounding pathophysiologic factors of ischemia/reperfusion injury, systemic venous drainage of the study segment, and immune rejection/immunosuppression, each of which may alter absorptive function, to study the isolated effects of extrinsic denervation on post-transplantation enteric physiology.[11,14] We showed that net ileal and net jejunal absorption of water, electrolytes, and bile salts decreased early after this model of extrinsic denervation but returned toward normal 8 weeks later, suggesting an adaptation by the extrinsically denervated jejunum and ileum.[13,14] In contrast, jejunal[19] and ileal[20] absorption of glutamine, arginine, leucine, and glucose from the bowel in vivo, in concentrations in which absorption was mediated primarily by active transport, were decreased both early (2 weeks) and late (8 weeks) after this model. Mechanistically, we further investigated these findings by examining transport kinetics from brush border membrane vesicles in innervated and extrinsically denervated canine small bowel. Active, carrier-mediated transport of glutamine (as well as glutamine absorption in vivo from the jejunum) remained decreased at 8 weeks. This decrease was secondary to a decrease in V_{max}, which is a function of the number of carrier molecules per gram of tissue rather than a change in Km, which measures changes in carrier affinity.[19] In the present study our aim was to expand our past work on the effects of extrinsic denervation on postjejunoileal transplantation enteric physiology to address the role of extrinsic neural input in the regional adaptive response of the ileum to subtotal intestinal resection.

Adaptation of the remaining small bowel after intestinal resection or bypass is a well-known event[4] demonstrated both structurally and functionally. Structurally, adaptation appears to occur via hyperplasia, with observed increases in villous height, crypt depth, and mucosal wet and dry weight, as well as mucosal protein, DNA and RNA content, and mucosal mass in the intestinal remnant. Functionally, the remaining bowel adapts by an increase in absorption per unit length of small bowel. Cellular changes can be detected within hours of resection, but the entire process can take months to complete. Although the

mechanism of these adaptive responses, even the relationship between histologic changes and functional changes, remains poorly defined, many factors appear to contribute. Intraluminal nutrients,[4] adequate enteric or plasma delivery of glutamine, the primary fuel source of the enterocyte,[5,6] pancreatobiliary secretions,[4,7] growth hormone,[6] enteroglucagon,[7] epidermal growth factor,[8] and peptide YY[17] have all been recognized as important enterotrophic factors that help to maintain normal structure and function of the gut and promote adaptation after resection or bypass.

The extent of adaptation appears to be related to the length and the site of the remnant small intestine. The greater the extent of resection, the greater the adaptive response. However, clinical observations, as well as experimental models of adaptation, show that proximal resection is better tolerated than distal resection. Cosnes et al.[21] found that distal resection in humans results in greater steatorrhea and increased stool moisture than proximal resection. They also demonstrated that vitamin B_{12} absorption was preserved after proximal but not distal resection. Thompson et al.[22] showed gross and microscopic evidence of a greater degree of structural adaptation in the ileal remnant after proximal resection compared to the jejunal remnant after resection in dogs; Nygaard[23] and Booth et al.[24] reported similar findings in rats. It is unclear whether this phenomenon is due to intrinsic differences or differences in adaptive capacity of the jejunum and the ileum. Transposition studies suggest that some of the observed differences relate to changes in luminal nutrient load.[25-28] Some difference can be attributed to the observation that several specific absorptive functions of the ileum cannot be assumed by the jejunum. Also, there are regional differences in the responses to specific nutrients such as zinc and glutamine, known intestinal growth factors.[29-30] Further, the distributions of various gastrointestinal hormones and growth factors are known to change throughout the gastrointestinal tract, which may also explain the variable response to resection. Finally, the well-established variation in regional motility may also differentially affect adaptation. Whatever the cause, there is a greater extent of adaptation in the remnant segment of bowel after proximal resection than after distal resection.

In the current study we examined the role of extrinsic neural input to the ileum in the regional adaptive response of the ileum to subtotal intestinal resection, a model of the enteric physiology of segmental ileal small bowel transplantation. Based on our previous work and that of others,[31,32] we hypothesized that the adaptive response of augmented net absorption and mucosal hyperplasia would occur in the extrinsically denervated ileum but would show a blunted re-

sponse when compared to resection-only control animals. However, our results showed no significant alteration in ileal net absorption of water, electrolytes, or simple nutrients after proximal 50% enterectomy, with or without extrinsic denervation, either early (2 weeks) or late (12 weeks) postoperatively. The lack of a decrease in net absorption after proximal 50% resection and no measurable functional adaptive response in the control animals suggests that a functional adaptation for the solutes tested in this group is physiologically unnecessary. This finding may be due to the moderate nature of the short gut syndrome produced by a 50% enterectomy. Although clinically our model of short gut is substantiated by weight loss and diarrhea, our model may be too mild for our method of quantifying absorption of water, electrolytes, and simple nutrients or to evaluate the more complex process of digestion and absorption of more complex carbohydrates, proteins, and fats.

However, the lack of change in net absorption of water, electrolytes, and simple solutes in the extrinsically denervated ileum 2 weeks after proximal 50% enterectomy differs from our findings of a decrease in net absorption of the extrinsically denervated ileum when the jejunum remains in situ.[14] Based on our previous work, this maintenance of baseline absorption in the setting of extrinsic denervation and proximal jejunal resection suggests a functional adaptive response to resection that is present at 2 weeks and maintained at 12 weeks. We would speculate that early adaptation probably occurs through changes in enteric neural modulation of mucosal absorptive function, but we have no objective data to support this speculation at this time. The morphometric changes of increased villous height, muscle width, and crypt depth measured at 12 weeks postoperatively in the extrinsically denervated group suggest a structural adaptive response. Unfortunately the design of our study did not allow for ileal biopsy at 2 weeks postoperatively; thus correlating functional and structural adaptive responses at 2 weeks is not possible.

Our findings suggest that extrinsic denervation actually enhanced adaptation after 50% proximal enterectomy. Based on our previous work, which indicates a level of functional disability induced by extrinsic denervation, it is likely that the greater extent of functional disability in absorption induced by extrinsic denervation over and above the functional disability introduced by 50% proximal enterectomy triggers a greater adaptive response compared to neurally intact dogs that underwent proximal resection alone. It is important to note that although extrinsic denervation causes some gut dysfunction, it does not appear to affect the ability of the gut to adapt to compensate for that dysfunction. This ob-

servation suggests that the extrinsic denervation inherent in the procedure of small bowel transplantation may not affect the ultimate functional outcome of the transplanted segment.

In contrast to our findings, Lauronen et al.[32] recently concluded that autotransplantation blunts the adaptive ability of the ileum, showing that both functional and structural adaptation occurred in pigs after proximal small bowel resection but not in pigs that underwent proximal small bowel resection with concomitant full autotransplantation of the remaining ileum. The differences between the findings of Lauronen et al. and ours may be reflected by the differences in the animal models used (dog vs. pig) or in the models of transplantation—our model is devoid of ischemia/reperfusion, maintains intestinal venous flow through the portal venous system, and specifically isolates the effects of extrinsic denervation. The porcine model of Lauronen et al.[32] involved a full autotransplantation with its multiple confounding factors.

Despite the lack of objective decreases in net absorption of water, electrolytes, bile salts, and simple nutrients, extrinsic denervation of the ileum results in diarrhea and a persistent weight loss after proximal 50% enterectomy. In spite of the diarrhea, only minor insignificant changes occur in net electrolyte absorption or in net absorption of simple nutrients. This impressive diarrhea and weight loss remains largely unexplained. Our past work failed to show a generalized global abnormality in absorption or a marked steatorrhea after in situ neural isolation of the entire jejunoileum[10]; similarly, more focused evaluations of regional absorption of water and electrolytes showed only modest (albeit significant) decreases that would not likely explain most of this diarrhea. These findings suggest that the diarrhea may arise from the large intestine. Indeed this canine model of jejunoileal extrinsic denervation also necessitates denervation of the proximal large intestine, and preliminary work in our laboratory (unpublished data) confirms that extrinsic denervation of the proximal large intestine of the dog decreases net absorption of water and electrolytes with values returning to normal 8 weeks later.

In summary, this study suggests that the extrinsically denervated ileum can acutely (within 2 weeks) and chronically (at 12 weeks post denervation) adapt to the loss of the jejunum both functionally and structurally. These findings may have important implications in the field of human segmental small bowel transplantation.

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Long- or Short-Limb Gastric Bypass?

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The aim of this study was to determine whether longer limb length improved results of gastric bypass in patients who were morbidly obese (body mass index <50 kg/m^2) or superobese (body mass index >50 kg/m^2). A total of 242 patients were followed for a mean of 5.5 years. The standard operation was a Roux-en-Y gastric bypass with a 40 cm Roux limb and a 10 cm afferent limb. The long-limb operation had a 100 cm Roux limb and a 100 cm afferent limb. Morbidly obese patients did not benefit from a long-limb bypass. The final body mass index was 28.6 ± 4.7 kg/m^2 in the short-limb group and 28.5 ± 3.8 kg/m^2 in the long-limb group. The superobese patients did benefit from a long-limb bypass. Final body mass index was 35.8 ± 6.7 kg/m^2 in the short-limb patients and 32.7 ± 5.1 in the long-limb patients ($P = 0.049$). A subgroup of 20 patients, all of whom had a body mass index greater than 60 kg/m^2, benefited the most from long-limb bypass. No macronutritional side effects unique to the long-limb bypass were encountered. (J GASTROINTEST SURG 2001;5:525-530.)

KEY WORDS: Obesity, gastric bypass, Roux-en-Y

In a recent report on outcomes after gastric bypass,[1] we noted a difference in results between patients who were morbidly obese before surgery (body mass index [BMI] <50 kg/m^2) as compared to those who were superobese (BMI >50 kg/m^2). In the former group 93% of patients achieved a satisfactory result after a mean of 5.5 years of follow-up, whereas in the superobese group only 57% had a similar result. A satisfactory result was defined as a BMI less than 35 kg/m^2 or an excess weight loss of at least 50%. An excellent result was defined as a BMI less than 30 kg/m^2 and 60% of the morbidly obese patients achieved this result as compared to only 26% of the superobese patients.

During this study it became obvious that the results were different in the morbidly obese vs. the superobese patients. This finding has been previously reported by others.[2]

We reasoned that a longer intestinal bypass may be indicated in superobese patients and elected, at a certain date, to increase the bypass length of intestine in all patients, both the morbidly obese and superobese. This report compares the results in these patients with a minimal follow-up of 3 years to a maximum of 8.4 years (5.5 ± 1.5 years). Of the 274 patients operated on, 242 (88.3%) have been followed.

METHODS

More than 10 years ago we established a weekly follow-up clinic for postoperative obese patients. All patients were seen twice during the first postoperative month, monthly for 3 months, and then every 3 months for 1 year. Thereafter patients were seen semiannually or for specific complaints. This was a retrospective study of obese patients who underwent gastric bypass at least 3 years prior to assessment. Patients were evaluated over a 6-month period. Those not followed up during that period were classified as lost to follow-up. No changes in preoperative evaluation, selection of candidates for surgery, or postoperative follow-up were instituted at the time of change in the operative procedure.

The technique of isolated gastric bypass has been previously reported.[3] A small 4 cm long pouch in the lesser curvature of the stomach was created adjacent to a 28 or 30 Maloney bougie with a V. Mueller PI-90 stapler (MMM Company, St. Paul, Minn.) using 4.8 mm staples. This stapler makes two double rows of staples with an interval of free tissue in between that permits division by sharp dissection or cautery. The staple line of the pouch was oversewn with PDS sutures and the staple line of the gastric side was inverted. Omentum was sutured between the staple
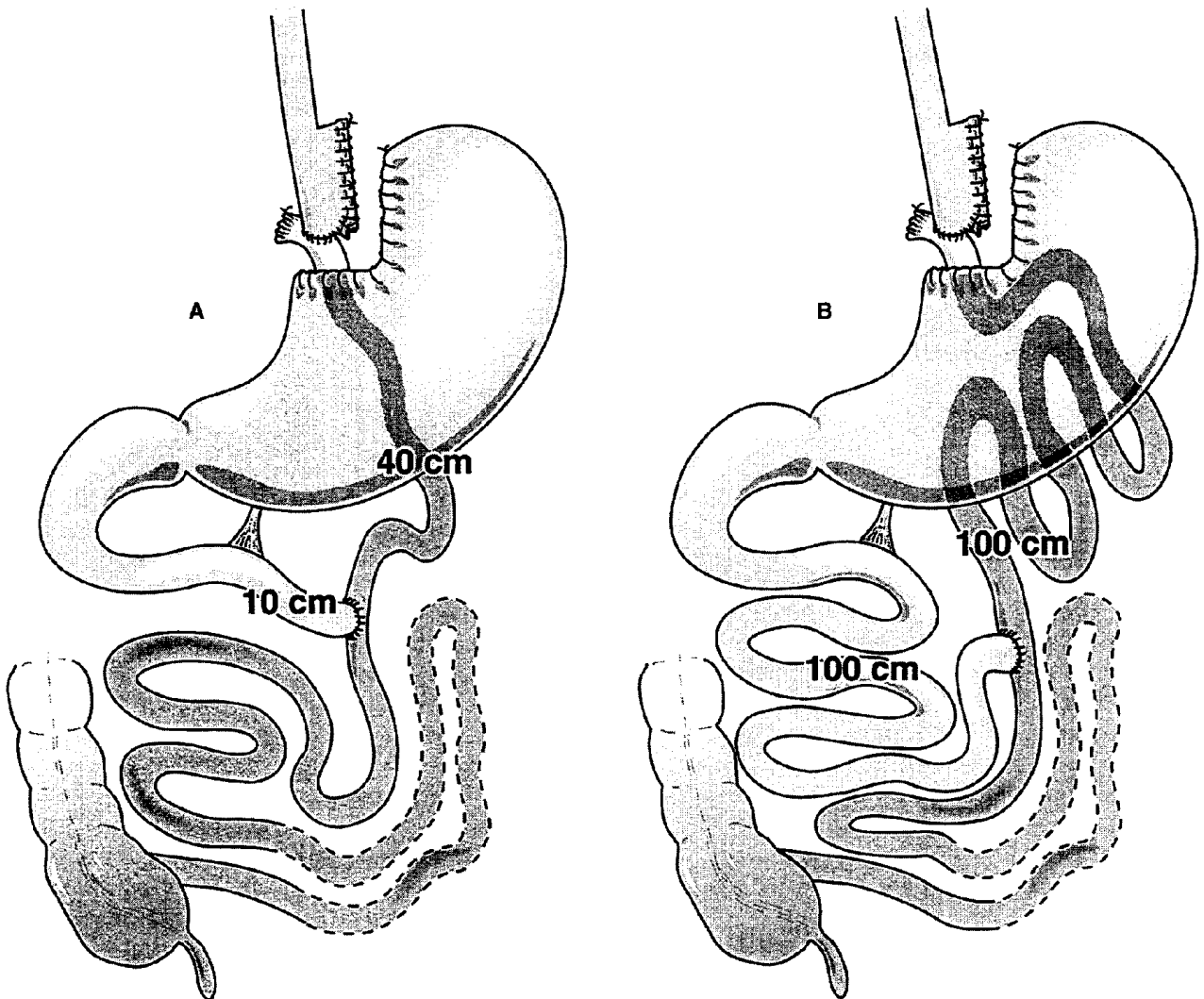
Fig. 1. A, Standard short-loop gastric bypass with a 15 ml gastric pouch, short afferent loop (10 cm), and 40 cm Roux-en-Y limb. B, Long-limb gastric bypass with a 100 cm afferent limb and a 100 cm Roux-en-Y limb.

lines. A proximal loop of jejunum was divided 10 cm from the ligament of Treitz, and the distal end was advanced in a retrocolic, retrogastric position to create a 40 cm Roux-en-Y limb, which was anastomosed with a 15 ml gastric pouch. This was the operation performed in the short-limb group (Fig. 1, *A*). The long-limb operation was created by dividing the jejunum 100 cm distal to the ligament of Treitz and making the Roux limb also 100 cm (Fig. 1, *B*). The gastric pouch–jejunal anastomosis was made 1 to 1.2 cm in diameter using a single layer of running absorbable sutures (PDS 3-0).

In our previous experience when we used a running nonabsorbable suture for this anastomosis to limit oral intake, we frequently found the suture within the lumen on endoscopy and a wide-open anastomosis. We formerly performed upper endoscopy in all obese patients after surgery at 6 weeks, 6 months, and 1 year. At 1 year the anastomosis was wide open in the vast majority of patients, even when nonabsorbable sutures were used. Nonabsorbable sutures when found in the lumen can cause symptoms. In the present study we used absorbable suture material and accept a wide-open anastomosis. In other studies we reported that a pouch made in this fashion is small and remains small over time.[1]

A modification of the Reinhold classification was used to document results (Table I).[4] We compared the results of the patients classified before surgery as morbidly obese with those who were superobese and whether they had the short- or long-limb operation. In each group we further assessed success on an individual basis. The results are reported as means ± standard deviation. A *t* test was used to compare the mean of the variables between long- and short-limb operations for the morbidly obese group and the superobese group, and separately for a subgroup of patients with a BMI greater than 60 kg/m^2. A chi-square test was used to compare the individual results in the two groups.

RESULTS

Of the 274 patients who underwent surgery, 242 (88.3%) were followed up. Of these, 146 were morbidly obese (BMI <50 kg/m^2) and 96 were superobese (BMI >50 kg/m^2). One hundred sixty-two of the 242 had the short-limb operation (67%) and 80 had the long-limb operation. Of the 146 morbidly obese patients, 96 had the short-limb operation (66%) and 50 had the long-limb operation. Of the 96 superobese patients, 66 had the short-limb operation (69%) and 30 had the long-limb operation.

The final mean result in the 96 morbidly obese patients who had the short-limb operation was excellent (BMI <30 kg/m^2) (Table II). The individual results were excellent in 55 patients (57%), good in 34 (35%), and failure in seven (8%). The long-limb operation produced a similar excellent result. These patients were followed for significantly less time (*P* <0.0001), that is, 75.6 vs. 43.6 months. Individual results were classified as excellent in 32 (64%), good in 16 (32%), and failure in two (4%). The final mean result in the 66 superobese patients who had the short-limb operation was failure (BMI >35 kg/m^2). The individual results were classified as excellent in 16 (24%), good in 15 (22%), and failure in 35 (53%) patients. The final mean result in the superobese patients who had the long-limb bypass was good (BMI 30 to 35 kg/m^2). Individual results were excellent in eight patients (27%), good in 12 (40%), and failure in 10 (33%).

The long-limb operation in the superobese group achieved a significantly lower mean BMI than the

Table I. Basis for evaluation of postoperative results

Results	Final body mass index (kg/m^2)	Final excess body weight (%)
Excellent	<30	0-25
Good	30-35	26-50
Failure	>35	>50

Table II. Results of long- and short-limb bypass in morbidly obese and superobese patients

	Short-limb bypass	Long-limb bypass	*P* value
Morbidly obese (mean preoperative BMI = 44 ± 3 kg/m^2)			
No. of patients	96	50	
Final BMI (kg/m^2)	28.6 ± 4.7	28.5 ± 3.8	NS
Follow-up (mo)	75.6 ± 12.5	43.6 ± 6.2	0.0001
Superobese (mean preoperative BMI = 56 ± 6 kg/m^2)			
No. of patients	66	30	
Final BMI (kg/m^2)	35.8 ± 6.7	32.7 ± 5.1	0.049
Follow-up (mo)	76.2 ± 12	46.3 ± 6.7	0.0003

Table III. Results of long- and short-limb bypass in 20 patients with a body mass index greater than 60 kg/m^2

	Short-limb bypass (N = 13)	Long-limb bypass (N = 7)	P value
Preoperative BMI (kg/m^2)	65.1 ± 5.7	63.1 ± 1.7	NS
Final BMI (kg/m^2)	39.8 ± 5.9	33.6 ± 2.9	0.047
Follow-up (mo)	76.2 ± 12.6	45.4 ± 7.5	<0.0001

short-limb operation. Comparison of individual results in the superobese patients did not show a statistically significant difference at the 5% level.

Patients with a BMI greater than 60 kg/m^2 achieved a significantly lower final BMI with the long-limb bypass (Table III).

DISCUSSION

The length of the small intestine beyond the ligament of Treitz measured in vivo is reported to be 3 to 3.5 m.[5] Autopsy measurements range from 3 to 8.6 m.[6] The lengths measured in vivo in the reports referred to herein are considerably longer, up to 7 m. This discrepancy is probably due to the techniques used to measure intestinal length at operation and variations between men and women.

Brolin et al.[7] performed a prospective randomized trial consisting of a standard gastric bypass of a 15 cm segment of jejunum beyond the ligament of Treitz anastomosed to a 75 cm Roux limb compared to a long-limb bypass with a 30 cm afferent limb and a 150 cm Roux limb. The mean weight loss was greater at 24 and 36 months in the long-limb group but not at 48 months when the number of patients followed decreased. The mean BMI was significantly lower at the 24-month period only. The final values for BMI at 36 months were 45 ± 14 kg/m^2 in the short-limb group and 37 ± 6 kg/m^2 in the long-limb group. The mean preoperative BMI was 63.4 ± 10 kg/m^2 in the short-limb group and 61.6 ± 9 kg/m^2 in the long-limb group. Persistent diarrhea and protein malnutrition did not occur in either group. The patients in this series were very heavy (BMI >60 kg/m^2) preoperatively, and the difference between long- and short-limb results was clinically important.

Freeman et al.[8] have recommended a long-limb gastric bypass for all patients. They compared a short-limb gastric bypass (45 to 135 cm) to a long-limb bypass (180 to 225 cm). The preoperative BMI was 46 ± 2 standard error of the mean [SEM] kg/m^2. Sixty-five percent of patients were available for follow-up at 2 years. The final BMI for both groups was 30 ± 1 (SEM) kg/m^2, for the long-limb group 29 ± 1 (SEM) kg/m^2, and the short-limb group 31 ± 1 (SEM)

kg/m^2. Because of diarrhea, they now recommend that the long limb not exceed 180 cm. The results in this study were not as impressive because morbidly obese and superobese patients were both included and the mean preoperative BMI was only 46 kg/m^2, but the percentage of weight lost at 2 years was significantly greater in the long-limb group.

Murr et al.[9] compared partial biliopancreatic bypass with very, very long gastric bypass for superobesity. They favored the very, very long gastric bypass because it was associated with fewer late complications, especially refractory malnutrition and liver failure, in patients undergoing partial biliopancreatic bypass. The preoperative BMI was 67 ± 3 kg/m^2 in the 26 patients undergoing very, very long gastric bypass who were followed for a mean of 24 months (range 18 to 60 months). Only one patient was lost to follow-up. The final BMI was 42 ± 2 kg/m^2 without nutritional complications. The operation was accomplished by dividing the proximal jejunum 40 to 60 cm beyond the ligament of Treitz, advancing the distal end as the Roux limb, which was 300 to 400 cm in length, and reanastomosing the biliopancreatic limb 100 cm proximal to the ileocecal valve.

Sugerman et al.[10] performed a distal gastric bypass in patients who failed the standard procedure. The small bowel was divided much more distally than in previous reports, 250 cm from the ileocecal valve, creating a 145 cm alimentary limb and a 150 cm common limb with the same length (250 to 400 cm) biliopancreatic limb. This operation required revision to a longer common limb in 3 of 22 patients because of malnutrition. These investigators advise this operation only in superobese patients who have failed standard gastric bypass and have significant comorbidity. The initial BMI in these 22 patients was 57 ± 2 kg/m^2 and fell to 37 ± 2 kg/m^2 at 1 year and to 32 ± 2 kg/m^2 at 5 years in 10 patients available for follow-up at that late date.

In our study the preoperative BMI in the superobese group was 56 ± 6 kg/m^2 and the final BMI was 32.7 ± 5.1 kg/m^2 when the long limb was used. This is a much less drastic intestinal bypass than was advised by Sugerman et al.,[10] yet the results are very similar in terms of weight loss. In agreement with the reports by

Murr et al.[9] and Brolin et al.,[7] who also retained most of the jejunum in the alimentary limb, none of our patients developed protein malnutrition or required ongoing treatment for diarrhea.

The risks of protein malnutrition and diarrhea cannot be predicted on the basis of the length of the common channel alone after Roux-en-Y gastric bypass. The Murr operation, which is well tolerated by superobese patients, had only a 100 cm common channel, whereas the Sugerman operation (distal gastric bypass), which was less well tolerated, had a 150 cm common channel.

Neither persistent vomiting nor diarrhea has occurred in these patients. In the past we have found malnutrition (kwashiorkor or marasmus) only in patients with severe diarrhea or persistent vomiting. This is associated with a contraction of the body cell mass.[11] Patients who developed malnutrition had an intestinal bypass or gastroplasty, not a gastric bypass. The capacity of the jejunum to absorb water, electrolytes, and nutrients exceeds that of the ileum except for vitamin B_{12} and bile salts, which are selectively absorbed in the ileum. The nutritional hazards of not keeping jejunum in the alimentary limb but also of shortening the alimentary limb and the common limb have been described by Sugerman et al.[10] It is not clear why Freeman et al.[8] could not exceed 180 cm length of Roux limb without risk of serious diarrhea, whereas Murr et al.[9] used a 300 to 400 cm Roux limb. This discrepancy may be due to the fact that Freeman et al. were dealing with patients who were not as obese.

Although this and other studies emphasize freedom from protein-calorie malnutrition in patients after gastric bypass, freedom from the microdeficiencies of iron, vitamin B_{12}, folic acid, and even calcium are much less certain. Brolin et al.[12] and others[13-15] have reported iron deficiency and/or anemia in more than 50% of patients followed for at least 4 years after gastric bypass or subtotal gastrectomy.

We found that routine use of oral iron even when combined with vitamin C to enhance absorption did not solve the problem of iron deficiency and anemia.[15] These studies showed that patient compliance was an important factor inasmuch as oral iron is poorly tolerated by many. However, severe anemia occurs rarely and can be successfully treated using intravenous iron. We are still collecting data from patients in the long- and short-limb gastric bypass groups reported herein until all patients have been followed for at least 5 years. A preliminary report at 33 months showed no difference in iron deficiency or anemia between these two groups of patients.[15]

Vitamin B_{12} deficiency is also common after gastric bypass but is readily treated prophylactically by oral or parenteral crystalline B_{12}, which is well tolerated.[16] Folic acid deficiency is less common and easily avoided with a daily multivitamin preparation. The incidence of osteoporosis after gastric bypass is unknown, but one must agree with Mason[17] that lifelong follow-up is essential to detect early indicators of bone calcium deficiency before the damage is done.

CONCLUSION

We conclude from these studies that patients who are morbidly obese do not benefit from lengthening the Roux limb in a gastric bypass beyond 40 cm. Superobese patients do benefit from a longer limb Roux-en-Y. The final mean BMI in these patients is significantly closer to normal at 43.6 months than that in the short-limb group. For patients with a BMI greater than 60 kg/m², the evidence from our own study and from the literature[7,9] strongly suggests that a long Roux limb is advantageous. In our experience a 100 cm Roux limb and 100 cm afferent limb yielded results similar to those of Murr et al.[9] and Brolin et al.,[7] namely, loss of more than 50% of excess weight without macronutritional compromise or chronic diarrhea.

Further prospective studies will be necessary to confirm our findings and to establish ideal Roux limb lengths for superobese patients and that especially challenging group with a BMI >60 kg/m².

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Effects of Endogenous Acetaldehyde Production by Disulfiram and Ethanol Feeding on Rat Pancreas

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Exogenous acetaldehyde infusion can induce pancreatitis-like injury of the pancreas in some isolated pancreas models, whereas in vivo such treatment has failed to induce pancreatitis. In vivo *exogenous* acetaldehyde may not be effective because it is rapidly metabolized. The aim of this study was to investigate whether *endogenous* acetaldehyde accumulates in the pancreas after ethanol feeding when acetaldehyde metabolism is blocked by disulfiram, and whether this treatment can induce pancreatitis-like injury in the rat. The liver was studied for comparison. In part I of the experiment, adult male Wistar rats were given water (n = 24), ethanol (n = 24), disulfiram (n = 24), and ethanol plus disulfiram for 1 week (n = 24) or 3 weeks (n = 24) and for 3 weeks with (n = 6) and without (n = 6) hypovolemia. In part II of the experiment, rats were given water (n = 6), ethanol (n = 6), and high-dose disulfiram (n = 6) and ethanol plus high-dose disulfiram (n = 6). Ethanol and acetaldehyde concentrations in blood, liver, and pancreas were measured. Animal behavior was monitored, and weight changes, plasma amylase activity, water content, and histomorphology of the pancreas and liver were studied without knowing the group. No increases in plasma amylase activity and no histomorphologic changes in the pancreas were observed under light or electron microscopy in part I of the experiment. In part II, treatment with ethanol induced acetaldehyde accumulation in the liver (33.6 ± 2.6 μmol/L), but to a lesser degree in the blood (9.6 ± 1.6 μmol/L) and pancreas (5.0 ± 1.2 μmol/L). Ethanol plus disulfiram induced marked accumulation of acetaldehyde in the liver (83.2 ± 15.9 μmol/L), blood (280.0 ± 47.4 μmol/L), and pancreas (43.6 ± 4.7 μmol/L). When tissue acetaldehyde levels reached 30 to 40 μmol/L, we found a decrease in zymogen granules along with formation of small intracytoplasmic vacuolizations in the acinar cells and accumulation of lipid droplets in the hepatocytes, whereas physiologic signs of pancreatitis (hyperamylasemia, edema) or increases in liver enzymes did not develop. High levels of acetaldehyde accumulate in the liver and pancreas with the treatment described. Although this was accompanied by lipid degeneration of the hepatocytes and some subcellular changes in the acinar cells, physiologic signs of pancreatitis did not develop. Thus acetaldehyde accumulation alone, or in combination with hypovolemia, is not responsible for the induction of acute pancreatitis. (J GASTROINTEST SURG 2001;5:531-539.)

KEY WORDS: Acetaldehyde, acute alcoholic pancreatitis, disulfiram, liver changes, histopathology

Ethanol-induced pancreatitis has been studied experimentally and clinically for many years, yet its mechanism remains unclear.[1] Previously acetaldehyde, the first stable product of ethanol oxidation, has been suggested to play an important role in the pathogenesis of acute alcoholic pancreatitis.[2-6] One possible mechanism is that acetaldehyde may be oxidized by xanthine oxidase and release free radicals during the oxidation.[7] Although subcellular changes have been described in rats chronically treated with ethanol, no one has been able to induce acute pancreatitis in rats using ethanol and exogenous acetaldehyde. Because aldehyde dehydrogenase has been demonstrated to exist in the human and pig pancreas,[8,9] we hypothesized that the rat pancreas contains aldehyde dehydrogenase, which rapidly metabo-

lizes acetaldehyde to nontoxic acetate. Disulfiram is an inhibitor of aldehyde dehydrogenase, which results in the accumulation of endogenous acetaldehyde in blood after exposure to ethanol.[10-12] The present studies were carried out to investigate whether a high dose of ethanol with or without disulfiram treatment could induce endogenous acetaldehyde accumulation in the rat, and whether such accumulation is associated with the development of physiologic signs of pancreatitis (edema, hyperamylasemia). For comparison, we also studied the liver to determine whether any changes in that organ could be observed with this treatment.

MATERIAL AND METHODS

Adult male Wistar rats, weighing 250 to 300 g (National Experimental Animal Centre, Kuopio, Finland), were used. Animals were housed in light-dark cycle–regulated, air-conditioned (23° C), and air humidified (60%) quarters, and given free access to drinking water and standard food pellets (Ewos, Södertelje, Sweden). The rats were weighed at the beginning (weight 1) and at the end (weight 2) of the experiment. During the last 24 hours of the experiment, the animals were fasted in separate cages but had free access to drinking water. The experiment was approved by the Animal Committee of Tampere University and performed in accordance with the "Guidelines for the Care and Use of Laboratory Animals" (NIH publication No. 86-23, revised 1985).

Part I

The animals were randomly allocated to the following groups.

Reference Group (n = 6). Rats in this group were sacrificed and samples were obtained before the beginning of the experiment.

Group I (n = 24): Controls. Rats in this group were fed tap water, 1 ml/100 g (twice a day, total 11 times), for 6 days by cannula. In addition, they were given free access to food and tap water.

Group II (n = 24): Ethanol. These rats were fed 33% (weight/volume [w/v]) ethanol, 1 ml/100 g (twice a day, total 11 times), for 6 days by cannula. In addition, they were allowed free access to food and a drinking solution of 8% (w/v) ethanol/water.

Group III (n = 24): Disulfiram. These rats were fed tap water, 1 ml/100 g (twice a day, total 11 times), and 4 mg/ml disulfiram (Antabus; Dumex-Alpharma, Copenhagen, Denmark) 0.25 ml/100 g (once a day, total 6 times), for 6 days by cannula. In addition, they had free access to food and tap water.

Group IV (n = 24): Ethanol + Disulfiram (I). These rats were fed 33% (w/v) ethanol, 1 ml/100 g

(twice a day, total 11 times), and 4 mg/ml disulfiram, 0.25 ml/100 g (once a day, total 6 times), for 6 days by cannula. In addition, they had free access to food and a drinking solution of 8% (w/v) ethanol/water.

Group V (n = 24): Ethanol + Disulfiram (II). These rats were fed 33% (w/v) ethanol, 1 ml/100 g (twice a day, total 41 times), and 4 mg/ml disulfiram, 0.25 ml/100 g (once a day, total 21 times), for 3 weeks by cannula. In addition, they had free access to food and a drinking solution of 8% (w/v) ethanol/water.

Rats in groups I to V were divided into three subgroups (n = 8 in each subgroup), and samples were obtained at 1, 24, and 72 hours after the last treatment, respectively.

Group VI (n = 6): Hypovolemia + Ethanol + Disulfiram. Hypovolemia was induced by withdrawing 1.6 ml/100 g of blood by venesection under general anesthesia (Mebunat, Orion, Finland), 60 mg/kg body weight injected intraperitoneally. After venesection, ethanol and disulfiram were given as in group V. Immediately after the last administration, hypovolemia was induced for a second time in a similar manner. Samples were collected at 72 hours after the last ethanol and disulfiram treatment.

Group VII (n = 6): Hypovolemia-Control Group. Hypovolemia was induced as in group VI, but these rats were fed tap water, 1 ml/100 g (twice a day), instead of ethanol. In addition, they had free access to additional tap water.

Part II

The animals (n = 24) were randomly allocated to one of four groups and samples were drawn at 1 hour after the last treatment.

Water Group (n = 6). These rats were fed tap water, 1 ml/100 g (twice a day, total 11 times), for 6 days by cannula. In addition, they had free access to food and tap water.

Disulfiram Group (n = 6). These rats were fed tap water, 1 ml/100 g (twice a day, total 11 times), and 10% disulfiram, 320 mg/kg/day (once a day, total 6 times), for 6 days by cannula. In addition, they had free access to food and tap water.

Ethanol Group (n = 6). These rats were fed 33% (w/v) ethanol, 1 ml/100 g (twice a day, total 11 times), for 6 days by cannula. In addition, they had free access to food and a drinking solution of 8% (w/v) ethanol/water.

Ethanol + Disulfiram Group (n = 6). These rats were fed with 33% (w/v) ethanol, 1 ml/100 g (twice a day, total 11 times), and with 10% disulfiram, 320 mg/kg/day (once a day, total 6 times), for 6 days by cannula. In addition, they had free access to food and an 8% (w/v) ethanol/water solution for drinking.

Sampling

Part I. With the rats under general anesthesia, laparotomy was performed and blood samples were drawn from the inferior vena cava into polystyrene tubes containing heparin, 100 units/ml. Plasma was centrifuged at 2000 rpm for 10 minutes. The plasma samples were stored at $-70°$ C until assay. The pancreas was excised, and a small piece of tissue was cut and rapidly fixed in 10% (w/v) formalin for light microscopic examination; another specimen approximately 1 mm³ was then cut and immediately fixed in 2.5% glutaraldehyde for electron microscopy. To measure the water content, the pancreas was weighed before and after dehydrating at 150° C for 24 hours.

Part II. Specimens were taken as described in part I. In addition, 0.2 ml of the blood sample was immediately mixed with 1.8 ml of cold distilled water (0° C) and vibrated to induce hemolysis; specimens were then stored in liquid nitrogen for later measurement of acetaldehyde and ethanol. A small piece of tissue was rapidly freeze-clamped from the liver and from the pancreas, and stored in liquid nitrogen for measurement of acetaldehyde. Additional 1 mm³ specimens of liver were cut and immediately fixed in 2.5% glutaraldehyde for histologic analysis under a light microscope and an electron microscope.

Measurement and Morphologic Analysis

Plasma amylase, alanine aminotransferase, and bilirubin were measured using a Hitachi 704 analyzer (Hitachi Corp., Kyoto, Japan; reagents from Boehringer Mannheim, Mannheim, Germany). Alkaline phosphatase was measured with the same instrument using homemade reagents.[13] Blood hemolysates and tissue powder treated with 0.6 mol/L perchloric acid containing 25 mmol/L thiourea (to block artifactual acetaldehyde formation) were used for head-space analysis of ethanol and acetaldehyde by gas chromatography as previously described.[14] To correct for artifactual acetaldehyde formation in the blood, calculations were made with a correction curve using control blood to which ethanol had been added.

For light microscopic examination, the formalin-fixed specimens were cut and stained with hematoxylin and eosin. For electron microscopy, tissues were fixed in 2.5% glutaraldehyde for 2 to 6 hours, and then postfixed with ice-cold 1% osmium tetroxide in 0.1 mol/L phosphate buffer (pH 7.4) for 1 hour, dehydrated in ethanol, and flat-embedded with Epon 812 (Ladd Research Industries, Inc., Burlington, Vt.). After polymerization, thick sections were cut and stained with toluidine blue for light microscopic preview. Suitable pieces were then cut into ultrathin sections with an Ultratome III (model 8800, LKB, Wallac, Sweden), mounted on precoated grids, and observed under an electron microscope (JEM-1200X, JEOL Ltd., Tokyo, Japan). Morphologic analyses were carried out without knowing which group each specimen came from.

Data are expressed as mean ± standard error (SE). Analysis of variance and Student's t test were used. Differences were considered significant at $P < 0.05$.

RESULTS
Part I

During the experiment, the behavior of the rats was normal in groups I and III, but animals in groups II, IV, V, and VI showed marked anorexia, weakness, and confusion. In group II, the symptoms rapidly disappeared after the cessation of the ethanol administration. In the groups treated with ethanol plus disulfiram (groups IV, V, and VI), the symptoms were most severe and continued for the duration of the experiment. In groups IV and V, two rats died without any obvious cause of death found at autopsy.

The weight changes in all groups are shown in Fig. 1. During the experiment, the rats treated with ethanol plus disulfiram showed a marked weight loss. The water content of the pancreas is shown in Fig. 2. There were no significant differences in the water content of the pancreas among groups I to V, regardless of sampling time. Hypovolemia with and without ethanol plus disulfiram exposure (groups VI and VII) resulted in increased water content in the pancreas, which did not differ between the hypovolemic rats with and without ethanol plus disulfiram treatment.

The serum amylase activity in all groups is shown in Fig. 3. Although the amylase activity in group VI (hypovolemia + ethanol + disulfiram) was higher than that in group VII (hypovolemia-control), it was still within the reference level. There were no significant differences in serum amylase activity among groups I to V, regardless of the sampling time. Alanine aminotransferase, bilirubin, and alkaline phosphatase were within the reference levels in all groups.

No edema of the pancreas, acinar cell necrosis, leukocyte infiltration, hemorrhage of the pancreas, or changes in the acinar cell subcellular structure were observed in any of the groups under light or electron microscopy.

Part II

During this experiment, the behavior of the rats was normal in the Water and Disulfiram groups. Some confusion was observed in the Ethanol group, but the symptoms rapidly disappeared after cessation of high-dose ethanol treatment. Rats in the Ethanol + Disulfiram group showed marked anorexia, weakness, and confusion. Moreover, these symptoms continued throughout the entire experiment.
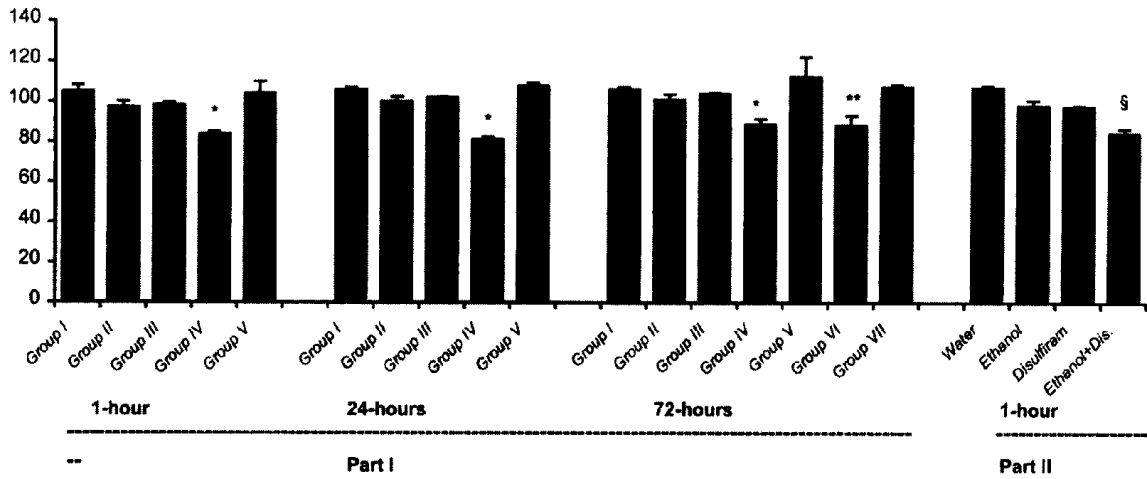
Fig. 1. Weight changes in all groups of rats (mean ± SE). Weight 1: beginning of experiment; weight 2: end of experiment. * = $P < 0.01$, compared to groups I, II, and III, respectively; ** = $P < 0.01$, compared to group VII; § = $P < 0.01$, compared to Water, Ethanol, and Disulfiram groups.
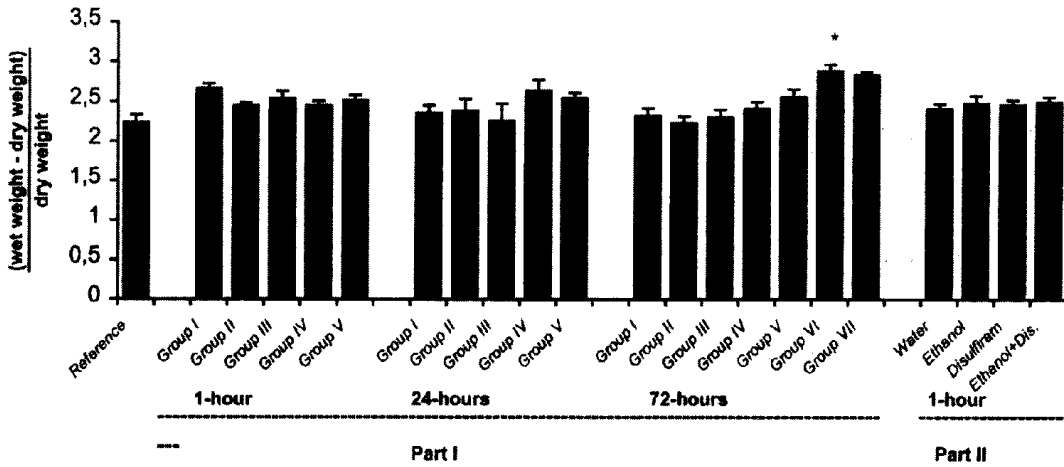

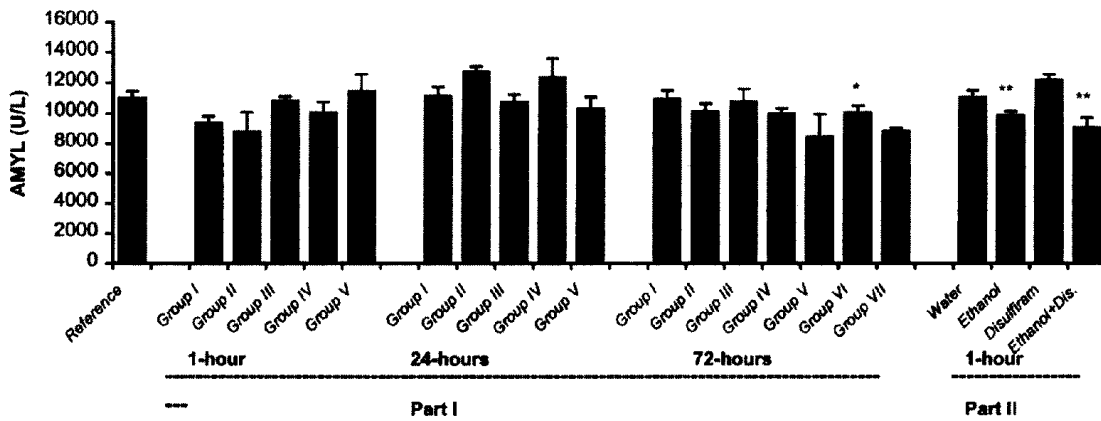**Fig. 2.** Water content of pancreas in all groups (mean ± SE). * = $P < 0.01$, compared to Reference group.

Fig. 3. Serum amylase activity in all groups (mean ± SE). * = $P < 0.01$, compared to group VII; ** = $P < 0.01$, compared to Water and Disulfiram groups.

The weight changes in all groups are shown in Fig. 1. Compared with the Water, Disulfiram, and Ethanol groups, the weight 2/weight 1 ratio was significantly lower in the Ethanol + Disulfiram group ($P < 0.01$). The water content of the pancreas did not differ significantly among the four groups (see Fig. 2). The serum amylase activity decreased a little in the Ethanol and Ethanol + Disulfiram groups compared with the Water and Disulfiram groups (see Fig. 3). There were no significant differences among any of the groups with regard to alanine aminotransferase, bilirubin, and alkaline phosphatase levels.

Ethanol and acetaldehyde were not found in the blood samples of the rats treated with water and those given water plus disulfiram. The blood ethanol concentration was elevated in the Ethanol (26.2 ± 3.1 mmol/L) and Ethanol + Disulfiram (41.8 ± 8.5 mmol/L) groups, but the difference between these two groups was not significant. In the Ethanol group, the acetaldehyde concentration was markedly elevated in the liver (33.6 ± 2.6 μmol/L), but was less elevated in the blood (9.6 ± 1.6 μmol/L), and the smallest increase was in the pancreas (5.0 ± 1.2 μmol/L) ($P < 0.05$ in the pancreas compared to the liver; $P < 0.01$ in the pancreas compared to blood). In the Ethanol + Disulfiram group, the acetaldehyde concentrations were higher than in the Ethanol group in each organ studied (liver 83.2 ± 15.9 μmol/L, blood 280.0 ± 47.4 μmol/L, and pancreas 43.6 ± 4.7 μmol/L; $P < 0.01$ for each organ comparison).

Morphologic analyses showed normal pancreas in the Water, Disulfiram, and Ethanol groups. No acinar cell necrosis, leukocyte infiltration, or hemorrhage of the pancreas was found in any of the specimens in any of these three groups. A decrease of zymogen granules by one half or more was found in four of six rats in the Ethanol + Disulfiram group, and small intracytoplasmic vacuolizations appeared in the pancreas in three of six rats in the Ethanol + Disulfiram group on light and electron microscopy (Figs. 4 and 5). The liver was normal in the Water and Disulfiram groups. Lipid deposits in the hepatocytes
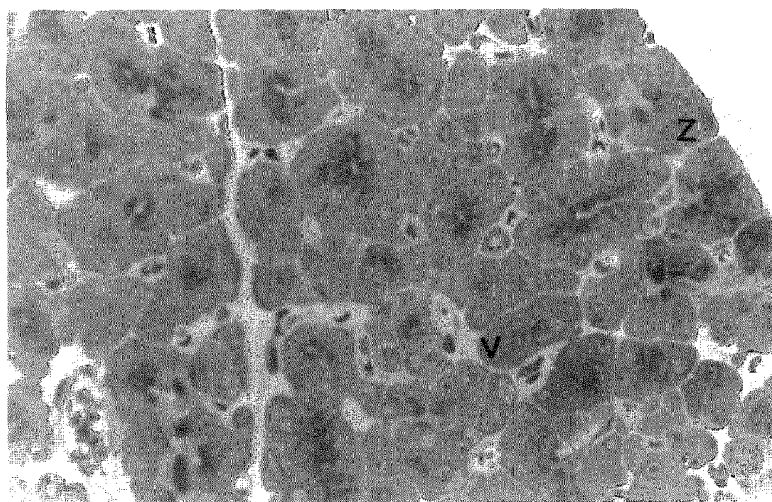

Fig. 4. Light micrograph of acinar cell from a rat treated with ethanol + disulfiram shows only a few zymogen granules (Z) and small intracytoplasmic vacuolizations (V). (Toluidine blue stain; ×400.)
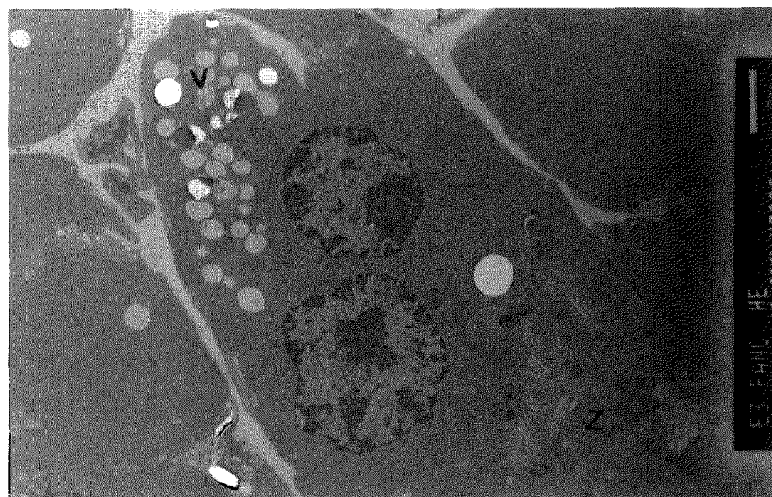
Fig. 5. Electron micrograph of acinar cell from a rat treated with ethanol + disulfiram shows a decrease in zymogen granules (Z) and small intracytoplasmic vacuolizations (V). (×5000.)
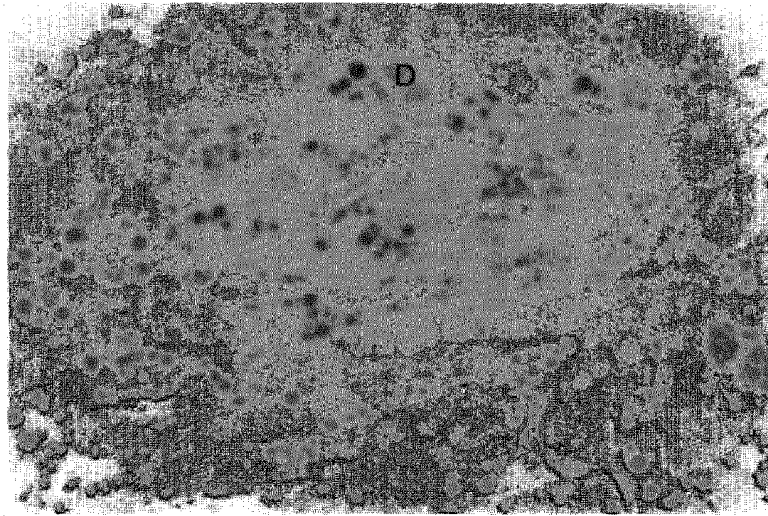
Fig. 6. Light micrograph of liver shows lipid deposits (D) in hepatocytes in Ethanol and Ethanol + Disulfiram groups. (×200.)
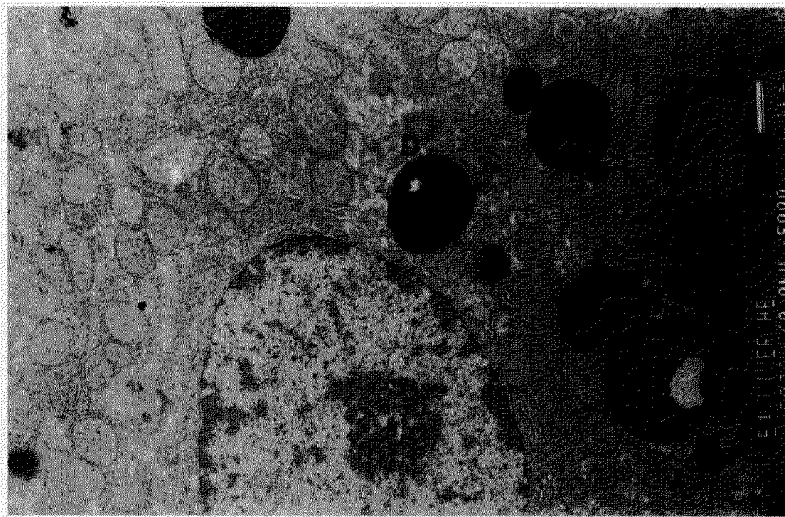
Fig. 7. Electron micrograph of hepatocyte shows lipid deposit (D) in hepatocytes in Ethanol and Ethanol + Disulfiram groups. (×5000.)

were found in three of six rats in the Ethanol group. All six rats in the Ethanol + Disulfiram group demonstrated an abundant number of lipid droplets in the hepatocytes (Figs. 6 and 7).

DISCUSSION

Ethanol metabolites may be involved in the pathogenesis of acute alcoholic pancreatitis.[2-4] Acetaldehyde, the first oxidation product of ethanol, could be oxidized by xanthine oxidase and released free radicals during the oxidation.[7] In tissues the xanthine oxidase presents as the inactive enzyme xanthine dehy-

drogenase, which can be converted to xanthine oxidase by several stimuli including high-dose acetaldehyde and ischemia.[15-21] Previous experiments have demonstrated that the exogenous acetaldehyde is able to induce pancreatic impairment.[3,5,6,22] Some investigators used acetaldehyde to infuse the isolated canine pancreas prepared together with secretin stimulation and found that the pancreas became edematous and hemorrhagic, and its secretory volume decreased.[3] Nordback et al.[5,6] also demonstrated that acetaldehyde infusion together with organ ischemia could initiate acute pancreatitis in the isolated pancreas of dogs and found that the toxic oxygen metabolites mediated

the injury. In whole-body animal experiments, it is still unclear whether exogenous acetaldehyde is injurious to the pancreas. Some investigators have demonstrated that intraperitoneal injections of acetaldehyde in the rat could increase serum amylase activity and induce structural changes in the acinar cells.[22] But others have reported that infusion of acetaldehyde into the splenic artery in the pig did not induce histomorphologic changes in the pancreas or affect the amylase activity in the serum.[23] Inadequate experimental exposure of the pancreas to acetaldehyde may cause these differences in results because acetaldehyde boils at room temperature and is readily metabolized by several mammalian tissues including red blood cells.[24,25] The appearance of acetaldehyde-induced pancreatitis-like acute injury of the pancreas is thus still limited in isolated pancreas models.

Disulfiram is an inhibitor of aldehyde dehydrogenase and can thus block acetaldehyde oxidation to acetate. This results in the accumulation of endogenous acetaldehyde when exposure to ethanol continues.[10-12] Plasma acetaldehyde accumulation results in reactions such as flush in the neck, hypotension, anorexia, weakness, vertigo, confusion, among others.[26,27] In part I of our experiment, we used high-dose ethanol plus disulfiram in a dose equivalent to human therapy and found typical disulfiram-ethanol reactions. Although we did not measure the acetaldehyde concentration in part I of our experiment, the symptoms suggested the accumulation of endogenous acetaldehyde in the rats treated with ethanol plus disulfiram. Even though we treated the groups with ethanol and disulfiram for either 6 days or 21 days, with or without induction of hemorrhagic shock to promote the relative ischemia, and obtained samples at three different time points, we did not find any signs of pancreatitis (increased serum amylase activity, edema, histomorphologic changes on gross examination or light microscopy, or acinar cell changes on electron microscopy). The mild increase in the water content of the pancreas in both hypovolemic groups (with and without ethanol + disulfiram) demonstrated that hypovolemia was effective in inducing capillary "leakage," but this was not ameliorated by treatment with ethanol plus disulfiram. In part II of the experiment, a very high "supratherapeutic" dose of disulfiram was used to ensure maximal acetaldehyde accumulation as verified by acetaldehyde measurements. Still signs of acute pancreatitis could not be induced in the pancreas, although some intracellular changes were observed, similar to previous studies.[22]

In our previous clinical studies we found that short-term high-dose ethanol exposure did not induce pancreatic injury,[28] and the occurrence and severity of acute alcoholic pancreatitis depended on the duration

and degree of alcohol abuse in patients with alcoholic pancreatitis.[29-31] Maybe a much longer period of alcohol exposure than was used in the present experiments is needed to induce pancreatitis. Because not all patients develop pancreatitis after high-dose, long-term ethanol exposure, other simultaneous cofactors may be important in the induction of acute clinical pancreatitis. These may be exogenous (diet, drugs, dehydration, other diseases) or endogenous (genetic variability). Because xanthine oxidase is activated in tissues from its dehydrogenase form by ischemia, and acetaldehyde may serve as a substrate to xanthine oxidase resulting in the release of toxic oxygen metabolites,[5,21] hypovolemia-induced relative ischemia was tested as one possible exogenous cofactor that may be needed for pancreatitis to develop. In fact, excessive alcohol consumption is well known to result in dehydration (i.e., partial hypovolemia). In the present experiments, hypovolemia induced some edema of the pancreas, but this was not accompanied by pancreatitis either in the rats treated with ethanol or in those treated with ethanol plus disulfiram. Thus hypovolemia alone is hardly such a cofactor.

After a relatively long period of continuous high-dose (12.6 g/kg body weight/day, intubation + drinking) alcohol exposure, elevated endogenous acetaldehyde concentrations were found in the liver but only slight increases in the pancreas and blood. Acetaldehyde levels increased remarkably with disulfiram treatment in all tissues studied. This is a new finding in the pancreas, but one that has been well described previously in blood.[10-12,32] When tissue acetaldehyde levels reached 30 to 40 μmol/L, subcellular structural changes appeared in the liver and in the pancreas. These changes were accumulation of lipid droplets in the hepatocytes and vacuoles in the acinar cells. Neither pancreatitis nor hepatitis developed. The biochemical results did not reveal such a severe subclinical cellular injury that it would have resulted in enzyme leakage from either the pancreas or the liver, because both the amylase and alanine aminotransferase concentrations remained normal. Also, we are not aware of any case reports where alcohol in disulfiram-treated patients would have induced pancreatitis.

In addition to acetaldehyde, fatty acid ethyl esters, which are the result of nonoxidative metabolism of ethanol, have also been proposed as mediators of ethanol-induced damage to the pancreas. Concentrations found in intoxicated individuals have induced some edema, intracytoplasmic vacuolization, and intrapancreatic activation of trypsin, but no cellular leakage of amylase into serum during a 24-hour period.[33] Thus accumulation of either acetaldehyde or fatty acid ethyl esters may result in similar vacuolization of the acinar cells. Pancreatitis with acinar cell

necrosis, however, has not yet been induced by ethanol or any of its metabolites.

Acetaldehyde has also previously been considered responsible, at least in part, for the impaired excretion of hepatocytes resulting in lipid accumulation.[34] In the acinar cells, ethanol increases the expresssion of mRNA in digestive enzymes and increases the intracellular lipase content.[35] Acetaldehyde may also decrease the volume of secretory proteins in the acini.[36] Thus it is possible that acetaldehyde disturbs acinar cell secretion mechanisms in a way that may, under certain preconditions, result in acinar cell injury. In the future it would be interesting to study whether the currently observed changes in cellular structure represent a disturbance in the balance between acinar cell protein synthesis, packaging, and exocytosis that may be followed by colocalization of lysosomal and digestive proenzymes, which is found in the initial mechanism of cellular injury in cerulein pancreatitis and duct obstruction pancreatitis models.[37]

CONCLUSION

The study suggests that high concentrations of endogenous acetaldehyde can be achieved in the pancreas, liver, and blood with the treatment described. Subcellular changes develop both in hepatocytes and in acinar cells beginning with acetaldehyde levels of 30 to 40 μmol/L. On the other hand, such remarkable accumulation of acetaldehyde does not induce acute pancreatitis in the rat during 1 to 3 weeks of exposure. Thus a much longer exposure to acetaldehyde, other metabolites of ethanol, or other cofactors (besides the currently studied hypovolemia) are needed for ethanol-induced pancreatitis. Such cofactors may well be endogenous genetic variabilities or other thus far unstudied exogenous conditions.

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Bile Acid Absorption After Near-Total Proctocolectomy in Dogs: Ileal Pouch vs. Jejunal Pouch–Distal Rectal Anastomosis

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Bile acid malabsorption is often present in patients after near-total proctocolectomy and ileal pouch–anal canal anastomosis, suggesting ileal dysfunction. Experiments were performed in dogs to compare bile acid absorption after a modified procedure, in which a jejunal pouch was interposed between the terminal ileum and the distal rectum, with that after a conventional ileal pouch operation. Fecal bile acid output (equivalent to hepatic bile acid biosynthesis) and composition were determined by gas chromatography/mass spectrometry in five jejunal pouch dogs and in five ileal pouch dogs more than 6 months after operation. Fecal bile acid output in the jejunal pouch dogs (mean ± standard deviation) was 215 ± 59 mg/day (10.1 ± 2.7 mg/kg-day), a value similar to that obtained in the ileal pouch dogs (261 ± 46 mg/day [12.8 ± 3.1 mg/kg-day]; $P > 0.05$). These values were also similar to those reported by others for healthy unoperated dogs, indicating that increased bile acid biosynthesis occurring in response to bile acid malabsorption was not present. Fecal bile acids in pouch dogs were completely deconjugated and extensively 7-dehydroxylated (jejunal pouch = 90.4% ± 3.9% dehydroxylated; ileal pouch = 88.6% ± 6.6% dehydroxylated) and consisted predominantly of deoxycholic acid derivatives. We conclude that when either a jejunal pouch or an ileal pouch is used as a rectal substitute in dogs, an anaerobic pouch flora develops that efficiently deconjugates and dehydroxylates bile acids, rendering them membrane permeable. The resultant passive absorption of unconjugated bile acids appears to compensate for any loss of active ileal absorption of conjugated bile acids, and bile acid malabsorption does not occur. (J GASTROINTEST SURG 2001;5:540-545.)

KEY WORDS: Bile acid absorption, proctocolectomy, ileal pouch–anal canal anastomosis, jejunal pouch–anal canal anastomosis

In healthy adults, most bile acids are absorbed in conjugated form by active transport in the terminal ileum.[1] In patients who have undergone near-total proctocolectomy and ileal pouch–anal canal anastomosis, bile acid malabsorption is often present, suggesting ileal dysfunction.[2-6]

We hypothesized that preservation of an intact ileum would decrease the bile acid malabsorption that was expected to be present in dogs that had undergone a conventional ileal pouch procedure. To accomplish this, we interposed a jejunal pouch between the terminal ileum and the anal canal. We found in past tests that jejunal pouch dogs have slower small intestinal transit and fewer postprandial bowel movements than ileal pouch dogs.[7] Although both groups of dogs were shown to be similar in that they had greater numbers of anaerobic and aerobic bacteria in their pouches than are present in the ileum of healthy dogs, jejunal pouch dogs have higher basal and postprandial levels of peptide YY in

their sera.[7,8] Peptide YY is secreted by the ileal mucosa. These results suggest that ileal mucosal function, hence bile acid absorption, is better preserved in jejunal pouch dogs than in ileal pouch dogs. Also, active absorption of conjugated bile acids might occur in the relatively sterile terminal ileum of the jejunal pouch dogs prior to the entrance of ileal chyme into the jejunal pouch, where bile acids would be deconjugated by anaerobic bacteria and, hence, only passively absorbed there.

We report herein measurements of bile acid absorption in dogs with jejunal pouches as compared to measurements in dogs that had undergone a conventional ileal pouch procedure. Our experimental results indicate that neither procedure induced bile acid malabsorption in dogs. Analysis of fecal bile acid suggested that the anaerobic flora in both pouches deconjugated and dehydroxylated bile acids. Such bacterial biotransformations render bile acids membrane permeable, permitting their rapid passive absorption and maintaining the enterohepatic circulation of bile acids.

METHODS
Animal Preparation

Ten mongrel dogs underwent near-total proctocolectomy, preserving only the distal rectum and the anal canal. Five dogs (mean weight ± standard error of the mean [SEM] = 20.4 ± 0.5 kg) had a J-shaped pouch constructed from the distal 30 cm of jejunum and interposed between the unaltered terminal ileum and the distal 2 cm of rectum, as previously described.[7] Five dogs (mean weight ± SEM = 20.0 ± 0.5 kg) had a J-shaped pouch constructed from the terminal 30 cm of ileum and anastomosed to the distal 2 cm of rectum, as in the conventional ileal pouch–anal canal anastomosis. Electrodes were implanted on the intestines of both types of dogs, but they were not used in the studies reported here. All dogs were allowed to recover from the operation for 6 months, after which fecal bile acid output was measured. Electrical, motility, bacteriologic, histologic, and clinical observations were made in both groups of dogs during the 6 months. The results of these tests were reported recently.[7]

Fecal Bile Acid Determinations

Twenty-four-hour stool collections were made for 2 days in the week before the dogs were killed. Three volumes of reagent-grade isopropanol were added, and the feces were homogenized. An aliquot of the well-mixed homogenate was sent to the University of California, San Diego, for analysis.

Thin-layer chromatography of the supernate using a solvent system for conjugated bile acids[9] indicated that all bile acids were in unconjugated form. A 200 μl aliquot of the supernate was used for determination of bile acid composition by gas chromatography/mass spectrometry. To this aliquot was added 40 μl of an internal standard. The internal standard contained nordeoxycholic acid (3α, 12α-dihydroxy-5β-cholan-23-oic acid) at a concentration of 1.0 mmol/L. The isopropanol was evaporated to dryness using a nitrogen stream and reconstituted in 1.0 ml of chloroform-methanol (2:1 volume:volume). To this was added 1 ml of ethereal diazomethane. After 15 minutes for methyl esterification, samples were taken to dryness again. Peracetates were prepared by the addition of 1.0 ml acetylation solution (acetic acid-acetic anhydride-perchloric acid, 14:10:0.1 volume:volume). After 90 minutes' incubation, saturated NaCl was added (1.0 ml) and the aqueous phase was extracted three times with an equal volume of ethyl acetate. The pooled ethyl acetate extracts containing the methyl ester peracetates of the bile acids were then evaporated.

Samples were dissolved in isoamyl acetate for analysis by gas chromatography/mass spectrometry. A Hewlett-Packard 5970 Mass Selective Detector equipped with Hewlett-Packard 5890 Ion Detector and Chemstation (Unix) software was used. The capillary column (30 meters with an internal diameter of 0.25 mm) had a 35% phenyl methyl silicone stationary phase (SPB-35 [Supelco, Bellefonte, Pa.]). Chromatography was performed isothermally at 268° C using helium as a carrier gas. Quantification was based on peak area generated by the total ion mass; it was assumed that all compounds ionized equally. Compound identification was based on relative retention time of compounds and mass spectrometric characteristics compared to those of known reference compounds.[10]

Total bile acid output was obtained by summarizing all peaks. Peaks were assigned to the cholic family (possessing a hydroxy group at C-12) or the chenodeoxycholic acid family (lacking a substituent at C-12). These values were used to calculate primary bile acid synthesis. In addition, the extent of bacterial 7-dehydroxylation was calculated by summing all peaks lacking a hydroxy group at C-7 and dividing this by the total bile acid mass in the corresponding family. A small amount of murideoxycholic acid (3α, 6β-dihydroxy-5β-cholan-24-oic acid) was present in some samples. This compound was assumed to arise from hepatic 6β-hydroxylation of lithocholic acid[11] that had been absorbed from the intestine during transport through the hepatocyte. Therefore this compound was included in the chenodeoxycholic acid family. Values were calcu-

Table I. Bile acid metabolism in dogs after near-total proctocolectomy and enteric pouch–distal rectal anastomosis

Biosynthesis of bile acids	Ileal pouch (n = 5)	Jejunal pouch* (n = 5)
Total fecal bile acid output (mg/day)	261 ± 46	215 ± 59
Total fecal bile acid output (mg/kg-day)	12.8 ± 3.1	10.1 ± 2.7
Cholic acid output (mg/day)	227 ± 36	192 ± 53
Chenodeoxycholic acid output (mg/day)	34 ± 12	22 ± 6
Cholic acid (% of total bile acid synthesis)	86.1 ± 2.7	89.0 ± 1.8

*Values (mean ± standard deviation) do not differ from ileal pouch values; $P > 0.05$.

Table II. Bacterial metabolism of fecal bile acids in dogs undergoing proctocolectomy and enteric pouch–distal rectal anastomosis

Bacterial metabolism of bile acids	Ileal pouch (n = 5)	Jejunal pouch (n = 5)*
Cholic acid, 7-dehydroxylation (mg/day)	211 ± 56	174 ± 48
Chenodeoxycholic acid, 7-dehydroxylation (mg/day)	29 ± 12	19 ± 6
Total primary bile acid, 7-dehydroxylation (mg/day)	240 ± 68	193 ± 56
Cholic acid (% dehydroxylated)	90.3 ± 7.4	91.1 ± 4.7
Chenodeoxycholic acid (% dehydroxylated)	79.8 ± 8.1	84.6 ± 2.0
Both primary bile acids (% dehydroxylated)	88.6 ± 6.6	90.4 ± 3.9

*Values (means ± standard deviation) do not differ from ileal pouch; $P > 0.05$.
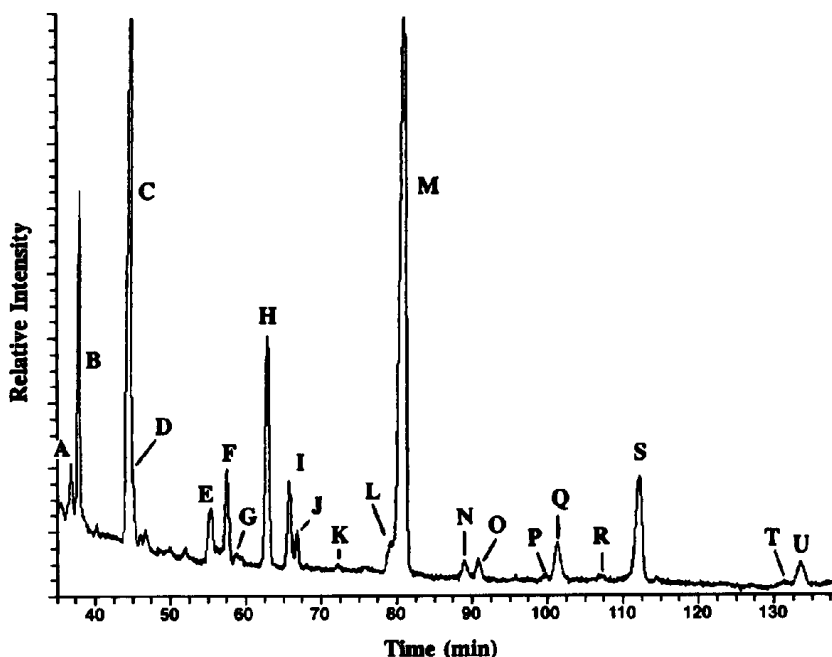
Fig. 1. Representative chromatogram of the fecal bile acid and sterol profile of an ileal pouch dog. Peak identities and percentage composition are as follows: B = coprosterol, 8.8; C = cholesterol, 25.5; D = 5α-cholestane-3β-ol, 1.5; E = campesterol, 2.2; F = lithocholic acid, 3.7; G = stigmasterol, 0.5; H = nordeoxycholic acid (internal standard); I = β-sitosterol, 3.4; J = stigmastanol, 1.4; K = 3-oxo, 12α-hydroxy-5β-cholan-24-oic acid, 0.2; L = allodeoxycholic acid, 1.5; M = deoxycholic acid, 36.5; N = chenodeoxycholic acid, 1.3; O = 3α, 12β-dihydroxy-5β-cholan-24-oic acid, 1.2; P = 3α, 12β-dihydroxy-5α-cholan-24-oic acid, 0.2; Q = 3α-hydroxy, 12-oxo-5β-cholan-24-oic acid, 0.2; R = 3α, 6β-dihydroxy-5β-cholan-24-oic acid, 0.1; S = cholic acid, 7.0; T = 3β, 7α, 12α, 6 = trihydroxy-5α-cholan-24-oic acid, 0.1; and U = 3α, 12α dihydroxy-7-oxo-5β-cholan-24-oic acid, 1.8. Peak A = α-tocopherol acetate (a dietary constituent), 0.2.

Table III. Fecal bile acid composition in dogs after near-total proctocolectomy and ileal pouch or jejunal pouch–distal rectal anastomosis

Substituents	Trivial name	Ileal pouch (n = 5 dogs)	Jejunal pouch (n = 5 dogs)	Healthy controls* (n = 4 dogs)
Cholic acid and its bacterial metabolites				
Bile acids retaining a substituent at C-7				
3α-OH, 7α-OH, 12α-OH	Cholic acid	6.7 ± 4.1	6.0 ± 3.8	13.1 ± 11.4
3α-OH, 7αO, 12α-OH	7-oxo-deoxycholic	2.3 ± 1.9	1.7 ± 1.2	3.8 ± 3.7
5α, 3β-OH, 7α-OH, 12α-OH	"Alloisocholic acid"	0.3 ± 0.3	0.3 ± 0.1	ND
Bile acids that have undergone 7-dehydroxylation				
3α-OH, 12α-OH	Deoxycholic acid	67.0 ± 6.0	70.7 ± 2.8	68.0 ± 10.7
3 = O, 12α-OH		0.6 ± 0.2	0.4 ± 0.1	ND†
3α-OH, 12β-OH	12-epideoxycholic acid	0.8 ± 1.2	1.9 ± 0.7	ND
3α-OH, 12-O		3.0 ± 1.7	3.8 ± 1.0	5.8 ± 4.0
5α, 3α-OH, 12α-OH	Allodeoxycholic acid	3.2 ± 0.8	3.2 ± 0.4	ND
5α, 3α-OH, 12β-OH		1.2 ± 1.2	1.1 ± 0.6	ND
Chenodeoxycholic acid and its bacterial metabolites				
Bile acids retaining a substituent at C-7				
3α-OH, 7α-OH	Chenodeoxycholic acid	2.4 ± 0.7	1.9 ± 0.4	ND
Bile acids that have undergone 7-dehydroxylation				
3α-OH	Lithocholic acid	10.8 ± 3.2	8.7 ± 1.2	7.0 ± 2.7
3α-OH, 6β-OH	Murideoxycholic acid	0.5 ± 0.8	0.5 ± 0.4	ND

Values are means ± standard deviation; ND = not detected.
*Data from Horie et al.[13] from Beagle dogs ingesting commercial chow pellets. Horie et al. also reported that 2.3% of bile acids could not be identified.
†3-oxo bile acids such as this one are not determined by the enzymatic high-pressure liquid chromatography detector used by Horie et al.[13] The detector features an immobilized 3α-hydroxysteroid dehydrogenase that dehydrogenates bile acids having a 3α-hydroxy group.

lated for each collection, the mean of the two values for each dog was determined, and the overall means for each group of dogs (n = 5) were calculated and expressed as the mean ± standard deviation of the mean. Differences having a P value <0.05 using the Wilcoxon rank-sum test were considered significant.

RESULTS
Bile Acid Synthesis

Bile acid synthesis in jejunal pouch dogs did not differ significantly from that in ileal pouch dogs (Table I). Moreover, total bile acid synthesis in pouch dogs was nearly identical to that previously reported for healthy dogs using identical methodology.[12] Bile acid synthesis in both types of dogs was predominantly as cholic acid, averaging 88% of total bile acid synthesis.

Bacterial Metabolism of Bile Acids

Fecal bile acids were entirely in unconjugated form and had undergone extensive 7-dehydroxylation in both jejunal and ileal pouch dogs. Bacterial modifications of bile acids in the fecal samples are summarized in Table II. The extent of dehydroxylation was identical in the two experimental groups, and both cholic

acid and chenodeoxycholic acid were dehydroxylated to the same extent.

Fig. 1 shows a representative gas chromatogram of fecal bile acids, and Table III summarizes fecal bile acid composition obtained using this methodology. A variety of secondary bile acids were present because of other bacterial modifications. These were of the following three types: (1) epimerization (the change from an α-OH group to a β-OH group) at C-3 and/or C-12; (2) dehydrogenation (oxidation of a hydroxy group to an oxo group) at C-3, C-7, or C-12; and (3) A/B ring isomerization (formation of allo [5α] bile acids). Murideoxycholic acid (3α, 6β-dihydroxy-) was present in most samples (see Discussion) in trace proportions. Table III also gives the values reported by Horie et al.[13] for fecal bile acids in healthy dogs. The pattern of bile acids in our dogs did not differ from that reported by Horie et al.[13] for healthy dogs.

DISCUSSION

Neither surgical procedure induced bile acid malabsorption. When expressed as mg/kg-day, the daily synthesis rate of our dogs was nearly identical to the value reported by Horie et al.[13] for four healthy beagles. Because the body weights of our dogs were twice

as great as those of Horie's dogs, the total fecal bile acid outputs were twice as great.

The fecal bile acid output was similar in both experimental groups. Although the output of bile acids was approximately 20% less in the jejunal pouch dogs than in the ileal pouch dogs, the difference was not statistically significant because of the variability between animals. We cannot rule out a type II statistical error, but to do so would require 20 to 30 animals in each group, numbers that are impracticable.

Fecal bile acids in all dogs had undergone complete deconjugation and extensive 7-dehydroxylation. Dehydroxylation is mediated only by anaerobic bacteria, a flora present in both types of dogs.[7] Others have also found that deoxycholic acid is the predominant fecal bile acid found in ileal pouch dogs.[14] These changes and the similarity of the fecal bile acid composition of our dogs to that reported for the dog with an intact colon by Horie et al.[13] indicate that both types of neorectal pouch had developed an anaerobic flora differing little from the colonic flora of a healthy dog.

In dogs with an interrupted enterohepatic circulation of bile acids because of bile acid malabsorption[14] or biliary diversion,[15] a several-fold compensatory increase in hepatic bile acid biosynthesis occurs. The absence of any increase in compensatory bile acid synthesis in our pouch dogs indicates that total bile acid conservation by active and passive mechanisms was the same, on average, for the two surgical procedures. Because unconjugated di- and monohydroxy bile acids can be absorbed rapidly by passive membrane transport,[13,15-22] it is likely that a major fraction of the secreted bile acids was absorbed passively after undergoing bacterial deconjugation-dehydroxylation. An increased flux of unconjugated bile acids from the intestine can be detected by the finding of an increased level of unconjugated bile acids in systemic venous plasma, and such has been reported to be present in patients with ileal pouch–anal canal anastomosis.[2]

No information is available on what fraction of bile acid absorption occurred by active transport of conjugated bile acids. In retrospect, it would have been possible to quantify active bile acid absorption by the ileal transport system by using a deconjugation-dehydroxylation–resistant conjugated bile acid such as cholylsarcosine[23] or the radioactive conjugated bile acid analogue ^{75}Se-selenohomocholyltaurine.[24-26] Decreased absorption of this conjugated bile acid homologue from ileal pouches has been reported to be present in patients with malfunctioning pouches.[27]

The bacterial modifications of bile acids observed in these studies (7-dehydroxylation, epimerization, dehydrogenation, A/B juncture isomerization) are well known to occur in animals possessing an anaerobic cecum.[27] One novel fecal bile, murideoxycholic acid, was observed. We suggest that this compound was

formed in the hepatocyte by 6β-hydroxylation of lithocholic acid, as is well known to occur in other species.[13] Kurata[28] reported many years ago, using paper chromatographic analysis, that lithocholic acid undergoes 7α-hydroxylation (to form chenodeoxycholic acid) in the dog, but his methodology is unlikely to have detected a small proportion of 6β-hydroxylation.

It seems unlikely that the presumed increased flux of unconjugated bile acids across the pouch epithelium had deleterious effects. No changes in mucosal morphology in the dogs were seen by light microscopy.[7] Considerable deconjugation of bile acids in the distal small intestine occurs in healthy humans[1] and healthy rats.[29] Unconjugated bile acids, such as chenodeoxycholic acid, have been administered orally[30] or infused into the proximal small intestine[31] without inducing apparent structural or functional changes. Conjugated and unconjugated dihydroxy bile acids induce mucosal damage in animals when perfused at concentrations approaching their critical micellar concentration,[32] but the solubility of deoxycholic acid at the pH present in the pouch is likely to be too low to cause cytotoxicity.[33]

The change in bile acid metabolism induced by near-total proctocolectomy and pouch construction in our dogs differs from that reported in patients undergoing such a procedure. Among patients with an ileal pouch, bile acid malabsorption of a moderate degree is present in most patients based on recovery of fecal bile acids[3,5,6] or whole-body retention of SeHCAT, a conjugated bile acid analogue.[4] Bile acid dehydroxylation is much less extensive in most ileal pouch patients than in healthy subjects or in the ileal pouch dogs described here.[2-6,34] Others have reported that the ratio of anaerobic to aerobic flora in the feces of ileal pouch patients is smaller than in the feces of healthy subjects[35] and pouch dogs.[7] As a consequence of decreased bacterial 7-dehydroxylation, cholic acid, which is the major bile acid biosynthesized in humans, remains the major fecal bile acid in ileal pouch patients. Cholic acid, because of its extra hydroxy group, is absorbed passively at a much slower rate than deoxycholic acid,[16-18,20-22] which was the predominant fecal bile acid in our pouch dogs.

CONCLUSION

After enteric pouch–anal canal anastomosis, dog pouches develop an anaerobic fecal flora. Their fecal bile acids consist mostly of deoxycholic acid and its bacterial derivatives. Rapid, passive absorption of deoxycholic acid and its derivatives likely occurs and prevents bile acid malabsorption. Human pouches, in contrast, do not develop such an abundant anaerobic fecal flora. Cholic acid is deconjugated by bacteria in human pouches, but is not dehydroxylated. It remains the dominant fecal bile

acid. Unconjugated cholic acid is not efficiently absorbed by passive mechanisms, and so bile acid malabsorption occurs.

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Hypoosmotic Stress Stimulates Growth in HepG2 Cells via Protein Kinase B–Dependent Activation of Activator Protein-1

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Although hypoosmotic stress-induced cell swelling activates phosphatidylinositol-3-kinase, its impact on the downstream signal protein kinase B and cell growth is unknown. Activator protein-1 is in part phosphatidylinositol-3-kinase dependent, and is important in proliferation. We hypothesized that cell swelling modulates proliferation in HepG2 cells via the protein kinase B–dependent activation of activator protein-1. HepG2 cells pretreated with or without LY294002 were exposed for up to 30 minutes to hypoosmotic medium (160 mOsm/L). Tumor necrosis factor–alpha (1.4 nmol/L) or normoosmolar medium (270 mOsm/L) served as positive and negative controls, respectively. Western immunoblots measured cytoplasmic phosphorylated and total protein kinase B. Electromobility shift assays measured nuclear activator protein-1. Methylene blue assays measured cell proliferation at 24, 48, and 72 hours after stimulation. Hypoosmotic stress phosphorylated protein kinase B by 10 minutes. Subsequently, hypoosmotic exposure stimulated activator protein-1 by 30 minutes. Pulse exposure to hypoosmotic stress potentiated HepG2 proliferation by 72 hours as compared to both negative controls and LY-inhibited cells (n = 4 per group, $P = 0.009$ and $P = 0.004$, respectively; $P < 0.001$ analysis of variance. All three activation events were abolished with LY294002 pretreatment. In HepG2 cells, hypoosmotic stress-induced swelling stimulates proliferation via protein kinase B–mediated activation of activator protein-1. These data delineate a possible mechanism linking changes in cell volume to growth in human liver cancer. (J GASTROINTEST SURG 2001;5:546-555.)

KEY WORDS: HepG2, cell volume, PKB, AP-1, proliferation

Over the past 30 years, the number of cases of hepatocellular carcinoma in the United States has nearly doubled, with an annual incidence of 1 to 7 per 100,000. At the present time, surgery (resection or transplantation) offers the only hope for prolonged survival, and survival rates in these patients range from 11% to 46%.[1] Studies investigating the mechanisms of liver carcinogenesis may provide insight into newer treatment strategies.

Several investigations have associated cell swelling with hepatocarcinogenesis.[2-4] In mice, the administration of the hepatocarcinogen diethylnitrosamine and flumequine resulted in centrilobular hepatocyte swelling and increased proliferation.[5] However, this association has been questioned by at least one other study in which the inhibition of H6 hepatoma growth in vivo by amiloride was linked to intracellular Na+ content in the absence of a change in cell volume.[6] The association of cell swelling with hepatocarcinogenesis is not surprising, as various studies have implicated an increase in hepatocyte volume as playing a role in proliferative signaling.[7-9]

The phosphatidylinositol-3-OH-kinase/protein kinase B (PI-3-K/PKB) pathway represents one proliferative cascade. The PI-3-K–dependent activation of PKB begins with the binding of 3′ phosphorylated phosphoinositide lipids to its pleckstrin homology domain, with subsequent phosphorylation at Thr308 and Ser473 by upstream moieties such as PDK1.[10] Although initially believed to function in preventing

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apoptosis, recent studies have confirmed the role of PKB in cell proliferation. Studies in BaF/3 cells transfected with mutant PI-3-K confirmed both decreased PKB phosphorylation and cell proliferation in the absence of an altered rate of apoptosis.[11] In cultured primary vascular smooth muscle cells, IGF-I–induced migration and proliferation were dependent on the coactivation of PI-3-K and PKB.[12] In murine T cells, interleukin-3 receptor mutants Mc4 and Mc5, which did not proliferate with interleukin-3 exposure, showed decreased PKB activation as compared to wild-type controls.[13] In CV1 cells transfected with mutant FKHR1, a member of the forkhead family of transcription factors that is normally phosphorylated and exported from the nucleus on mitogenic stimulation, both PKB-mediated phosphorylation and export were abolished.[14] In addition, cultured bovine tracheal smooth muscle cells demonstrated the involvement of the PI-3-K/PKB pathway in proliferation.[15] As data accumulate demonstrating the correlation between PI-3-K and PKB in proliferative signaling, investigators now describe the PI-3-K/PKB coactivation step in growth regulation.[16]

Activator protein-1 (AP-1) is another proliferative intracellular messenger. At phosphorylation on the c-jun subunit by JNK, this transcription factor (composed of c-jun and c-fos proteins) enters the nucleus to bind and initiate various genes involved in cell cycle progression. Rat hepatocyte studies both in vitro and in vivo demonstrate that these events result in transcription of immediate early genes of growth.[17] Recent studies demonstrate that PI-3-K can activate AP-1.[18,19] A change in volume has been shown to activate PI-3-K, suggesting that hepatocyte swelling may play a role in the promotion of liver cancer. In this study, HepG2 cells exposed to hypoosmotic stress demonstrated a proliferative response involving the above-mentioned second messengers. Specifically, hypoosmotic stress induced an initial phosphorylation of PKB followed by the nuclear translocation of AP-1. These signaling events correlated with increased cell proliferation by 72 hours.

MATERIAL AND METHODS
Cell Lines and Reagents

HepG2 cells (American Type Culture Collection [ATCC], Rockville, Md.) were maintained at 37° C in 5% CO_2 in Eagle's minimum essential medium (MEM) with Earle's salts (ATCC) and supplements (1% nonessential amino acids, 10% fetal calf serum, 1% penicillin/streptomycin, and 1% sodium pyruvate [Gibco BRL, Rockville, Md.]). For cytoplasmic and nuclear protein extractions, cells were plated in six-well plates (Becton Dickinson, Franklin Lakes, N.J.) at 1 ×

10^6 cells/well. For proliferation assays, cells were plated in 96-well plates (Dow Corning, Corning, N.Y.) at 5 × 10^3 cells/well. Tumor necrosis factor-alpha (TNF-α) was obtained from Sigma (St. Louis, Mo.).

Experimental Conditions

Two days after plating, HepG2 cells at 60% confluence were studied. For cytoplasmic and nuclear extractions, cells were serum starved for 4 hours to induce quiescence. Hypoosmolar medium was created by diluting serum-free Eagle's MEM (270 mOsm/L) with sterile water at a ratio of 2:1 for a final osmolarity of 160 mOsm/L according to the method of Sadoshima et al.[20] and confirmed by osmometry (Precision Systems, Natick, Mass.). HepG2 cells were stimulated over various time periods by gently replacing old Eagle's MEM with hypoosmolar medium and incubating them at 37° C in 5% CO_2. TNF-α (1.4 nmol/L) served as positive controls. Serum-free Eagle's MEM served as controls. All replacement media were preincubated to 37° C in 5% CO_2. Parallel experiments were performed as above; however, these cells were pretreated for 1 hour with either LY294002, an inhibitor of PI-3-K (50 μmol/L) (Calbiochem, La Jolla, Calif.), or an identical dimethylsulfoxide (DMSO; Sigma) vehicle control. As the vehicle for LY294002 and alone as a vehicle control, DMSO was added to cell culture media at a 1:1000 dilution.

Cytoplasmic and Nuclear Protein Extraction

Cytoplasmic proteins were extracted by membrane lysis at 4° C using RIPA buffer (50 mmol/L Tris, pH 7.4, 150 mmol/L NaCl, 0.25% deoxycholate, 1% NP-40, 1 mmol/L EGTA, 1 mmol/L NaF, 1 μl/ml aprotinin, 1 μl/ml leupeptin, 1 μl/ml pepstatin, 1 mmol/L Na_3VO_4, 1 mmol/L NaF, and 1 mmol/L PMSF) and subsequent centrifugation.

Nuclear proteins were isolated using a modified version of the method of Dignam et al.[9,21] Briefly, cells were lysed by adding ice-cold buffer I (HEPES, 10 mmol/L; $MgCl_2$, 1.5 mmol/L; KCl, 10 mmol/L; and protease inhibitor [Sigma]), scraped, and dounce homogenized. The nuclear membrane was removed by serial washing and centrifugation in buffer II (HEPES, 10 mmol/L; KCl, 10 mmol/L; EDTA, 0.1 mmol/L; EGTA, 0.1 mmol/L; and protease inhibitor), and the nuclear proteins were isolated by centrifugation in buffer III (HEPES, 20 mmol/L; glycerol, 10%; KCl, 400 mmol/L; EDTA, 1 mmol/L; and EGTA, 1 mmol/L). For both nuclear and cytoplasmic extracts, protein concentrations in each sample were quantified using the Lowry assay (Bio-Rad Laboratories, Inc., Hercules, Calif.).

Western Immunoblot Analysis

For the detection of PKB, rabbit polyclonal immunoglobulin G (IgG) antibodies specific for total PKB (New England Biolabs, Beverly, Mass.) were used. For the detection of phosphorylated PKB, these same membranes were stripped using standard stripping formula per antibody vendor's recommendations (2-mercaptoethanol, 10 mmol/L; Tris-HCl, 62.5 mmol/L at pH 7.6; and SDS 2%) and rabbit polyclonal IgG antibodies specific for phosphorylated PKB (Ser473) (New England Biolabs) were used. Protein extracts from PDGF-treated NIH 3T3 cells served as a control for the phosphorylated PKB (New England Biolabs) antibody binding. Protein bands were visualized using a commercially available chemiluminescence kit (Bio-Rad) and autoradiographic film exposure (Eastman Kodak, Rochester, N.Y.).

Electromobility Shift Assay

[gamma-^{32}P] Adenosine triphosphate (ATP) was used to label a commercially available AP-1 double-stranded consensus oligonucleotide probe (Promega, Madison, Wis.). Briefly, 3.5 pmole of oligonucleotide and 10 μCi of [gamma-^{32}P]ATP were incubated with T4 polynucleotide kinase (1 U) at 37° C, and then purified by column chromatography (Bio-Rad) to yield 50,000 cpm/μl. For cold competition samples, unlabeled AP-1 oligonucleotide probes were prepared by omitting the radioisotope.

The binding reaction was a modification of the method of Sen and Baltimore[22]; 10 μg nuclear protein extract was incubated in a buffer (20 mmol/L HEPES, 50 mmol/L KCl, 0.1 mmol/L EDTA, 1 mmol/L DTT, 200 μg/ml bovine serum albumin, and 5% glycerol), with 2 μg poly dI/dC and 1 μl radiolabeled oligonucleotide probe. The binding reaction was incubated for 30 minutes at room temperature, then run on 6% polyacrylamide gels with 0.25× TBE (22 mmol/L Tris-HCl, 22 mmol/L boric acid, and 0.5 mmol/L EDTA). Autoradiographic films were exposed to the gels for 24 hours at −70° C and then developed.

Cell Proliferation Assay

Using HepG2 cells at 60% confluence 2 days after plating, experiments were performed as described earlier with or without preinhibition with LY294002. Following exposure to experimental conditions, cells were washed with phosphate-buffered saline solution and replenished with Eagle's MEM plus 10% fetal calf serum. Methylene blue proliferation assays[23] were performed 24, 48, and 72 hours following experimental conditions as follows. Cells were washed twice with

phosphate-buffered saline, fixed with 10% phosphate-buffered formalin, then washed twice with 10 mmol/L borate buffer (pH 8.5) and stained with 100 μl of filtered 1% methylene blue in 10 mmol/L borate buffer. These cells were then washed with borate buffer, and absorbed dye was eluted from the cells by adding 100 μl of 1:1 volume:volume ethanol: 0.1N HCl to each well. Dye absorbance was then read at 650 nm (Microplate Reader, Molecular Devices, Sunnyvale, Calif.).

Statistical Analyses

All experiments were performed in triplicate. To quantify both Western immunoblots and electromobility shift assays, densitometries were measured using the Stratagene Eagle-Eye system (Stratagene, La Jolla, Calif.). Relative densitometries with respect to time 0 band strengths were then calculated for each experimental time course, and statistical significance between paired blots (i.e., experimental conditions vs. control, or LY294002 exposed vs. nonexposed) was confirmed using Student's t test. For methylene blue assay, mean absorbance values and standard deviations were calculated for eight replicate wells for each treatment in four experiments. Statistical significance was determined by Student's t test and analysis of variance.

RESULTS
Phosphorylation of Protein Kinase B by Hypoosmolar Medium and TNF-α Is Inhibited by LY294002

Although hypoosmotic stress-induced cell swelling has been shown to activate PI-3-K, its impact on the downstream moiety of PKB in HepG2 cells is unknown. Therefore Western immunoblots were used to detect total or phosphorylated PKB within cytoplasmic protein extracts after hypoosmotic stress. Experiments examining HepG2 cells exposed to hypoosmolar medium (160 mOsm/L) for 0, 5, 10, 30, 45, and 60 minutes demonstrated an initial increase in phosphorylated PKB at 10 minutes with a maximum level at 30 minutes (data not shown). Subsequent experiments then focused on 0, 10, and 30 minutes of stimulation as time points representing baseline, initial, and maximum PKB activation, respectively. The relative densitometries of these blots demonstrated statistically significant increases in phosphorylation of PKB initially at 10 and maximally at 30 minutes (Student's t test, * = P <0.05; Fig. 1, A and B). When cells were pretreated for 1 hour with LY294002 (50 μmol/L), an inhibitor of PI-3-K-mediated PKB phosphorylation, there were persistently undetectable levels of phosphorylated PKB on exposure to hypoosmolar medium over time (see Fig.

Time (min)	0	10	30	LY
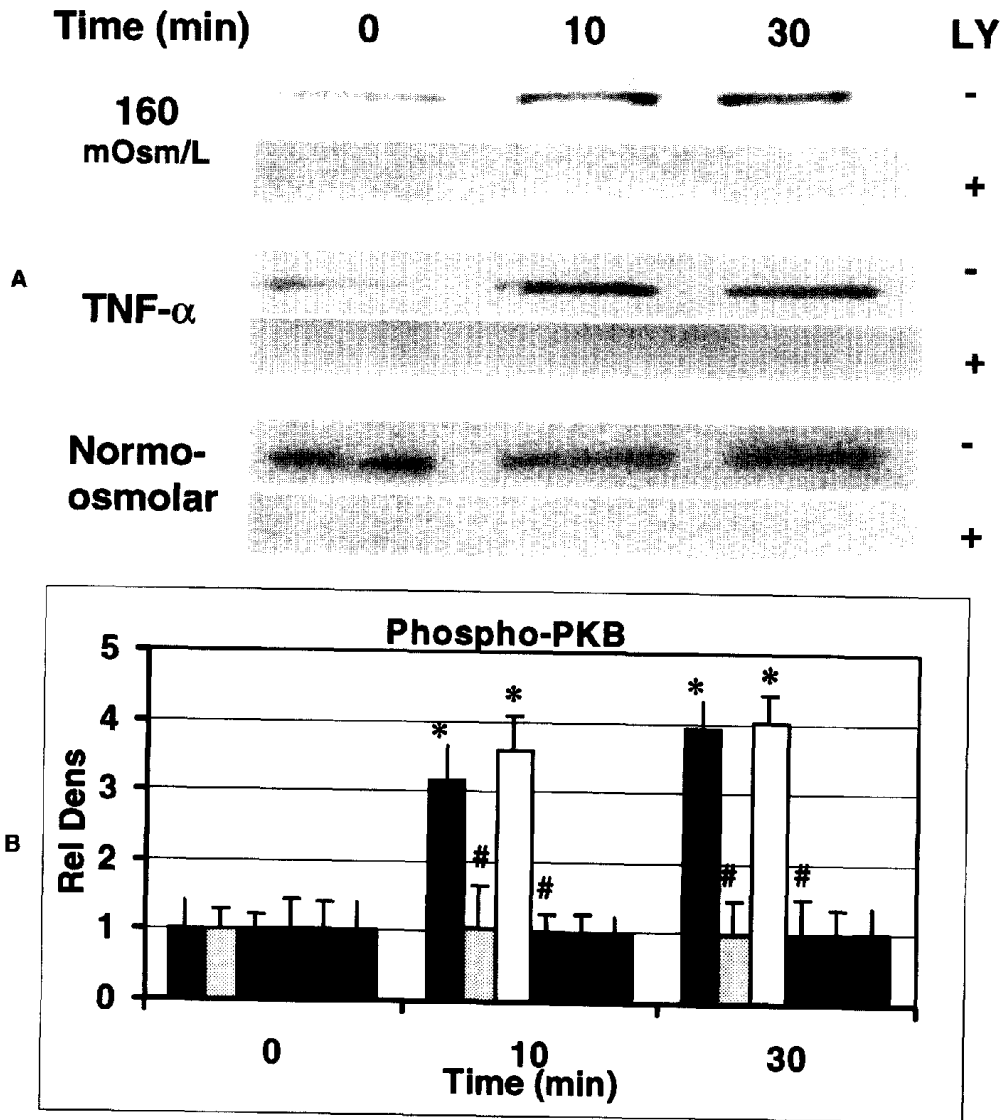
Fig. 1. Phosphorylation of PKB by hypoosmotic stress. After 4 hours of serum starvation, HepG2 cells were pretreated for 1 hour with or without 50 μmol/L LY294002 (LY+ or −, respectively), then exposed to hypoosmolar serum-free medium (160 mOsm/L) for the indicated times. A, Equal amounts of cytoplasmic proteins were separated by Western immunoblots on 10% SDS-polyacrylamide gels, proteins were transferred, and membranes were probed for phosphorylated PKB (Ser473). As positive controls, parallel experiments were performed using 1.4 nmol/L TNF-α in lieu of hypoosmolar medium. As negative controls, parallel experiments were also performed using normoosmolar serum-free medium (270 mOsm/L). B, Densitometries were measured and relative values with respect to time 0 band strengths were calculated. Statistical significance was determined by Student's t test (1) between treated and normoosmolar controls for each time interval (* = P <0.05) and (2) between LY nonpretreated and pretreated (# = P <0.05) cells for each experimental condition. All experiments were performed in triplicate (solid = LY294002^{−} and 160 mOsm/L; dotted = LY294002^{+} and 160 mOsm/L; vertical stripe = LY294002^{−} and TNF-α; horizontal stripe = LY294002^{+} and TNF-α; diagonal stripe = LY[−] and normoosmolar; checkered = LY[+] and normoosmolar).

1), and this inhibition was not reduced over time. The relative densitometries of these blots demonstrated a statistically significant decrease in PKB phosphorylation between the LY-nonpretreated and LY-pretreated cells at both 0 and 30 minutes (Student's t test, # = P

<0.05; see Fig. 1). When these same immunoblots were reprobed for *total* PKB, levels remained stable from 0 to 30 minutes demonstrating that hypoosmolar medium, either alone or following LY294002, had no effect on total PKB (Fig. 2, *A* and *B*).
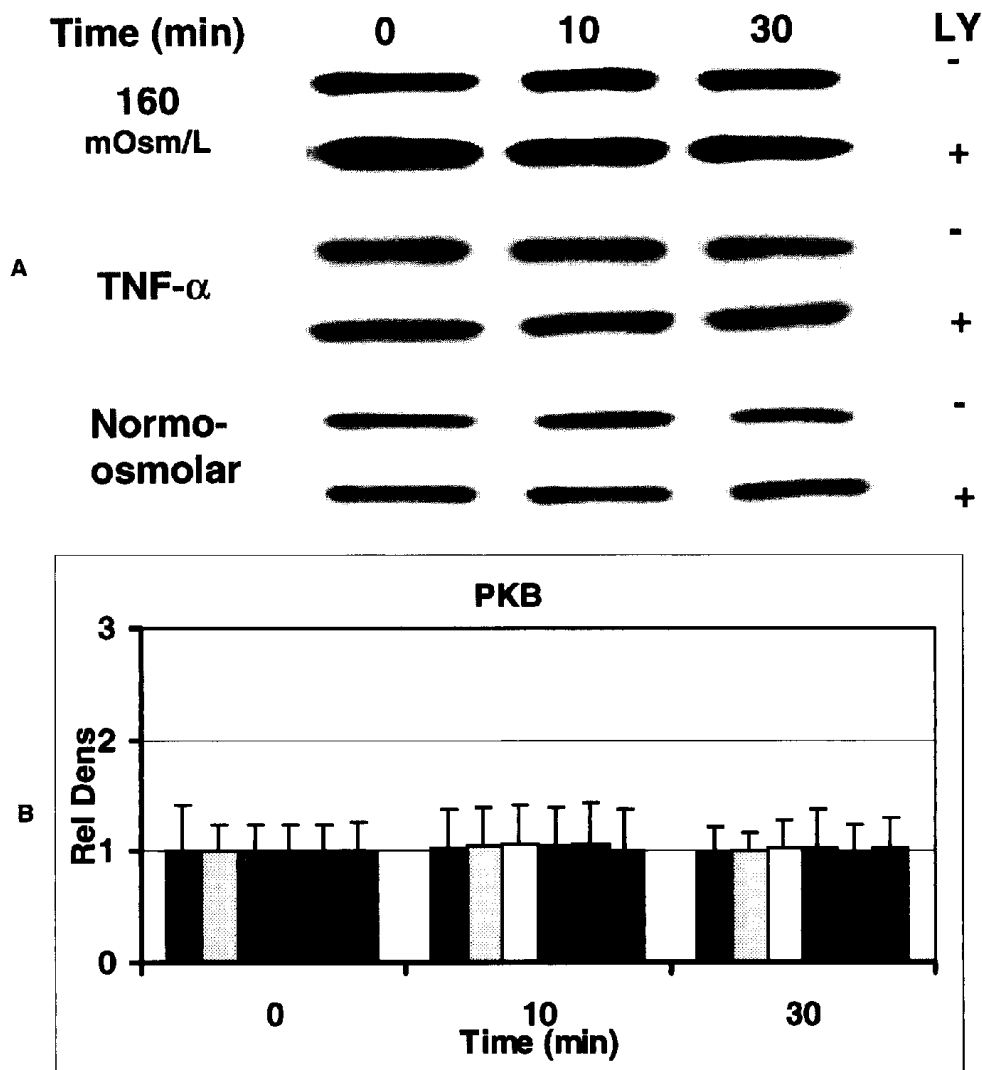
Fig. 2. Total PKB levels following hypoosmotic stress. After 4 hours of serum starvation, HepG2 cells were pretreated for 1 hour with or without 50 μmol/L LY294002 (LY+ or −, respectively), then exposed to hypoosmolar serum-free medium (160 mOsm/L) for the indicated times. A, Equal amounts of cytoplasmic proteins were separated by Western immunoblots on 10% SDS-polyacrylamide gels, proteins were transferred, and membranes were probed for PKB. As positive controls, parallel experiments were performed using TNF-α (1.4 nmol/L) in lieu of hypoosmolar medium. As negative controls, parallel experiments were also performed using normoosmolar serum-free medium (270 mOsm/L). B, Densitometries were measured and relative values with respect to time 0 band strengths were calculated. Statistical significance by Student's t test was not found (1) between treated and normoosmolar controls for each time interval and (2) between LY nonpretreated and pretreated cells for each experimental condition. All experiments were performed in triplicate (solid = LY294002^{−} and 160 mOsm/L; dotted = LY294002^{+} and 160 mOsm/L; vertical stripe = LY294002^{−} and TNF-α; horizontal stripe = LY294002^{+} and TNF-α; diagonal stripe = LY[−] and normoosmolar; checkered = LY[+] and normoosmolar).

TNF-α is a potent mitogen found to activate PI-3-K and PKB in various cells. Positive control experiments using TNF-α (1.4 nmol/L) were performed in parallel to confirm the appropriate HepG2 response. Cells stimulated with TNF-α alone showed increased levels of phosphorylated PKB from 0 to 30 minutes, and this increase was abolished with preinhibition with LY294002 (see Fig. 1). When these same im-

munoblots were reprobed for total PKB, cells showed unchanging levels of total PKB, with or without preinhibition (see Fig. 2).

HepG2 cells pretreated with or without LY294002 and then exposed to normoosmolar medium (270 mOsm/L) served as negative controls. These cells demonstrated unchanging levels of both phosphorylated and total PKB (see Figs. 1 and 2).
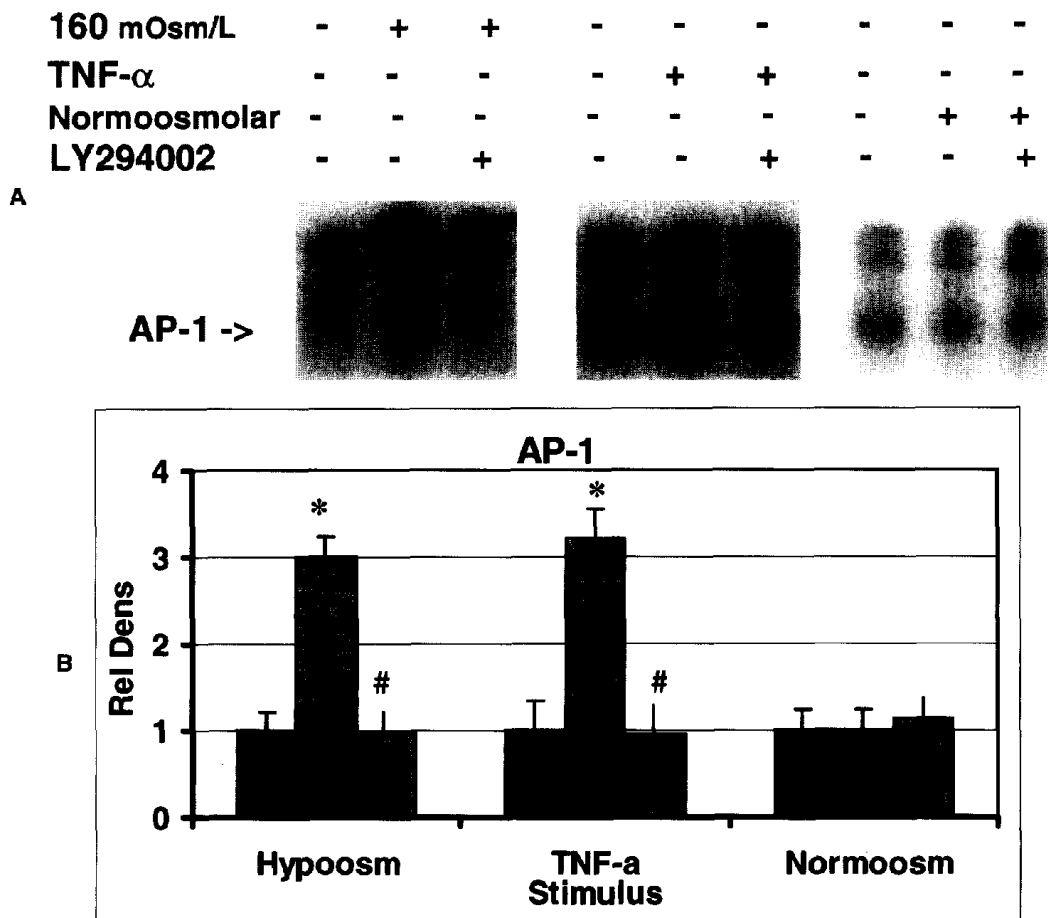
Fig. 3. Activation of AP-1 following hypoosmotic stress. After 4 hours of serum starvation, HepG2 cells were exposed to hypoosmolar serum-free medium (160 mOsm/L) for either 0 (lane 1) or 30 minutes (lane 2). A, Equal amounts of nuclear proteins were probed with [gamma-^{32}P]ATP–labeled AP-1 consensus oligonucleotides and were separated by electromobility shift assays on 6% Tris-borate-EDTA gels. As positive controls, parallel experiments were performed using TNF-α (1.4 nmol/L) in lieu of hypoosmolar medium for 0 and 30 minutes (lanes 4 and 5, respectively). As negative controls, parallel experiments were also performed using normoosmolar serum-free medium (270 mOsm/L) for 0 and 30 minutes (lanes 7 and 8). For all three stimulation conditions, another group of cells were preinhibited for 60 minutes with LY294002 (lanes 3, 6, and 9). B, Densitometries were measured and relative values with respect to time 0 band strengths were calculated. Statistical significance was determined by Student's t test (1) between cells either not exposed or exposed to experimental conditions for 30 minutes (* = P <0.05) and (2) between LY nonpretreated and pretreated cells (# = P <0.05) for each experimental condition. All experiments were performed in triplicate (solid = LY294002^{−} and stimulus[−]; vertical stripe = LY294002^{−} and stimulus[+]; diagonal stripe = LY294002^{+} and stimulus[+]).

Translocation of Activator Protein-1 by Hypoosmolar Medium and TNF-α Is Inhibited by LY294002

Although the PI-3-K/PKB cascade is known to translocate AP-1 following exposure to mitogens, the relationship between these two signals in HepG2 cells following hypoosmotic stress is unknown. Electromobility shift assays were performed on nuclear protein extracts from HepG2 cells to detect activated AP-1. Initial experiments in HepG2 cells stimulated for 0, 10, 30, and 60 minutes with hypoosmolar medium demon-

strated nuclear AP-1 by 10 minutes with maximum levels at 30 minutes (data not shown). Subsequent experiments focused on 0 and 30 minutes of stimulation as time points representing baseline and maximum AP-1 translocation, respectively. The relative densitometries of these electromobility shift assays demonstrated statistically significant increases in AP-1 shift band densities in cells exposed for 30 minutes to hypoosmotic stress (Student's t test, * = P <0.05; Fig. 3, A and B). As positive controls, HepG2 cells exposed to TNF-α also exhibited increased nuclear AP-1 at 30 minutes. In
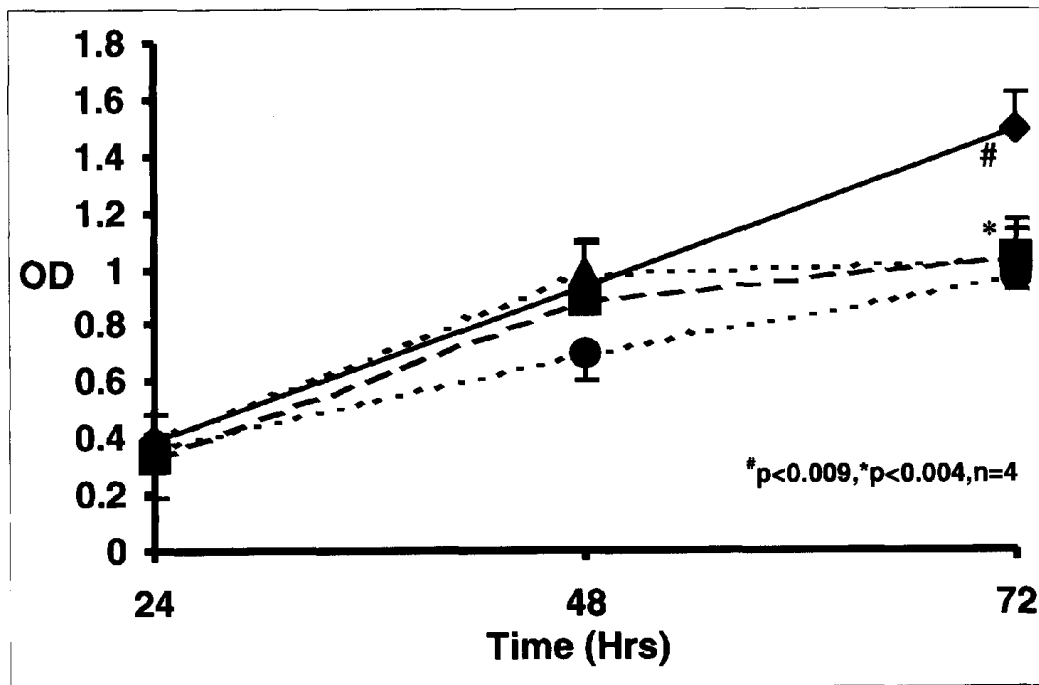
Fig. 4. Potentiation of HepG2 growth by hypoosmotic stress. Two days after plating, HepG2 cells at 60% confluence were exposed for 10 minutes to hypoosmolar serum-free medium (160 mOsm/L) in the presence (triangles) or absence (diamonds) of LY294002 (50 μmol/L). Parallel experiments were performed by exposing cells to 10 minutes of normoosmolar serum-free medium (270 mOsm/L) either in the presence (circles) or absence (squares) of LY294002. Cell numbers were determined at 24, 48, and 72 hours by methylene blue assays reading absorbances at 650 nm. Statistical significance was determined by t test and analysis of variance (n = 4).

both hypoosmotic and TNF-α treatment groups, preinhibition for 1 hour with LY294002 blunted the increase in nuclear AP-1. The relative densitometries of these electromobility shift assays demonstrated statistically significant decreases in AP-1 shift band densities in cells pretreated with LY294002 prior to hypoosmotic stress (Student's t test, # = P <0.05; see Fig. 3). Parallel negative control cells undergoing normoosmolar medium change alone failed to induce nuclear AP-1 above initial baseline levels (see Fig. 3). Cold competition assays using unlabeled AP-1 oligonucleotide probes demonstrated abrogation of shift bands with hypoosmotic stress and TNF-α (data not shown).

HepG2 Proliferation Potentiated by Hypoosmolar Medium Is Inhibited by LY294002

To investigate the proliferative impact of hypoosmotic stress-induced activation of PKB and AP-1, methylene blue proliferation assays were performed on cells 24, 48, and 72 hours after exposure to the same experimental conditions used in the cytoplasmic and nuclear analyses above. At 72 hours following a

10-minute exposure to hypoosmolar medium (160 mOsm/L), HepG2 cells showed statistically significant increases in optical densities as compared to cells exposed to normoosmolar medium (negative controls) (P = 0.009, n = 4). Similarly, HepG2 cells exposed to hypoosmolar medium alone demonstrated statistically significant increases in optical densities as compared to cells exposed to LY294002 (50 μmol/L) and hypoosmolar medium (P = 0.004, n = 4). Cells that were exposed to both LY294002 and normoosmolar medium showed both persistent growth and no difference in optical densities at 72 hours as compared to negative controls. When compared to negative controls, LY294002-hypoosmolar medium, and LY294002-normoosmolar medium as a group, cells stimulated with hypoosmolar medium showed a statistically significant increase in proliferation at 72 hours (P <0.001 analysis of variance, n = 4) (Fig. 4).

DISCUSSION

In the first part of this study, hypoosmotic stress stimulated the phosphorylation of PKB at Ser473 by 10 minutes in HepG2 cells (see Fig. 1). Because Ser473

phosphorylation is essential for the proliferative activity of PKB,[10] this event shows that PKB may be initiating its growth response through osmosignaling.

The early phosphorylation of PKB following hypoosmotic stress is consistent with the role of PKB as both an early proliferative signal and a direct target of PI-3-K.[10-14] The close correlation between PKB and PI-3-K activity has led some investigators to describe the PI-3-K/PKB coactivation step in growth regulation.[15,16] Studies correlating hepatocellular swelling with PI-3-K cascade activation support our data. Swelling induced by hypoosmotic stress, glutamine, or proline activates both PI-3-K and p70S6 kinase downstream in primary hepatocytes.[24] Similarly, hepatocytes exposed to insulin demonstrate increased proliferation in a p70S6 kinase-dependent fashion, suggesting that hormone-induced swelling may induce PI-3-K proliferative activity.[25] In H4IIE rat hepatoma cells, swelling-mediated NF-κB activation was abolished by PI-3-K inhibition with LY294002, suggesting PI-3-K involvement in transcriptional activation.[26]

Our data demonstrate that hypoosmotic stress induces the translocation of AP-1 in HepG2 cells (see Fig. 3). The role of cell swelling in the activation of AP-1 has been reported by others. In the setting of liver regeneration following partial hepatectomy, activated JNK phosphorylates AP-1 at its c-jun activation domain. Furthermore, pretreatment with anti-TNF-α antibody prior to partial hepatectomy inhibited both DNA synthesis and JNK activation.[27] Because TNF-α has been shown to cause hepatocyte swelling,[28] part of its mechanism of JNK/AP-1 activation in regenerating livers may be through increasing cell volume. In another study, lipopolysaccharide potentiated the human growth factor (HGF)–induced activation of JNK and AP-1 with resultant increases in hepatocyte replication.[29] Since endotoxin induces a hepatocyte volume increase,[30] this lipopolysaccharide-mediated AP-1 activation may also be due to swelling.

Our data suggest that swelling-mediated translocation of AP-1 is dependent on the activation of the PI-3-K/PKB pathway. A number of studies have already confirmed the involvement of PI-3-K/PKB in AP-1 activation. In JB6 cells, both 12-O-tetradec-anoylphorbol-13-acetate and epidermal growth factor–induced activation of AP-1 were inhibited by pretreatment with wortmannin or LY294002.[18] In both HepG2 and KB cells, the interleukin-1–induced activation of AP-1 was abrogated by PI-3-K inhibition via wortmannin and in a dominant negative mutant of the p85 subunit.[19] Similar studies in murine T cells showed that AP-1 activation was also inhibited by both wortmannin and in a dominant negative mutant of the p85 regulatory subunit of PI-3-K.[31] In

contrast, one study of HGF–stimulated HepG2 cells revealed that PI-3-K inhibition by wortmannin increased AP-1 activity.[32] However, as this study used a different stimulus and inhibitor, these findings likely represent an independent mechanism from the one presented here.

In the third part of this study, HepG2 cells exposed to hypoosmotic stress demonstrated statistically significant increases in growth by 72 hours as compared to cells exposed to negative control conditions with normoosmolar medium (Fig. 4). These data demonstrate for the first time that swelling alone may act as a potent mitogenic stimulus that influences cancer cell growth. When HepG2 cells were preinhibited with LY294002 prior to hypoosmotic stimulation, the decline in cell growth at 72 hours was statistically significant as compared to noninhibited cells (see Fig. 4). These results suggest that hypoosmotic stress-induced growth potentiation is at least partly dependent on PKB and AP-1 activation. The similar growth patterns between cells exposed to normoosmolar medium with and without LY294002 confirmed that preinhibition did not have a toxic effect on baseline HepG2 growth. Therefore the observed effects of LY294002 were likely due to its effects on hypoosmotic stress-induced cell signaling. The possibility of swelling-induced hepatocyte growth was initially suggested by liver regeneration studies; hepatocyte volume in the regenerating rat livers following partial hepatectomy increased by 25% at 6 hours and then returned to control values by 12 hours. In addition, DNA synthesis in these livers increased with amino acid uptake–induced swelling.[33] A more direct study describing swelling-induced growth potentiation involved *in vitro* and *in vivo* experiments on system A in rat hepatocytes. When system A, the sodium-dependent neutral amino acid transporter that is activated following partial hepatectomy, was inhibited 60 minutes prior to partial hepatectomy, DNA synthesis decreased by 45%, liver mass by 46%, and DNA synthesis by 56% at 24 hours.[33] These data demonstrated that cell volume increase is an important factor in hepatocyte proliferative competence.

Although no studies have shown a direct relationship between swelling and hepatocyte proliferation, studies in other cell lines have suggested that physical perturbation may induce cell cycle progression and growth. The calcium channel blockers bepridil and nifedipine decreased both cell volume and proliferation in ras+ NIH 3T3 fibroblasts,[34] suggesting a correlation between cell hydration and growth. In fetal spleen cells, mitogens were shown to increase both cell volume and DNA synthesis, again linking cell swelling with proliferation.[35]

CONCLUSION

Our data describe a possible mechanism in which HepG2 growth may be modulated via osmosignaling. In this study, hypoosmotic stress-induced swelling increases the normal proliferative state of these cells by the activation of the PI-3-K/PKB cascade with the subsequent induction of AP-1. Hypoosmotic stress has already been shown to stimulate ERK-1 and 2-mediated translocation of NF-κB, another proliferative transcription factor.[9] Whether this mechanism represents a proliferative response that is active in all liver cells or is specific to human hepatoma cells is unknown. In order to delineate completely this signaling cascade, studies in different human cancer cell lines, using chemical and genetic inhibitors at all levels of the PI-3-K pathway, are warranted.

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The Gut and Food Intake: An Update for Surgeons

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Food intake is the simplest and most obvious measure of gastrointestinal function, yet it rarely receives more than cursory attention from surgeons. In this review we cover recent findings on relationships between gut function and appetite regulation mediated via neuropeptides influenced by afferent and efferent vagal activity. Evidence from the new discipline known as neurogastroenterology elucidates gastric and intestinal signals involved in the elicitation of hunger, satiety, and aversion. Discovery of the adipose-tissue–derived hormone, leptin, has energized the field of metabolism spawning increasing numbers of publications related to interactions between leptin and insulin release and glucose disposal, as well as appetitive behavior. Peptides such as cholecystokinin (CCK), the proglucagon-derived peptides, glucagon-like peptides 1 and 2 (GLP-1 and GLP-2), and the recently identified powerful intake-stimulating molecule, orexin, are examples of potential targets for drug development and studies of surgical pathophysiology. A major conclusion of this work is that the considerable redundancy and overlap between mediators of caloric intake subserving survival of the species, while beneficial after foregut surgery, contribute to the complexity of treating the global epidemic of obesity. Possibly knowledge derived from basic research in neurogastroenterology can translate into advances in surgical treatment of obesity. (J GASTROINTEST SURG 2001;5:556-567.)

KEY WORDS: Appetite regulation, gastrointestinal surgery, neurogastroenterology, glucagon-like peptide-1, cholecystokinin, vagus nerve

Food intake not only has a vital nutritive function, but it is also an essential component of the quality of life of any individual. As gastrointestinal surgeons, we exclusively diagnose and treat diseases that either have a direct impact on food intake or have the potential to do so pursuant to our treatment. Remarkably, food intake is ignored in surgical texts, even those specializing in metabolism and surgical nutrition. In routine clinical practice we limit ourselves to the question of whether the patient is "tolerating" a liquid or solid diet, without attempting to assess the quantity or quality of food intake. Heretofore we might be forgiven for our preoccupation with mechanical problems such as stenosis, stricture, and dysmotility, but with recent advances in neuroscience it behooves us to broaden our knowledge base to include appetite regulation and ingestive behavior.

PEPTIDES AND INGESTIVE BEHAVIOR

Our understanding of the regulation of food intake is rapidly increasing with frequent reports of "new peptides" influencing ingestion (Table I). These peptides act as hormones and transmitters and have traditionally been described as having single functions, being organ specific, synthesized, or active in the brain, the gut, or more recently, adipose tissue (leptin). It has become increasingly clear, however, that many of the peptides are ubiquitous and multifunctional with considerable redundancy and overlap, which is understandable in view of the primal importance of nutrition for the survival of all organisms. A sophisticated system of neurocircuitry and neurochemistry serves to monitor signals indicating substrate levels in various tissues including the liver, adipose tissue, and blood. When these signals reach the

Table I. Some peptides and hormones implicated in the control of food intake

Increased food intake	Reduced food intake
Agouti-related hormone (AGRH)	Amylin
Aldosterone	Bombesin
Dynorphin	Calcitonin
β-Endorphin	Calcitonin gene-related peptide (GGRP)
β-Casomorphin	Cocaine and amphetamine-regulated transcript (CART)
Corticosterone	Cholecystokinin (CCK)
Galanin	Enterostatin
Growth hormone-releasing hormone (GHRH)	Gastrin-releasing peptide
Orexin A and B	Glucagon
Neuropeptide Y (NPY)	Glucagon-like peptide-1 (GLP-1)
Peptide YY (PYY)	Glucagon-like peptide-2 (GLP-2)
	Insulin
	Leptin (ob protein)
	α-Melanocyte-stimulating hormone (α-MSH)
	Neurotensin (NT)
	Oxytocin
	Somatostatin
	Thyrotropin-releasing hormone (TRH)
	Urocortin
	Vasopressin

cognitive level they are experienced as "hunger" or "satiety," ultimately resulting in ingestive behaviors such as eating and drinking.

THE FOREGUT AS A SMALL BRAIN

Although the close relationship between the brain and the gut has been known for many years, it is only recently that the field of neurogastroenterology has been recognized as a specialty in its own right.[1] Novel peptides originally thought to be found only in the feeding centers of the brain have just been identified in the intestine,[2] just as gut peptides such as insulin, cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), and glucagon-like peptide-2 (GLP-2) have been found to have important functions in the brain and nerve tissue.[3-6] Similarly, neuropeptide Y (NPY), a potent stimulator of food intake in the brain of rodents, has also been found in the gut[7,8] (for a thorough review of the role of NPY in appetite regulation see Kalra et al.[8]). These findings, along with the realization of the complex in-

teractions between the different organs of the gastrointestinal tract, underlie the concept of the gut as a "small brain."

Food intake is an intricate process involving cognitive and psychosocial components, as well as autonomous physiologic ones. *Hunger* is regulated by a central feeding drive modulated by peripheral satiety signals that are activated during and after a meal.[9-12] In humans, it is also common for psychological factors to override or modulate physiologic signals that regulate food intake. These factors can limit intake, even in the presence of hunger, and can sustain intake despite sensations of fullness or lack of hunger.[13] Thus there are considerable interactions between physiologic and psychological signals in regulating ingestive behavior.

"Satiety," the opposite of hunger, refers to the state of repletion or the feeling of having consumed enough, whereas the verb "to satiate" means to satisfy the appetite or to feed to repletion. *Nimiety*, on the other hand, is defined as being filled to excess, an unpleasant aversive state. Blundell et al.[9] have emphasized that although there is an overlap between satiation and satiety, they should be separated and considered to operate independently. Satiation (the process by which feeding is brought to an end) and satiety (the end result causing inhibition of food intake) may be regulated by similar or separate processes.

In a recent study using positron emission tomography, increased regional blood flow was present in the vicinity of the hypothalamus, insular cortex, paralimbic and limbic areas, and other areas of the brain when subjects reported hunger. In contrast, 30 minutes after the intake of a liquid meal during satiety, regional blood flow was increased in the ventromedial and dorsolateral prefrontal cortex and inferior parietal lobule.[14] It is reasonable to assume that these "brain signals" are mediated by signals from the gut.

Recently much of the focus of research in the area of energy intake has focused on leptin. Leptin is a hormone secreted from adipose tissue. Circulating plasma concentrations of leptin reflect fat mass. In rodents lack of leptin or a defective leptin receptor is associated with obesity. Administration of leptin inhibits food intake and decreases weight in obese rodents lacking circulating leptin. However, in the majority of obese humans plasma leptin concentrations are high. Administration of leptin to obese humans does not seem to have a major effect on food intake. Thus there are differences between the effects of leptin in genetically obese mice and obese humans. There is a growing body of evidence suggesting an interaction between leptin and gastrointestinal signals in the regulation of food intake in the central nervous system of rodents.[15,16]

The gut, as the portal of entry of all nutrients, naturally has a fundamental role in appetite regulation. Several of the signals arising from the gastrointestinal tract that influence food intake are altered in "surgical" disease states and after surgical intervention. The aim of this report was to review the current literature on relationships between gut function and appetite regulation focusing on foregut signals, most of which are mediated via the vagus nerve and interactions with the "old" gut peptide CCK and the "new" proglucagon-derived peptides GLP-1 and GLP-2.

GASTRIC SIGNALS

It is obvious that the stomach has an important role in the regulation of food intake, yet the mechanisms are only partly understood. Best appreciated are mechanisms related to mechanoreceptors involved in the reservoir and propulsive functions of the stomach, where distention is the adequate stimulus influencing all types of motility. Although less well characterized, gastric chemoreceptors have a fundamental role in all aspects of gastric physiology involved in appetite regulation. For example, noncaloric liquid saline empties exponentially from the stomach, whereas nutrients empty rapidly during the first few minutes and thereafter at a steady linear rate until the stomach is completely empty. There is evidence that the pylorus meters the energy content of the food: a fixed number of calories empties into the duodenum per unit of time, regardless of the composition of the food.[17] At the same time, a host of other endogenous and exogenous factors influence emptying rates.

Gastric distention produces a feeling of satiety in humans.[18-20] Balloon distention of the stomach induces an unpleasant sensation rather than the hedonic feeling of satisfaction normally experienced after a meal. Intragastric balloons may reduce food intake in obese subjects, but the effect is very short-lived.[21] A pleasant postprandial-like satisfying feeling is achieved if lipid is infused into the duodenum at the same time that the gastric balloon is inflated.[22] Viscous substances such as xylitol and guar gum delay gastric emptying and also decrease food intake. However, this effect is short-lived unless nutrients are included with the guar gum.[23,24]

Hunger and fullness are often interpreted as being reciprocal, although they are mediated by different mechanisms. After a fatty meal, postprandial fullness but not hunger ratings were related to intragastric content measured by ultrasonography.[25] After ingestion of a meal containing olive oil and low-calorie beef soup, postprandial fullness was related to gastric content of soup, whereas hunger was related to the gastric oil content.[18,19] In a recent study, postprandial fullness but not hunger was found to be closely re-

lated to antral distention after 350 ml of 20% glucose measured as antral area by means of ultrasound.[26] These findings suggest that gastric distention by food may play a major role in eliciting satiation, whereas reduction of hunger feelings after a meal most likely results from an interaction of nutrients with receptors in the small intestine and postabsorptive signals from the liver and/or central nervous system. Interestingly, infusion of food into the totally denervated stomach in rats decreased food intake, implying a humoral mechanism independent of neural effects.[27] Gastrin-releasing peptide (GRP) and somatostatin are released from the antrum of the stomach. These two peptides have been shown to have satiating effects after food intake in humans and laboratory animals.[28,29] It is not clear whether those effects are systemic or local. Recently leptin has been found in rodent and human gastric mucosa,[30,31] although it is not clear whether this has any implications for food intake.

The vagus nerves have extensive projections to the gastrointestinal tract, both afferent and efferent. Information from the gut is in large part relayed to the central nervous system via afferent vagal fibers that project to the nucleus tractus solitarius. Fibers from the nucleus tractus solitarius project further to different nuclei of the hypothalamus. Circulating factors such as gut peptides and signals relaying information regarding the size of adipose tissue (leptin and insulin) are thought to enter the brain at the arcuate nucleus, which projects fibers to the paraventricular nucleus of the hypothalamus and the lateral hypothalamus. The neurons in the arcuate nucleus, paraventricular nucleus, and lateral hypothalamus (LH) contain several neuropeptides known to influence energy intake, such as NPY, orexin, cocaine and amphetamine–regulated transcript (CART), α-melanocyte–stimulating hormone (α-MSH), GLP-1, CCK, and agouti-related protein (AGRP).[3,32-35]

Gastric distention results in an increased discharge rate of vagal afferent fibers.[36] The receptive fields of these fibers are localized to small regions (<3 mm) of the stomach, and the fibers show a short response latency suggesting that they may be involved in the feedback control of propulsive and retropulsive grinding forces necessary to achieve the small particle size needed for nutrients to pass the pylorus.[36] c-fos expression is increased in the nucleus tractus solitarius and dorsal motor nucleus after gastric distention in rats.[37] Furthermore, c-fos–like immunoreactivity is increased in the dorsal motor ganglion and hypoglossus nucleus after feeding in rats, implying afferent signaling from the stomach to the central nervous system via the vagus nerve during and after food intake.[38] Close arterial infusion of picomolar doses of CCK increases the discharge rate in these gastric vagal mechanosensitive afferent fibers.[36] This synergistic ef-

fect of CCK and gastric distention will be explored further below. A newly discovered peptide, urocortin, from the corticotropin-releasing factor family has recently been shown to inhibit food intake and gastric emptying in lean and obese *ob/ob* mice when given peripherally.[39] This lends further support to the concept of the stomach having a central role in the control of food intake.

As surgeons, on a daily basis we encounter a type of "gastric" anorexia—that is, anorexia seen during postoperative ileus. The motility of the stomach can be impaired for 24 to 48 hours after abdominal surgery.[40,41] In contrast, the small intestine regains motility earlier, perhaps within a few hours of surgery. Thus the small intestine is ready to process food early after surgery, but the stomach is not ready to accommodate food intake. This makes a case for early enteral nutrition through a jejunostomy when gastric emptying is thought to be impaired for a long period of time.

INTESTINAL SIGNALS

Infusion of lipid into the small bowel before and during a meal induces a feeling of satiety and a reduction in food intake. If the same lipid solution is infused intravenously, no effects on satiety or eating behavior can be demonstrated.[42] This suggests a nutrient receptor in the small intestine, which may be vagal since the effects are blocked by vagotomy.[43] Also, if visceral afferent nerve fibers are defunctionalized by capsaicin (a selective neurotoxin derived from red hot chili peppers), rats overconsume a high-fat diet, suggesting a role for capsaicin-sensitive afferent fibers in providing negative feedback resulting in early termination of meals.[44] Since nutrient infusions into the intestine inhibit gastric emptying and intestinal transit,[45,46] it could be argued that the satiety-inducing effect of lipid infusion is secondary to gastric signals. However, infusion of lipid into the duodenum suppresses food intake even in animals equipped with esophageal or gastric fistulas that allow food to empty without causing gastric distention.[47,48] This suggests that intestinal factors alone can induce satiety. However, there is likely an additive effect between gastric and intestinal signals.

The length of intestine in contact with nutrients and the duration of nutrient stimulation may be important determinants of intestinal satiety.[49,50] Guar gum added to a lipid-rich soup induces satiety that lasts for several hours, much longer than the soup alone. The rate of gastric emptying was the same for guar gum with and without lipid, suggesting a prolonged exposure of the intestine to nutrients or an increased distention of the small bowel after intake of guar gum. It is possible that guar gum delays small

intestinal absorption, prolonging the time during which nutrients are in contact with the absorptive mucosa of the small intestine, thus inducing a prolonged period of postprandial satiety.[24,51,52] This suggests that nutrient-sensitive receptors in the small intestine can signal satiety.
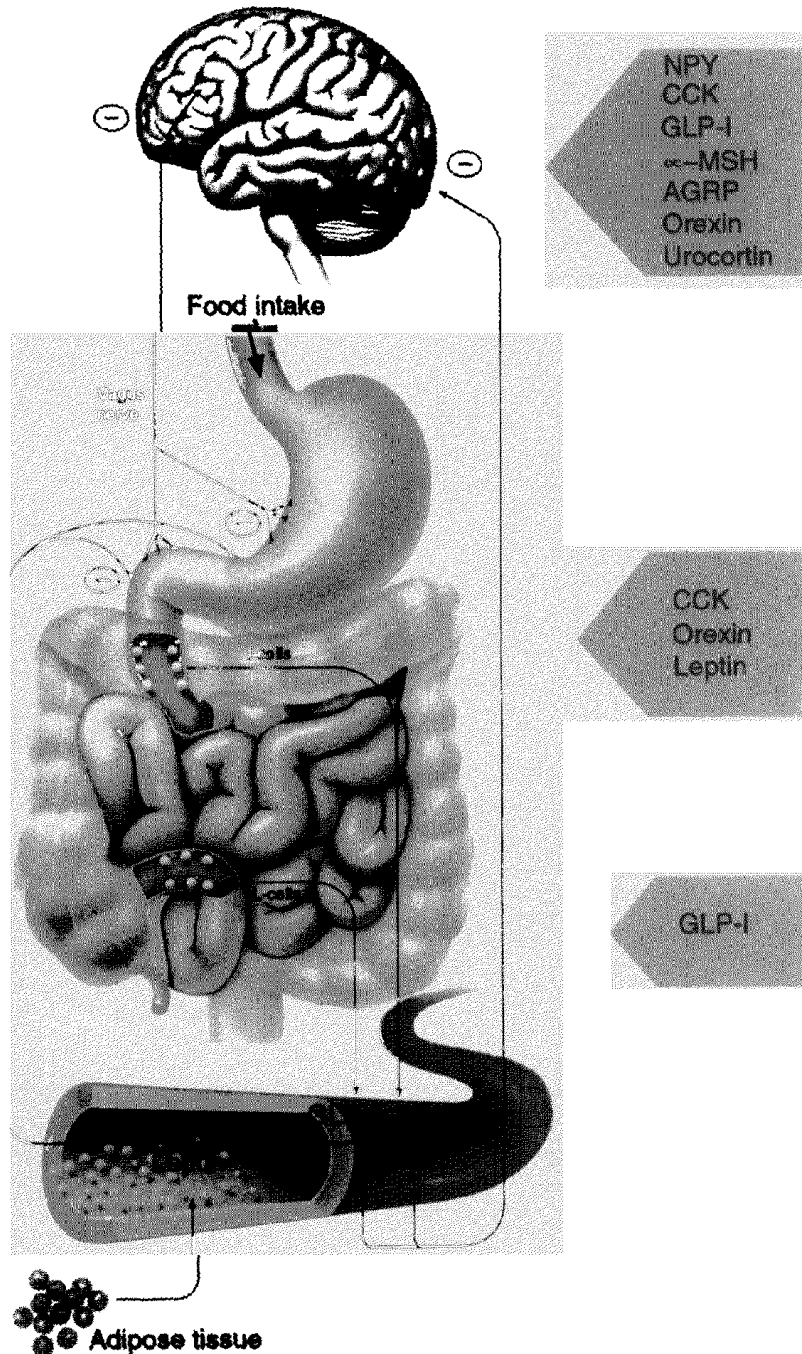
Nutrient-sensitive fibers have been demonstrated in rats.[53] Vagal afferent fibers have been found to project from the duodenal villi to the nodose ganglion.[54] In addition, load-sensitive vagal afferent fibers have been demonstrated in the upper duodenum. These fibers respond briskly to small loads of isotonic saline solution or inflation of a small balloon in the duodenum. The fibers also respond to close intra-arterial injection of CCK in a similar manner to the vagal afferent fibers seen in the stomach. CCK pretreatment sensitizes these duodenal vagal afferent fibers to subsequent duodenal loads, indicating that CCK and duodenal loads act synergistically to stimulate these mechanosensitive fibers to a greater degree than stimulation by CCK or duodenal loads alone.[55,56] Since endocrine cells located in the upper duodenum release CCK in response to intraluminal nutrients, it is possible that CCK acts in an endocrine or paracrine manner, stimulating gastric and duodenal vagal fibers and other neurons in the stomach and intestine,[57] thus influencing food intake through central nervous mechanisms (fig. 1).

GUT PEPTIDE SIGNALS
Cholecystokinin

CCK is found in the I-cells (an endocrine cell mainly found in the duodenum and jejunum)[58,59] and is released into the circulation after food intake. The family of CCK peptides exists in several bioactive molecular forms with 4 to 58 amino acid residues. All forms of native CCK have the bioactive C-terminal portion of the molecule in common and CCK-8 and CCK-33 have been reported to have biologic actions after food intake.[60,61] There are two types of CCK receptors that have been classified as CCK-A and CCK-B on the basis of their affinity for structurally and functionally related peptides.[62,63]

Several studies in humans have demonstrated that CCK infusion reduces food intake[64-67] and CCK receptor blockade, using the experimental drug MK-309, increases food intake.[68] However, it seems as if a gastric preload is necessary to achieve a satiating effect with CCK-33. CCK-33 infused to high physiologic levels in fasting humans had no influence on satiety or food intake,[69] whereas infusion after a banana preload decreased food intake in both obese and lean subjects.[70]

The relationship between decreases in plasma levels of CCK and reports of hunger has also been in-

fig. 1. Schematic illustration of sites of action of the gut peptides cholecystokinin *(CCK)* and glucagon-like peptide-1 *(GLP-1)* influencing satiety through vagal afferent signals from the gut and direct effects on central feeding centers. CCK and GLP-1 are found throughout the gut, whereas CCK is found in higher concentrations in the proximal gut and GLP-1 in the distal gut. To the right are peptides with appetitive effects at different levels (NPY = neuropeptide Y; α-MSH = α-melanocyte–stimulating hormone; AGRH = agouti-related hormone). Leptin receptors have also been demonstrated in the brain.

vestigated in humans. Considerable between-subject variations in postprandial CCK concentrations and sensations of hunger and fullness were present.[71] It has been suggested that nausea might contribute to the reduction of food intake after CCK infusion, but this was refuted in a recent article.[72]

The concept that a preload of nutrients is necessary to unveil a satiating effect of CCK is consistent with neurophysiologic studies. As mentioned earlier, intra-arterial injection of CCK close to the stomach and duodenum of rats potentiates the response of afferent gastric and duodenal vagal fibers,[56] suggesting

that afferent fibers show polymodal sensitivity to both CCK and mechanical stimulation. Also, a combination of a gastric distending load and CCK acts synergistically to produce greater discharge rates than those of either stimulus alone.[73] Thus there seems to be a dose-response relationship between gastric distention, CCK, and the vagal afferent discharge rate. Less gastric distention is needed to achieve similar vagal afferent discharge rates in the presence of high levels of CCK. This may be important for dosing CCK and similar peptides in order to inhibit food intake.

Administration of CCK antibodies and receptor antagonists results in increased food intake, suggesting that CCK may be an inhibitor of food intake.[74-76] Peripheral CCK acts on CCK-A receptors in the antrum of the stomach and is involved in the inhibition of gastric emptying after CCK administration.[77] CCK-A receptors have also been shown to be present in the subdiaphragmatic vagus nerve.[78] Studies in gastrectomized rats have shown that extragastric CCK inhibits food intake,[79] suggesting a direct effect on the vagus nerve of CCK released from the duodenum after food intake. Also, feeding results in the release of CCK locally in the brain where it can act on CCK-B receptors indicating an additional pathway for the action of CCK on food intake.[4] Many of the studies of CCK and food intake have used doses of CCK in the pharmacologic range making it difficult to draw clear conclusions regarding a physiologic role for CCK in the control of food intake.

Proglucagon-Derived Peptides (GLP-1 and GLP-2)

GLP-1 and GLP-2 are peptides that are produced in the L-cells (endocrine cells mainly found in the ileum and colon) of the intestinal mucosa and are secreted after intake of a mixed meal. GLP-1 and GLP-2 arise as the result of proteolytic cleavage of proglucagon in the L-cells of the gut.[80,81] The amino acid sequence of GLP-1 and GLP-2 is highly conserved indicating a physiologically important function of the peptides.[82,83]

To date, only one GLP-1 receptor type is known. GLP-1 receptors have been found in the central nervous system, lung, stomach, skeletal muscle, and fat cells.[84-87] In the brain, GLP-1 immunoreactive cell bodies are present in the caudal portion of the solitary tract, and in the dorsal and ventral parts of the medullary reticular nucleus, corresponding to regions that receive afferents from the gastrointestinal tract via the vagus nerve.[3] GLP-1 immunoreactive nerve fibers have been found in the paraventricular nucleus and the periventricular strata,[3,88,89] as well as in thalamic nuclei, and in the brainstem.[89,90] GLP-1 binding

sites have been found in the sensory circumventricular organs including the subfornical organ and area postrema. Because the various regions of the circumventricular organs lack a perivascular blood-brain barrier, free exchange of molecules between the blood and cerebrospinal fluid is possible.[91-93]

At physiologic plasma levels, GLP-1 is insulinotropic and glucagonostatic and thus lowers blood glucose concentrations. It inhibits meal- and pentagastrin-induced gastric acid secretion and increases insulin-induced glucose clearance. Furthermore, GLP-1 in slightly supraphysiologic doses delays gastric emptying of liquid[94] and solid meals,[95] as well as plain water (unpublished observation), suggesting that GLP-1 is an "ileal brake" hormone.[94] There is evidence that the effect of GLP-1 on gastric secretion and motility is mediated via the vagus nerve in both animals and humans[96-98] and the blood glucose-lowering effect of GLP-1 is partly mediated through the peptide's inhibitory effect on gastric emptying.[99]

GLP-1 has a direct central action on feeding behavior. Several reports have demonstrated that intracerebroventricular injection of GLP-1 in rats inhibits food and water intake[100-102] and leads to increased *c-fos* gene expression in the paraventricular nucleus.[102] Intracerebroventricular administration of the GLP-1 receptor antagonist, exendin (9-39)amide, originally found in the venom of the lizard *Heloderma suspectum*, to satiated but not fasted rats, resulted in increased food intake.[102] Furthermore, administration of exendin (9-39)amide twice daily for 10 days not only increased food intake but also resulted in a significant weight gain.[103] No effect on food intake has been found after intraperitoneal injection of GLP-1,[102] suggesting a central nervous rather than peripheral mode of action in rats. However, the very short half-life of GLP-1 in rodents may account for the lack of effect of peripheral GLP-1 on food intake.

To date, seven studies have shown that GLP-1 increases satiety and decreases food intake in normal-weight,[104-106] diabetic,[107,108] and obese humans,[109,110] whereas one study failed to demonstrate any effect of GLP-1 on food intake or appetite.[111] These studies range from 2 to 48 hours in duration with either intravenous or subcutaneous infusions. The GLP-1 infusion resulted in slightly supraphysiologic plasma concentrations in all of these studies and was similar to the concentrations seen in studies investigating the possible role of GLP-1 in the treatment of non-insulin-dependent diabetes mellitus. No nausea or other side effects have been associated with GLP-1 infusion at the rates used in the preceding studies. As with CCK, many studies have used pharmacologic doses of GLP-1 making it difficult to ascertain whether the effect of peripheral GLP-1 on food intake is physiologic. The use of the GLP-1 receptor antagonist ex-

endin (9-39)amide in humans may shed further light on the effect of endogenous GLP-1 on food intake.

Indirect data also suggest effects of GLP-1 on food intake. It has been proposed that the meal response of GLP-1 is attenuated in obese humans, implying that lower postprandial GLP-1 concentrations may result in a shorter intermeal period.[112-114] It is of interest that genetic studies in families with morbid obesity have shown a linkage with islet 1 locus (Isl-1) on chromosome 5q. Isl-1 is a positive regulator of proglucagon gene transcription and may thus influence GLP-1 secretion,[115] possibly resulting in decreased plasma GLP-1 concentrations with a concomitant increase in food intake.

The effects of GLP-2 are not as clearly characterized as those of GLP-1. GLP-2 has been shown to have trophic effects on the intestinal mucosa of rodents[116] and is currently being investigated as a treatment for intestinal failure in humans.[117] In pigs, GLP-2 inhibits antral motility[118] and has been shown to inhibit gastric acid secretion in humans.[119] Recently a GLP-2–containing neuronal pathway connecting the nucleus tractus solitarius and dorsomedial hypothalamic nucleus has been found in rats, and GLP-2 receptor mRNA was found in the dorsomedial hypothalamic nucleus. Central administration of GLP-2 resulted in a 35% decrease in food intake compared to placebo.[5] Thus, in terms of gastric function, it would seem as if both GLP-1 and GLP-2 contribute to the "ileal brake" effect and that the two peptides have similar effects on food intake. The physiologic role of GLP-2 in humans has yet to be determined.

INTERACTION BETWEEN DIFFERENT TYPES OF PERIPHERAL SIGNALS

Two recent publications have shown synergistic effects of CCK and leptin in the control of food intake in rodents. Concomitant administration of leptin centrally and CCK peripherally decreased food intake more than the administration of either peptide alone.[120] In a second study, synergy between leptin and CCK on food intake was confirmed, and it was also shown that the combination of leptin and CCK caused greater c-fos activation in brain areas associated with food intake than with either peptide alone.[121] Taken together, these reports suggest that the proposed "long-term" satiety agent, leptin, also may modulate intermeal satiety by interacting with gut signals, which are mainly thought to affect intermeal satiety. In addition, it has been shown that CART-derived peptides are found in vagal afferent neurons sensitive to CCK, implying that CART may mediate the satiety effect of CCK.[122] In analogy with CCK, there is evidence that GLP-1 and leptin interact in the brain to influence food intake,[123,124] suggesting the in-

teraction of short-term and long-term mechanisms for regulating appetite.

SURGICAL IMPLICATIONS

As seen in this review, signals arising from the foregut seem to influence short-term (intermeal) appetite and food intake interval. In addition, there is evidence that gastrointestinal peptides such as CCK and GLP-1 interact with signals involved in the long-term regulation of food intake such as leptin from adipose tissue and the afferent vagal system of the gut and liver. There are two opposite clinical problems that create the need for understanding relationships between the gut and food intake: undernutrition and obesity. The former has been in the domain of gastrointestinal surgeons throughout history, whereas the latter has only relatively recently become the object of our interest. Major foregut surgery such as gastrectomy, pancreatoduodenectomy, and vagotomy obviously greatly interferes with many appetite signals, stimulatory as well as inhibitory, arising from the gut. However, the scarcity of long-term clinical consequences such as involuntary weight loss postoperatively can be interpreted as evidence of considerable reciprocity between the systems regulating hunger and satiety. Alternatively, because of their crucial importance for survival, hunger signals exhibit considerable redundancy. The liver, via glycogen for short-term regulation, and proteins of the lean body for long-term homeostasis are likely peripheral sources of such signals.

General Surgery

Postlaparotomy ileus is a short-term inhibitor of food intake likely with only minor consequences for overall energy balance. However, with the recent demonstration of significant improvements in patient well-being as shown by the elimination of the traditional preoperative fast in favor of energy-rich liquids up to 2 hours before the time of operation[125] and early postoperative enteral feeding with rapid mobilization,[126] it is conceivable that reduced gastric ileus contributes to more rapid postoperative recovery. This mechanism has not yet been evaluated, however. A significant component of postoperative anorexia is also attributable to cytokine release caused by the surgical trauma of the wound. Reduction of the cytokine response through minimally invasive laparoscopic surgical approaches[127] might explain the characteristically earlier return to full function seen after laparoscopic surgery, although the energy consequences of cytokine release in this context have also not yet been studied.

Gastrectomies of varying extent for ulcer disease or cancer have not been well studied with respect to their

appetitive effects, although there is a reasonable body of literature on their nutritional sequelae. The results of gastric resection on gut peptide levels show increased plasma concentrations of enteroglucagon, insulin, and neurotensin after a meal.[128] These studies have been performed with respect to the dumping syndrome and not appetite. Vagotomy, on the other hand, as is obvious from this review, has been studied extensively, although there is a paucity of information on the effects of vagotomy on peptides in patients. Motility and mechanisms of acid secretion including polypeptide analysis after vagotomy have been investigated in the context of ulcer disease without analysis of effects on ingestion. Our own studies of truncal vagotomy in a small series of obese patients concerned insulin release[129] and food and liquid intake[130] without determinations of other gastrointestinal polypeptides.

Patients with short bowel but preserved colon often show evidence of functional adaptation and normal gastric emptying compared to patients with short bowel and jejunostomy. Recent data demonstrate that patients with a resected ileum but an intact colon have elevated fasting GLP-1 and GLP-2 plasma concentrations and greater meal-stimulated responses of the two peptides compared to nonoperated control subjects. Possibly this reflects the trophic effects of GLP-2 on gut mucosa and inhibitory effects of GLP-1 on gastric emptying.[131]

Antiobesity Surgery

Gastrointestinal surgery for severe obesity, on the other hand, deals with a different threat to the quality and duration of life. Its goal is to enhance satiety and/or reduce hunger signals to create undernutrition.[132] Purely gastric restrictive procedures, such as vertical banded gastroplasty or gastric banding, increase gastric and/or esophageal distention from solids in an attempt to elicit early satiety. Signals from distention are neural or neurohumoral. The human esophagus is well characterized with respect to neuropeptide distribution.[133] Recent positron emission tomography studies of esophageal afferents have identified brain loci stimulated by distention.[134] Interestingly, these loci are in proximity to loci stimulated by appetitive behavior.[14] Hypothetically, esophageal signals could participate in regulating (inhibiting) food intake. Our observations during endoscopy of reduced esophageal sensitivity in severely obese patients might indicate a "satiety defect" in obese patients (unpublished observations). Gastric distention of the cardia does not yet seem to have been evaluated with respect to neural or humoral signals relevant to appetitive behavior.

As indicated earlier, truncal vagotomy alone, without drainage, has been studied as a treatment for obe-

sity[135] in a small group of patients. Because of the modest weight loss, vagotomy was abandoned as a single procedure. However, based on our findings of a reduction in calorically dense liquid intake after vagotomy,[130] the hallmark of the "soft calorie syndrome," the primary cause of failure of gastric restrictive procedures,[136] we *added* vagotomy to gastroplasty in another small group of patients. We found that the vagotomy potentiated the weight loss of the vertical banded gastroplasty procedure.[137] The mechanisms are not known, although motility factors as well as neurohumoral signals are certainly involved.

Gastric bypass relies on gastric restriction much like gastroplasty in the initial phase of weight loss (6 to 18 months), subsequently relying on the bypass to maintain the loss. The maintenance phase accounts for the superior performance of gastric bypass over gastroplasty, whether by banding or a combination of banding and stapling. The mechanisms of the diversionary component of gastric bypass are partly absence of a pyloric "meter" or "brake" allowing rapid transit via the gastrojejunostomy and partly the maldigestion caused by the absence of acid and pepsin and the grinding-mixing forces of the stomach. Thus undigested food rapidly shunted into the jejunum causes nimiety via mechanoreceptors and possibly satiety via chemoreceptors. A few studies have analyzed patterns of peptide release after gastric antiobesity operations.[138-141] After vertical banded gastroplasty, changes in meal-stimulated plasma concentrations of gut hormones are few, whereas after gastric bypass hormones mainly found in the upper gut are decreased and hormones found in the distal gut such as enteroglucagon are elevated. GLP-1 has not specifically been measured after gastric bypass, but since plasma concentrations of enteroglucagon are elevated after gastric bypass, and enteroglucagon and GLP-1 are secreted in parallel from the L-cells of the gut, it is reasonable to assume that plasma concentrations of GLP-1 might also be elevated, thus influencing hunger sensations.

Intestinal bypass operations used clinically, experimenting with varying lengths of jejunum and ileum in continuity,[142] have been studied with respect to gastrointestinal polypeptide patterns. Although these operations are no longer performed as primary procedures for surgical treatment of obesity, their effects on gut peptides are relevant and the information provided can be used in developing other strategies for weight control. As we have recently demonstrated, GLP-1 concentrations are elevated both short term (9 months) and long term (20 years) after jejunoileal bypass, indirectly giving evidence of the satiety effects of GLP-1 after jejunoileal bypass.[143,144]

The most extensive of the gastrointestinal operations for obesity are biliopancreatic bypass (BPD)[145]

and its modification whereby a duodenal switch is added (BPD-DS).[146] Sarson et al.[147] performed an analysis of peptide patterns in the early 1980s, but such studies do not seem to have been repeated since the discovery of many of the "newer" peptides. Studies of gastric emptying of solids and liquids and of enteroglucagon levels in patients[148] after the duodenal switch give some indirect indication of the mechanisms accounting for weight loss after BPD-DS.

We conclude that detailed studies of the neurohumoral effects of antiobesity surgery should help in developing drugs for the treatment of this serious disease and also, by increasing the understanding of the phenomenon of satiety, contribute to refining operations for obesity. We also conclude that surgeons can contribute substantially to the new field of neurogastroenterology, just as this field will likely improve outcomes of gastrointestinal surgery.

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Letter to the Editors

To the Editors:

I have been requested by the Board of Trustees of The Society for Surgery of the Alimentary Tract to write a letter of apologia. At issue is the suggestion that my group and I have published two manuscripts with sufficient similarity to approximate duplicate publication.[1,2] This is a most serious concern, and I view it as such. Clearly, neither I nor any of my group desire or need duplicate publication. If any impropriety occurred, it was certainly not intentional, and I do apologize.

Both manuscripts were derived from our prospective database initiated in 1983, and both were related to infectious complications following pancreaticoduodenectomy. It is correct that sections of the materials and methods of these two manuscripts are essentially identical. However, I believe it is equally true that we provide substantially different data between the two papers. The first paper referred to 161 patients, and the second to 240 patients. The two papers together contained 20 tables. The first manuscript contained eight tables and the second manuscript 12 tables. Although they contained different numbers, three of the total 20 tables have the same title and similar data. In retrospect, we clearly should have informed the editors of the existence of the *Annals of Surgery* manuscript at the time of presentation of the manuscript to the JOURNAL OF GASTROINTESTINAL SURGERY.

I do acknowledge my serious oversight in not comparing the manuscripts in parallel, although I do believe that there is room for genuine debate over the extent of similarity. I do wish to emphasize that the responsibility is mine and not that of my coauthors.

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