State-of-the-Art Address

The Pathogenesis of Cholesterol Gallstones—A Review

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Cholesterol is extremely insoluble in water. As a result, cholesterol secretion into bile is highly regulated and once secreted into bile, solubility is maintained by cholesterol transporters. Understanding the physicochemical relationships between cholesterol and its transporters is key to understanding gallstone formation. Stone formation requires excess cholesterol secretion into bile, which produces cholesterol supersaturation. Many of the epidemiologic risk factors for stone formation such as obesity, femaleness, and age can now be linked to specific abnormalities of cholesterol metabolism. Cholesterol supersaturation is necessary but not sufficient for cholesterol crystal formation. Crystallization also requires a gallbladder motility defect and/or the presence of procrystallizing factors such as mucous glycoprotein or immunoglobulins. The latter seem to be stimulated by gallbladder inflammation. Stone growth is the final step in stone formation and the least well understood. Recent work suggests that cholesterol stones form as agglomerations of cholesterol crystals and that pigmentation is secondary. (J GASTROINTEST SURG 1998;2:109-125.)

Cholesterol cholecystolithiasis is an extremely common problem. There are approximately 500,000 new cases of symptomatic cholesterol cholelithiasis in the United States annually. In order for cholesterol gallstones to form, bile must become supersaturated with cholesterol, the supersaturated cholesterol must nucleate into cholesterol monohydrate crystals, and the nucleated crystals must be formed into a macroscopic stone. This review will describe relevant aspects of the physical chemistry of biliary lipids, their secretion into bile under normal and abnormal circumstances, and the current concepts of cholesterol gallstone formation.

PHYSICAL CHEMISTRY OF BILIARY LIPIDS—CHOLESTEROL, PHOSPHOLIPIDS, AND BILE SALTS

Cholesterol

Cholesterol gallstones, by definition, are at least 70% cholesterol by weight. Cholesterol is a sterol that is obtained in the diet or synthesized, mainly in the liver. It is an amphipath, that is, it has hydrophilic (polar) and hydrophobic (nonpolar) components. The molecule consist of three parts (Fig. 1): a sterol nucleus and a side chain, both of which are hydrophobic, and a hydrophilic hydroxyl group. The latter is internalized, thus reducing its beneficial effect on aqueous solubility. As a result of its composition and structure, cholesterol is very sparingly soluble in water; it has an aqueous solubility of 10^{-8} mol/L.[1]

Phospholipids

The major phospholipid of bile is phosphatidylcholine (lecithin). Lecithin has backbone consisting of glycerol, a molecule with three carbon atoms, linked to which are two fatty acid chains and a choline group. The choline group contains the hydrophilic portion of the molecule; the two fatty acid chains are hydrophobic. Unlike the hydroxyl group of the cholesterol molecule, the hydrophilic group of lecithin is external and a very strong polar group. Spatially, lecithin is a linear amphipathic molecule, with a hy-

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drophilic head and a hydrophobic body and tail (see Fig. 1).

Bile Salts

Bile salts are synthesized from cholesterol in the liver. In the course of synthesis, hydroxyl groups are added to the sterol ring of the cholesterol molecule and a carboxyl group to the side chain. The spatial orientation of these polar hydroxyl and carboxyl groups, in most physiologic bile salts, is such that they align along one side of the molecule (see Fig. 1). The bile salt molecule may therefore be thought of as a plate, one side of which is hydrophilic and the other hydrophobic.

The behavior, in aqueous solution, of these three amphipathic biliary lipids depends on their structure. At any concentration above its very low aqueous solubility, cholesterol self-aggregates into solid cholesterol monohydrate crystals. Phospholipids have a somewhat higher aqueous solubility than cholesterol. However, at relatively low concentrations, phospholipids also associate out of solution. They form a molecular *bilayer* in which the hydrophobic ends of the molecules are turned inward away from the aqueous environment and the hydrophilic ends are turned out toward the aqueous environment. This familiar structure is the basis of cell membranes. These sheets of molecular bilayers will close naturally to form hollow spheres known as vesicles. Vesicles can be seen by specialized light microscopy, such as phase-contrast microscopy, and are very easily seen by electron microscopy. Although vesicles may be very small and may not aggregate with each other to form larger vesicles for some time, vesicles are not in solution but are a solid phase. They are highly deformable and therefore are often called "liquid crystals."

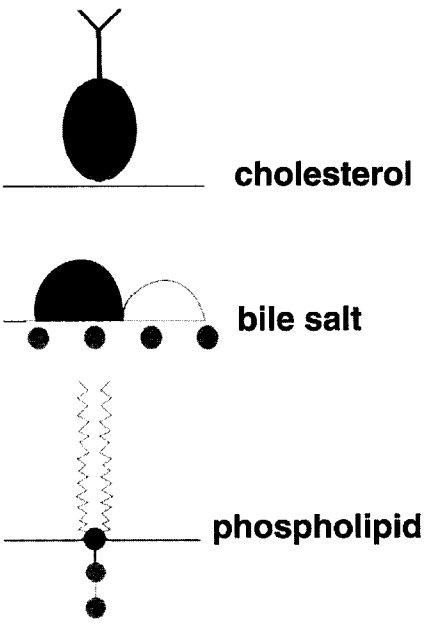

Fig. 1. Structural representation of the major biliary lipids. Polar portions of the molecules are shown in light shading. Nonpolar portions are shown in darker shading.

Bile salt monomers are quite soluble in water. Their aqueous solubility, which is approximately 10^{-3} mol/L, is much higher than that of cholesterol or phospholipid monomers. Above this concentration, referred to as the critical micellar concentration (CMC), associative behavior begins and bile salts aggregate into *simple* micelles. The exact CMC varies depending on the type of bile salt and other conditions. Micelles are molecular associations of bile salts in which molecules are aligned to present the hydrophilic surface to the aqueous environment. The number of molecules per simple micelle is between 4 and 25. Micelles are much smaller than vesicles and are too small to be seen by electron microscopy. Unlike self-aggregates of cholesterol or phospholipid, micelles are fully in solution.

Associations Among Biliary Lipids

Any of the three biliary lipids may associate with each other. Phospholipid molecules may be incorporated into simple bile salt micelles to form mixed micelles. These molecular complexes are considerably larger than simple bile salt micelles. In an environment in which an excess of bile salt is present, phospholipid vesicles will be totally incorporated into mixed micelles. Cholesterol is readily incorporated into the phospholipid bilayer of vesicles and there becomes associated with the highly hydrophobic fatty acid chains. Similarly, cholesterol may be incorporated into micelles. Its solubility in simple micelles is low, but mixed micelles have a much greater capacity to solubilize cholesterol because of the high affinity of cholesterol for the fatty acid chains of phospholipids. The incorporation of cholesterol into vesicles and micelles greatly enhances its transport in aqueous solutions such as bile.

Phases, Equilibrium, and the Equilibrium Phase Diagram

Each of the forms in which cholesterol can exist in bile is referred to as a "phase." Thus the phases of cholesterol are the monomeric phase, solid cholesterol crystals, the vesicular phase, in which cholesterol

is incorporated into phospholipid vesicles, and the micellar phase, in which cholesterol is incorporated into mixed micelles (Fig. 2).

Movement of cholesterol molecules between phases is possible and is governed by the energetics of the system. For instance, cholesterol monomers may move into the vesicular phase or the micellar phase. Cholesterol molecules may move out of a supersaturated vesicular phase to form cholesterol crystals.

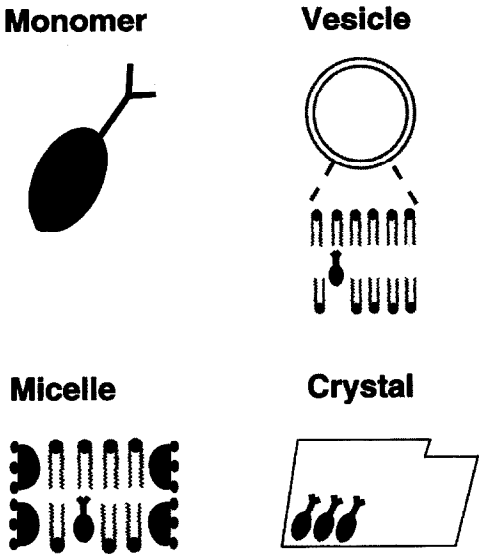

Fig. 2. Phases of cholesterol in bile.

However, eventually all movement stops and a state of equilibrium is reached. Understanding equilibrium is key to comprehending the physicochemical changes that lead to gallstone formation. Equilibrium is a condition in which all acting influences are canceled by others, resulting in a stable, balanced, or unchanging system. The phases present at equilibrium are predicted by the equilibrium phase diagram (Fig. 3). The monomeric phase is not depicted in this diagram. By definition, a solution is supersaturated with respect to cholesterol when a solid phase is present at equilibrium—either vesicles or solid cholesterol monohydrate crystals or both. Unsaturated solutions contain only monomers and micelles. Although aqueous solutions may be constructed which are supersaturated on the basis that vesicles alone are present at equilibrium, the range of bile composition in humans is such that bile is either unsaturated or if supersaturated has cholesterol crystals with or without vesicles. Whether bile is supersaturated with cholesterol can be determined by measuring the concentration of the lipids in bile and plotting its relative composition on the phase diagram. Because this is cumbersome, the information on the phase diagram was mathematically converted to a cholesterol saturation index. If bile has a cholesterol saturation index greater than 1.0, it is saturated with cholesterol and will contain cholesterol crystals when that bile comes to equilibrium.

It is of critical importance in understanding the pathogenesis of cholesterol gallstones to be aware that bile is not secreted in a state of equilibrium. At the

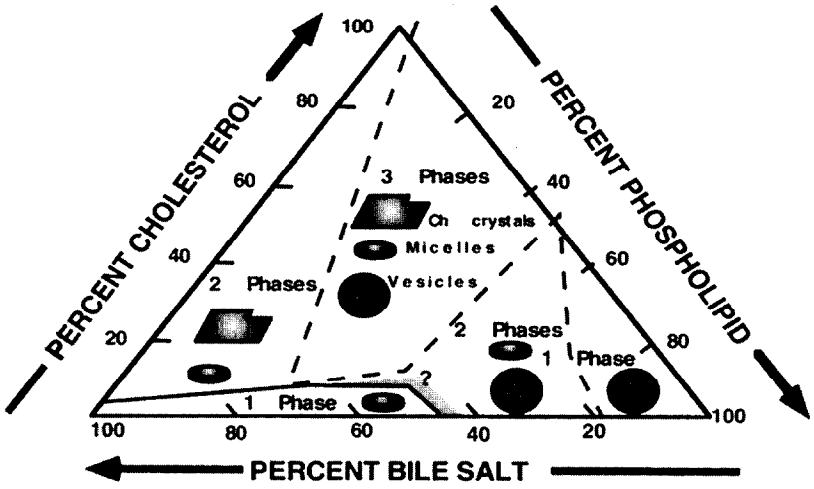

Fig. 3. Equilibrium phase diagram for bile salt-lecithin-cholesterol-water at a concentration of 10% solids, 90% water. The monomeric phase is not depicted as a phase since it exists at the same concentration throughout. The one-phase zone contains only micelles. There are several other zones but only the two on the left above the one-phase zone apply to human gallbladder bile, and both contain cholesterol monohydrate crystals at equilibrium. (Adapted from Donovan JM, Carey MC. Separation and quantification of cholesterol 'carriers' in bile. Hepatology 1990;12:94S-104S.)

time of secretion, there are no cholesterol crystals in bile. Some supersaturated biles reach equilibrium and contain cholesterol crystals while still in the biliary tree, but others do not. As we shall see later in this review, whether cholesterol crystals form in supersaturated bile in the biliary tree depends on certain kinetic factors.

SECRETION AND TRANSPORT OF CHOLESTEROL IN BILE

Normal Secretion and Transport

Hepatic cholesterol is derived either from preformed cholesterol taken up from the blood by the liver cell or from synthesis of cholesterol within the hepatocyte (Fig. 4). Hepatic cholesterol may be exported into bile directly, used for bile salt synthesis, or converted into cholesterol esters. Bile salts are secreted directly into bile, and cholesterol esters are either exported from the liver into serum or stored as such in the liver. Many of the enzymes and receptors involved in these steps are known. Certain of the risk factors associated with gallstone formation are known to affect the activity of these enzymes or expression of receptors governing these steps (see Fig. 4).

Whether bile is supersaturated is largely determined at the moment it is secreted into the canaliculus, although some modification may occur as a result of lipid absorption in the gallbladder. Cholesterol is secreted into bile as cholesterol-phospholipid vesicles.[2,3] Most biliary cholesterol appears to be preformed, although approximately 20% is synthesized. There is considerable evidence that bile salts are necessary for the "budding-off" of vesicles from the canalicular membrane, and this corresponds to the observation made years ago that bile salts stimulate the secretion of cholesterol and phospholipids into bile.[4,5] Bile salts are probably secreted into the canaliculus in monomeric form and associate to form simple micelles.

The presence of vesicles and micelles in the same aqueous compartment provides the opportunity for lipid exchange to occur between cholesterol carriers, alluded to previously. We have referred to the process by which vesicles are altered in the presence of micelles as vesicular "maturation" (Fig. 5). It is likely that maturation occurs as a result of direct contact between cholesterol carriers as well as movement of cholesterol monomers between carriers. The net effect is incorporation of vesicular lipid into simple micelles to make mixed micelles. In unsaturated bile there is micellar excess and eventually all vesicular lipid becomes incorporated into micelles (see Fig. 5). There seems to be a tendency for vesicular phospholipids to be incorporated into micelles more readily than vesicular cholesterol. As a result, during maturation, vesicles become enriched in cholesterol. When the cholesterol-to-phospholipid ratio of vesicles exceeds 1.0, vesicles tend to become unstable and nucleate cholesterol crystals. In unsaturated bile, cholesterol enrichment of vesicles is of no consequence since eventually all vesicular lipid is incorporated into micelles.

The Pathway of Crystal Formation in Supersaturated Bile

Secretion of supersaturated bile is similar to that of unsaturated bile, the major difference being that the relative amount of cholesterol secreted into bile is greater. Lipid shifts, that is, maturation, proceed as with unsaturated bile, the difference being that residual vesicles still are present at the point that micelles have taken up cholesterol and phospholipids to capacity (Fig. 6). For reasons already given, such vesicles are enriched in cholesterol. Furthermore, these vesicles develop areas on their surface that are particularly enriched in cholesterol. The subsequent steps resulting in the appearance of cholesterol monohydrate crystals consist of vesicular aggregation and fusion, nucleation, and crystal growth. Vesicular aggregation and fusion in supersaturated bile nucleating cholesterol crystals was first shown by Halpern et al.[6,7] Aggregation and fusion are events that tend to bring cholesterol-enriched zones on the vesicular surface into apposition, greatly facilitating nucleation of cholesterol monohydrate crystals (Fig. 7). Initially crystals that have nucleated are very small and unstable, but with crystal growth they attain a stable size and eventually a size at which they are microscopically detectable. Sometimes the initial crystal is the classical cholesterol monohydrate crystal, but at other times the initial crystals are filaments, coils, or tubes that subsequently are transformed into the classical cholesterol monohydrate crystals.[8]

THE THREE STAGES OF GALLSTONE FORMATION

Supersaturation

Supersaturation is due to excessive cholesterol secretion, and there are many responsible mechanisms. Bile could theoretically become supersaturated as a result of excessive cholesterol secretion or reduced secretion of cholesterol carriers—bile salts or phospholipids. Formerly it was thought that reduced bile salt

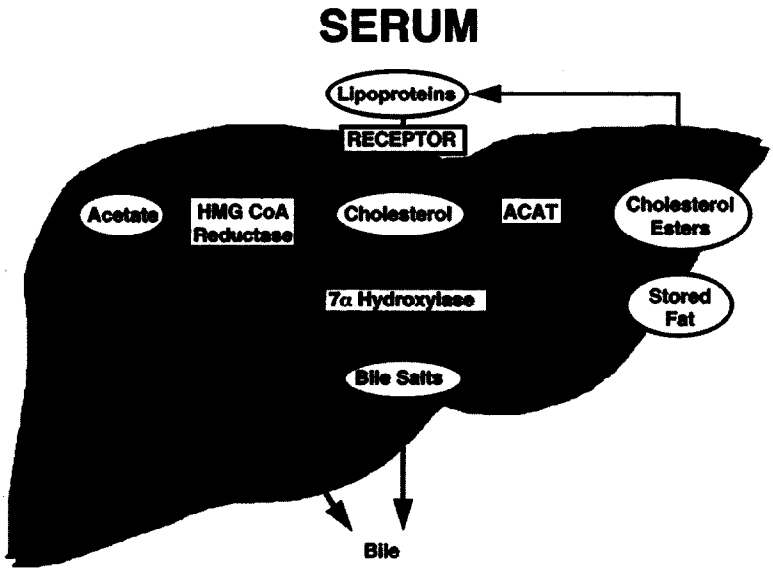

Fig. 4. Intermediary metabolism of hepatic cholesterol. A central cholesterol pool is shown. Cholesterol enters the pool from synthesis or receptor-mediated uptake from the blood. It leaves the pool to be exported directly into bile, to be used for bile salt synthesis or converted into cholesterol esters, which are stored in the liver or exported. Some of the enzymes and receptors involved in these steps are shown. Bile salts stimulate cholesterol and phospholipid secretion. Biliary-destined cholesterol is packaged into phospholipid vesicles prior to excretion into bile.

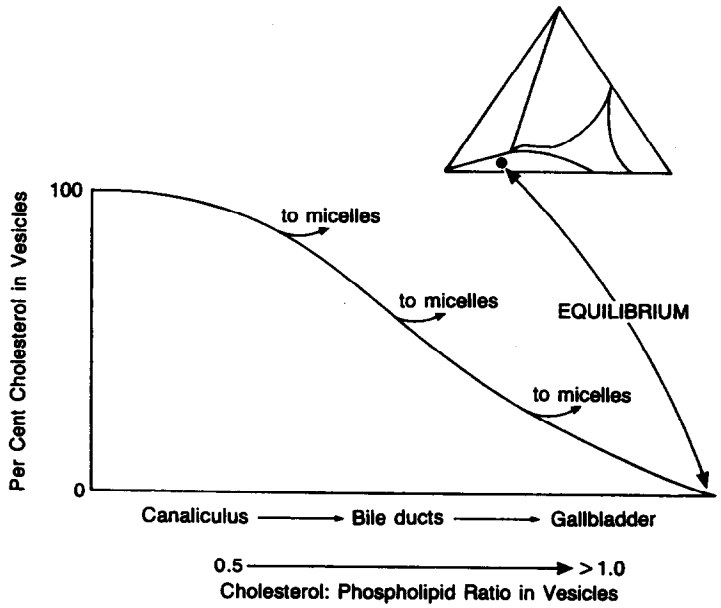

Fig. 5. Maturation of vesicles in unsaturated bile—Case 1. Micellar excess. Cholesterol and phospholipid are secreted in vesicular form. Micellation of these lipids takes place in the biliary tree. There is preferential micellation of phospholipid, with the result that the cholesterol: phospholipid ratio of the residual vesicles rises. However, eventually all cholesterol and phospholipid are incorporated into micells and at equilibrium only this phase exists. (From Strasberg SM, Harvey PRC. Biliary cholesterol transport and precipitation: Introduction and overview of conference. Hepatology 1990;12:1S-5S.)

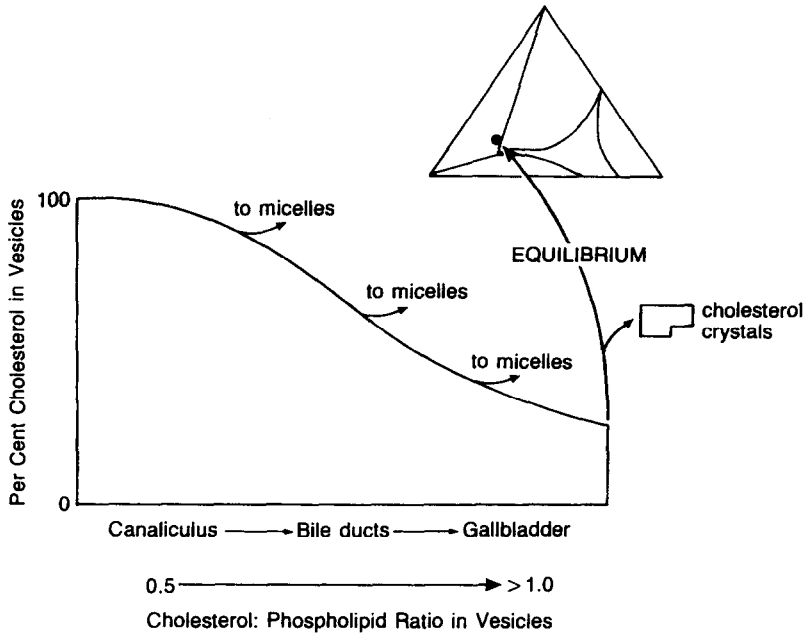

Fig. 6. Maturation of vesicles in saturated bile—Case 2. Micellar insufficiency. The same process of micellation takes place including preferential micellation of phospholipid. The residual "mature" vesicles are enriched in cholesterol relative to phospholipid (high cholesterol:phospholipid ratio). These vesicles persist after all micelles are saturated with cholesterol. Through processes of vesicle aggregation and fusion, cholesterol microcrystal formation is promoted. At equilibrium, cholesterol monohydrate crystals are present. (From Strasberg SM, Harvey PRC. Biliary cholesterol transport and precipitation: Introduction and overview of conference. Hepatology 1990;12:1S-5S.)

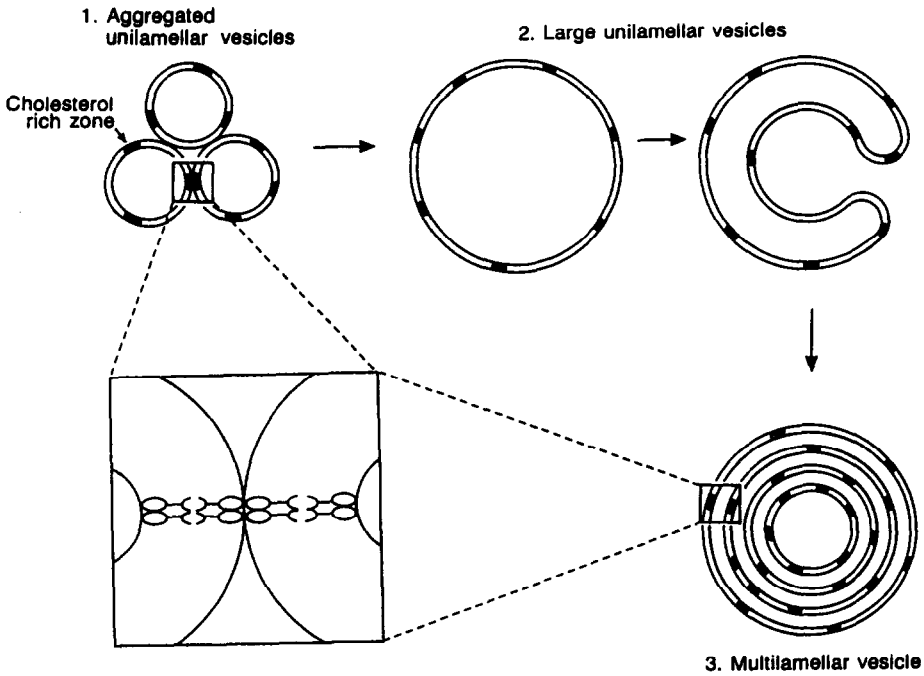

Fig. 7. Schematic depicting how aggregation and fusion provide ideal conditions for contact between cholesterol-rich zones on unilamellar or multilamellar vesicles.

secretion was an important mechanism. Indeed, thin patients do have smaller bile salt pools and their bile salt secretion rates may be low, possibly because of inappropriately low bile salt synthesis rates. However, it now seems more likely that the small bile salt pool is secondary to stone formation. The enterohepatic circulation of bile salts is more rapid in these patients, increasing the return of bile salts to the liver, which secondarily reduces synthesis rates producing a smaller pool size. This leads to a new steady state in which there is a smaller pool and more rapid cycling, but normal secretion rates and eventually normal synthesis rates. Therefore, although theoretically events other than cholesterol hypersecretion could induce cholesterol saturation, in actuality, supersaturation usually appears to be due to cholesterol hypersecretion. However, there are multiple mechanisms producing cholesterol hypersecretion and many of these have been shown to be related to known risk factors for cholesterol stone formation.

Age. Supersaturation of bile increases with age[9] because of increased cholesterol secretion.[9,10] Bile salt secretion is not decreased, although the bile salt pool becomes smaller.[10] The effect of age is independent of the influence of obesity, although the effect of these factors is additive.[9] The cellular mechanisms responsible for increasing cholesterol secretion with age are not completely known. Recently it was shown that cholesterol 7 alpha-hydroxylation, the rate-limiting step in bile salt synthesis from cholesterol, was significantly decreased in older patients compared to middle-aged subjects.[11] This suggests that increased cholesterol secretion in older subjects is linked to decreased utilization of hepatic cholesterol for bile salt synthesis.

Obesity. The linkage between cholesterol gallstones and obesity has been known for years. There is a linear correlation between body weight and cholesterol secretion into bile.[12] Recently Stahlberg et al.[13] demonstrated that the main defect is excessive cholesterol synthesis and that neither reduced bile salt synthesis nor reduced esterification of cholesterol contributes to the excessive cholesterol secretion. Livers of gallstone patients contain an intracellular vesicular fraction rich in lecithin.[14] The cholesterol-to-phospholipid ratio in this vesicular fraction and the cholesterol-to-phospholipid ratio in bile canalicular membranes and in bile are correlated. In persons who are obese, there is more rapid transport of this fraction into bile.[14]

Rapid weight loss in the obese patient also contributes to gallstone formation since during rapid weight loss there is a sharp increase in cholesterol secretion into bile.[12] Altered gallbladder motility and accelerated crystallization rates may also contribute to gallstone formation during rapid weight loss.[15]

Femaleness and Pregnancy. Femaleness is a strong risk factor for supersaturation of bile. Estrogen promotes secretion of cholesterol into bile.[16] Premarin has this effect as a result of enhancing hepatic lipoprotein uptake and inhibiting bile salt synthesis.[17] During the last two trimesters of pregnancy, cholesterol secretion into bile increases relative to bile salt and phospholipid secretion, with the result that the cholesterol saturation index rises.[18] Studies in the hamster suggest that this is due, at least in part, to increasing cholesterol synthesis rates late in pregnancy.[19] There is also a progressive decrease in the percentage of chenodeoxycholic acid in the bile salt pool during pregnancy because of decreasing chenodeoxycholic acid synthesis rates,[18] and chenodeoxycholic acid is an inhibitor of cholesterol synthesis. Pregnant women and those taking contraceptive steroids have a slower rate of gallbladder emptying[20,21] and prolonged gastrointestinal transit time.[22] As will be discussed below, slower gallbladder emptying is a critical kinetic factor in the formation of gallstones.

Diet. Diet plays an important role in cholesterol supersaturation. Vegetarians do not form cholesterol gallstones. Cholesterol gallstones are common in populations eating a Western diet, which is relatively high in animal fat. The incidence of cholesterol gallstones rises in populations shifting to higher consumption of fat in the diet. However, the relationship between diet and gallstones is complex. In a key contribution, Kern[23] recently showed that patients who form stones and control subjects handle cholesterol feeding very differently. Cholesterol absorption decreases in both control subjects and patients with stones. Cholesterol synthesis decreases in both groups, as expected, because of negative feedback inhibition. However, bile salt synthesis and pool size tend to increase in control subjects, but in patients with gallstones bile salt synthesis and pool size actually decrease. Biliary cholesterol secretion increases only in the group with gallstones. In other words, persons with gallstones respond appropriately to a high-cholesterol diet in terms of negative feedback reduction in inhibition of cholesterol synthesis but respond inappropriately by not diverting dietary cholesterol into the bile salt synthesis pathway—diverting it instead into the bile. This strongly suggests that a genetic mechanism is at work.

Genetic Mechanisms. Until recently, potential genetic mechanisms involved in gallstone formation received little attention. In an important series of studies, it was demonstrated that there is a large interstrain variability in gallstone formation in cholesterol-

fed mice.[24] Genetic analysis demonstrated that susceptibility to gallstone formation was a dominant trait, determined by at least two genes.[24] One, a major gene named Lith 1, is mapped to mouse chromosome 2. Susceptible strains failed to downregulate cholesterol synthesis during cholesterol feeding.[24] Although this is unlike the response of humans with cholesterol gallstones,[23] the approach used in these studies shows promise for application in humans with gallstone disease.

Deoxycholate Enrichment of the Bile Salt Pool. Another factor inducing supersaturation appears to be enrichment of the bile salt pool with the secondary bile salt deoxycholate. Persons with stones often have higher deoxycholate levels in bile.[25] There seems to be a correlation between cholesterol and deoxycholate secretion rates into bile.[26] Feeding low doses of deoxycholate results in increased cholesterol saturation of bile.[27] Presumably deoxycholate is more efficient in stimulating cholesterol secretion by the liver than other bile salts. Not all studies have found an increase in deoxycholate levels. This may be due to the fact that some patients with stones have deoxycholate excess whereas others do not, as recently shown by Berr et al.[28] Therefore, if only small numbers of patients are studied, the differences in the size of the deoxycholate pool between patients with stones and control subjects might be missed. The methodologic problem of sample size also applies to other areas of this multifactorial disease.

Recent efforts have focused on understanding the mechanism by which the bile salt pool is enriched in these persons. Women of normal weight seem to have slower intestinal transit, which provides more time for conversion of cholate to deoxycholate in the bowel (Fig. 8).[29] On the other hand, Berr et al.[28] found that increased cholic acid 7 alpha-dehydroxylation activity of the intestinal microflora was the more important factor in producing deoxycholate excess (Fig. 9).

Colectomy. Since too much conversion of cholate to deoxycholate results in supersaturated bile, it is paradoxical that colectomy, a procedure that results in virtual obliteration of the deoxycholate component of the bile salt pool, also results in supersaturation. We have shown that colectomy in patients who have ulcerative colitis results in a rapid increase in the cholesterol saturation index and the appearance of cholesterol crystals in bile.[30]

Obviously, supersaturation of bile may result from a very complex set of events. There are many points in intermediary metabolism of cholesterol at which a slight increase or decrease in enzymatic activity or receptor expression could result in cholesterol supersaturation (see Fig. 4). Furthermore, as has been noted, intestinal as well as hepatic events can be responsible for supersaturation.

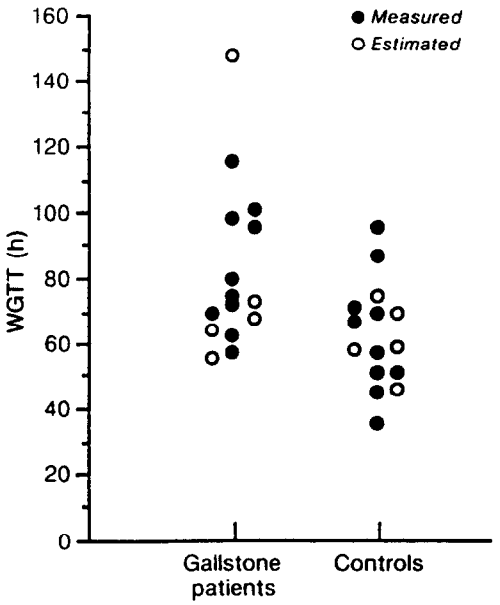

Fig. 8. Whole-gut transit times *(WGTT)* in normal-weight women with gallstones and in age-matched controls. (From Heaton KW, Emmett PM, Symes CL, Braddon FE. An explanation for gallstones in normal-weight women: Slow intestinal transit. Lancet 1993;341:8-10.)

Accelerated Crystallization

The second stage of stone formation is crystallization from supersaturated bile. It was through the application of physical chemistry to the clinical problem of cholesterol cholelithiasis, principally stimulated by the studies of Donald M. Small of Boston University, that the modern concepts of supersaturation of human bile and its role in gallstone formation came about. Initial studies suggested that gallbladder bile from patients with gallstones was supersaturated and control bile was not.[31] However, it rapidly became clear that many normal persons without cholesterol gallstones also have supersaturated bile.[32-34] One may summarize many studies performed in patients living in industrialized Western countries as follows. Virtually all patients with cholesterol gallstones have supersaturated bile. Some normal persons have unsaturated bile but many have supersaturated bile. There are many more stone-free persons with supersaturated bile than patients with supersaturated bile and cholesterol gallstones. In other words, supersaturation is needed for stone formation but supersaturation does not guarantee stone formation. It should be noted that the situation in Japan may be quite different, perhaps for genetic reasons.[35] In that country it appears that patients who form stones have highly supersaturated bile, whereas persons without stones have unsaturated bile. The difference is shown in Fig.

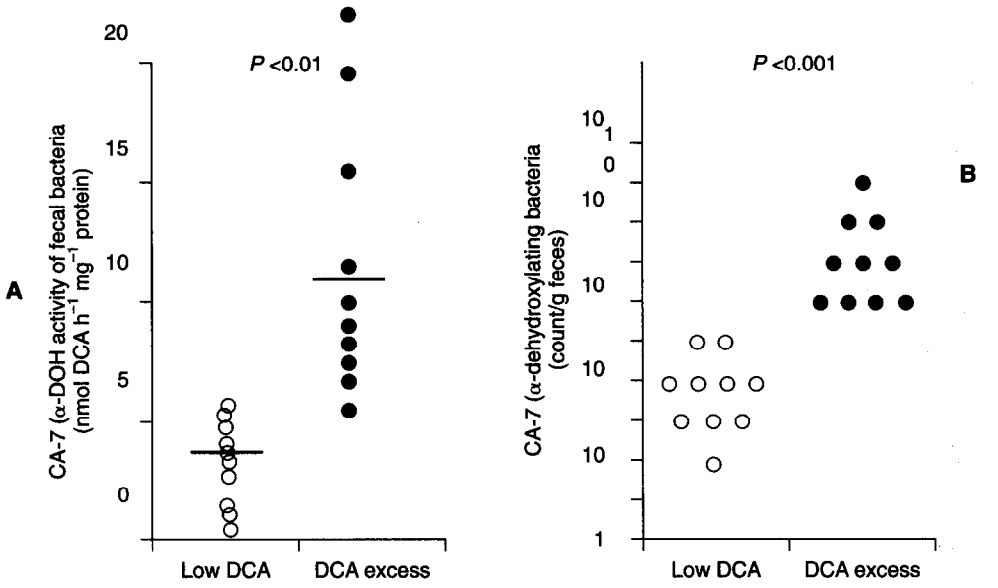

Fig. 9. A, Activity of enzyme that converts cholate to deoxycholate in the intestine. Note the much higher activity in patients with deoxycholate excess. **B,** Number of bacteria exhibiting converting activity in the two groups of patients. (From Berr F, Kullak-Ublick GA, Paumgartner G, Munzing W, Hyelmon PB. 7-Alpha-dehydroxylating bacteria enhance deoxycholic acid input and cholesterol saturation of bile in patients with gallstones. Gastroenterology 1996;111:1611-1620.)

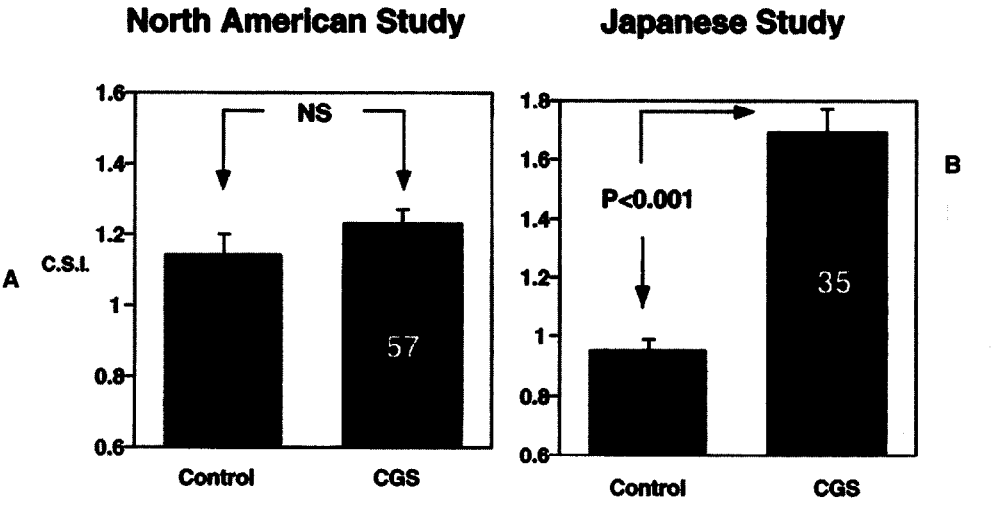

Fig. 10. A, Cholesterol saturation index (CSI) in gallbladder bile from control subjects and patients with cholesterol gallstones *(CGS)* from a recent study in Canadian patients.[36] Note there is little difference in the CSI. **B,** Same information from a recent Japanese study[35] in which there was a clear difference in the CSI between groups, and control subjects had unsaturated bile. Numbers within bars indicate the number of patients in each study group.

10, which compares this Japanese study with recent results from a Canadian population.[36]

Why Does Supersaturated Bile From Patients With Stones Develop Cholesterol Crystals in the Gallbladder and Supersaturated Bile From Control Subjects Does Not? As noted earlier in this review, all supersaturated bile will have cholesterol crystals at equilibrium. The short answer to the preceding question is that supersaturated bile from patients with stones reaches equilibrium while in the biliary tree and conversely supersaturated bile from control subjects does not. There are two mechanisms that permit bile to reach equilibrium within a pathophysiologically relevant time frame. The first is a defect in gallbladder motility, which results in prolonged retention of bile in the gallbladder. This provides time for maturation, aggregation, fusion, nucleation, and crystal growth to occur. The second is a defect in kinetics, which results in acceleration of the steps in the crystallization pathway. As a result maturation, aggregation, and so forth occur more rapidly.

Cholesterol Crystallization and the Gallbladder Motility Defect. In the nineteenth century Virchow claimed that stasis was important in stone formation. However, a possible role for a motility defect was not established until it was shown by Everson et al.[21] that during pregnancy, gallbladder emptying was reduced and residual volumes were increased. Pomerantz and Shaffer[37] then showed that some patients with cholesterol stones have a motility defect, and this has been confirmed by others. Subsequently it was found that obesity[38,39] also exerts an effect on stone formation via impairment of gallbladder motility. Confirmatory evidence comes from animal models in which it was shown that the motility defect precedes the crystal formation.[40]

The origin of the motility defect has been studied extensively by Behar et al.[41] They have reported that smooth muscle from gallbladders with cholesterol stones exhibits an impaired response to cholecystokinin. Human gallbladder muscle exposed to bile containing excess cholesterol demonstrates reduced contractility compared with muscle strips from specimens obtained from patients with pigment stones. Since impaired contractility is present in muscle strips from patients with crystals but no stones, it must precede gallstone formation.[41] High-cholesterol feeding in animal models results in increased cholesterol content and cholesterol/phospholipid mole ratio in plasma membranes of gallbladder muscle and reduced muscle cell contraction in response to cholecystokinin octapeptide. Incubation of normal gallbladder muscle cells with cholesterol-rich liposomes produces similar effects. These are reversible if muscle cells are subsequently incubated with cholesterol-free liposomes.[42] Therefore supersaturation of bile with cholesterol appears to expose gallbladder myocytes to higher cholesterol levels. Presumably this occurs through absorption of cholesterol by the gallbladder and results in impaired motility. Why these events selectively affect only some persons with supersaturated bile is uncertain.

Cholesterol Crystallization and the Kinetic or "Nucleation" Defect. The defining study in this area was published in 1979 by Holan and Holzbach.[33] They introduced a technique for measuring the rapidity of crystal formation from bile, initially cleared of all detectable crystals by ultracentrifugation. The measurement, initially called "nucleation time" but now more appropriately referred to as "crystal detection time," was used to establish that gallbladder bile from patients with cholesterol stones produced crystals much more rapidly than equally supersaturated bile from control subjects. A very similar set of observations was made simultaneously by Sedaghat and Grundy.[43]

A kinetic defect accelerating crystallization must act by influencing one of the elements in the crystallization pathway leading to equilibrium (vesicle maturation, aggregation, fusion, nucleation, and crystal growth) characterized above. It seemed possible that the kinetic defect was either impairment of a normal mechanism that retarded progress toward equilibrium or the introduction of an abnormal mechanism that accelerated the process or both. Substances that potentially influence these mechanisms have been referred to as antinucleating or pronucleating factors. These terms are no more appropriate than "nucleating time," since such factors might act at any of the multiple steps leading to equilibrium and not just at the nucleation step. They are better referred to as procrystallizing and anticrystallizing factors. Both procrystallizing and anticrystallizing factors have been identified.

Procrystallizing Factors. The existence of a potent procrystallizing factor in human gallbladder bile obtained from cholesterol gallstone patients was demonstrated in our laboratory in 1983.[44] This study was followed by two others that examined whether calcium, bilirubin,[45] or mucous glycoprotein[46] was the potent procrystallizing factor. The rationale for performing these studies was that mucous glycoprotein secretion had been shown to be an important element in crystal formation in animal models[47] and calcium bilirubinate seemed to be implicated in early stages of stone formation by the observation that cholesterol stones had pigmented centers. We found no evidence that calcium or bilirubin was involved in acceleration of crystallization in human bile.[45] Although mucous glycoprotein did certainly accelerate crystallization,[46] it did

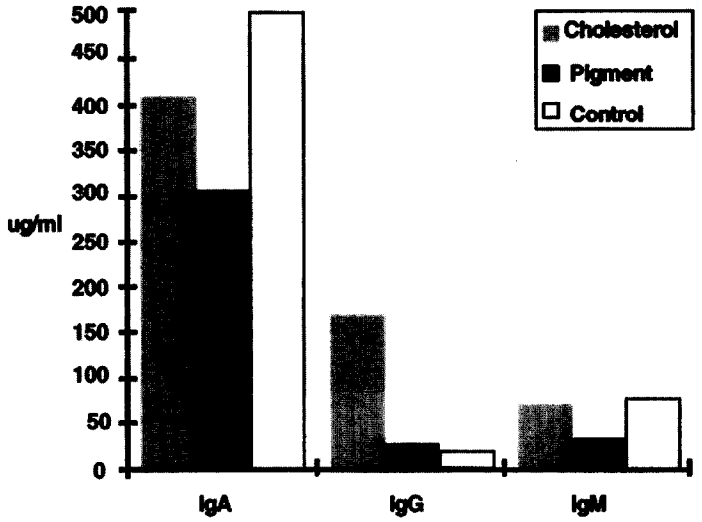

Fig. 11. Immunoglobulin concentrations in control subjects and patients with cholesterol or pigment stones. Note the marked difference in immunoglobulin G *(IgG)* concentrations in the group with cholesterol stones. The effect is not simply secondary to the effect of stones since patients with pigment stones are not affected.

not appear to explain our particular observations regarding the procrystallizing factor in human gallbladder bile, since there was little difference in biliary mucous glycoprotein concentration between these patients with stones and control subjects. However, when we examined proteins other than mucous glycoproteins, it was found that these proteins accelerated crystallization when they were obtained from patients with stones.[48] Total protein concentrations in bile were also higher in patients with stones.[48] It was concluded that the potent procrystallizing factor[44] was a protein other than mucous glycoprotein.[48] These studies were followed by reports by Groen et al.,[49,50] which confirmed these findings and also introduced a new way to separate biliary proteins based on their affinity to concanavalin A (Con A). A major obstacle to the study of biliary proteins was the separation of proteins from lipids, and the Con A methodology facilitated this separation. Con A technology, however, was later found not to be as clean as was first thought.[51]

The combination of Con A technology and the identification of biliary proteins as procrystallizing agents stimulated many studies that attempted to identify these agents. Over the succeeding 6 or 7 years, many procrystallizing proteins were identified including immunoglobulins,[52,53] aminopeptidase N,[50,54] phospholipase C,[55] alpha acid glycoprotein,[56] haptoglobin,[57] and an 84 kd glycoprotein.[58] Of these, immunoglobulin G (Fig. 11) and alpha acid glycoprotein have been shown to be in greater abundance in bile from patients with cholesterol gallstones. So many different proteins were identified that Ahmed et al.[59] explored whether procrystallization was simply some effect of proteins in general; they reported that polar proteins are procrystallizing and nonpolar proteins had the opposite effect. Although this might provide a simple explanation for the diverse findings, we repeated the experiments of Ahmed et al. and were unable to confirm their results.

In an attempt to determine the relative importance of the different proteins, comparative studies were performed, but these often came to differing conclusions regarding the relative importance and potency of these compounds.[60,61] Many of the discrepancies in results appear related to differences in experimental methods including various ways of measuring the procrystallizing effect. One serious methodologic problem seems to be that the Con A technique imperfectly separates lipids from protein. In fact, cholesterol microcrystals, which could greatly affect results of crystallization assays, were found in the Con A eluate unless a bile salt wash was used.[51] There are other serious methodologic problems in experiments in which proteins are "subtracted" from bile by various techniques including digestion with proteases; detailed consideration is beyond the scope of this review. Perhaps such problems explain why some proteins such as fibronectin were only briefly thought to be procrystallizing.[62] The problems of interpreting findings in this field became the subject of editorials.[63,64]

Conflicting findings were not limited to which pro-

teins were procrystallizing but also posed the question of whether an increase in total protein was present, as first described by Gallinger et al.[48] Again methodologic problems were common; total protein measurements in bile are difficult to perform because of the presence of interfering substances such as bilirubin. Adding to the confusion is the fact that a single method may be employed in more than one way. For instance, there is not one "fluorescamine" technique but a number of ways of measuring biliary proteins using this agent. Unless there is initial great dilution of the specimen, as described by Harvey et al.,[65] fluorescamine is interfered with by bilirubin and erroneous results are possible. If initial dilution is achieved, then "the fluorescamine technique" compares well with amino acid analysis,[48,65] which is considered the "gold standard."

Another problem in detecting the increase in protein concentration is that the relationship between the total protein concentration and gallstone formation is not a simple one in which every patient with gallstones has a higher concentration than every person without them. As a result, large numbers of patients must be compared to detect the difference, and this difference is not likely to be detected when the numbers are small.[66] This problem is similar to the one described earlier regarding detection of whether or not patients with gallstones have an increase in deoxycholate pool size. Nonetheless, many studies have now confirmed the relationship between protein concentration and stone formation.[58,67-70] Notably, total biliary protein increases in animal models before stone formation.[71] Multiple gallbladder stones seem to be associated with shorter nucleation time and higher biliary concentrations of total protein and glycoprotein than solitary stones.[69] Furthermore, total protein concentration is higher in gallbladder bile from patients with crystals but no stones[72] and higher in gallstone patients who have crystals in their bile at the time of cholecystectomy,[67] both of which indicate that the high protein concentration in humans is not secondary to stone formation. A high protein concentration might simply be due to a general increase in the concentration of bile, which by increasing the chance for contact between cholesterol carriers would accelerate maturation of vesicles and aggregation and perhaps other steps in the crystallization pathway. Indeed, as shown by Roslyn et al.,[73] there is an early increase in the bile concentration in animal models of gallstones because of enhanced absorption of water and electrolytes. The importance of bile concentration on crystallization has also been demonstrated by van Erpecum et al.[74] The increase in protein concentration might also be due to protein secretion by the gallbladder. Our data suggest that both increased water absorption and protein secretion may be important.[72]

Despite the confusion and controversy in this area, it is now possible, based on a number of recent studies, to synthesize an attractive description of events in the gallbladder, which link pronucleating proteins, cholesterol supersaturation, and deoxycholate enrichment of the bile salt pool. Paradoxically, to simplify concepts regarding the role of procrystallizing proteins, it is necessary to recognize that their relationship to stone formation is complex. Just as there is not a single event determining supersaturation, there is not a single procrystallizing protein determining rapid crystallization. The common feature of many of the procrystallizing proteins such as immunoglobulins and mucous glycoprotein is that they are secreted as a result of inflammation. Cholesterol supersaturation appears to be the insult capable of initiating this inflammation. Cholesterol supersaturation is capable of inducing gallbladder inflammation in the prairie dog model of cholelithiasis.[75] Mucous glycoprotein synthesis is also stimulated by cholesterol supersaturation.[76] When pig gallbladders are subjected to varying conditions of supersaturation and pool composition, those exposed to both high saturation and deoxycholate enrichment show rapid recruitment of plasma cells, immunoglobulin secretion, and mucous glycoprotein secretion.[36] Immunoglobulin G is present in higher concentration in patients with cholesterol gallstones compared to control subjects or patients with pigment stones, and only the patients with cholesterol gallstones have both supersaturated bile and a deoxycholate-enriched pool[36] (Figs. 12 and 13). Coupled with the information on motility given previously, the following may be summarized as a current view of events.[36] Cholesterol supersaturation, especially when coupled with deoxycholate excess, leads to gallbladder inflammation and secretion of procrystallizing proteins, which in turn lead to crystal formation in the gallbladder. The associated dysmotility extends the time for crystallization to occur.

Although the role of procrystallizing proteins seems secure, their method of action is only partly explored. They could of course act at any point on the crystallization pathway. In an important recent study using dynamic light scattering, transmission electron microscopy, and fluorescent biochemical assays, Afdhal et al.[77] showed that mucin greatly accelerated vesicle fusion. Yamashita et al.[78] have demonstrated that the procrystallizing effect of haptoglobin is that it increases the cholesterol content of vesicles. More studies of this type are needed to further refine our understanding of proteins and crystallization.

Anticrystallizing Factors. A parallel set of studies

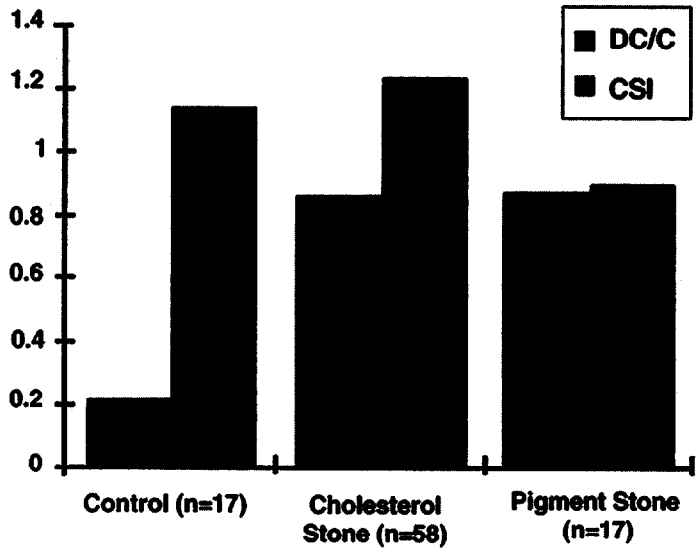

Fig. 12. Cholesterol saturation index *(CSI)* and ratio of deoxycholate/cholate *(DC/C)* in bile of control subjects and patients with cholesterol or pigment stones. Note that only patients with cholesterol stones fulfill the dual conditions of deoxycholate enrichment and supersaturation with cholesterol.

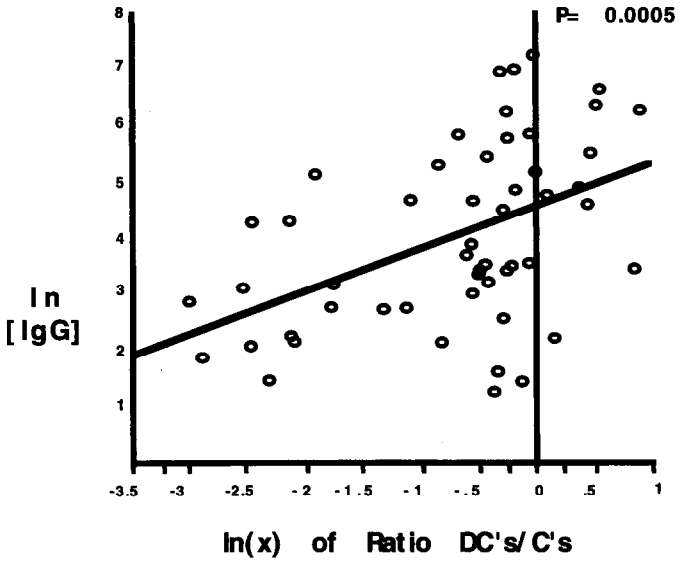

Fig. 13. Relationship between immunoglobulin G *(IgG)* concentration and deoxycholate enrichment in patients with a gallbladder bile cholesterol saturation index (CSI) ranging from 1.0 to 1.6. There is a highly significant correlation between these variables in patients with supersaturated bile within this CSI range, which encompasses almost all patients in North America with cholesterol gallstones DC's/C's = ratio of deoxycholate/cholate).

have determined that there are also anticrystallizing agents in bile. Most of these studies have emanated from Holzbach's group in Cleveland; these investigators have shown that apolipoprotein A,[79] a 120 kd glycoprotein,[80] and a 15 kd protein[81] all have anticrystallizing effects. Busch et al.[82] have isolated several anticrystallizing proteins that appear to bind to cholesterol crystals, suggesting that they are crystal growth inhibitors. Anticrystallizing activity has been induced in rats by feeding them curcumin or capsaicin.[83] It has been proposed that the more rapid crystallization of abnormal bile is due to a lack of these normal substances in bile; this may be true but it remains to be shown whether patients with cholesterol gallstones lack these substances. Stimulation of production of anticrystallizing agents, for example, by dietary modification or additives, is potentially a means of preventing gallstones.

Stone Formation From Crystals

The third and final stage of cholesterol stone formation occurs when cholesterol crystals form into stones.

Sludge, Sediment, and Stone Formation. The earliest reference to bile "sludge" that we have found is by Harris[84] in 1969. The term was used in the 1970s in the liver transplantation literature to refer to necrotic collagen that obstructed bile ducts[85]; this problem was largely solved by irrigating bile out of the donor biliary tree and the term is now seldom used in this fashion. Sludge also refers to material that precipitates in bile ducts in association with cholangiohepatitis[86] or stents. The term was also used simultaneously to describe echogenic material that layered out in the gallbladder in some patients who had been fasting. This is now the common use of the term and it should be remembered that the term sludge used in this way is a contraction of "echogenic gallbladder sludge," that is, material detectable in the gallbladder on ultrasonography.

Sludge must be distinguished from "sediment." Sediment refers to solid material detectable by microscopy. Bile normally contains sediment but it is sparse and consists mainly of dead cells. Pathologic sediment contains microcrystals of cholesterol or calcium bilirubinate or both. Pathologic sediment is usually not detectable by ultrasonography, but if sludge is detected there will always be pathologic sediment.

Sludge is usually composed of bilirubinate microcrystals and mucus[87,88]; however, sometimes cholesterol crystals are also present.[89,90] This is not surprising since the conditions leading to sludge formation, such as fasting, lead to concentration of bile, one of the mechanisms by which crystallization is accelerated. Sludge is a precursor of bilirubinate stones[91,92] and it has been proposed as a precursor to cholesterol stones. This was an attractive hypothesis but there is little direct evidence that it applies to most instances of cholesterol stone formation. Stones appear to form in sludge only after it has been present for some weeks or months, and sludge persists for some time after stones have formed in the sludge. Yet sludge is an uncommon ultrasound finding in a nonfasting population, many of whom will form cholesterol gallstones. On the other hand, pathologic sediment certainly always precedes stone formation since crystals are necessary for stones to form. As can be seen, the term sludge is an unfortunate one and is best reserved for describing clinical phenomena. Even then it is necessary to qualify what type of sludge is being discussed.

The Wolpers Model of Cholesterol Stone Formation. A schema for formation of cholesterol stones has been devised by Wolpers and Hofmann.[93] They used cholecystography, macroscopic examination, radiography, scanning electron microscopy, and chemical analysis of gallstones from these and other patients obtained at cholecystectomy. Solitary gallstones form from free-floating crystal laminae of cholesterol. These laminae aggregate loosely and undergo external compaction and internal remodeling by movement of cholesterol molecules to form compact spheroids. Multiple cholesterol gallstones form without a precursor phase. Rather, innumerable, very thin cholesterol crystals appear that very abruptly aggregate to form spheres of up to 1 mm in diameter. A second aggregation takes place within 3 months in which these spheres coalesce to form mulberry stones. Mulberry stones are transformed into either faceted stones or barrel stones over a period of 3 years. We have recently described a similar progression for the formation of multiple stones.[94] The smallest spheroids, which we termed "lithons," contained no calcium bilirubinate. Mulberry stones appear to be aggregations of lithons.[94] Earlier studies performed by Sanabria et al.[95,96] demonstrated that cholesterol gallstones were porous to large molecules and provided evidence that pigmentation was secondary. These studies provide independent confirmation of the Wolpers model and extend the model.

In summary, there have been many advances in the understanding of cholesterol gallstone formation in recent years. The keys to stone formation are supersaturation and crystallization, both of which are influenced by many variables. A major step has been the linking of supersaturation to gallbladder inflammation and secretion of procrystallizing agents. At least in some patients, it appears that deoxycholate excess

as well as supersaturation is needed to trigger inflammation in the gallbladder. What separates other patients with stones from patients without stones but with supersaturated bile is not yet apparent.

REFERENCES

1. Chijiiwa K, Kiyosawa R, Nakayama F. Cholesterol monomer activity correlates with nucleation time in model bile. Clin Chim Acta 1988;178:181-191.
2. Ulloa N, Garrido J, Nervi F. Ultracentrifugal isolation of vesicular carriers of biliary cholesterol in native human and rat bile. Hepatology 1987;7:235-244.
3. Cohen DE, Angelico M, Carey MC. Quasielastic light scattering evidence for vesicular secretion of biliary lipids. Am J Physiol 1989;257:G1-G8.
4. Hardison WG, Apter JT. Micellar theory of biliary cholesterol excretion. Am J Physiol 1972;222:61-67.
5. Entenman C, Holloway RJ, Albright ML, Leong GF. Bile acids and lipid metabolism. II. Essential role of bile acids in bile phospholipid excretion. NRDL-TR-68-102. Res Dev Technical Rep 1968;16:1-12.
6. Halpern Z, Dudley MA, Lynn MP, Nader JM, Breuer AC, Holzbach RT. Vesicle aggregation in model systems of supersaturated bile: Relation to crystal nucleation and lipid composition of the vesicular phase. J Lipid Res 1986;27:295-306.
7. Halpern Z, Dudley MA, Kibe A, Lynn MP, Breuer AC, Holzbach RT. Rapid vesicle formation and aggregation in abnormal human biles. A time-lapse video-enhanced contrast microscopy study. Gastroenterology 1986;90:875-885.
8. Konikoff FM, Chung DS, Donovan JM, Small DM, Carey MC. Filamentous, helical, and tubular microstructures during cholesterol crystallization from bile. Evidence that cholesterol does not nucleate classic monohydrate plates. J Clin Invest 1992;90:1155-1160.
9. Valdivieso V, Palma R, Wunkhaus R, Antezana C, Severin C, Contreras A. Effect of aging on biliary lipid composition and bile acid metabolism in normal Chilean women. Gastroenterology 1978;74(5 Pt 1):871-874.
10. Einarsson K, Nilsell K, Leijd B, Angelin B. Influence of age on secretion of cholesterol and synthesis of bile acids by the liver. N Engl J Med 1985;313:277-282.
11. Bertolotti M, Abate N, Bertolotti S, et al. Effect of aging on cholesterol 7 alpha-hydroxylation in humans. J Lipid Res 1993;34:1001-1007.
12. Bennion LJ, Grundy SM. Effects of obesity and caloric intake on biliary lipid metabolism in man. J Clin Invest 1975;56:996-1011.
13. Stahlberg D, Rudling M, Angelin B, et al. Hepatic cholesterol metabolism in human obesity. Hepatology 1997;25:1447-1450.
14. Ahmed HA, Jazrawi RP, Goggin PM, Dormandy J, Northfield TC. Intrahepatic biliary cholesterol and phospholipid transport in humans: Effect of obesity and cholesterol cholelithiasis. J Lipid Res 1995;36:2562-2573.
15. Andersen T. Liver and gallbladder disease before and after very-low-calorie diets. Am J Clin Nutr 1992;56:235S-239S.
16. Bennion LJ, Mott DM, Howard BV. Oral contraceptives raise the cholesterol saturation of bile by increasing biliary cholesterol secretion. Metabolism 1980;29:18-22.
17. Everson GT, McKinley C, Kern F Jr. Mechanisms of gallstone formation in women. Effects of exogenous estrogen (Premarin) and dietary cholesterol on hepatic lipid metabolism. J Clin Invest 1991;87:237-246.
18. Kern F Jr, Everson GT, DeMark B, et al. Biliary lipids, bile acids, and gallbladder function in the human female. Effects of pregnancy and the ovulatory cycle. J Clin Invest 1981;68:1229-1242.
19. Reichen J, Karlaganis G, Kern F Jr. Cholesterol synthesis in the perfused liver of pregnant hamsters. J Lipid Res 1987;28:1046-1052.
20. Braverman DZ, Johnson ML, Kern F Jr. Effects of pregnancy and contraceptive steroids on gallbladder function. N Engl J Med 1980;302:362-364.
21. Everson GT, McKinley C, Lawson M, Johnson M, Kern F Jr. Gallbladder function in the human female: Effect of the ovulatory cycle, pregnancy, and contraceptive steroids. Gastroenterology 1982;82:711-719.
22. Lawson M, Kern F Jr, Everson GT. Gastrointestinal transit time in human pregnancy: Prolongation in the second and third trimesters followed by postpartum normalization. Gastroenterology 1985;89:996-999.
23. Kern F Jr. Effects of dietary cholesterol on cholesterol and bile acid homeostasis in patients with cholesterol gallstones. J Clin Invest 1994;93:1186-1194.
24. Khanuja B, Cheah YC, Hunt M, et al. Lith l, a major gene affecting cholesterol gallstone formation among inbred strains of mice. Proc Natl Acad Sci USA 1995;92:7729-7733.
25. Pomare EW, Heaton KW. Bile salt metabolism in patients with gallstones in functioning gallbladders. Gut 1973;14:885-890.
26. Leiss O, von Bergmann K. Comparison of biliary lipid secretion in non-obese cholesterol gallstone patients with normal, young, male volunteers. Klin Wochenschr 1985;63:1163-1169.
27. Di Donato P, Carubbi F, Ponz de Leon M, Carulli N. Effect of small doses of deoxycholic acid on bile cholesterol saturation in patients with liver cirrhosis. Gut 1986;27:23-28.
28. Berr F, Kullak-Ublick GA, Paumgartner G, Munzing W, Hylemon PB. 7-Alpha-dehydroxylating bacteria enhance deoxycholic acid input and cholesterol saturation of bile in patients with gallstones. Gastroenterology 1996;111:1611-1620.
29. Heaton KW, Emmett PM, Symes CL, Braddon FE. An explanation for gallstones in normal-weight women: Slow intestinal transit. Lancet 1993;341:8-10.
30. Harvey PR, McLeod RS, Cohen Z, Strasberg SM. Effect of colectomy on bile composition, cholesterol crystal formation, and gallstones in patients with ulcerative colitis. Ann Surg 1991;214:396-401.
31. Admirand WH, Small DM. The physicochemical basis of cholesterol gallstone formation in man. J Clin Invest 1968;47:1043-1052.
32. Holzbach RT, Marsh M, Olszewski M, Holan K. Cholesterol solubility in bile. Evidence that supersaturated bile is frequent in healthy man. J Clin Invest 1973;52:1467-1479.
33. Holan KR, Holzbach RT, Hermann RE, Cooperman AM, Claffey WJ. Nucleation time: A key factor in the pathogenesis of cholesterol gallstone disease. Gastroenterology 1979;77:611-617.
34. Gollish SH, Burnstein MJ, Ilson RG, Petrunka CN, Strasberg SM. Nucleation of cholesterol monohydrate crystals from hepatic and gall-bladder bile of patients with cholesterol gall stones. Gut 1983;24:836-844.
35. Shoda J, He BF, Tanaka N, et al. Increase of deoxycholate in supersaturated bile of patients with cholesterol gallstone disease and its correlation with de novo syntheses of cholesterol and bile acids in liver, gallbladder emptying, and small intestinal transit. Hepatology 1995;21:1291-1302.

36. Sanabria JR, Upadhya A, Mullen B, Harvey PR, Strasberg SM. Effect of deoxycholate on immunoglobulin G concentration in bile: Studies in humans and pigs. Hepatology 1995; 21:215-222.
37. Pomerantz IS, Shaffer EA. Abnormal gallbladder emptying in a subgroup of patients with gallstones. Gastroenterology 1985;88:787-791.
38. Marzio L, Capone F, Neri M, Mezzetti A, De Angelis C, Cuccurullo F. Gallbladder kinetics in obese patients. Effect of a regular meal and low-calorie meal. Dig Dis Sci 1988;33:4-9.
39. Vezina WC, Paradis RL, Grace DM, et al. Increased volume and decreased emptying of the gallbladder in large (morbidly obese, tall normal, and muscular normal) people. Gastroenterology 1990;98:1000-1007.
40. Fridhandler TM, Davison JS, Shaffer EA. Defective gallbladder contractility in the ground squirrel and prairie dog during the early stages of cholesterol gallstone formation. Gastroenterology 1983;85:830-836.
41. Behar J, Lee KY, Thompson WR, Biancani P. Gallbladder contraction in patients with pigment and cholesterol stones. Gastroenterology 1989;97:1479-1484.
42. Yu P, Chen Q, Biancani P, Behar J. Membrane cholesterol alters gallbladder muscle contractility in prairie dogs. Am J Physiol 1996;271:G56-G61.
43. Sedaghat A, Grundy SM. Cholesterol crystals and the formation of cholesterol gallstones. N Engl J Med 1980;302:1274-1277.
44. Burnstein MJ, Ilson RG, Petrunka CN, Taylor RD, Strasberg SM. Evidence for a potent nucleating factor in the gallbladder bile of patients with cholesterol gallstones. Gastroenterology 1983;85:801-807.
45. Gallinger S, Harvey PR, Petrunka CN, Strasberg SM. Effect of binding of ionised calcium on the in vitro nucleation of cholesterol and calcium bilirubinate in human gall bladder bile. Gut 1986;27:1382-1386.
46. Harvey PR, Rupar CA, Gallinger S, Petrunka CN, Strasberg SM. Quantitative and qualitative comparison of gallbladder mucous glycoprotein from patients with and without gallstones. Gut 1986;27:374-381.
47. Lee SP, LaMont JT, Carey MC. Role of gallbladder mucus hypersecretion in the evolution of cholesterol gallstones. J Clin Invest 1981;67:1712-1723.
48. Gallinger S, Harvey PR, Petrunka CN, Ilson RG, Strasberg SM. Biliary proteins and the nucleation defect in cholesterol cholelithiasis. Gastroenterology 1987;92:867-875.
49. Groen AK, Stout JP, Drapers JA, Hoek FJ, Grijm R, Tytgat GN. Cholesterol nucleation-influencing activity in T-tube bile. Hepatology 1988;8:347-352.
50. Groen AK, Noordam C, Drapers JA, Egbers P, Jansen PL, Tytgat GN. Isolation of a potent cholesterol nucleation-promoting activity from human gallbladder bile: Role in the pathogenesis of gallstone disease. Hepatology 1990;11:525-533.
51. Harvey PR, Upadhya GA, Strasberg SM. Cholesterol microcrystals associated with concanavalin A–binding glycoproteins contribute artifactually to nucleating activity assays. J Lipid Res 1995;36:2661-2669.
52. Harvey PR, Upadhya GA, Strasberg SM. Immunoglobulins as nucleating proteins in the gallbladder bile of patients with cholesterol gallstones. J Biol Chem 1991;266:13996-14003.
53. Upadhya GA, Harvey PR, Strasberg SM. Effect of human biliary immunoglobulins on the nucleation of cholesterol. J Biol Chem 1993;268:5193-5200.
54. Offner GD, Gong D, Afdhal NH. Identification of a 130-kilodalton human biliary concanavalin A binding protein as aminopeptidase N. Gastroenterology 1994;106:755-762.
55. Pattinson NR, Willis KE. Effect of phospholipase C on cholesterol solubilization in model bile. A concanavalin A-binding nucleation-promoting factor from human gallbladder bile. Gastroenterology 1991;101:1339-1344.
56. Abei M, Kawczak P, Nuutinen H, Langnas A, Svanvik J, Holzbach RT. Isolation and characterization of a cholesterol crystallization promoter from human bile. Gastroenterology 1993;104:539-548.
57. Yamashita G, Corradini SG, Secknus R, et al. Biliary haptoglobin, a potent promoter of cholesterol crystallization at physiological concentrations. J Lipid Res 1995;36:1325-1333.
58. Lipsett PA, Fox-Talbot MK, Falconer SD, et al. Biliary nonmucin glycoproteins in patients with and without gallstones. J Surg Res 1995;58:386-390.
59. Ahmed HA, Petroni ML, Abu-Hamdiyyah M, Jazrawi RP, Northfield TC. Hydrophobic/hydrophilic balance of proteins: A major determinant of cholesterol crystal formation in model bile. J Lipid Res 1994;35:211-219.
60. de Bruijn MA, Mok KS, Out T, Tytgat GN, Groen AK. Immunoglobulins and alpha 1-acid glycoprotein do not contribute to the cholesterol crystallization-promoting effect of concanavalin A-binding biliary protein. Hepatology 1994; 20:626-632.
61. Abei M, Schwarzendrube J, Nuutinen H, et al. Cholesterol crystallization-promoters in human bile: Comparative potencies of immunoglobulins, alpha 1-acid glycoprotein, phospholipase C, and aminopeptidase N1. J Lipid Res 1993; 34:1141-1148.
62. Miquel JF, Von Ritter C, Del Pozo R, Lange V, Jungst D, Paumgartner G. Fibronectin in human gallbladder bile: Cholesterol pronucleating and/or mucin "link" protein? Am J Physiol 1994;267:G393-G400.
63. Afdhal NH, Smith BF. Cholesterol crystal nucleation: A decade-long search for the missing link in gallstone pathogenesis. Hepatology 1990;11:699-702.
64. Harvey PR, Strasberg SM. Will the real cholesterol-nucleating and antinucleating proteins please stand up? Gastroenterology 1993;104:646-650.
65. Harvey PR, Upadhya GA, Toth JL, Strasberg SM. Fluorometric assay of protein in native human bile. Clin Chim Acta 1989;183:147-154.
66. Yamazaki K, Powers SP, LaRusso NF. Biliary proteins: Assessment of quantitative techniques and comparison in gallstone and nongallstone subjects. J Lipid Res 1988;29:1055-1063.
67. Jungst D, Lang T, von Ritter C, Paumgartner G. Role of high total protein in gallbladder bile in the formation of cholesterol gallstones. Gastroenterology 1991;100:1724-1729.
68. Hahm JS, Sung IK, Yang SC, et al. Biliary proteins in patients with and without gallstones. Korean J Intern Med 1992;7:18-24.
69. Tudyka J, Kratzer W, Kuhn K, Mason R, Wechsler JG, Adler G. Solitary versus multiple gallstones: The importance of total biliary protein concentration and other factors. Hepatogastroenterology 1995;42:638-644.
70. Tudyka J, Wechsler JG, Kratzer W, et al. Gallstone recurrence after successful dissolution therapy. Dig Dis Sci 1996;41:235-241.
71. Moser AJ, Abedin MZ, Roslyn JJ. Increased biliary protein precedes gallstone formation. Dig Dis Sci 1994;39:1313-1320.
72. Strasberg SM, Toth JL, Gallinger S, Harvey PR. High protein and total lipid concentration are associated with reduced metastability of bile in an early stage of cholesterol gallstone formation. Gastroenterology 1990;98:739-746.

73. Roslyn JJ, Conter RL, DenBesten L. Altered gallbladder concentration of biliary lipids during early cholesterol gallstone formation. Dig Dis Sci 1987;32:609-614.
74. van Erpecum KJ, van Berge Henegouwen GP, Stoelwinder B, Schmidt YM, Willekens FL. Bile concentration is a key factor for nucleation of cholesterol crystals and cholesterol saturation index in gallbladder bile of gallstone patients. Hepatology 1990;11:1-6.
75. Haley-Russell D, Husband KJ, Moody FG. Morphology of the prairie dog gallbladder: Normal characteristics and changes during early lithogenesis. Am J Anat 1989;186:133-143.
76. Afdhal NH, Williams N, Offner GD, et al. Cholesterol supersaturation is the stimulus for gallbladder mucin hypersecretion in the prairie dog [abstr]. Gastroenterology 1993; 104:A868.
77. Afdhal NH, Niu N, Nunes DP, et al. Mucin-vesicle interactions in model bile: Evidence for vesicle aggregation and fusion before cholesterol crystal formation. Hepatology 1995; 22:856-865.
78. Yamashita G, Secknus R, Chernosky A, Krivacic KA, Holzbach RT. Comparison of haptoglobin and apolipoprotein A-I on biliary lipid particles involved in cholesterol crystallization. J Gastroent Hepatol 1996;11:738-745.
79. Kibe A, Holzbach RT, LaRusso NF, Mao SJ. Inhibition of cholesterol crystal formation by apolipoproteins in supersaturated model bile. Science 1984;225:514-516.
80. Ohya T, Schwarzendrube J, Busch N, et al. Isolation of a human biliary glycoprotein inhibitor of cholesterol crystallization. Gastroenterology 1993;104:527-538.
81. Secknus R, Yamashita G, Ginanni Corradini S, et al. Purification and characterization of a novel human 15 kd cholesterol crystallization inhibitor protein in bile. J Lab Clin Med 1996;127:169-178.
82. Busch N, Lammert F, Marschall HU, Matern S. A new subgroup of lectin-bound biliary proteins binds to cholesterol crystals, modifies crystal morphology, and inhibits cholesterol crystallization. J Clin Invest 1995;96:3009-3015.
83. Hussain MS, Chandrasekhara N. Biliary proteins from hepatic bile of rats fed curcumin or capsaicin inhibit cholesterol crystal nucleation in supersaturated model bile. Indian J Biochem Biophys 1994;31:407-412.
84. Harris RC. Bile plugs and bile sludge. Pediatrics 1969;44:145-146.
85. McMaster P, Herbertson B, Cusick C, Calne RY, Williams R. Biliary sludging following liver transplantation in man. Transplantation 1978;25:56-62.
86. Chen CL, Wang KL, Chuang JH, Lin JN, Chu MF, Chang CH. Biliary sludge-cast formation following liver transplantation. Hepatogastroenterology 1988;35:22-24.
87. Allen B, Bernhoft R, Blanckaert N, et al. Sludge is calcium bilirubinate associated with bile stasis. Am J Surg 1981;141:51-56.
88. Bernhoft RA, Pellegrini CA, Broderick WC, Way LW. Pigment sludge and stone formation in the acutely ligated dog gallbladder. Gastroenterology 1983;85:1166-1171.
89. Messing B, Bories C, Kunstlinger F, Bernier JJ. Does total parenteral nutrition induce gallbladder sludge formation and lithiasis? Gastroenterology 1983;84:1012-1019.
90. Lee SP, Nicholls JF. Nature and composition of biliary sludge. Gastroenterology 1986;90:677-686.
91. Eberle F, Rettenmaier G. Gallbladder sludge: A sonographically recognizable stage of lithogenesis. Gastroenterol 1984;22:82-87.
92. Conter RL, Roslyn JJ, Pitt HA, DenBesten L. Carbohydrate diet-induced calcium bilirubinate sludge and pigment gallstones in the prairie dog. J Surg Res 1986;40:580-587.
93. Wolpers C, Hofmann AF. Solitary versus multiple cholesterol gallbladder stones. Mechanisms of formation and growth. Clin Invest 1993;71:423-434.
94. Strasberg SM, Harvey PRC, Taylor DR, Soloway RD. Multiple crops of cholesterol gallstones in the same gallbladder indicates that cholesterol precipitates first and pigmented centers and layers develop during remodeling [abstr]. Gastroenterology 1996;110:A477.
95. Sanabria JR, Upadhya GA, Harvey RP, Strasberg SM. Diffusion of substances into human cholesterol gallstones. Gastroenterology 1994;106:749-754.
96. Sanabria JR, Gordon ER, Harvey PR, Goresky CA, Strasberg SM. Accumulation of unconjugated bilirubin in cholesterol pellets implanted in swine gallbladders. Gastroenterology 1996;110:607-613.

Extended Lymphadenectomy Is Associated With a Survival Benefit for Node-Negative Gastric Cancer

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The purpose of this study was to determine whether extended lymph node (D2) dissection is associated with a survival benefit for patients with histologically node-negative gastric cancer at a single institution in the United States. Review of the prospective gastric database at Memorial Sloan-Kettering Cancer Center from July 1985 to August 1995 identified 774 patients who had undergone curative gastric resection. Of these, 247 patients (32%) were identified with histologically negative lymph nodes by hematoxylin-eosin staining. Data are expressed as median (range). Overall survival was compared by log-rank test. The overall 5-year survival rate for the entire cohort was 79%. The extent of lymph node dissection did not predict survival over the entire cohort. However, when stratified for tumor (T) stage, D2 dissection offered a survival advantage for T3 tumors. The 5-year survival rate for patients with T3 tumors undergoing a D2 dissection (n = 15) was 54% compared to 39% for those undergoing a D1 dissection (n = 53, $P < 0.05$). D2 dissection is associated with improved survival in advanced T stage, node-negative gastric cancer. With adequate staging, results of gastric resection for adenocarcinoma in Western countries begin to approximate those seen in Japan. Excision of N2 lymph nodes may also remove microscopic metastatic disease, contributing to the survival benefit. (J GASTROINTEST SURG 1998;2:126-131.)

In the United States the survival rate for resectable gastric cancer is poor, with an overall 5-year survival rate ranging from only 15% to 30%.[1,2] In contrast, Japanese investigators report superior results, with 5-year survival rates approaching 50%.[3] Although distribution of tumor- or patient-dependent prognostic factors between the East and West do not explain these differences in outcome,[4] the Japanese assert that gastric resection with routine extended lymph node dissection (D2) contributes to their improved results.

The rationale for improved survival after a D2 dissection is twofold. One argument is based on the surgical removal of metastatic disease in regional lymph nodes prior to local growth and widespread systemic metastases. In addition, D2 dissection improves staging accuracy, resulting in a survival advantage for all stages (stage migration or the "Will Rogers phenomenon"). The relative contribution of these two factors is unknown.

Recent data from Europe suggest that D2 dissection provides a significant survival benefit for patients with node-negative gastric cancer.[5] Although it seems initially counterintuitive that patients without lymph node metastases should benefit from a D2 dissection, it was subsequently demonstrated that up to 45% of these histologically negative lymph nodes harbored occult micrometastases detected by immunohistochemical techniques.[6] Based on these data and the above-mentioned rationale of improved staging accuracy and removal of microscopic lymph node metastases, we believe that a survival benefit after D2 dissection may be detected in patients with node-negative gastric cancer.

The purpose of this study was to determine whether D2 dissection provides a survival benefit for patients with histologically node-negative gastric cancer at a single institution in the United States. In ad-

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dition, other factors influencing morbidity and survival in this subset of patients are reported.

PATIENTS AND METHODS

A review of the prospective database for gastric adenocarcinoma at Memorial Sloan-Kettering Cancer Center between July 1985 and August 1995 identified 774 patients who underwent curative gastric resection. Of these, 247 patients (32%) were identified with histologically negative lymph nodes by hematoxylin-eosin staining and form the basis of this study.

A standard gastrectomy encompassing N1 (perigastric) lymph nodes was defined as a D1 dissection, whereas complete removal of N2 (main arteries of the celiac axis, splenic hilus/pancreatic tail) lymph nodes was considered a D2 dissection. In addition to comparing survival based on the extent of lymphadenectomy (D1 vs. D2), the following factors were analyzed for prognosis: (1) sex, (2) age, (3) tumor site (gastroesophageal junction/proximal third/body/antrum), (4) tumor grade, (5) tumor size, and (6) tumor stage.

Data are reported as median (range) and comparisons were by unpaired *t* test. Survival was calculated by the Kaplan-Meier method and results were compared by means of log-rank test. Those variables determined to be significant by univariate analysis were analyzed by multivariate analysis. Multivariate analysis was calculated by the Cox proportional hazard model with statistical significance defined as $P < 0.05$.

RESULTS

The median age of patients with node-negative gastric cancer was 67 years (range 26 to 92 years) with a male predominance of 2 to 1 (166:81). Distribution of primary site, procedure, tumor stage, and tumor grade are summarized in Table I. The median tumor size was 3.3 cm (range 0.1 to 15 cm).

One hundred sixty-eight patients underwent a D2 dissection and 79 underwent a D1 dissection. For the entire cohort the median number of lymph nodes per specimen was 19 (range 4 to 84). Patients undergoing a D2 dissection had significantly more lymph nodes identified per specimen (22 [range 4 to 84]) compared to those who had a D1 dissection (12 [range 4 to 64]; $P < 0.01$).

The median follow-up time was 27 months. The length of hospital stay was 14 days (range 5 to 81 days) with an in-hospital operative mortality rate of 3.2%. The overall 5-year survival for the entire cohort was 79% (Fig. 1).

The results of the univariate and multivariate analysis for factors predictive of outcome are depicted in Table II. By univariate analysis, primary site, tumor size (≤7 cm vs. >7 cm), and T stage were predictors of outcome. When proportional hazards analysis was performed, T stage and primary site remained significant predictors of survival.

The extent of lymph node dissection did not predict survival over the entire cohort. However, when stratified for T stage, D2 dissection offered a survival advantage for T3 tumors (Fig. 2). The 5-year survival rate for patients with T3 tumors undergoing a D2 dissection (n = 15) was 54% compared to 39% for those undergoing a D1 dissection (n = 53, Table III).

Table I. Summary: Node-negative gastric cancer

Factors predictive of outcome	No. of patients
Primary site	
Antrum	89
Body	38
Proximal	41
Gastroesophageal	75
Diffuse	4
Procedure	
Distal subtotal gastrectomy	110
Esophagogastrectomy	78
Proximal gastrectomy	28
Total gastrectomy	31
Tumor stage	
T1	105
T2	74
T3	68
Tumor grade	
Well	34
Moderate	90
Poor	94
Unknown	29

Table II. Survival analysis for node-negative gastric cancer

Factor	Univariate	Multivariate
Lymphadenectomy (D1 vs. D2)	NS	NS
Grade	NS	NS
Sex	NS	NS
Age (≤70 yr vs. >70 yr)	NS	NS
Size (≤7 cm vs. >7 cm)	$P < 0.05$	NS
Site	$P < 0.05$	$P < 0.05$
T stage	$P < 0.05$	$P < 0.05$

NS = not significant.

Table III. Analysis of D1 vs. D2

	D1 (n = 79)	D2 (n = 168)	*P* value
No. of lymph nodes/specimen	12 (range 4-64)	22 (range 4-84)	0.001
Length of hospital stay	14 days (range 6-81)	14 days (range 5-59)	NS
5-year survival			
T1	90% (n = 43)	93% (n = 62)	NS
T2	87% (n = 21)	94% (n = 53)	NS
T3	39% (n = 15)	54% (n = 53)	0.05

NS = not significant.

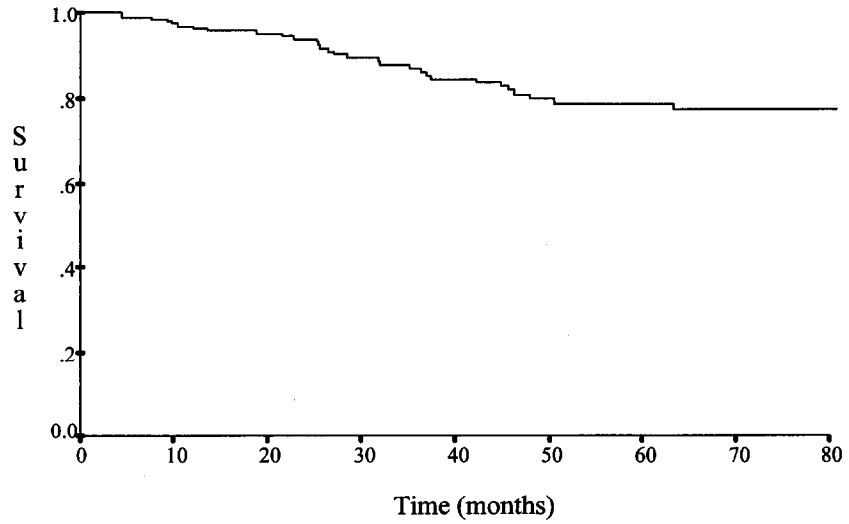

Fig. 1. Overall survival for patients with node-negative gastric cancer.

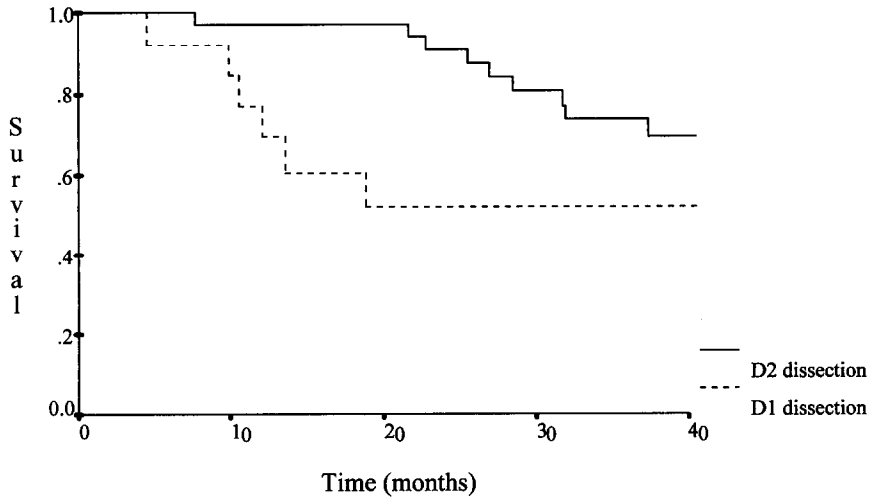

Fig. 2. D2 dissection was associated with improved survival for node-negative gastric cancer patients with T3 tumors.

DISCUSSION

In Japan the standard of care for patients with gastric cancer includes gastrectomy with an extended lymph node dissection encompassing draining lymphatic tissue beyond the perigastric area. This contrasts with the surgical practice in the United States, where D1 dissection predominates.[1] Although the safety of D2 dissection has been documented in some centers,[5,7] an increase in morbidity and mortality has also been reported, usually associated with pancreaticosplenic resections.[8,9] The reluctance of surgeons in Western countries to use more radical resections is based on the lack of evidence that extended resections contribute to a survival benefit.

Outside of Japan D2 dissection does not confer a survival benefit for all stages of gastric cancer.[10] However, there do appear to be subsets of patients who may benefit from extended dissection. The German Gastric Carcinoma Study group comparing standard lymph node dissection with radical lymph node dissection in 2394 patients reported that a D2 dissection did not have any effect on survival for earlier (stages IA and IB) or more advanced (stages IIIB and IV) tumors. However, for stage II disease, D2 dissection was associated with an improvement in 5-year survival from 26.8% to 55.2% and for stage IIIA disease the 5-year survival rate was significantly increased from 25.3% to 38.4%. In addition, D2 dissection was an independent prognostic factor for stage II and stage IIIA disease by multivariate analysis.[5] Similarly D2 dissection has been reported to significantly improve 5- and 10-year survival rates for the subset of node-negative gastric cancer patients.[11]

Our data suggest that for the entire cohort of node-negative gastric cancer patients, D2 dissection is not associated with a survival benefit. However, when stratified by T stage, D2 dissection was associated with improved survival for T3 lesions. Since the rationale for D2 dissection is based on a combination of improved staging accuracy and removal of metastatic disease in lymph nodes, and because T stage predicts nodal status, it is not surprising that only patients with the more invasive lesions in this cohort would benefit from an extended lymph node dissection.

By increasing the number of lymph nodes evaluated, the chance of identifying a positive lymph node increases. This has led to the recommendation that for all gastric cancers, a minimum of 15 lymph nodes be removed and pathologically examined for adequate staging. This is clinically relevant and we have demonstrated that survival statistics can be altered by as much as 20% by examining 15 or more lymph nodes in stage II and IIIA gastric cancer (unpublished data). However, there are diminishing returns of histologically examining increasing numbers of lymph nodes if the true lymph node positivity rate is low. For T1 and T2 lesions the node positivity rate is approximately 2.5% and 10%, respectively.[6,12] It would therefore take hundreds of patients with these early lesions to demonstrate any meaningful stage migration by examining an increased number of lymph nodes. In contrast, for T3 and T4 lesions the rate of node positivity increases dramatically and the yield when additional lymph nodes are examined is much higher. Therefore stage migration will occur at a higher rate and be reflected in an improved survival as a result of improved staging accuracy.

For node-negative gastric cancer, it is counterintuitive that an extended lymph node dissection should affect survival in that ostensibly normal lymph nodes are removed. Standard pathologic examination of lymph nodes is based on hematoxylin-eosin staining of a single section through the hilus. Recent data demonstrate that immunohistochemical evaluation of histologically negative lymph nodes detects micrometastatic disease with varying frequency and this rate of positivity is proportional to the T stage. In patients with node-negative gastric cancer, lymph nodes of T1 tumors had associated micrometastatic disease in only 13% to 15%. This positivity increased as the T stage increased from T2 (22%) to T3 (45%). Importantly, cytokeratin positivity was an independent factor for survival.[6] In a related study Maehara et al.[13] studied a cohort of early gastric cancer patients who died of recurrence. These investigators identified cytokeratin-positive cell in lymph nodes in 22.2% of patients with previously diagnosed node-negative disease. Again, cytokeratin positivity was associated with a worse outcome.

In addition to stage migration, D2 dissection may contribute to improved survival by excision of micrometastatic tumor that is not seen on routine pathologic evaluation. With more advanced T stage, the chance of micrometastatic disease increases, thus making it more likely that a D2 dissection would be associated with an improved outcome by stage. The fact that in our series D2 dissection benefited only patients with the more advanced lesions of node-negative gastric cancer supports this rationale.

In most Western countries, lymph nodes are regarded as *indicators* rather than *governors* of outcome for gastric adenocarcinoma. According to this philosophy, extended lymph node dissection merely improves the accuracy of tumor staging.[14] With adequate staging, Western results of gastric resection for

adenocarcinoma begin to approximate those seen in Japan. Excision of N2 lymph nodes may also remove microscopic metastatic disease, contributing to the survival benefit as well. Although the Dutch[8] and Medical Research Council[9] trials evaluating D2 dissection report high mortality rates (10% to 13%), our operative mortality rate was 3.2%. The major difference is that we do not routinely perform pancreaticosplenic resections as part of our D2 dissections.

Although our data suggest that extended lymphadenectomy contributes to improved survival in a select group of patients, the limitations of this study should be addressed. This is a retrospective study and the subset analysis is based on relatively few patients. Although the results are encouraging, more definitive conclusions regarding extent of lymphadenectomy will be based on results of prospective randomized trials.[8,9]

In conclusion, we demonstrated that D2 dissection was associated with improved survival in patients with advanced T stage, node-negative gastric cancer. These data offer a strong argument in favor of performing routine D2 dissection for gastric carcinoma with advanced primary lesions in the absence of obvious gross nodal metastasis.

REFERENCES

1. Wanebo HJ, Kennedy BJ, Chmiel J, Steele G, Winchester D, Osteen R. Cancer of the stomach: A patient care study by the American College of Surgeons. Ann Surg 1993;218:583-592.
2. Breaux JR, Bringaze W, Chappuis C, Cohn I. Adenocarcinoma of the stomach: A review of 35 years and 1,710 cases. World J Surg 1990;14:580-586.
3. Nakamura K, Ueyama T, Yao T, Xuan ZX, Ambe K, Adachi Y, Yakeishi Y, Matsukuma A, Enjoji M. Pathology and prognosis of gastric carcinoma: Findings in 10,000 patients who underwent primary gastrectomy. Cancer 1992;70:1030-1037.
4. Bonenkamp JJ, van de Velde CJH, Kampschoer GHM, Hermans J, Hermanek P, Bemelmans M, Gouma DJ, Sasako M, Maruyama K. Comparison of factors influencing the prognosis of Japanese, German and Dutch gastric cancer patients. World J Surg 1993;17:410-415.
5. Siewert JR, Roder JD, Bottcher K, Busch R, Hermanek P, Meyers HJ. Prognostic relevance of systemic lymph node dissection in gastric carcinoma. Br J Surg 1993;80:1015-1018.
6. Siewert JR, Kestlmeier R, Busch R, Bottcher K, Roder JD, Muller J, Fellbaum C, Hofler H. Benefits of D_2 lymph node dissection for patients with gastric cancer and pN_0 and pN_1 lymph node metastases. Br J Surg 1996;83:1144-1147.
7. Smith JW, Shiu MH, Kelsey L, Brennan MF. Morbidity of radical lymphadenectomy in the curative resection of gastric carcinoma. Arch Surg 1991;126:1469-1473.
8. Bonenkamp JJ, Songun I, Hermans J, Sasako M, Welvaart K, Plukker JTM, van Elk P, Obertop H, Gouma DJ, Taat CW, van Lanschot J, Meyer S, de Graaf PW, von Meyenfeldt MF, Tilanus H, van de Velde CJH. Randomised comparison of morbidity after D1 and D2 dissection for gastric cancer in 996 Dutch patients. Lancet 1995;345:745-748.
9. Cuscheri A, Fayers P, Fielding J, Craven J, Bancewicz J, Joypaul V, Cook P. Postoperative morbidity and mortality after D1 and D2 resections for gastric cancer: Preliminary results of the MRC randomised controlled surgical trial. Lancet 1996;347:995-999.
10. Dent DM, Madden MV, Price SK. Randomised comparison of R1 and R2 gastrectomy for gastric carcinoma. Br J Surg 1988;75:110-112.
11. Baba H, Maehara Y, Takeuchi H, Inutsuka S, Okuyama T, Adachi Y, Akazawa K, Sugimachi K. Effect of lymph node dissection on the prognosis in patients with node-negative early gastric cancer. Surgery 1994;117:165-169.
12. Namieno T, Koito K, Higashi T, Sato N, Uchino J. General pattern of lymph node metastasis in early gastric carcinoma. World J Surg 1996;20:996-1000.
13. Maehara Y, Oshiro T, Endo K, Baba H, Oda S, Ichiyoshi Y, Kohnoe S, Sugimachi K. Clinical significance of occult micrometastasis in lymph nodes from patients with early gastric cancer who died of recurrence. Surgery 1996;119:397-402.
14. Roder JD, Bonenkamp JJ, Craven J, van de Velde CJH, Sasako M, Bottcher K, Stein HJ. Lymphadenectomy for gastric cancer in clinical trials: Update. World J Surg 1995;19:546-553.

Discussion

Dr. D. Glotzer (Boston, Mass.). I wonder if you looked at the extent of the gastrectomy in these two groups. Did the patients who underwent D2 dissection actually have a more extensive gastrectomy? Could this be an alternative explanation for the better survival in the T3 group.

Dr. L.E. Harrison. There was no difference in survival between those patients undergoing a subtotal distal vs. a total gastrectomy or a proximal gastrectomy.

Dr. S. Hosking (Poole, England). How does the surgeon decide whether to perform a D1 or a D2 resection? This is crucial to the interpretation of your results.

Dr. Harrison. Our gastric cancer database is prospective, but it is not randomized. Therefore procedures are done at the surgeon's discretion. In our experience there were a number of surgeons who did not perform a D1 dissection. At the present time the vast majority of our gastrectomies include extended lymphadenectomies.

Dr. Hosking. So the surgery has changed throughout. Your thesis is that a D2 resection makes a difference, yet your lymph node harvest was as low as four nodes. I find it hard to understand how a D2 resection yields such a low node harvest.

Dr. Harrison. What is reported is the number of lymph nodes identified. That is a function of not only the surgeon but also the pathologist. We know that Japanese pathologists are very diligent in going through the specimens and detect up to 60 lymph nodes per specimen.

Dr. G. Aranha (Maywood, Ill.). It seems that tumors at

the gastroesophageal junction behave more like esophageal rather than gastric cancers. Did you find the majority of those tumors to be more advanced? Also, do you use any special procedures such as endoscopic ultrasound before surgery to accurately predict the T stage of the primary tumor?

Dr. Harrison. Endoscopic ultrasound was used only in the setting of a protocol in which we were considering preoperative chemotherapy. Otherwise, it is not standard. In terms of gastroesophageal junction tumors, we have recently compared the survival of proximal vs. distal gastric cancers and, independent of stage, proximal gastric cancers have a worse prognosis.

Dr. J. Buyske (Philadelphia, Pa.). We occasionally see patients in transfer who have not had an extended lymphadenectomy. Would you recommend reoperation?

Dr. Harrison. We do not routinely take patients back to restage them. We think that the risk outweighs the benefit in this case.

Dr. D. Fromm (Detroit, Mich.). Could you explain how removing negative lymph nodes improves survival? Couldn't your results be explained entirely by stage migration? In other words, some tumors may have skipped the D1 nodes and landed in D2 nodes, thereby accounting for your results.

Dr. Harrison. We believe that most of the benefit we are seeing is the result of stage migration. We would certainly stage the patients much better than if we just took out the perigastric lymph nodes. If you take histologically negative lymph nodes and then perform immunohistologic chemical studies, up to 45% of those nodes will be positive in T3 lesions. There might be a subset of these patients in whom one is actually removing regional disease.

Dr. C. Shatney (San Jose, Calif.). I think it is important to note that we are analyzing and commenting on a small number of patients. If I recall, the T3 group, which is the only one showing a difference, was 15 vs. maybe 50. So, let's not get too carried away. Furthermore, there are other factors that you have not mentioned such as splenectomy, which must have been done frequently for en bloc cancer resection as well as node procurement. That has clearly been shown to have an effect on survival in gastric cancer. Did you look at the influence of splenectomy as well as the amount of blood loss, both of which have been shown to be survival factors? More important, where are you going to go from here? Are you actually going to carry out a prospective randomized study, which will get us away from some of these assumptions that we have to make with retrospective studies.

Dr. Harrison. The small number of T3 lesions represents the biology of the disease. As the T stage increases, so does nodal positivity. Therefore this is a highly select group of patients with T3 lesions that are truly N0. We did not analyze the data according to blood loss; however, in the past we have done the analysis for splenectomy and have shown that survival is worse. The plans are just to continue our approach with the D2 dissection.

Dr. P. Crooks (Los Angeles, Calif.). Did any of your patients receive chemotherapy?

Dr. Harrison. There were patients within this group who received chemotherapy pre- and postoperatively at different time points. There was no difference in survival between the groups. So we included all of them in a single cohort.

Dr. L.F. Rikkers (Madison, Wis.). I notice on your survival curves, even though there was a statistical difference, at 40 months they were beginning to approach one another. There are different statistical tests that can be used when analyzing Kaplan-Meyer survival curves . . . some emphasize early differences in the curve and some emphasize late differences. Can you tell me which test you used and is there really a true significant difference in survival?

Dr. Harrison. We used log rank to compare survival between the two curves and those plateaus were maintained at 5 years.

Laparoscopic Management of Small Bowel Obstruction: Indications and Outcome

Enrique Luque-de León, M.D., Alejandro Metzger, M.D., Gregory G. Tsiotos, M.D., Richard T. Schlinkert, M.D., Michael G. Sarr, M.D.

Our aim was to evaluate the feasibility of a laparoscopic, minimal access approach for the management of patients with small bowel obstruction. Forty patients underwent laparoscopic treatment of radiologically documented or suspected small bowel obstruction based on history and/or motility study. None had chronic abdominal or pelvic pain. The operation was completed laparoscopically in 14 patients (35%) and with laparoscopic-assisted procedures in 12 (30%); 14 (35%) required conversion to open celiotomy because of dense adhesions (precluding complete inspection or adhesiolysis), small bowel necrosis in the setting of small bowel obstruction, or neoplasia. Three iatrogenic enterotomies occurred while "running" the bowel. There were three (7%) postoperative procedure-related complications (wound infection, intra-abdominal abscess, ileus). The combined group of patients treated laparoscopically or with laparoscopic-assisted procedures had a shorter hospital stay than those converted to open celiotomy (4 ± 0.6 vs. 7 ± 0.7 days; $P < 0.003$). At median follow-up of 12 months, 21 of 26 patients managed laparoscopically or with laparoscopic-assisted procedures remain asymptomatic; all 21 patients with an operatively confirmed site of mechanical obstruction managed by a minimal access approach remain asymptomatic. Laparoscopic treatment of small bowel obstruction is effective, leads to a shorter hospital stay, and has good long-term results. A minimal access approach to treatment of small bowel obstruction should be considered in *selected* patients. (J GASTROINTEST SURG 1998;2:132-140.)

Small bowel obstruction (SBO) is a common entity. Not infrequently, at the time of celiotomy the definitive management involves only a relatively simple adhesiolysis and, on occasion, transection of a single obstructing adhesive band. In retrospect, many of these patients could have been managed by a minimal access, "laparoscopic" approach.

Several years ago we became interested in the potential use of a minimal access, laparoscopic approach in the operative management of selected patients with radiologically documented SBO or with a history suggestive of partial, intermittent SBO. Gynecologic surgeons have used similar laparoscopic techniques for more than three decades, but most of their work has focused on the evaluation and treatment of adhesions in relation to indeterminate chronic abdominal and pelvic pain.[1,2] Over the past 5 years, our experience with this approach in the management of radiologically documented or suspected SBO *(not chronic abdominal pain)* has grown, and our indications have matured. In this article we report our experience with a minimal access approach to the management of 40 patients with preoperatively established acute, progressive, or intermittent SBO or symptoms suggestive of partial or recurrent SBO.

PATIENTS AND METHODS

Between November 1991 and August 1996, 40 patients underwent laparoscopy for the management of established or suspected SBO. There were 19 women and 21 men whose median age was 64 years (range 18 to 90 years); 32 patients (80%) had undergone previous abdominal operations including 20 with one, eight with two, and four with three or more prior operative interventions.

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Table I. Operative indications

Indication	No.	(%)
Radiologically documented SBO		
Acute complete	4	(10)
Nonresolving	10	(25)
Recurrent, partial	13	(33)
Suspected SBO*		
History strongly suggestive (no objective findings)	4	(10)
Motility studies suggestive of mechanical obstruction	4	(10)
Unclear, atypical clinical picture; possible obstruction	5	(13)
TOTAL	40	(100)

*Based on symptoms of SBO—not chronic abdominal pain.

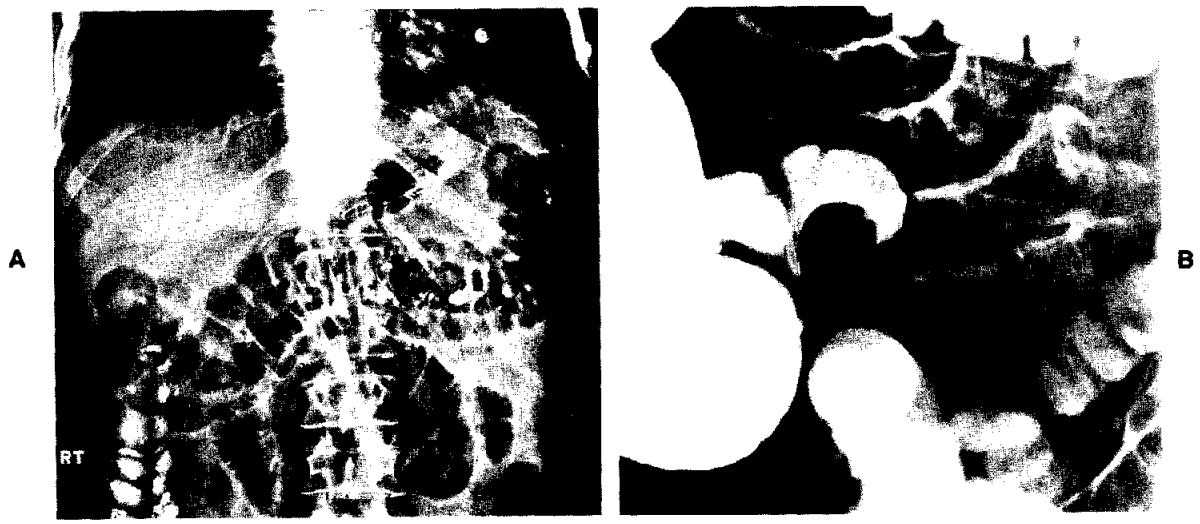

Fig. 1. Radiographic features of patients with small bowel obstruction. **A,** Plain abdominal x-ray film showing small bowel dilatation. The patient had progressive, nonresolving obstruction caused by a single adhesive band; after laparoscopic division, the patient remains asymptomatic 4 years later. **B,** Hypaque enema demonstrates bowel obstruction 5 cm proximal to the ileocecal valve with multiple dilated bowel loops proximally. Multiple adhesions and a thick band with an internal hernia caused the obstruction. The patient was treated with laparoscopy only and remains asymptomatic 3 years later.

Operative Indications

Patients were divided retrospectively into two categories—those with a radiologic diagnosis of SBO and those in whom there was a strong preoperative suspicion of SBO (Table I). In the former group, diagnosis was achieved through a combination of clinical and radiologic parameters. Plain abdominal x-ray films, ultrasound images, or CT scans showed at some time either small bowel dilatation, wall thickening, abnormal distribution of intraluminal gas, and/or air-fluid levels (Fig. 1, *A*); in many of these patients, a point of partial or complete obstruction was demonstrated after antegrade or retrograde oral administration of a contrast agent (Fig. 1, *B*). Patients in this category were subdivided according to the nature of the obstruction into the following groups: acute complete SBO (n = 4), nonresolving partial SBO (n = 10), and multiply recurrent partial SBO (n = 13).

In the second group, patients' histories and symptoms were highly suggestive of partial, intermittent SBO or chronic partial SBO; these symptoms included intermittent episodes of nausea and vomiting, abdominal distention, and/or episodes of colicky abdominal pain. *No* patient had chronic, persistent abdominal or pelvic pain alone. Attempts to demonstrate objectively a site of obstruction were repeatedly unsuccessful. Included in this category were four patients with a history strongly suspicious for partial, in-

termittent SBO but without objective findings, four patients with extensive diagnostic workups in whom only the upper gut manometric studies[3] suggested mechanical obstruction (idiopathic intestinal pseudo-obstruction was included in the differential diagnosis in three of these), and five patients who despite thorough investigations had an unclear diagnosis and in whom laparoscopy was performed to rule out SBO, which was included in the differential.

Operative Technique

Patients were placed in the supine position with abducted arms and supports mounted to allow safe tilting and lateral rotation of the operating table. Two video monitors were used; the video monitor to the patient's right was positioned inferiorly at the level of the hip and the monitor to the left positioned superiorly at the level of the shoulder (Fig. 2). This positioning forms a plane parallel to the root of the small bowel mesentery and allows the operating surgeon to look and work in the same direction as the camera orientation. The configuration of the operating room arrangement was flexible to permit modifications during the operation.

Patients were prepared and draped in a way that allowed conversion to an open procedure when necessary. All interventions were performed under general endotracheal anesthesia with a nasogastric tube and urinary catheter in place. *Because nitrous oxide as an anesthetic gas has been found to produce bowel dilatation, its use was specifically avoided in most patients.* A pneumoperitoneum (15 cm H_2O) was established using an open technique with a modified Hasson-type[4] balloon cannula (Origin Medsystems Inc., Menlo Park, Calif.) inserted via a 1.5 cm periumbilical vertical incision. A 30-degree angled-view laparoscope was used, and two additional 5 mm trocars, introduced under direct vision, were usually placed in the right upper and left lower quadrants (see Fig. 2). A fourth or rarely a fifth trocar was required to allow better retraction, improve visualization, or facilitate intraperitoneal adhesiolysis in a few cases. Adhesions to the anterior abdominal wall were dissected using scissors, electrocautery hook, or blunt avulsion and the operation proceeded until *complete* visualization of the small bowel could be obtained.

Evaluation of the entire jejunoileum was attempted in a systematic fashion starting at the ileocecal junction, looking at the right (lower) monitor, with the patient in a Trendelenburg position. The bowel was carefully inspected and systematically "run" in a retrograde fashion with a "hand-to-hand" technique, which involved grasping the bowel by its antimesenteric border alternately between two large, atraumatic graspers. Extreme care was necessary while "running" the acutely obstructed bowel because traumatic iatrogenic enterotomies can occur (see below). Inspection was facilitated by tilting and rotating the operating table. As the proximal jejunum was approached, the patient was placed in a reverse Trendelenburg position, and visualization shifted to the left-sided (upper) monitor. We considered this systematic exploration of the *entire* jejunoileum an essential part of the procedure such that when complete inspection of the small bowel was not feasible, conversion to an open procedure was strongly considered. Use of a 5 mm laparoscope placed through one of the lateral 5 mm trocars was often necessary to obtain a better view of certain regions.

When adhesions or obstructing bands were encountered, they were usually lysed with scissors, thereby avoiding potential thermal injury to adjacent bowel. As with the open operative approach for SBO,

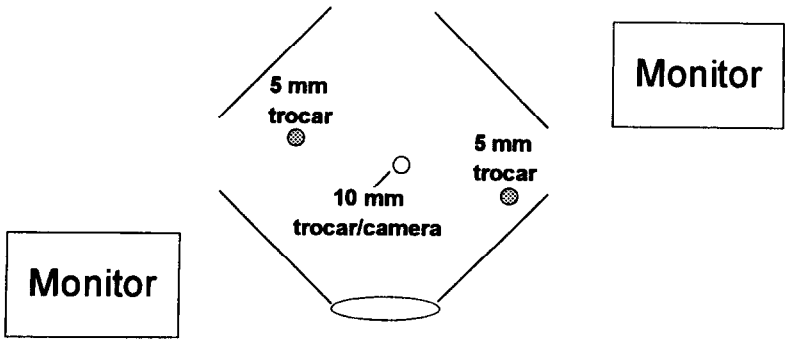

Fig. 2. Diagram depicting positions of the video monitors and trocars. A right-sided (lower) monitor and a left-sided (upper) monitor help with inspecting the bowel from the distal ileum to proximal jejunum. Placing 5 mm trocars in the right upper quadrant and left lower quadrant maximizes the distance from the trocars to the ileocecal valve and the ligament of Treitz, respectively.

when a point of obstruction was not clearly identified, lysis continued until all suspicious adhesions or bands responsible for the symptomatology were dissected. Similarly, we evaluated the entire jejunoileum, even if a convincing obstruction was found in the ileum.

Abnormalities requiring bowel resection or stricturoplasty prompted performance of laparoscopic-assisted procedures. These were carried out by removing the 10 mm laparoscope and placing a 5 mm laparoscope in another port. The abnormal bowel was then grabbed with a Babcock clamp placed through the 10 mm periumbilical port and pulled through the umbilical incision; the incision was enlarged just enough (usually 2 to 3 cm) to allow exteriorization and extracorporeal repair of the bowel (Fig. 3). The bowel was then returned to the abdominal cavity, the fascial defect closed or occluded with the balloon trocar, and the pneumoperitoneum reestablished so that a final inspection could be performed. In patients in whom conversion to laparotomy was deemed necessary, a midline incision was usually performed.

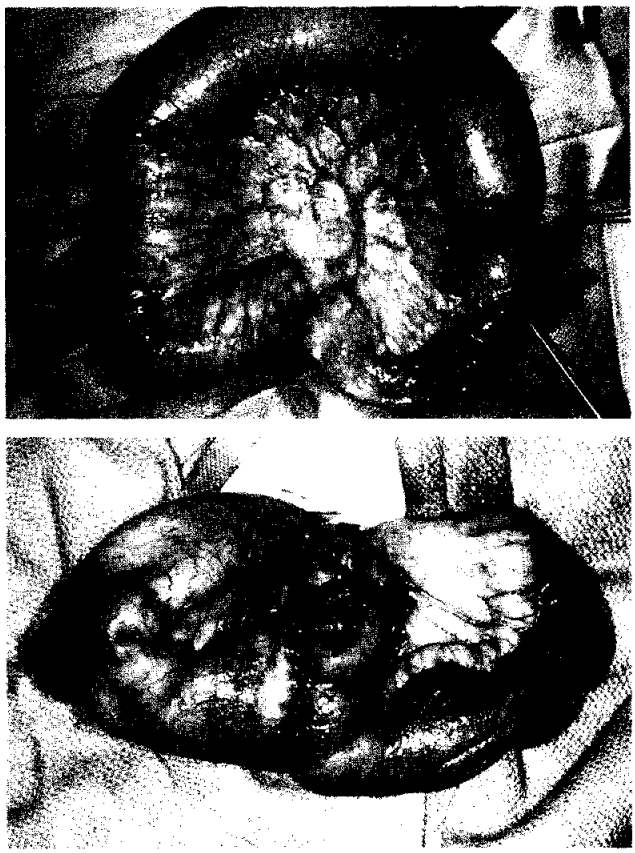

Fig. 3. Laparoscopic-assisted procedure in a patient with recurrent partial obstruction. **A,** Exteriorized bowel showing strictures due to NSAID lesions with proximal bowel dilatation. **B,** Segmental resection of the involved bowel and primary anastomosis were performed extracorporeally.

Data Analysis

Data are expressed as mean ± standard error of the mean or median and range when appropriate. We recorded past medical and surgical history, signs and symptoms, laboratory and radiographic findings, operative technique and findings, intraoperative and postoperative complications, and final outcome. Because of stratification, group samples were small, and descriptive analyses were used for most variables. Comparisons were made between the combined group of patients treated with laparoscopic and laparoscopic-assisted procedures (minimal access approach) and those in whom an open celiotomy was required; the subgroups of operative indications were similarly compared (see Table I). Length of stay was compared by means of a Mann-Whitney U test. P values <0.05 were considered significant.

RESULTS

Laparoscopic Operations

Management of SBO was achieved by laparoscopy alone in 14 patients. Single or multiple adhesions were found in five and eight patients, respectively (Table II), but an obvious site of obstruction (with proximal bowel dilatation) was clearly identified in only 10 of these 13 patients. One remaining patient had not undergone prior abdominal interventions and, despite a suggestive history and motility study, no evidence of SBO was found; small serosal nodules prompted appendectomy, but the appendix was microscopically normal. There was one intraoperative iatrogenic enterotomy requiring a laparoscopic-assisted procedure for repair (Fig. 4). Mean operating time was 108 ± 9 minutes (range 55 to 160 minutes). Mean hospital stay was 2.9 ± 0.7 days. One patient

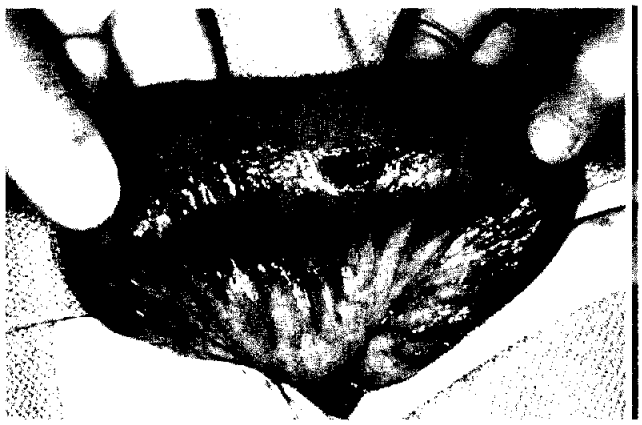

Fig. 4. Iatrogenic enterotomy produced during (laparoscopic) "run" of the small bowel, repaired through a laparoscopic-assisted procedure.

Table II. Intraoperative findings by method of treatment

Laparoscopic	No.	Laparoscopic-assisted	No.	Open celiotomy	No.
Adhesive/bands	13	Adhesions/bands	3	Adhesions/bands	10
Single (n = 5)		Single (n = 1)		Unable to lyse all (n = 3)	
Multiple (n = 8)		Multiple (n = 2)		Unable to "run" bowel (n = 2)	
No pathologic findings	1	Strictures	5	Obliterated abdominal cavity (n = 2)	
		Radiation (n = 2)		Bowel necrosis (n = 2)	
		Crohn's disease (n = 1)		Bowel ischemia (n = 1)	
		Previous anastomosis (n = 1)		Stricture (Crohn's)	1
		NSAID lesions (n = 1)		Tumor	2
		Meckel's diverticulum	1	Ileal carcinoid (n = 1)	
		No pathologic findings	3	Ileal adenocarcinoma (n = 1)	
				Mesenteric adenitis	1
TOTAL	14		12		14

who had undergone an extensive laparoscopic adhesiolysis was readmitted 2 weeks after hospital discharge because of an intraperitoneal abscess, which was drained percutaneously without further complications. The remaining 13 patients had an uneventful course.

Laparoscopic-Assisted Operations

Laparoscopic-assisted procedures were performed in 12 patients. Mechanical obstruction was confirmed in nine (see Table II). Three patients had dense adhesions requiring laparoscopic assistance for complete adhesiolysis (n = 1), assessment of a questionable stricture (n = 1), and repair of an iatrogenic enterotomy (n = 1). Five patients had strictures due to radiation (n = 2), Crohn's disease (n = 1), a previous anastomosis (n = 1), and stenosing ulcers believed related to nonsteroidal anti-inflammatory drugs (NSAIDs) (n = 1). One patient had an area of posterior fixation and kinking in the ileum related to a Meckel's diverticulum with a broad base. Resections and/or stricturoplasties were performed extracorporeally. The other three patients had a history and motility study suggestive of SBO but no other preoperative objective findings; after a normal diagnostic laparoscopy with assurance of no mechanical obstruction by inspection of the entire jejunoileum, the ileum was exteriorized to obtain a full-thickness biopsy to evaluate the presence of histopathologic features of idiopathic intestinal pseudo-obstruction. There were no intraoperative complications in this group. One patient developed a postoperative superficial wound infection. Mean operating time was 135 ± 9 minutes (range 80 to 185 minutes). Mean hospital stay was 5.4 ± 0.1 days.

Open Celiotomy

Conversion to an open celiotomy was necessary in 14 patients (see Table II). Dense, extensive adhesions required conversion in seven patients because of inability to completely and safely lyse all adhesions (n = 3), inability to adequately inspect ("run") the whole bowel (n = 2), or insufficient access to the abdominal cavity to allow laparoscopic evaluation (n = 2); after adhesiolysis, segmental resections were required in three of these seven patients because of matted loops with perforations, a contained abscess, and a stricture at a previous enteroenterostomy. In the remaining seven patients, open celiotomy was necessary because of an intraperitoneal abnormality that was deemed unmanageable by a laparoscopic-assisted technique. Adhesive bands causing small bowel necrosis in two patients prompted conversion for a satisfactory and safe segmental resection; bowel ischemia in another patient proved reversible after open adhesiolysis and untwisting of the affected segment. One patient had Crohn's disease and two had obstructing neoplasms (carcinoid, adenocarcinoma); a formal open celiotomy in these patients was deemed necessary to perform an adequate resection. The last patient had an atypical clinical presentation, and laparoscopy was performed mainly as a diagnostic procedure; large mesenteric lymph nodes were found, and open celiotomy was believed necessary to allow complete intra-abdominal manual exploration.

Two incidental enterotomies occurred during the laparoscopic stage preceding open celiotomy but were not the reason for conversion. Mean operating time was 208 ± 23 minutes (range 105 to 422 minutes). There were four postoperative complications of a medical nature (e.g., cardiopulmonary) but these were not technique related. The mean hospital stay was 7.4

Table III. Rate of conversion to open celiotomy and long-term outcome according to operative indication

Operative indication	Method of treatment				CR (%)	Outcome (% asymptomatic)*
	Total	Lap	L-A	Cel		
Radiologically documented SBO						
Acute complete	4	1	—	3	75	100
Nonresolving partial	10	4	2	4	40	100
Recurrent partial	13	5	6	2	15	91
Suspected SBO						
Suggestive history (no objective evidence)	4	2	—	2	50	50
Suggestive motility studies	4	1	3	0	0	25
Atypical picture (possible SBO)	5	1	1	3	60	100
TOTAL	40	14	12	14		

Lap = laparoscopic; L-A = laparoscopic-assisted; Cel = celiotomy; CR = conversion rate (to open celiotomy).
*At 12 months' (median) follow-up; only patients treated laparoscopically or with laparoscopic-assisted procedures.

± 0.7 days, which was longer than the mean stay of the combined group of patients treated by laparoscopy alone or laparoscopic-assisted procedures (4.0 ± 0.6 days; $P < 0.003$).

Follow-up

Median follow-up for the 26 patients treated with a laparoscopic or laparoscopic-assisted technique was 12 months (range 2 to 56 months). Twenty-one patients (81%) remain free of their initial symptoms. Five patients continue to have intermittent symptoms of abdominal pain, distention, nausea, vomiting, constipation, or a combination thereof. Three of them had no pathologic findings noted at laparoscopy and carry the diagnosis of intestinal pseudo-obstruction. The other two had had previous abdominal procedures, and although adhesiolysis was performed, no obvious point of obstruction was noted at that time. Thus, of the 21 patients managed by a minimal access approach who were found to have a definite site of mechanical SBO, all remain symptom free postoperatively.

Table III summarizes the conversion rate to open celiotomy and the long-term outcome according to the indication for operation. The highest rate of conversion to open celiotomy (three of four patients) occurred in those patients with acute, complete SBO. Conversely, patients undergoing laparoscopy because of suggestive symptoms and an abnormal motility study consistent with SBO were all managed laparoscopically (conversion rate = 0%); however, as stated previously, no pathologic findings were noted and three of the four patients have persistent symptoms. The largest group consisted of patients with recurrent partial SBO. The laparoscopic approach in this group was most effective as 11 (85%) of 13 patients were managed without open celiotomy, and 10 (91%) of these 11 remain asymptomatic. Patients with nonresolving partial SBO had a conversion rate of 40% and a long-term effectiveness of 100%.

Another interesting subgroup includes the eight patients without previous abdominal operations who were subjected to laparoscopy because of radiologically documented or suspected SBO. Laparoscopic findings in this subgroup included neoplasm-related obstruction, Crohn's stricture, obstructing Meckel's diverticulum, and mesenteric adenitis; no pathologic findings were encountered in three patients, all of whom are believed to have intestinal pseudo-obstruction. Interestingly, one patient had two spontaneous adhesive bands that were the cause of obstruction. Besides the two patients with neoplasms and the patient with mesenteric adenitis, all of whom were treated by open celiotomy, the remaining patients were managed with laparoscopic or laparoscopic-assisted procedures.

DISCUSSION

As our experience with laparoscopic procedures in abdominal surgery has improved and expanded, our ability and confidence in handling the bowel via a minimal access approach has also matured. The use of laparoscopic techniques to manage patients with documented or suspected SBO is an obvious natural extension of this expertise.[5] Adequate access to the right upper quadrant for a laparoscopic cholecystectomy in patients with a history of previous intraperitoneal operation often requires takedown of intraperitoneal adhesions to obtain adequate visualization and exposure. A similar minimal access approach for SBO requires a modification of similar techniques.

Such an approach, if successful, should prevent the need for a formal celiotomy large enough for manual exploration and thereby minimize hospital stay, decrease postoperative discomfort and morbidity, and possibly minimize subsequent adhesion formation to the undersurface of the abdominal wall.

Our experience with a primary minimal access approach to the operative management of 40 selected patients with radiologically documented or suspected SBO was successful in 26 patients (65%). Others have reported their experience with a laparoscopic minimal access approach in the operative management of SBO.[6-21] Several of these previous reports have been limited to either acute SBO[9-12,16,20] or postoperative adhesions[13,15,17]; success rates in series with 10 or more patients have averaged approximately 70% with a range of 29% of 87%.[10,12,15,17,18,20] Several of these previous reports have included patients with a syndrome of chronic abdominal pain believed (but not proved) to be secondary to intraperitoneal adhesions.[13] We *specifically excluded* such patients from our series; our patients represent only those with radiologically documented SBO or symptoms suggestive of recurrent or chronic SBO.

Our success with managing patients with acute SBO on a semiemergent basis was limited (one of four patients); three of these four patients had obviously necrotic or ischemic bowel precluding, in our opinion, a safe laparoscopic or laparoscopic-assisted approach for fear of intestinal trauma and intraperitoneal spillage of intestinal contents. Possibly a better selection of patients would have increased the success rate (see below—Indications for a Minimal Access Approach).

Because of dilated and fragile thin-walled bowel, the risk of traumatic iatrogenic enterotomies is increased during both trocar insertion and bowel manipulation. For these reasons and because of fear of intraperitoneal adhesions fixing segments of bowel to the undersurface of the abdominal wall, we strongly believe that access into the peritoneal cavity to establish the pneumoperitoneum should be obtained by an open, Hasson-type approach.[4] We prefer a vertical periumbilical incision because this location is optimal both for intraperitoneal inspection during evaluation of the bowel and for potential laparoscopic-assisted exteriorization of bowel for extracorporeal resection, lysis of difficult adhesions between bowel loops, or stricturoplasty. In addition, if conversion to open celiotomy is necessary, a midline extension of the original periumbilical incision generally provides the best operative exposure. If appropriate access cannot be obtained because of adhesions from a previous midline incision, one can attempt to gain access laterally, but again an open approach with full visualization seems prudent.

Overzealous retraction of thin-walled small bowel fixed intraperitoneally during manipulation may also lead to iatrogenic enterotomies as occurred in three of our patients. The incidence of iatrogenic enterotomies in other reported series has ranged from 3% to 21%.[10,12,15,17,18,20] Nontraumatic bowel clamps rather than the "dissecting" graspers commonly used during laparoscopic cholecystectomy are suggested. In addition, when "running" the bowel between the two manipulating bowel clamps, *both* clamps should remain in view at all times. When one clamp leaves the visual field, it is difficult to appreciate the amount of traction being applied; also, if an enterotomy should occur, it may not be appreciated. One of our patients was readmitted several weeks postoperatively with an intraperitoneal abscess. He had undergone an extensive laparoscopic adhesiolysis, and presumably a small enterotomy was made that we did not recognize. Repair of an iatrogenic enterotomy does not necessarily require conversion to open celiotomy and can be accomplished either by intracorporeal suturing[12] or by extracorporeal repair by exteriorizing the involved bowel.

Very little is known about the long-term success of laparoscopic adhesiolysis for SBO from the literature. Our follow-up ranged from 2 to 56 months. All 21 patients with radiologically documented or highly suspected mechanical SBO managed by a minimal access approach (i.e., laparoscopic or laparoscopic-assisted) in whom the site of obstruction was identified remain free of their preoperative symptoms. Francois et al.[13] followed 17 patients with SBO managed by a minimal access technique for a mean of 28 ± 5 months; six developed recurrent SBO requiring reoperation (four of them within the first 3 months of follow-up). Fourteen patients (82%) have since remained asymptomatic. The single cases reported by Bastug et al.,[7] and Silva and Cogbill[8] have remained asymptomatic at 3 weeks and 14 months of follow-up, respectively. Most of the other series also represent anecdotal experience, and none have reported follow-up results. Thus our series is important and justifies this approach based on a large series with good follow-up.

Indications for a Minimal Access Approach

Our experience suggests that an attempt at laparoscopic management seems appropriate in patients with acute SBO provided they are not markedly distended, which would compromise safe insertion of trocars, jeopardize establishment of a pneumoperitoneum, and limit work space. Other even more ideal candidates include patients with a nonresolving, partial SBO or a recurrent, chronic SBO demonstrated on contrast study. In addition, patients with a history

strongly suggestive of recurrent, intermittent SBO or those in whom functional "pseudo-obstruction" is suspected but in whom a mechanical obstruction cannot be excluded also appear to be appropriate candidates, and a minimal access approach may obviate the need for a formal celiotomy with manual evaluation and exploration. Contraindications to this approach include a documented history of severe or extensive dense adhesions, a frozen abdomen, or obviously necrotic, obstructed bowel.

CONCLUSION

A minimal access approach certainly appears to be appropriate for selected patients with radiologically documented or suspected SBO and may prevent the need for a formal open celiotomy in up to 65% of patients in our experience. Management of the site of obstruction may be amenable to a fully laparoscopic adhesiolysis or to a laparoscopic-assisted bowel resection, stricturoplasty, or adhesiolysis depending on the bowel abnormality and laparoscopic expertise of the surgeon in performing complex intracorporeal suturing. This minimal access approach appears to minimize the length of the incision (and thus postoperative pain and disability), shorten the hospital stay, and speed convalescence. Long-term relief of SBO has been excellent.

We wish to acknowledge the help of Deborah Frank in the preparation of this manuscript.

REFERENCES

1. Howard IM. The role of laparoscopy in chronic pelvic pain: Promise and pitfalls. Obstet Gynecol Surv 1993;48:357-387.
2. Daniell JF. Laparoscopic enterolysis for chronic abdominal pain. J Gynecol Surg 1989;5:61-66.
3. Frank JW, Sarr MG, Camilleri M. Use of gastroduodenal manometry to differentiate mechanical and functional intestinal obstruction: An analysis of clinical outcome. Am J Gastroenterol 1994;89:339-344.
4. Hasson HM. A modified instrument and method of laparoscopy. Am J Obstet Gynecol 1971;110:886-887.
5. Schlinkert RT, Sarr MG, Donohue JH, Thompson GB. General surgical laparoscopic procedures for the "non-laparoscopist." Mayo Clin Proc 1995:70:1142-1147.
6. Clotteau JE, Premont M. Occlusion sur bride traitee par section sous coelioscopie. Presse Med 1990;19:1196.
7. Bastug DF, Trammell SW, Boland JP, Mantz EP, Tiley EH. Laparoscopic adhesiolysis for small bowel obstruction. Surg Laparosc Endosc 1991;1:259-262.
8. Silva PD, Cogbill TH. Laparoscopic treatment of recurrent small bowel obstruction. Wis Med J 1991;90:169-170.
9. Keating J, Hill A, Schroeder D, Whittle D. Laparoscopy in the diagnosis and treatment of acute small bowel obstruction. J Laparoendosc Surg 1992;2:239-244.
10. Levard H, Mouro J, Schiffino L, Karayel M, Berthelot G, Dubois F. Traitement coelioscopique des occlusions aigues du grele. Ann Chir 1993;47:497-501.
11. Adams S, Wilson T, Brown AR. Laparoscopic management of acute small bowel obstruction. Aust N Z J Surg 1993;63:39-41.
12. Franklin ME, Dorman JP, Pharand D. Laparoscopic surgery in acute small bowel obstruction. Surg Laparosc Endosc 1994;4:289-296.
13. Francois Y, Mouret P, Tomaoglu K, Vignal J. Postoperative adhesive peritoneal disease: Laparoscopic treatment. Surg Endosc 1994;8:781-783.
14. Reissman P, Ligumsky M, Bloom A, Durst AL. Laparoscopic adhesiolysis—A treatment for recurrent intestinal obstruction due to adhesions. Minim Invasive Ther 1994;3:103-104.
15. Parent S, Bresler L, Marchal F, Boissel P. Laparoscopic surgery for acute adhesions on the small bowel. J Chir 1995;132:382-385.
16. Sfairi A, Patel JC. Laparoscopic cure of acute bowel occlusion: Preliminary results. Presse Med 1995;24:1727-1730.
17. Federmann G, Walenzyk J, Schneider A, Bauermeister G, Scheele C. Laparoscopic therapy of ileus of the small intestine caused by adhesions or bands—Preliminary results. Zentralbl Chir 1995;120:377-381.
18. Eypasch E, Paul A, Köhler L, Troidl H. Laparoscopic surgery for intestinal obstruction. Zentralbl Chir 1995;120:382-386.
19. Konstantinidis K, Sabalis G, Vorias M. Small bowel obstruction treated laparoscopically. Surg Endosc 1995;9:627.
20. Ibrahim IM, Wolodiger F, Sussman B, Kahn M, Silvestri F, Sabar A. Laparoscopic management of acute small-bowel obstruction. Surg Endosc 1996;10:1012-1015.
21. Reissman P, Wexner SD. Laparoscopic surgery for intestinal obstruction. Surg Endosc 1995;9:865-868.

Discussion

Dr. G. Gowen (Philadelphia, Pa.). I am very pleased that you have given attention to SBO. Eighty percent of the time we can relieve SBO in properly selected patients using a long intestinal tube. Long-tube decompression has declined in use because of the frustration in getting the tube out of the stomach and into the small bowel. That problem has been corrected by the endoscopic placement technique, which we have been using now for 15 years. In addition, we have a better tube than before. We are conducting a prospective study in Philadelphia comparing the short tube with the long tube, placed endoscopically. We have included what you are describing here, and prior to a laparotomy we would actually perform a laparoscopy. As you point out, two thirds of the time laparoscopic enterolysis will prevent the need for a formal laparotomy. I would recommend that you follow Wangensteen's original observations and decompress the bowel every time before you relieve the obstruction.

Dr. B. Schirmer (Charlottesville, N.C.). I think this series is really one of the largest that has been presented and it really serves as a landmark study to substantiate this approach to patients with SBO. Three out of four of those patients with acute obstruction had to be converted to an open procedure. Do you have any additional criteria that would help you assess the appropriateness of a minimally invasive approach. Others have stated that perhaps the amount of CO_2 infused may provide some indiscretion, but can you preoperatively predict with any accuracy when you are going to be able to perform enterolysis laparoscopically.

Dr. E. Luque-de León. Two of the three patients who were converted had bowel necrosis. We thought it more appropriate to perform an open celiotomy. If the laparoscopy could be performed in the early stages of the established acute complete obstruction, I think that would be best.

Dr. Schirmer. Just a comment on one other group—that is, patients who are found to have, on upper gastrointestinal and small bowel follow through, a documented partial obstruction have often been rehospitalized for numerous episodes of partial SBO. This is really the approach to use in those patients and your data confirm that.

Dr. N. Soper (St. Louis, Mo.). Do you have any idea of the denominator of patients with these problems who came to the Mayo Clinic during this time period so we can get some idea of how select this group was? Also, given the fact that you have shown that the patients with the longest hospital stay are those who were started with the laparoscopic approach and later converted to an open procedure, what are your criteria for not initially attempting the laparoscopic approach?

Dr. Luque-de León. I do not have the denominator. We would not pursue laparoscopy in patients who have massive dilatation of the small bowel or in those patients who have been preoperatively documented to have severe adhesions either by previous operations or other means.

Dr. J. Bender (Baltimore, Md.). You mentioned that two patients had their enterotomies repaired after conversion to celiotomy. Were those enterotomies noted before or after conversion?

Dr. Luque-de León. They were noted before the conversion.

Dr. L. Way (San Francisco, Calif.). I think there is a great deal of technical expertise and fine judgment that is involved in these good results. I am aware, just from personal referrals, of a number of patients in Northern California in whom accidental enterotomies occurred and went undetected, resulting in the death of several of these patients. It would be a mistake to underestimate some of the technical pitfalls and the risk of these accidental enterotomies.

Dr. Luque-de León. We suggest the loop of bowel that is being "run" should be kept within view on the monitor to reduce the risk of an enterotomy.

Dr. Way. These complications seem to be clustered among patients who have pelvic pain, being operated on following previous pelvic operations, either by gynecologists or general surgeons attempting to solve this problem laparoscopically. In one of your introductory slides you stated that such patients were systematically excluded from this analysis. Is this operation being done, however, in your medical center and do you have an opinion concerning its value? I am concerned that very serious complications are occurring as a result of this clinical entity.

Dr. Luque-de León. We are not performing this operation for these indications, or for chronic abdominal or pelvic pain syndromes.

Dr. A. Bastidas (Palo Alto, Calif.). You suggested that laparoscopy provided a shorter length of stay. Do you have any data from concurrent patients who were not considered for laparoscopy to validate this statement?

Dr. Luque-de León. I do not have those data for comparison.

Comparison of Generic (SF-36) vs. Disease-Specific (GERD-HRQL) Quality-Of-Life Scales for Gastroesophageal Reflux Disease

Vic Velanovich, M.D., F.A.C.S.

The Gastroesophageal Reflux Disease–Health-Related Quality-of-Life (GERD-HRQL) scale was developed to objectively quantify symptom severity. It was compared to a "gold standard" health survey, the SF-36. Forty-three patients treated either medically or surgically for gastroesophageal reflux disease were asked to complete both the GERD-HRQL and the SF-36. They were asked the following: (1) Which questionnaire do you like best? (2) Which questionnaire was easier to understand? (3) Which questionnaire was more reflective of the problems you have with reflux disease? (4) Given the choice, which questionnaire would you rather fill out? Patients were asked to state their overall satisfaction with their present reflux symptom conditions. Multivariate analysis showed that the only significant predictor of patient satisfaction was the total GERD-HRQL score ($P < 0.00001$). There were differences in the SF-36 domains of physical function (88.7 vs. 65.3; $P = 0.004$) and general health (68 vs. 46.5; $P = 0.006$). There were no correlations between the total GERD-HRQL scores and the SF-36 domain scores. Fifty-nine percent of patients preferred the GERD-HRQL questionnaire, 62% felt it was easier to understand, 86% felt it was more reflective of their symptoms, and 67% said they would rather use it over the SF-36. The GERD-HRQL better assesses symptom severity for gastroesophageal reflux disease than the generic SF-36. (J GASTROINTEST SURG 1998;2:141-145.)

For many surgical diseases, traditional measures of clinical results, such as survival, complication rate, and recurrence, are being questioned as the relevant end points. For example, Rutkow[1] has analyzed the various end points for inguinal hernia repair, for which recurrence has been the customary outcome reported. He points out that technical difficulty, complication rate, rehabilitation time, recurrence rate, and socioeconomic factors such as hospital costs and time off from work may each be the most important end point depending on an individual patient's or surgeon's situation. Although outcome measures may take many forms, qualify of life is increasingly used.[2] In an effort to systematically incorporate quality-of-life measurements in surgical practice, Temple et al.[3] and Velanovich[4] have previously reported on the use of a generic quality-of-life scale, the SF-36, in measuring health status in clinical practice. It seems clear, therefore, that quality-of-life measurements are going to be an increasingly used end point in the evaluation of surgical care and their sound use investigated.

Quality-of-life measurements seem particularly useful in clinical problems where the primary goal is to relieve symptoms and a wide variety of treatment options exist. Gastroesophageal reflux disease (GERD) is a common problem that fits this description. It is a disease with a broad spectrum of symptom severity ranging from the occasional water brash to possibly adenocarcinoma. Accordingly, there are a variety of treatment options ranging from simple lifestyle changes to surgery. The problem has been assessing the efficacy of these treatments objectively. Short of treatments for the most severe complications of reflux, such as stricture, bleeding, and cancer, most treatments have been directed at alleviating symptoms. However, no uniform system has been developed to measure symptom severity. Traditional measures of symptom outcome have been the qualitative

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Table I. Gastroesophageal Reflux Disease–Health-Related Quality-of-Life Scale

Scoring Scale

0 = No symptoms
1 = Symptoms noticeable but not bothersome
2 = Symptoms noticeable and bothersome but not every day
3 = Symptoms bothersome every day
4 = Symptoms affect daily activities
5 = Symptoms are incapacitating—unable to do daily activities

Questions About Symptoms ***(circle one for each question)***

1.	How bad is your heartburn?	0 1 2 3 4 5
2.	Heartburn when lying down?	0 1 2 3 4 5
3.	Heartburn when standing up?	0 1 2 3 4 5
4.	Heartburn after meals?	0 1 2 3 4 5
5.	Does heartburn change your diet?	0 1 2 3 4 5
6.	Does heartburn wake you from sleep?	0 1 2 3 4 5
7.	Do you have difficulty swallowing?	0 1 2 3 4 5
8.	Do you have pain with swallowing?	0 1 2 3 4 5
9.	If you take medication, does this affect your daily life?	0 1 2 3 4 5
10.	How satisfied are you with your present condition?	Satisfied Neutral Dissatisfied

scale of "excellent, good, fair, poor," and so forth. The problem is that this type of reporting is prone to bias and misinterpretation.

The Gastroesophageal Reflux Disease–Health-Related Quality-of-Life (GERD-HRQL) scale was developed and tested to address these issues.[5] It is a disease-specific instrument that was developed with an emphasis on reliability, validity, practicality, and responsiveness to the effects of treatments. However, it has not been tested against a "gold standard" quality-of-life scale. The SF-36[6] is a well-tested and validated generic quality-of-life instrument. It has been used to assess outcomes in GERD.[7] The purpose of this study was to compare the GERD-HRQL against this gold standard.

MATERIAL AND METHODS

Forty-three patients who were being treated medically (n = 25) or who had already undergone surgical therapy (n = 18) for GERD were asked to complete both the GERD-HRQL and the SF-36 questionnaires. The patients were treated by a group of six general surgeons, and all surgeons had patients in the medically and surgically treated groups. Patients were also asked to rate their present level of satisfaction with their GERD symptoms. The questionnaires were mailed to each patient. It has been previously shown that mailed questionnaires are as valid as in-person responses or replies obtained by telephone.[8] The patients were provided with stamped, self-addressed envelopes to be mailed back to the service secretary. The secretary then provided the author with the data for statistical analysis.

The SF-36 measures eight domains of quality-of-life including physical functioning (PF), role-physical (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role-emotional (RE), and mental health (MH). The worst possible score is zero and the best possible score is 100, with 100 being the optimal level of function. The GERD-HRQL is a nine-item, Likert-type questionnaire that measures the severity of heartburn, heartburn lying down, heartburn standing up, heartburn after meals, change of diet, nocturnal heartburn, dysphagia, odynophagia, and effects of medications (Table I). The GERD-HRQL score is derived from simply adding the scores from each of the nine items. The best possible score is zero (i.e., asymptomatic in each item) and the worst possible score is 45 (incapacitated in each item).

In addition, patients were asked the following: (1) Which questionnaire do you like best? (2) Which questionnaire was easier to understand? (3) Which questionnaire was more reflective of the problems you have with reflux disease? (4) Given the choice, which questionnaire would you rather fill out?

Statistical analysis was performed using stepwise logistic regression analysis, linear regression analysis, and Student's *t* test. Bonferroni's correction was used because of multiple tests of significance.

Table II. Results of regression analysis of GERD-HRQL score with domains of the SF-36

Regression equation	*P* value	*r* (correlation coefficient)
GERD = 17.9-0.07PF	0.4	−0.14
GERD = 15.0-0.06RP	0.2	−0.23
GERD = 18.9-0.12BP	0.1	−0.27
GERD = 16.9-0.10GH	0.2	−0.23
GERD = 12.7-0.004VT	1.0	−0.01
GERD = 12.9-0.005SF	0.8	−0.05
GERD = 16.1-0.06RE	0.2	−0.22
GERD = 18.3-0.07MH	0.3	−0.19

PF = physical functioning; RP = role-physical; BP = bodily pain; GH = general health; VT = vitality; SF = social functioning; RE = role-emotional; MH = mental health.

RESULTS

When analyzing the GERD-HRQL and the eight domains of the SF-36, multivariate analysis showed that the only significant predictor of patient satisfaction was the GERD-HRQL ($P < 0.00001$; Figs. 1 and 2). There were statistically significant differences between satisfied and dissatisfied patients in the SF-36 domains of PF ($P = 0.004$) and GH ($P = 0.006$) but not in the other six domains. There were no correlations found by linear regression analysis between the GERD-HRQL score and any of the domains of the SF-36 (Table II). A total of 59% of patients preferred the GERD-HRQL, 62% felt it was easier to understand, 86% felt it better reflected their symptoms, and 67% said they would rather use it over the SF-36 (Fig. 3).

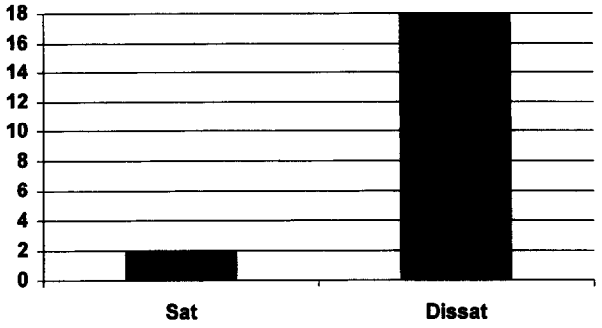

Fig. 1. Comparison of total GERD-HRQL scores for satisfied *(Sat)* and dissatisfied *(Dissat)* patients. The best possible score (i.e., completely asymptomatic) is 0; the worst possible score is 45 (i.e., incapacitated for all nine items). These scores were analyzed using multivariate analysis with the scores of the eight domains of the SF-36 (see Fig. 2).

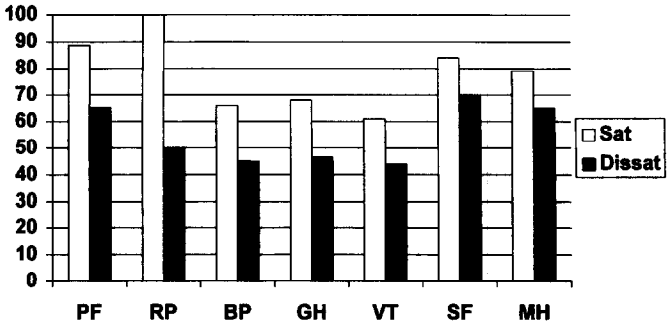

Fig. 2. Comparison of SF-36 scores for satisfied *(Sat)* and dissatisfied *(Dissat)* patients. No score was an independent predictor of patient satisfaction. By univariate analysis, the differences between satisfied and dissatisfied patients in the domains of physical functioning *(PF)* and general health *(GH)* were statistically significant (for other abbreviations, see Table II).

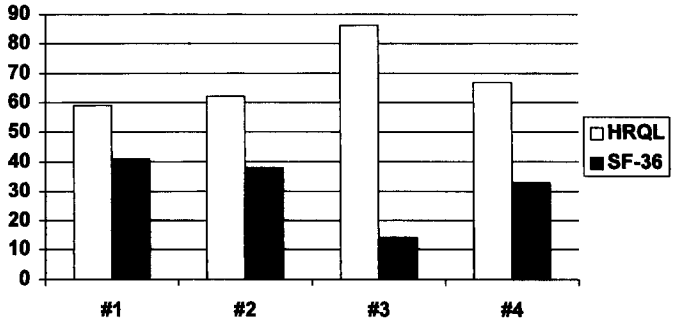

Fig. 3. Comparison of patients' answers to preference questions (see text for details).

DISCUSSION

Despite the tremendous interest in measuring quality of life, it is not a straightforward process.[9-11] It is important to determine what it is that is to be measured and what purpose the measurement is going to serve.[12] Once this is established, choices can be made as to the type of instrument. For example, generic quality-of-life scales are better suited for comparing outcomes across different populations and interventions, whereas disease-specific instruments may be better for assessing the responsiveness of a particular disease to a particular treatment.[13] Prior to evaluation of the GERD-HRQL, there had been no rigorous test instrument dedicated specifically to measuring the symptoms of reflux disease.[14] Thus in designing the GERD-HRQL instrument, emphasis was placed on reliability, validity, responsiveness, and practicality.[5]

Because the whole area of rigorous quality-of-life measurement in surgical disease is new, many "homegrown" instruments are being developed but not adequately tested. Therefore to further test the appropriateness of the GERD-HRQL for the purpose of measuring reflux-related symptom severity, it was evaluated against what is widely considered a "gold standard" with respect to quality-of-life surveys, the SF-36, a generic instrument. The measurement of satisfaction as an end point was chosen because the goal of both medical and surgical therapy for GERD is to relieve the patient's symptoms. Because symptoms are "patient centered,"[15] no surrogate physiologic or clinical end point is adequate.[5] Moreover, Trus et al.[16] and Velanovich and Karmy-Jones[17] have shown that there does not seem to be a relationship between physiologic measures of GERD and patient-perceived symptoms. This study shows that the GERD-HRQL is superior to the SF-36 for measuring symptom severity of GERD.

The SF-36 measures eight domains of health-related quality of life using 36 items. These include physical functioning (PF)—limitations related to health problems; role-physical (RP)—limitations in the ability of the patient to perform activities they find important because of physical limitations; bodily pain (BP)—physical pain suffered by the patient; role-emotional (RE)—the emotional effects of the patient's limitations in daily activities; general health (GH)—the patient's perceptions of his or her health; vitality (VT)—the patient's sense of vigor or malaise; social functioning (SF)—the ability of the patient to participate in social activities; and mental health (MH)—the patient's level of depression, anxiety, and so forth. Although none of these measures specifically address GERD symptom severity, clearly GERD can affect *all* of these domains of quality of life. Fig. 2 demonstrates that dissatisfied patients had lower scores in all domains compared to satisfied patients, even though statistical significance was reached in only the domains of PF and GH. Moreover, Hunter et al.[7] have demonstrated that SF-36 scores improved after laparoscopic antireflux surgery. This implies that GERD can affect the broad range of health-related quality of life. Clearly, however, the GERD-HRQL and the SF-36 measure different aspects of quality of life because there is *no* correlation between the total GERD-HRQL score and any of the SF-36 domain scores. Therefore other aspects of these instruments, such as responsiveness to treatment and practicality, become important.

This raises the question of the use of generic instruments for surgical diseases. Generic instruments have the advantage of being applicable to a broad range of diseases. However, for those diseases that affect a narrow spectrum of the patient's quality of life, a generic instrument may be too insensitive to measure changes brought about by treatment. In choosing an instrument to measure the effects of treatment, the responsiveness and sensitivity to change are very important aspects of the instrument.[9,12,18] In a study comparing a generic quality-of-life scale (the Psychological General Well-Being Index) with a more disease-specific scale (the Gastrointestinal Symptom Rating Scale in patients with upper gastrointestinal diseases), the disease-specific scale was more sensitive to change and could better discriminate between patient groups.[19] In addition, the SF-36 could not measure the difference between pretreatment and posttreatment quality of life in patients who had had hernia surgery[20] or breast cancer surgery.[21] This study demonstrated that it has poor discriminatory powers for satisfied and dissatisfied patients with GERD. Therefore some diseases that are amenable to surgical therapy but affect only a limited aspect of the patient's quality of life may not be best measured by such generic scales.

In conclusion, this study demonstrates that the GERD-HRQL, a short, disease-specific quality-of-life scale, is a better predictor of patient-perceived symptoms and satisfaction than the SF-36.

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REFERENCES

1. Rutkow IM. The importance of socioeconomic issues in surgical outcomes: What is a relevant end point? Eur J Surg 1995;161:545-548.
2. Kreder HJ, Wright JG, McLeod R. Outcome studies in surgical research. Surgery 1997;121:223-225.
3. Temple PC, Travis B, Sachs L, Strassers S, Choban P, Flancbaum L. Functioning and well-being of patients before and after elective surgical procedures. J Am Coll Surg 1995; 181:17-25.
4. Velanovich V. The use of a generic quality of life scale (SF-36) in a general surgical practice. Presented at the Sixty-Fifth Annual Scientific Meeting of the Southeastern Surgical Congress, Nashville, Tenn., February 3-5, 1997.
5. Velanovich V, Vallance SR, Gusz JR, Tapia FV, Harkabus MA. Quality of life scale for gastroesophageal reflux disease. J Am Coll Surg 1996;183:217-224.
6. The Medical Outcomes Trust. The SF-36 Health Survey. Boston: Medical Outcomes Trust, Inc., 1992.
7. Hunter JG, Trus TL, Branum GD, Waring JP, Wood WC. A physiologic approach to laparoscopic fundoplication for gastroesophageal reflux disease. Ann Surg 1996;223:673-687.
8. McHorney CA, Kosinski M, Ware JE Jr. Comparisons of the costs and quality of norms for the SF-36 health survey collected by mail versus telephone interview: Results from a national survey. Med Care 1994;32:551-567.
9. Guyatt GH, Feeny DH, Patrick DL. Measuring health-related quality of life. Ann Intern Med 1993;118:622-629.
10. Wilson IB, Cleary PD. Linking clinical variables with health-related quality of life: A conceptual model of patient outcomes. JAMA 1995;273:59-65.
11. Guyatt GH, Cook DJ. Health status, quality of life, and the individual. JAMA 1994;272:630-631.
12. Testa MA, Simonson DC. Assessment of quality of life outcomes. N Engl J Med 1996;334:835-840.
13. Patrick DL, Deyo RA. Generic and disease-specific measures in assessing health status and quality of life. Med Care 1989; 27(Suppl):S217-S232.
14. Glise H. Quality of life and cost of therapy in reflux disease. Scand J Gastroenterol 1995;30(Suppl 210):38-42.
15. Gill TM, Feinstein AR. A critical appraisal of quality of life measurements. JAMA 1994;272:619-626.
16. Trus TL, Laycock WS, Branum GD, Waring JP, Hunter JG. Quality of life scores correlate poorly with subjective and objective measurements of gastroesophageal reflux. Presented at the Thirty-Eighth Annual Meeting of The Society for Surgery of the Alimentary Tract, Washington, D.C., May 11-14, 1997.
17. Velanovich V, Karmy-Jones R. Measuring gastroesophageal reflux disease: Relationship between the health-related quality of life score and physiologic parameters. Presented at the Fortieth Annual Meeting of the Midwest Surgical Association, Huron, Ohio, August 11, 1997.
18. Fitzpatrick R, Ziebland S, Jenkinson C, Mowat A, Mowat A. Importance of sensitivity to change as a criterion for selecting health status measures. Qual Health Care 1992;1:89-93.
19. Dimeas E. Methodological aspects of evaluation of quality of life in upper gastrointestinal diseases. Scand J Gastroenterol 1993;29(Suppl 199):18-21.
20. Burney RE, Jones KR, Coon JW, Blewitt DK, Herm A. Core outcome measures for inguinal hernia repair. Surg Forum 1996;47:627-630.
21. Wapnir IL, Cody RP, Greco RS. Superior quality of life following lumpectomy—Axillary dissection in patients with stage I and stage II breast cancer. Surg Forum 1996;47:635-637.

Glucagon-Like Peptide 2 Is a Potent Growth Factor for Small Intestine and Colon

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Factors that stimulate gut mucosal proliferation may be beneficial during periods of gut disuse or atrophy. Recently glucagon-like peptide 2 (GLP-2) has been shown to stimulate small bowel growth. The purpose of our study was to compare the trophic effects of GLP-2 with those of neurotensin (NT), a potent gut trophic factor. Mice were randomized to receive either GLP-2, NT, or saline solution (control) for 10 days. The mice were killed on day 11, at which time the jejunum, ileum, and colon were removed, weighed, and DNA and protein content measured. Mice treated with GLP-2 showed a significant increase in the weight of the jejunum, ileum, and colon compared to both control and NT-treated mice. DNA content, a marker of cellular hyperplasia, was significantly increased in the small bowel and colon by treatment with GLP-2 and NT compared to control tissues. Small intestinal protein content, an indicator of cellular hypertrophy, was significantly increased by GLP-2 compared to both NT and control; protein content of the colon was greater in each of the treatment groups compared with control mice. We have demonstrated, for the first time, that GLP-2 stimulates colonic growth. In addition, GLP-2 is a potent trophic factor of normal small intestine with proliferative effects that are equal to or greater than those of NT. Administration of GLP-2 may be useful clinically to enhance small intestinal regeneration and adaptation during periods of disease and in the early phases of the short bowel syndrome. (J GASTROINTEST SURG 1998;2:146-150.)

The complex regulation of intestinal mucosal growth involves a multitide of factors including the actions of luminal nutrients, pancreatobiliary secretions, and enterotrophic agents. The gut trophic factors identified to date represent nonintestinal and intestinal hormones and peptides that have a common ability to stimulate intestinal growth resulting in increases of both mucosal mass[1-3] and absorptive capacity.[4,5] On a cellular level, intestinal growth factors stimulate both biosynthetic and proliferative processes, as demonstrated by measurable increases in DNA and protein synthesis and mitotic activity,[6] and are crucial to the maintenance of both structural and functional gut integrity.[7] A number of gut hormones or peptides, including peptide YY,[8,9] bombesin,[10] and neurotensin (NT),[11-13] have been shown to significantly stimulate gut mucosal growth both during periods of disuse (gut atrophy) and in the normal intestine. Recently the gut hormone glucagon-like peptide 2 (GLP-2) has been identified as a novel growth factor for the small intestine.[14]

GLP-2, a 33-amino acid member of a family of proglucagon-derived peptides that also includes enteroglucagon, is localized to and secreted from L-type enteroendocrine cells (concentrated in the ileum and proximal colon) following tissue-specific cleavage of a proglucagon precursor.[15] Prior to the identification of other proglucagon-derived peptides, enteroglucagon was thought to be the L-cell–secreted peptide that produced the marked trophic effects observed in the gut.[16] Recent findings suggest that GLP-2, and not enteroglucagon, may be the factor responsible for intestinal regeneration and adaptation after massive small bowel resection[14]; however, the role of

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GLP-2 in intestinal adaptation, its efficacy relative to other enterotrophic factors, and its effects on other parts of the gastrointestinal tract (e.g., the colon) have not been clearly defined. The purpose of our present study was to compare the effects of GLP-2 on proliferation of the small bowel and colon with that of NT, a potent gut trophic factor.[11-13,17,18]

MATERIAL AND METHODS

Animals

Ten-week-old female athymic nude mice (Balb/c, 23 to 27 g; Life Science, St. Petersburg, Fla.) were housed under specific pathogen-free conditions in a temperature-controlled (22° C) isolation unit with 12-hour light/dark cycles, in accordance with National Research Council recommendations.[19] The mice were fed a standard chow (autoclavable Rodent Chow No. 5010; Ralston Purina, St. Louis, Mo.) and sterile water, both given ad libitum.

Peptide Preparation

A stock solution of GLP-2 (a generous gift from Biomeasure, Inc., Medford, Mass.) or NT (Peninsula Laboratories, Belmont, Calif.) was prepared by first dissolving the amount of GLP-2 or NT needed for the study in 1 ml of sterile water with 1% (weight/volume) bovine serum albumin (BSA) (Sigma Chemical, St. Louis, Mo.) and then diluted to the required concentration with saline solution containing 1% BSA. Equal portions of this solution, sufficient for a single subcutaneous injection of all animals of a given group, were stored in plastic vials at −20° C. Saline solution containing 1% BSA (control) was aliquoted and stored at −20° C. To prolong absorption, control or peptide solutions were mixed 1:4 (volume:volume) with 16% (weight/volume) hydrolyzed gelatin (Sigma Chemical) prior to administration.

Experimental Protocol

Mice (n = 6 to 7/group) were weighed and randomized into three groups to receive subcutaneous injections of either GLP-2 (1.75 mg/kg twice a day), NT (600 μg/kg three times a day), or saline solution with BSA (control) for 10 days. The dosage of GLP-2 was selected based on the findings of Drucker et al.[14] demonstrating maximal trophic effects at this approximate concentration. The dosage of NT used in this study produces a maximal trophic effect on the gut of rats[12,13,17,18] and mice.[20] The last injections of GLP-2 and NT were given 12 hours and 8 hours, respectively, before sacrifice. At sacrifice (day 11), mice were weighed and the small intestine (from the ligament of Treitz to the cecum) and colon were harvested separately. The mesentery was trimmed and the luminal contents removed by gentle manual stripping and flushing with cold saline solution. The small intestine was bisected with the proximal half designated jejunum and the distal half designated ileum. All segments were blotted dry, weighed, and immediately frozen at −70° C until assayed for DNA and protein content.

DNA and Protein Determination

Full-thickness tissues were thawed and homogenized (Polytron, Kinematica GmbH, Kriens-Luzem, Switzerland). DNA content was measured by the Burton[21] modification of the diphenylamine procedure with calf thymus DNA used as the standard. Protein content was determined by the method of Lowry et al.,[22] with BSA as the standard.

Statistical Analysis

Intestinal weight, DNA, and protein content were expressed as mean ± standard error of the mean and analyzed using one-way classification analysis of variance with Fisher's least significant difference for multiple comparisons. A P value <0.05 was considered significant.

RESULTS

One mouse in the control group had an isolated anatomic abnormality of the colon; this colon was not used for further analysis. There were no differences in initial or final body weights in the groups treated with either GLP-2, NT, or saline solution (control).

GLP-2 Stimulates Colonic Growth

Treatment with GLP-2 significantly increased colon weight (89%), DNA content (87%), and protein content (108%) compared to control values (Fig. 1). Similar to our previous findings in the rat,[18] NT significantly stimulated all indices of colonic growth. Both agents produced similar increases in DNA and protein content; however, GLP-2 produced a greater increase in colonic weight compared to NT. There was no evidence from histologic sections of either edema or smooth muscle hypertrophy to account for differences in colonic weight between animals treated with GLP-2 or NT (data not shown). These findings are the first to demonstrate a trophic effect of GLP-2 on the normal colon.

GLP-2 Is a More Potent Enterotrophic Factor Than NT

In the jejunum, GLP-2 produced significant increases in weight (60%), DNA (56%), and protein content (93%) compared to control; treatment with NT increased DNA content by 42% and protein content by 38%, but weight was not significantly increased (Fig. 2). Comparison of the groups treated with GLP-2 or NT demonstrated significant increases in jejunal weight and protein content in the GLP-2–treated group. In the ileum, both GLP-2 and NT increased weight by 69% and 29%, DNA content by

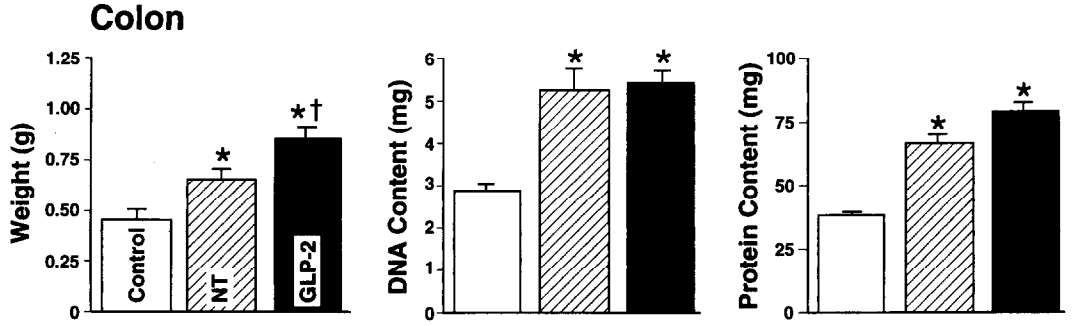

Fig. 1. Colonic weight, DNA, and protein content from mice treated with injection of saline solution (control; open bar), NT (600 μg/kg; single-hatched bar), or GLP-2 (1.75 mg/kg; closed bar). * = $P < 0.05$ vs. control; † = $P < 0.05$ vs. NT.

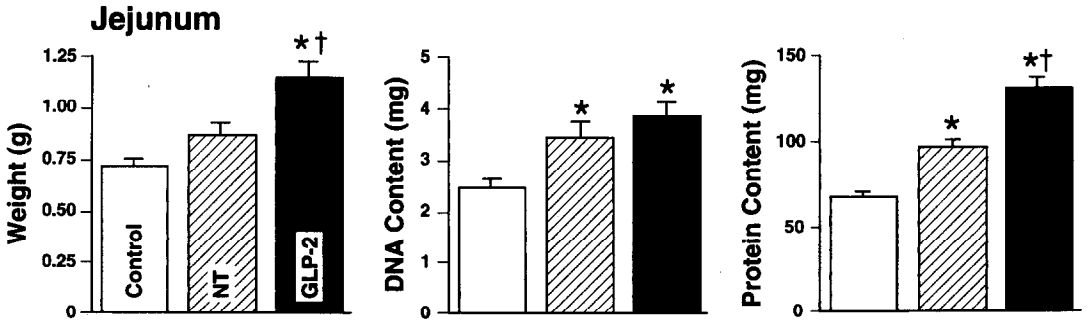

Fig. 2. Jejunal weight, DNA, and protein content from mice treated with injection of saline solution (control; open bar), NT (600 μg/kg; single-hatched bar), or GLP-2 (1.75 mg/kg; closed bar). * = $P < 0.05$ vs. control; † = $P < 0.05$ vs. NT.

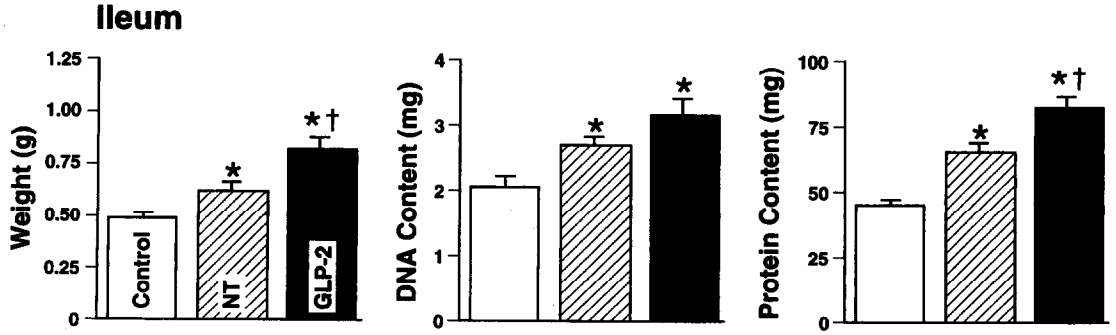

Fig. 3. Ileal weight, DNA, and protein content from mice treated with injection of saline solution (control; open bar), NT (600 μg/kg; single-hatched bar), or GLP-2 (1.75 mg/kg; closed bar). * = $P < 0.05$ vs. control; † = $P < 0.05$ vs. NT.

53% and 31%, and protein content by 82% and 44%, respectively (Fig. 3). In addition, treatment with GLP-2 produced significant increases in ileal weight and protein content compared to treatment with NT.

Taken together, these results confirm previous findings that both GLP-2[14] and NT[12,13,17,18] are significant enterotrophic factors. Moreover, the effects of GLP-2 on growth of the small bowel appear to be more pronounced than those of NT.

DISCUSSION

We have identified, for the first time, that GLP-2 significantly stimulates colonic growth. Similar to NT, which we have previously shown stimulates colonic mucosal growth in rats,[18] GLP-2 significantly increased indices of both mucosal hypertrophy (weight and protein content) and mucosal hyperplasia (DNA content), thus identifying GLP-2 as a potent growth factor for the colon. Compared to NT, GLP-2 significantly increased colonic weight without increasing DNA synthesis (DNA content), suggesting that GLP-2 has a more pronounced effect on colonic mucosal hypertrophy than on hyperplasia. In addition, GLP-2 demonstrated differential effects on mucosal hypertrophy as noted by an increase in colonic weight, but not protein content, as compared to the effects of NT. Colonic growth factors may be clinically useful in preserving colonic integrity and function during periods of disease. For example, Chu et al.[23] demonstrated that administration of bombesin, another enterotrophic gut peptide, significantly increased survival in a rat model of severe enterocolitis induced by methotrexate. This increased survival appeared to be due to the proliferative effects of bombesin on the intestinal mucosa. Treatment with enterotrophic agents, such as GLP-2, may eventually prove beneficial in preventing the often incapacitating colitis noted in some cancer patients receiving chemotherapy.

We have confirmed and extended the previous findings of GLP-2–induced growth of the small intestine.[14] Our results demonstrate that GLP-2 increases small intestinal weight by 60% to 69% and induces both hyperplasia and hypertrophy of the small bowel mucosa compared to control animals treated with saline solution. In addition, we have compared the trophic effect of GLP-2 with that of NT, a potent growth factor for the small bowel and colon.[11-13,17,18,24] Using dosages of NT (600 μg/kg) and GLP-2 (1.75 mg/kg) that have previously been shown to produce maximal intestinal growth in rats or mice,[12-14,17] GLP-2 produced significant increases in jejunal and ileal weight and protein content, but not in DNA content, compared to NT. This suggests that GLP-2, compared to NT and similar to its role in the growth of the colon, is preferentially a hypertrophic agent of the small bowel. Taken together, these results establish that GLP-2 is a potent enterotrophic agent and suggest that GLP-2 (at its maximal effective dosage) is a more powerful gut trophic factor than NT.

Administration of intestinal growth factors may be clinically useful to treat malabsorptive states of the small bowel that are due to a loss of normal mucosa (e.g., Crohn's disease) and to prevent the gut mucosal hypoplasia or atrophy that occurs commonly in patients on long-term total parenteral nutrition (TPN) or elemental diets.[25] Furthermore, enterotrophic agents may be useful during the early stages following massive small bowel resection to ameliorate or prevent the debilitating sequelae of the short bowel syndrome. In addition, analogous to the current clinical trials evaluating the administration of growth hormone and glutamine in patients dependent on TPN,[26] GLP-2, specifically, may represent an alternative or an adjunct to growth hormone to increase the mucosal mass and absorptive capacity of the gut remnant, allowing patients to become independent of TPN. Future studies will determine both the optimal dosage and treatment schedule for GLP-2 and whether GLP-2, alone or in combination with other factors such as glutamine, may be useful in the treatment of certain pathologic states of the small bowel such as Crohn's disease, the short bowel syndrome, and atrophy secondary to disuse.

Athymic nude mice were used in this study to correlate the effects of exogenous administration of GLP-2 with ongoing studies evaluating the trophic effects of endogenous GLP-2 produced from an endocrine tumor (STC-1). Similar to our previous results evaluating the effect of NT in nude mice,[20] we demonstrate that the athymic state in mice does not prevent stimulation of intestinal growth in response to trophic agents. In fact, the magnitude of intestinal growth in response to GLP-2 in our study was similar to that of Drucker et al.[14] who used non–immunologically compromised CD-1 mice.

CONCLUSION

Our findings suggest that GLP-2 is the most effective enterotrophic gut hormone or peptide identified to date. The proliferative effects produced by a maximal dose of GLP-2, noted in both the small bowel and colon, were equal to or greater than that of the maximal trophic dose of NT. Intestinal trophic agents such as GLP-2 may be useful in the treatment of pathologic states of both the small bowel and colon that result in a loss of overall mucosal surface area.

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REFERENCES

1. Williamson RC. Intestinal adaptation. N Engl J Med 1978; 298:1393-1402, 1444-1450.
2. Dowling RH, Booth CC. Structural and functional changes following small intestinal resection in the rat. Clin Sci 1967; 32:139-149.
3. Shin CS, Chaudhry AG, Khaddam MH, Penha PD, Dooner R. Early morphologic changes in the intestine following massive resection of the small intestine and parenteral nutrition therapy. Surg Gynecol Obstet 1980;15:246-250.
4. Flint JM. The effect of extensive resection of the small intestine. Bull John Hopkins Hosp 1912;13:127-144.
5. Dowling RH, Booth CC. Functional compensation after small-bowel resection in man. Lancet 1966;1:146-147.
6. Johnson LR. Trophic action of gastrointestinal hormones. In Thompson JC, ed. Gastrointestinal Hormones. A Symposium. Austin: University of Texas Press, 1975, pp 215-230.
7. Bristol JB, Williamson RCN. Nutrition, operations, and intestinal adaptation. JPEN J Parenter Enteral Nutr 1988;12: 299-309.
8. Chance WT, Zhang X, Balasubramaniam A, Fischer JE. Preservation of intestine protein by peptide YY during total parenteral nutrition. Life Sci 1996;58:1785-1794.
9. Gomez G, Zhang T, Rajaraman S, Thakore KN, Yanaihara N, Townsend CM Jr, Thompson JC, Greeley GH Jr. Intestinal peptide YY: Ontogeny of gene expression in rat bowel and trophic actions on rat and mouse bowel. Am J Physiol 1995; 268:G71-G81.
10. Chu KU, Evers BM, Ishizuka J, Townsend CM Jr, Thompson JC. Role of bombesin on gut mucosal growth. Ann Surg 1995; 222:94-100.
11. Wood JG, Hoang HD, Bussjaeger LJ, Solomon TE. Neurotensin stimulates growth of small intestine in rats. Am J Physiol 1988;255:G813-G817.
12. Evers BM, Izukura M, Townsend CM Jr, Uchida T, Thompson JC. Differential effects of gut hormones on pancreatic and intestinal growth during administration of an elemental diet. Ann Surg 1990;211:630-638.
13. Evers BM, Izukura M, Townsend CM Jr, Uchida T, Thompson JC. Neurotensin prevents intestinal mucosal hypoplasia in rats fed an elemental diet. Dig Dis Sci 1992;37:426-431.
14. Drucker DJ, Ehrlich P, Asa SL, Brubaker PL. Induction of intestinal epithelial proliferation by glucagon-like peptide 2. Proc Natl Acad Sci USA 1996;93:7911-7916.
15. Conlon JM. Proglucagon-derived peptides: Nomenclature, biosynthetic relationships and physiological roles. Diabetologia 1988;31:563-566.
16. Lund PK, Ulshen MH, Rountree DB, Selub SE, Buchan AM. Molecular biology of gastrointestinal peptides and growth factors: Relevance to intestinal adaptation. Digestion 1990;46:66-73.
17. Izukura M, Evers BM, Parekh D, Yoshinaga K, Uchida T, Townsend CM Jr, Thompson JC. Neurotensin augments intestinal regeneration after small bowel resection in rats. Ann Surg 1992;215:520-527.
18. Evers BM, Izukura M, Chung DH, Parekh D, Yoshinaga K, Greeley GH Jr, Uchida T, Townsend CM Jr, Thompson JC. Neurotensin stimulates growth of colonic mucosa in young and aged rats. Gastroenterology 1992;103:86-91.
19. Committee on Care and Use of the "Nude" Mouse. Guide for the care and use of the nude (thymus-deficient) mouse in biomedical research. ILAR News 1976;19:M1-20.
20. Sumi S, Evers BM, Townsend CM Jr, Yoshinaga K, Uchida T, Murakami M, Sato K, Ishizuka J, Thompson JC. Comparative effects of neurotensin and neuromedin N on growth of human pancreatic cancer, MIA PaCa-2. Surg Oncol 1993;2:267-272.
21. Burton K. A study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochemistry 1956;162:315-323.
22. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-275.
23. Chu KU, Higashide S, Evers BM, Rajaraman S, Ishizuka J, Townsend CM Jr, Thompson JC. Bombesin improves survival from methotrexate-induced enterocolitis. Ann Surg 1994;220:570-577.
24. Evers BM, Izukura M, Rajaraman S, Parekh D, Thakore K, Yoshinaga K, Uchida T, Townsend CM Jr, Thompson JC. Effect of aging on neurotensin-stimulated growth of rat small intestine. Am J Physiol 1994;267:G180-G186.
25. Shanbhogue LKR Molenaar JC. Short bowel syndrome: Metabolic and surgical management. Br J Surg 1994;81:486-499.
26. Byrne TA, Persinger RL, Young LS, Ziegler TR, Wilmore DW. A new treatment for patients with short-bowel syndrome. Growth hormone, glutamine, and a modified diet. Ann Surg 1995;222:243-255.

Complete Hepatic Resection of Metastases From Leiomyosarcoma Prolongs Survival

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Although liver resection has been shown to prolong survival in selected patients with metastases from colorectal cancer, the benefit for other metastatic tumors is unproved. To determine whether hepatic resection has a role in the management of metastatic leiomyosarcoma, medical records from 11 consecutive patients who underwent resection of isolated metastases from leiomyosarcoma between 1984 and 1995 were reviewed. All liver resections were for leiomyosarcomas originating in the viscera (n = 6) or retroperitoneum (n = 5). The average disease-free interval was 16 months. Five of 11 primary tumors were classified as low grade, whereas six were high grade. Hepatic resections included lobectomy or extended lobectomy (n = 4), segmentectomy and/or wedge resection (n = 5), and complex resection (n = 2). There were no operative deaths. Median survival of all patients after liver resection was 39 months. Patients who underwent complete resection of hepatic metastases (n = 6) had a significantly longer survival than those who had incomplete resections (n = 5) ($P = 0.03$, log-rank test). Furthermore, five of six patients who underwent complete resection are alive after hepatectomy with a median follow-up of 53 months. Therefore, in selected patients with isolated liver metastases from visceral and retroperitoneal leiomyosarcomas, complete resection of hepatic metastases results in prolonged survival. (J GASTROINTEST SURG 1998;2:151-155.)

Liver resection for hepatic metastatic disease has been shown to prolong survival in selected patients with some types of primary tumors. With metastases from colorectal cancer, liver resection results in a 5-year survival rate of 25% to 39%.[1-3] Similarly, resection of liver disease from metastatic neuroendocrine tumors has been reported to result in a 4-year survival rate of 74% and 5-year survival as high as 54%.[4,5] Although other primary malignancies occasionally develop isolated hepatic metastases, the benefit of surgical resection remains unproved.

Unlike soft tissue sarcomas of the extremity and trunk, which frequently develop isolated pulmonary metastases, visceral and retroperitoneal sarcomas often metastasize to the liver. In a recent series, 16% of all retroperitoneal and 62% of all visceral sarcomas developed metastases to the liver.[6] Gastrointestinal stromal tumors of smooth muscle origin, or leiomyosarcomas, account for virtually all visceral sarcomas, comprise approximately 25% of retroperitoneal sarcomas, and are the most common histologic subtype of sarcoma metastatic to the liver.[6,7] Although resection of isolated pulmonary metastases from other histologic types of soft tissue sarcoma has been shown to prolong survival,[8,9] the role of hepatic resection for metastatic leiomyosarcoma is unclear. Thus we reviewed our experience with liver resection for metastatic leiomyosarcoma to determine whether surgical resection resulted in prolonged survival.

MATERIAL AND METHODS

A combined retrospective and prospective database containing information about patients who underwent liver resection for malignant disease at the Johns Hopkins Hospital was utilized. Between 1984 and 1995, 241 patients were identified who underwent hepatic resection for metastatic disease. Of these, 11 patients had metastatic liver disease from leiomyosarcoma and form the basis of this report. Patients who

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underwent resection for primary leiomyosarcoma of the liver were excluded.

Results of all histologic examinations were evaluated by a single pathologist. Histologic grade (low grade [1], high grade [2 or 3]) was compared between primary tumor and liver metastases, and to the initial pathology report. The number of liver lesions and the size of the largest tumor were also recorded. Patients were grouped according to resection status either as completely or incompletely resected. Complete resections were those with negative gross and histologic surgical resection margins, and no signs of residual disease at the completion of the operation. Incomplete resections were those with positive histologic surgical margins and/or gross residual tumor remaining after resection.

Survival probability was calculated by the Kaplan-Meier method and survival curves were compared using the log-rank test. Student's t test and Fisher's exact test were used when appropriate. Statistical significance was defined as a P value of <0.05.

RESULTS

Liver Resection for Metastatic Disease

Between 1984 to 1995, 241 patients underwent liver resection for metastatic disease at The Johns Hopkins Hospital. Leiomyosarcoma (n = 11) was the third most common primary tumor, accounting for 4.5% of all liver resections for metastatic disease. The two most common types of resected metastatic disease were colorectal (75%) and neuroendocrine (7%).

Patient Demographics

The median age of the 11 patients who underwent hepatic resection for leiomyosarcoma was 57 years (range 30 to 69 years) as shown in Table I. Two patients were male and nine were female. The majority of the patients were white (n = 9), whereas two patients were black. There was no difference between completely resected and incompletely resected patients with regard to these demographic features.

Primary and Metastatic Tumor Characteristics

All 11 patients with metastatic leiomyosarcomas to the liver who underwent hepatic resection had primary retroperitoneal or visceral leiomyosarcomas (Table II). Five patients had retroperitoneal leiomyosarcomas, whereas the six visceral tumors included three gastric, two small intestinal, and one uterine/adnexal primary leiomyosarcoma. Five patients had low-grade (grade 1) and six patients had high-grade (grade 2 or 3) primary tumors. In one patient with a low-grade primary tumor, the hepatic metastasis was found to be high grade (grade 2) on rereview. In all other cases the primary tumor and the hepatic metastases were of identical grade.

There was no difference between patients undergoing complete and incomplete resections with regard to the primary tumor site or tumor grade (Table III). Among patients with complete resections (n = 6), three had retroperitoneal leiomyosarcomas, whereas three had visceral tumors. Among patients with incomplete resections, two had retroperitoneal tumors

Table I. Demographics for patients with metastatic leiomyosarcomas to the liver (n = 11)

Age (yr)	
Mean ± SEM	56 ± 3
Median	57
Range	30-69
Sex	
Male	2
Female	9
Race	
White	9
Black	2

SEM = standard error of the mean.

Table II. Leiomyosarcomas—Tumor characteristics and patient management

Site of primary leiomyosarcoma (n)	
Retroperitoneum	5
Gastric	3
Small intestine	2
Uterine/Adnexal	1
Primary tumor grade (n)	
Low grade	
Grade 1	5
High grade	
Grade 2	4
Grade 3	2
No. of liver lesions	
Mean	2.6
Range	1-6
Size of largest lesion (cm)	
Mean	3.8
Range	1.1-10
Hepatic resections	
Lobectomy or extended lobectomy	4
Segmentectomy ± wedge resection	5
Complex resection	2

n = number of patients.

Table III. Comparison of tumor characteristics between patients with complete vs. incomplete hepatic resections

	Complete (n = 6)	Incomplete (n = 5)
Primary tumor		
Site		
Retroperitoneal	3	2
Visceral	3	3
Grade		
Low	3	2
High	3	3
Metastatic disease		
Mean no. of lesions	2.4 (range 1-6)	2.7 (range 2-4)
Mean size of largest lesion (cm)	3.9 (range 1.1-8)	3.7 (range 1-10)
Operation		
% Major resection	67	40

and three had visceral tumors. Low-grade tumors were seen in three patients with complete resections and in two patients with incomplete resections. High-grade tumors were seen in three patients with complete resections and in three patients with incomplete resections.

The average interval between resection of the primary leiomyosarcoma and liver resection was 16 ± 4 months, with a range of 0 to 40 months. The majority of the patients had liver metastases detected within 10 months of the initial resection of the primary tumor. The average number of metastatic liver lesions in all patients was 2.6 (range 1 to 6), and the average size of the largest liver metastasis was 3.8 cm (range 1.1 to 10 cm) (see Table II). There was no difference in the number of liver lesions or the average size of the largest lesion between completely and incompletely resected patients (see Table III). In all cases hepatic lesions were metastases and not a direct extension into the liver from the primary tumor.

Patient Management

Of the 11 patients, none received adjuvant chemotherapy or radiation therapy after primary tumor resection. One patient received preoperative radiation and chemotherapy prior to liver resection, consisting of external beam radiation therapy (3000 cGy) to the liver, and chemotherapy consisting of adriamycin, dacarbazine, and etoposide. Three patients received adjuvant chemotherapy after liver resection. One patient received doxorubicin, dacarbazine, isfosfamide, and mesna (MAID), another received doxorubicin, dacarbazine, and etoposide, and the third received cytoxan and vincristine. Adjuvant therapy was initiated at the discretion of the physician who was treating each patient.

Hepatic resections consisted of lobectomy or extended lobectomy in four patients, anatomic segmentectomy with or without wedge resection in five patients, and complex nonanatomic resection in two patients (see Table II). Completely resected patients had a slightly higher percentage of major liver resections (see Table III). All patients were thought to be resectable preoperatively. Of the five patients with incomplete resections, three were thought to be complete but were found to have positive margins, and in the remaining two, only a small volume of unresectable residual disease was left behind.

Patient Outcomes

There were no operative deaths. Median follow-up was 53 months (range 23 to 98 months). The median survival of all 11 patients was 39 months. Five patients are currently alive at 23, 32, 37, 43, and 53 months, respectively, after hepatic resection. All five patients underwent complete resection of metastatic disease. Two of these patients (survival of 23 and 43 months, respectively) are currently disease free, whereas the remaining three are alive with disease. All three patients who received adjuvant chemotherapy after liver resection died. One of these patients had undergone complete resection (survival of 19 months), whereas the other two had had incomplete resections (survival of 22 and 24 months, respectively).

Patients were grouped according to resection status (complete = negative margins vs. incomplete = positive margins or residual disease) and analyzed by Kaplan-Meier and log-rank methods (Fig. 1). Patients with complete resections had a significantly longer survival than patients with incomplete resections ($P = 0.03$, log-rank test). Aside from resection status, no other variable was predictive of outcome in this se-

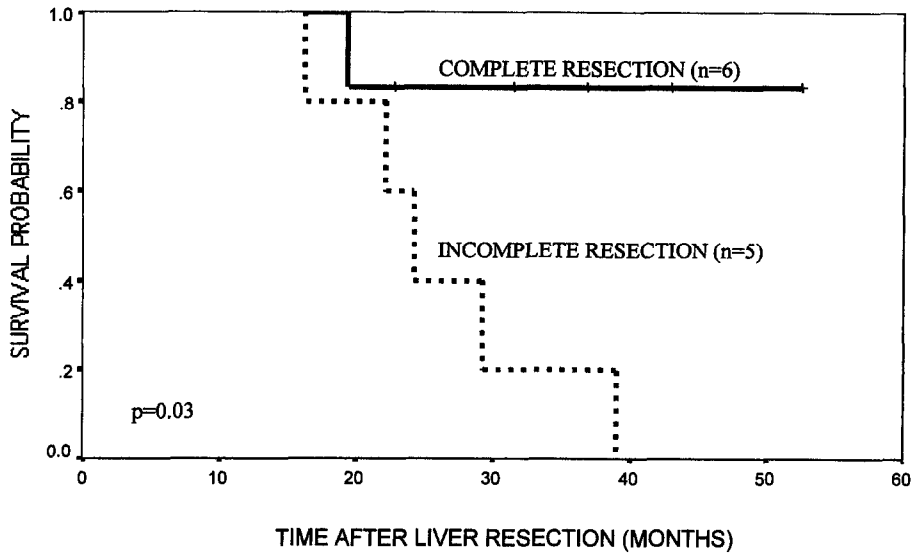

Fig. 1. Patient survival probability based on resection status. Complete = negative margins; incomplete = positive margins or residual disease after resection.

ries. There was no difference in survival between those with histologically high-grade tumors compared to those with low-grade tumors. Similarly, there was no difference in survival when patients were compared with regard to number of liver lesions, size of metastatic tumors, or extent of liver resection.

DISCUSSION

In our experience, metastatic disease from leiomyosarcoma was the third most commonly resected type of liver metastases but accounted for only 4.5% of liver resections for metastatic disease. We have found that the primary site of disease was found to include both visceral and retroperitoneal leiomyosarcoma. In this study we have shown in a highly select group of patients that complete resection of isolated liver metastases from visceral or retroperitoneal leiomyosarcoma can be performed safely and results in prolonged survival. Patients undergoing complete resection had a significantly longer survival compared to those undergoing incomplete resection. Furthermore, five of six patients who had complete resections are alive with a median follow-up of 53 months.

There have been several other series of hepatic resections for metastatic soft tissue sarcoma reporting combined experiences with various histologic types. Jacques et al.,[6] from Memorial Sloan-Kettering Cancer Center, reported that 7% of patients (n = 65) with soft tissue sarcomas developed liver metastases, the vast majority originating in the viscera. They found that only 14 (21.5%) of 65 patients with hepatic metastases underwent liver resection, and 13 of 14 were resected with histologically negative margins. Although the resected patients had a median survival of 30 months vs. 12 months in unresected patients, there was no statistical difference in survival and no patient in either group survived 5 years. In this report they reviewed the literature citing 34 other reported cases of resection of hepatic metastases from various types of soft tissue sarcomas.[6] By combining all known series, they determined in these 48 patients who underwent hepatic resection for soft tissue sarcoma metastases that the cumulative 5-year survival rate was 11%.[6] In another study, Hafner et al.[10] reported four patients who underwent liver resection for metastatic sarcoma and compared their survival to that of 19 similar patients who did not have surgery. Although the median survival was 54 months for resected patients vs. 20 months for unresected patients, this difference was not statistically significant. All of these reported studies have grouped all histologic types of metastatic sarcomas.

Our study specifically examines one histologic type, leiomyosarcoma, and comprises the largest series reported to date. In these other series of combined histologic types, leiomyosarcoma accounts for 85% of hepatic metastases from soft tissue sarcoma.[6] In contrast to our report, virtually all of the tumor primary sites of leiomyosarcoma reported in these other series were of visceral origin.[6,10] Primary visceral leiomyosarcomas have a better prognosis than

those of retroperitoneal origin, which are associated with a high rate of local recurrence.[6,11-14] Yet, based on this study, the prognosis after resection of liver metastases appears to be independent of whether the primary site was the viscera or the retroperitoneum.

Tumor grade has been shown to be highly predictive of prognosis and risk of metastatic disease in all soft tissue sarcomas.[7,15] We have shown, however, that histologic grade does not appear to correlate with survival in patients undergoing resection for already established metastatic disease. Although in other primary tumor types, such as colorectal cancer, histologic grade of liver metastases may portend a poorer prognosis, high-grade leiomyosarcoma does not appear to be a contraindication to resection. This study further demonstrates that even low-grade tumors have the ability to metastasize to the liver.

The median interval from resection of the primary tumor to liver metastasis was 10 months. Most of the patients presented with liver metastases within 10 months of their initial resection, which is similar to findings in other reported series.[6,10] Three patients presented with liver metastases at the time of their initial resection of the primary leiomyosarcoma. All of these patients underwent simultaneous hepatic resection in one stage. Two of these three patients are currently alive at 43 and 53 months, respectively, after resection. Therefore presentation with synchronous, resectable liver metastases does not appear to be a contraindication to surgical resection.

It is difficult to draw definitive conclusions from a study with so few patients. However, resectable hepatic metastases are uncommon in patients with leiomyosarcoma and a large series from a single institution is difficult to achieve. Clearly these patients constitute a highly select group. Because systemic chemotherapy or other treatment options have met with limited success in these patients, liver resection should be offered to patients with resectable liver metastases.

CONCLUSION

Patients who underwent complete hepatic resection of isolated metastases from leiomyosarcoma had a significantly longer survival than those who had an incomplete resection. Five of six patients who had a complete resection are now alive with a median follow-up of 53 months. These data demonstrate that in selected patients with isolated liver metastases from visceral and retroperitoneal leiomyosarcomas, complete resection of hepatic metastases results in prolonged survival.

REFERENCES

1. Hughes KS, Simon R, Songhorabodi S, Adson MA, Ilstrup DM, Fortner JG, Maclean BJ, Foster JH, Daly JM, Fitzherbert D. Resection of the liver for colorectal carcinoma metastases: A multi-institutional study of patterns of recurrence. Surgery 1986;100:278-284.
2. Rosen CB, Nagorney DM, Taswell HF, Helgeson SL, Ilstrup DM, van Heerden JA, Adson MA. Perioperative blood transfusion and determinants of survival after liver resection for metastatic colorectal carcinoma. Ann Surg 1992;216:493-505.
3. Scheele J, Stangl R, Altendorf-Hofmann A, Gall FP. Indicators of prognosis after hepatic resection for colorectal secondaries. Surgery 1991;110:13-29.
4. Kavolius J, Fong YF, Blumgart LH. Surgical resection of metastatic liver tumors. Surg Oncol Clin North Am 1996;5: 337-352.
5. Que FG, Nagorney DM, Batts KP, Linz LJ, Kvols LK. Hepatic resection for metastatic neuroendocrine carcinomas. Am J Surg 1995;169:36-43.
6. Jacques DP, Coit DG, Casper ES, Brennan MF. Hepatic metastases from soft-tissue sarcoma. Ann Surg 1995;221:392-397.
7. Shiu MH, Brennan MF. Surgical Management of Soft Tissue Sarcoma. Philadelphia: Lea & Febiger, 1989.
8. Gadd MA, Casper ES, Woodruff JM, McCormack PM, Brennan MF. Development and treatment of pulmonary metastases in adult patients with extremity soft tissue sarcoma. Ann Surg 1993;218:705-711.
9. van Geel AN, Pastorino U, Jauch KW, Judson IR, van Coevorden F, Buesa JM, Nielsen OS, Boudinet A, Tursz T, Schmitz PI. Surgical treatment of lung metastases: The European organization for research and treatment of cancer—Soft tissue and bone sarcoma group study of 225 patients. Cancer 1996;77:675-685.
10. Hafner GH, Rao U, Karakousis CP. Liver metastases from soft tissue sarcomas. J Surg Oncol 1995;58:12-16.
11. Herbsman H, Wetstein L, Rosen Y, Orces H, Alfonso AE, Iyer SK, Gardner B. Tumors of the small intestine. Curr Probl Surg 1980;17:121-182.
12. Pagtalunan N, Mayo CW, Dockerty MB. Primary malignant tumors of the small intestine. Am J Surg 1964;108:13-18.
13. Potter DA, Glenn J, Kinsella T, Glatstein E, Lack EE, Restrepo C, White DE, Seipp CA, Wesley K, Rosenberg SA. Pattern of recurrence in patients with high-grade soft tissue sarcomas. J Clin Oncol 1985;3:353-366.
14. McGrath PC, Neifield JP, Lawrence W, DeMay RM, Kay S, Horsley JS, Parker GA. Improved survival following complete excision of retroperitoneal sarcomas. Ann Surg 1984;200:200-204.
15. Torosian MH, Friedrich C, Godbold J, Hajdu SI, Brennan MF. Soft-tissue sarcoma: Initial characteristics and prognostic factors in patients with and without metastatic disease. Semin Surg Oncol 1988;4:413-419.

Is Outpatient Surgery Safe for the Higher Risk Patient?

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The trend toward outpatient surgery led to this study to determine the safety of elective outpatient laparoscopic surgery for the higher risk patient. One hundred consecutive higher risk patients from all patients scheduled for elective outpatient laparoscopic surgery were studied prospectively in a 256-bed community hospital. Seventeen percent of patients required admission. In each instance the need was readily evident in the perioperative observation period. Eighty-three percent of patients were stable and were successfully treated as outpatients. No patient who remained stable decompensated later, and none required readmission to treat complications resulting from outpatient status. The 2% readmission rate (for unrelated reasons) was comparable to the 2% readmission rate for low-risk patients. It was concluded that routine outpatient laparoscopic surgery is safe for elective higher risk patients. Problems requiring admission are readily evident during the period of observation and no patient who remains stable decompensates later. (J GASTROINTEST SURG 1998;2:156-158.)

Laparoscopy has enabled unprecedented shortening of hospital stay. This, combined with the economic constraints placed on health care delivery, has caused many laparoscopic operations to be changed to outpatient procedures.[1] Some surgeons have expressed doubt as to the wisdom of such practice, recommending that outpatient laparoscopy be reserved for selected healthy patients and advocating routine hospitalization of higher risk patients.[2]

Because hospitalization exposes patients to its own complications, this study was undertaken to determine whether routine outpatient laparoscopic surgery is a safe practice for patients who are deemed higher risk. To that end, the fate of 100 consecutive higher risk laparoscopic outpatients from the practice of the senior author (A.J.V.) was examined.

Both age and comorbidity have been demonstrated to be accurate predictors of risk,[3-5] so both were used to identify higher risk patients. The American Society of Anesthesiologists (ASA) classification of physical status was used to assess comorbidity, as it is relatively simple and has been shown to correlate well with outcome.[6] Laparoscopic cholecystectomy and laparoscopic inguinal hernia repair were selected as operations requiring approximately the same duration of general anesthesia and approximately the same extent of surgical dissection.

METHODS

One hundred consecutive higher risk patients scheduled for outpatient laparoscopic cholecystectomy or laparoscopic transabdominal preperitoneal inguinal hernia repair were studied, starting with the first such operation in March 1992. Table I presents the demographic data from these higher risk patients who underwent hernia repair and cholecystectomy. Higher risk was defined as age over 70 years or ASA classification of 3 or greater. Patient data were gathered prospectively. The ASA grading was noted independently by the anesthetist before surgery. Follow-up examination of all patients was carried out in the physician's office 2 weeks after surgery. For the purpose of this analysis, a retrospective review of all hospital charts a minimum of 3 months after surgery was added to document subsequent readmissions.

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Table I. Characteristics of higher risk patients

	Cholecystectomy	Hernia repair	Total
No. of patients	60	40	100
Mean age (yr)	67 (range 23-87)	72 (range 60-88)	69 (range 23-88)
ASA class ≥3 (No.)	43	23	66
% Male	33	88	55
Mean operative time (min)	36 (range 22-117)	48 (range 24-88)	41 (range 22-117)

All operations were performed by the same surgeon (A.J.V.) and assistant, under general inhalational anesthesia, at The Salvation Army Scarborough Grace Hospital, a 256-bed nonteaching community hospital on the periphery of a major urban center. Operative techniques and postoperative management have been described previously.[1] All 100 patients were seen in follow-up by the surgeon within 15 days of the operation. An outpatient is defined as a person who is discharged the same day that the operation takes place; an overnight stay, regardless of how short the duration, is classified as hospital admission. Differences were subjected to chi-square analysis and $P < 0.05$ was taken as an indication of significance.

RESULTS

The operations were performed over a 4½-year span; 100 higher risk patients were collected from a total pool of 431 consecutive elective outpatient laparoscopic operations. Comparison of higher and lower risk patients showed them to be similar on statistical analysis with respect to the following: rates of readmission, complications, and acute cholecystitis; ratios of hernia to gallbladder operations and males to females; duration of surgery; and length of hospital stay after admission for those admitted. Only admission and conversion rates were significantly higher for the higher risk patients. Table II shows these parameters in detail.

Of the 100 higher risk patients, age was a risk factor for 66 and comorbidity for 66; 32 patients had both risk factors. The reason for increased ASA classification was cardiovascular disease in 78% of the patients with comorbidity as a risk factor. Eighty-three patients were successfully treated as outpatients. Ten patients required conversion to open surgery and were admitted for this reason; seven other patients developed some form of instability during the perioperative period, also requiring admission to the hospital after surgery for reasons outlined in Table III. In each instance the instability was readily detected during the period of postoperative observation (average door-to-door time was 6 hours). Average length of stay was 2.8 days for the converted patients and 1.4 days for nonconverted patients. No higher risk patient, who was seen to be stable and therefore discharged as planned, developed any subsequent instability or complication because of his or her outpatient status. Table IV shows that the higher risk patients requiring admission to the hospital were similar to those treated successfully as outpatients, except for the conversion and readmission rates, which were higher in the admitted group. There were two readmissions from the group of admitted patients (pneumonia in one and a subphrenic abscess in the other, admitted 8 and 21 days postoperatively, respectively), whereas there were no readmissions from the outpatient group. This compares with seven readmissions (also 2%) in the lower risk patient pool.

Table II. Comparison of fate of higher risk and lower risk patients

	Higher risk	Lower risk	*P* value
No. of patients	100	331	—
% Admitted	17	6	<0.05
% Readmitted	2	2	>0.05
% Converted	10	2	<0.05

Table III. Reason for admission

Reason	No.
Chest pain	2
Congestive heart failure	1
Intraoperative hypotension	1
Malignant hyperthermia	1
Fever	1
Extenuating circumstances	1
Conversion to open surgery	10
TOTAL	17

Table IV. Comparison of higher risk outpatients and admitted patients

	Outpatients	Admitted	*P* value
No. of patients	83	17	—
Mean age (yr)	69 (range 23-88)	69 (range 24-87)	>0.05
Mean operative time (min)	40 (range 22-107)	51 (range 28-117)	>0.05
% Male	57	47	>0.05
% Converted	0	59	<0.05
% Readmitted	0	12	<0.05

DISCUSSION

This experience with 100 consecutive patients seems large enough to strongly suggest that routinely scheduling of higher risk laparoscopy patients for outpatient surgery is safe. Although the study represents only one physician's practice, the results parallel almost exactly a similar review of institutional practice from the senior author's hospital, where the higher risk cholecystectomy patients of all surgeons in the hospital were analyzed with similar findings.[7]

Quite understandably, these patients have more delicate pathophysiologic balances, which may be disturbed by the intervention, increasing the need for hospital admission. Indeed, previous reviews of outpatient cholecystectomy have demonstrated that factors predictive of need for hospital admission include the following: conversion, acute cholecystitis, longer operating time, age over 70 years, and ASA class 3 or higher.[8] Since this study limited itself exclusively to patients who met the last two criteria, a higher admission rate should not be surprising. Neither this nor any previous study has been able to identify patients in need of hospital admission with greater accuracy; that is, although higher risk patients do have a higher admission rate, the majority of apparently similar patients can be treated successfully as outpatients.

The major contributor to admission was the need for conversion to open surgery. A higher conversion rate in higher risk patients has been a consistent finding in other reviews.[1,7,8] The reason may be that older patients often present with conditions that make surgery more difficult because presumably their disease has been active for a longer period of time. There may also be a reluctance on the part of the surgeon to subject higher risk patients to prolonged anesthesia and a longer operative time, resulting in a lower conversion threshold.

Despite the higher admission rate for higher risk patients, it should be encouraging to any practitioner of outpatient surgery to learn that the patients who do develop some form of instability do so within the 6-hour period of perioperative observation allotted to outpatients and those who remain stable do not decompensate later. Furthermore, the readmission data reveal that postoperative admission in no way protects patients from subsequent problems, as evidenced by the fact that both readmissions occurred in the admitted group. Although Saunders et al.[2] recommend hospitalization of higher risk patients, their findings concur with ours—that is, the majority of complications observed by them did not seem preventable by overnight admission, as most of their minor complications became evident after 2 to 3 days and the major ones after 1 week.

These data add to the growing number of reports confirming the safety of "routine" outpatient surgery for the higher risk patient. Prior selection does not seem necessary, inasmuch as reasons for admission become evident perioperatively and patients who remain stable do not seem to decompensate later.

REFERENCES

1. Voitk AJ. Outpatient cholecystectomy. J Laparoendosc Surg 1996;6:79-81.
2. Saunders CJ, Leary BF, Wolfe BM. Is outpatient laparoscopic cholecystectomy wise? Surg Endosc 1995;9:1263-1268.
3. Wetchler BV. Outpatient anesthesia: The geriatric outpatient. Probl Anesth 1988;2:128-131.
4. Dawson B, Reed WA. Anaesthesia for adult surgical outpatients. Can Anaesth Soc J 1980;27:409-411.
5. Vacanti CJ, VanHouten RJ, Hill RC. A statistical analysis of the relationship of physical status to postoperative mortality in 68,388 cases. Anesth Analg 1970;49:564-568.
6. Dripps RD, Lamont A, Eckenhoff JE. The role of anesthesia in surgical mortality. JAMA 1961;178:261.
7. Voitk AJ. Is outpatient cholecystectomy safe for the higher risk elective patient? Surg Endosc 1997;11:1147-1149.
8. Voitk AJ. Routine outpatient cholecystectomy. Can J Surg 1995;38:262-265.

Resection of Locally Advanced Pancreatic Cancer After Downstaging With Continuous-Infusion 5-Fluorouracil, Mitomycin-C, Leucovorin, and Dipyridamole

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Patients with locally advanced pancreatic adenocarcinoma who receive conventional therapy with radiation with 5-fluorouracil (5-FU) have median survivals ranging from 8 to 12 months. Here we report our experience with a four-drug chemotherapeutic regimen that resulted in sufficient downstaging of tumor in some patients to justify surgical reexploration and resection. From April 1991 through April 1994, 38 patients received 5-FU as a continuous infusion (200 mg/m²/day), calcium leucovorin weekly by intravenous bolus injection (30 mg/m²), mitomycin-C every 6 weeks (10 mg/m² intravenously), and dipyridamole daily orally (75 mg) for locally advanced unresected pancreatic cancer. All of these patients were evaluable for response, toxicity, and survival. There were 14 partial responses and one complete response—a 39% response rate. The median survival for all patients was 15.5 months; the 1-year survival rate from time of initial diagnosis was 70%. Six of 15 responding patients had sufficient tumor regression to meet clinical criteria for resectability and reexploration, four of whom underwent a curative resection. The median survival of these six patients was 28 months from the time of original diagnosis. The 1-year survival was 83%, with one patient still alive and free of disease at 53 months. We believe this unique experience from a single institution justifies a prospective multi-institutional trial to evaluate the efficacy of this approach in a larger number of patients. (J GASTROINTEST SURG 1998;2:159-166.)

Pancreatic cancer afflicts 28,000 people per year in the United States and results in approximately the same number of deaths.[1] It is the fourth leading cause of cancer death overall and the second most common cause of death from gastrointestinal malignancies. Complete resection of the tumor offers the only hope of cure. The majority of the patients have advanced disease at the time of diagnosis and unfortunately are not candidates for resection. Tumor involvement of the adjacent major vascular structures, without apparent distant metastases, is one of the frequent causes of unresectability. These patients frequently receive a combination of chemotherapy and radiation, which may offer a modest prolongation of survival.

It is generally accepted that single-agent chemotherapy or radiation alone is ineffective as treatment for this neoplasm. However, the efficacy of 5-fluorouracil (5-FU) combined with supervoltage radiation was demonstrated as early as 1969 when Moertel et al.[2] studied 64 patients with locally advanced pancreatic cancer. Patients receiving radiation alone had a median survival of 6.3 months compared to 10.4 months for patients receiving 5-FU plus radiation. The Gastrointestinal Tumor Study Group confirmed these observations in 1981.[3] These investigators found that the median survival of patients receiving radiation alone was 4.5 months compared to 10 months for those who received both 5-FU and radiation. These prospective randomized studies established 5-FU and radiation as the standard treatment for patients with locally advanced unresectable pancreatic cancer.

More recently, 5-FU has been combined with biochemical modulators in an attempt to improve the

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therapeutic efficacy of chemotherapy in patients with various gastrointestinal malignancies. Several studies confirmed the superiority of the combination of 5-FU and calcium leucovorin (LV) over 5-FU alone in the treatment of colorectal cancer.[4,5] The overall response rate as well as the survival was better with the two-drug approach. The results have not been as good when these drugs were combined to treat pancreatic cancer. DeCaprio et al.[6] treated 43 patients with advanced disease with 5-FU and LV. The overall response rate was only 7%. These authors concluded that the combination of 5-FU and LV was no better than 5-FU alone. Recently, Isacoff et al.[7] treated 41 patients with advanced colorectal cancer with infusional 5-FU and LV, in conjunction with dipyridamole, and mitomycin-C. Twenty-five patients responded to therapy (61% response rate). There were 10 complete and 15 partial responses.[7] In light of these encouraging results, the same combination four-drug regimen was used to treat patients with advanced pancreatic cancer as well.

In the present study we report the ability of this regimen to downstage patients with locally advanced pancreatic cancer, and in some cases to convert patients with unresectable to surgically resectable disease.

MATERIAL AND METHODS

Chemotherapy

From April 1991 to May 1994, 38 patients with biopsy-proved locally advanced adenocarcinoma of the pancreas (stage II or III) were prospectively evaluated. Tissue for cytologic examination was obtained either by percutaneous fine-needle aspiration guided by CT scan (n = 12) or at exploratory laparotomy (n = 26). There were 22 women and 16 men whose median age was 65 years (range 42 to 83 years). Patients were required to have measurable disease on CT scan, white blood cell count $\geq 4000/mm^3$, platelet count $\geq 120{,}000/mm^3$, and a normal bilirubin level. All patients gave written informed consent for the chemotherapy protocol, which was approved by the UCLA Human Subject Protection Committee (protocol No. 91-01-024-2).

In the 26 patients who underwent laparotomy, chemotherapy was begun approximately 1 month postoperatively. All patients underwent surgical placement of an indwelling central venous catheter before the start of therapy. Treatment consisted of the following: (1) 5-FU, 200 mg/m^2/day, given as a continuous infusion using an ambulatory pump (Pharmacia Deltec CADD-1, St. Paul, Minn.); (2) LV, 30 mg/m^2, given intravenously as a bolus on day 1 and weekly thereafter; (3) mitomycin-C, 10 mg/m^2 (each dose not to exceed 15 mg; total not to exceed 60 mg), as a bolus injection on day 1 and then every 6 weeks; and (4) dipyridamole, 75 mg orally four times daily. Signs of clinical toxicity were monitored weekly. Complete blood counts were performed weekly and biochemical profiles to evaluate liver and kidney function were performed every 4 to 6 weeks. CT scans to assess tumor size were performed every 10 weeks.

Patients received continuous uninterrupted therapy until there were signs and symptoms of toxicity. Both 5-FU and LV were withheld when patients experienced grade III stomatitis (painful erythema and ulcers, inability to tolerate solids), grade II diarrhea (>6 watery bowel movements per day), nausea, or vomiting not controlled by antiemetics, and hand-foot syndrome (painful redness and burning of the palms and/or soles of the feet) resulting in functional disability. After symptoms of toxicity resolved, treatment was reinstituted at 75% of the initial 5-FU and LV doses. Treatment was withheld if patients developed granulocytopenia ($<1000/mm^3$) or thrombocytopenia ($<50{,}000/mm^3$). It was reinstituted at 100% of the original 5-FU and LV doses after patients recovered from hematologic toxicity. Mitomycin-C was reduced by 25% for all subsequent doses. The occurrence of hemolytic-uremic syndrome was assessed by the findings of an increase in the serum creatinine level by greater than 25%, progressive anemia in the face of an adequate reticulocyte response, and a rising lactate dehydrogenase. Dipyridamole was discontinued in patients with severe headaches or dizziness, and was resumed at doses of two or three times daily after symptoms resolved. Vitamin B_6 or E was used to treat the hand-foot syndrome as needed. Therapy was continued until there was objective evidence of disease progression or unacceptable toxicity developed.

Surgical Considerations

The surgical aspects of the study were not done as part of any experimental protocol. Patients were considered for reoperation when the CT scan showed the tumor had responded to the point where the lesion appeared to be resectable by standard criteria (no evidence of vascular involvement and no distant metastases). All of the patients who were offered the possibility of reoperation were informed of the unusual circumstances surrounding the management of their disease, and they gave their informed consent. They were specifically told that the tumor might still be found to be unresectable and that it was unknown whether resection would confer a longer survival than that to be expected from continued medical manage-

ment. Six patients were considered for reoperation after chemotherapy, and all of them had had the diagnosis made at operation at least 6 months earlier. Four of them had the initial exploratory operation performed at UCLA Medical Center; two patients underwent the first operation elsewhere. In all cases the operative notes from the first procedure were available for review, and the findings were discussed with the original surgeon. All of the reoperations were performed by one of two surgeons at UCLA Medical Center (5 by the senior author [H.A.R.]). After completely recovering from surgical resection, all patients resumed chemotherapy for an additional 4 months.

Response Criteria

A complete response to the chemotherapy was defined as no evidence of disease on CT scan. A partial response was defined as at least a 50% reduction in the volume of the cancer, calculated as the product of the cross-perpendicular diameters of tumor seen on CT scan. Stable disease was defined as less than a 25% decrease in the product of the cross-perpendicular diameters of tumor. Disease progression was defined as a greater than 25% increase in the product of the cross-perpendicular diameters of the tumor.

Off-Study Treatment Regimen

After the initial evaluation of the chemotherapy regimen in these 38 patients, 32 additional patients (20 males, 12 females) were treated through April 1997. Because the protocol was identical to that just outlined, and additional patients underwent reoperation after a response to treatment, those data are also presented.

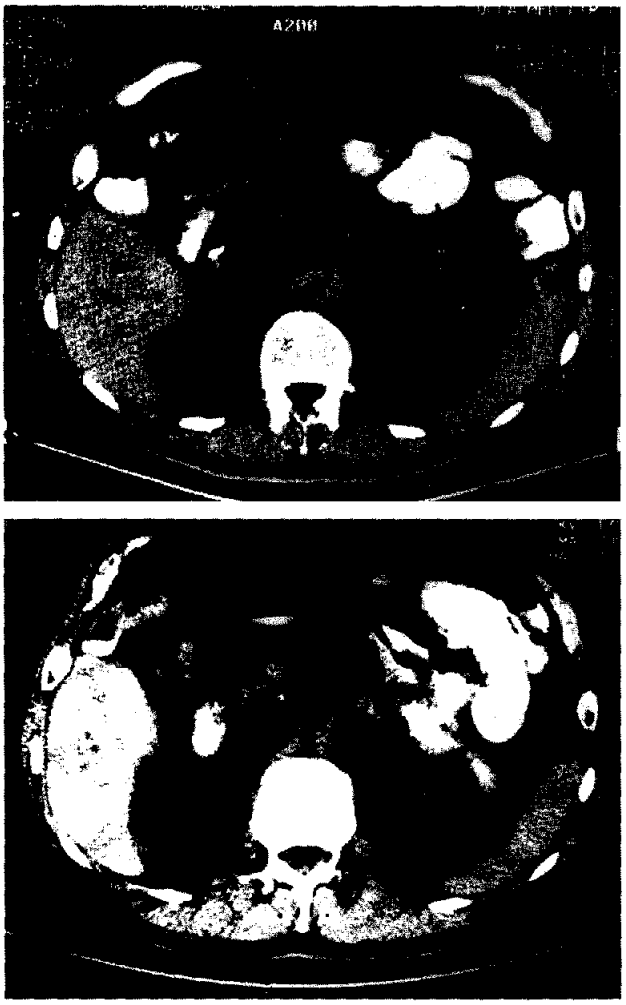

Fig. 1. A, CT scan from a patient with unresectable pancreatic cancer prior to the initiation of chemotherapy. There is involvement of the superior mesenteric vein by tumor. **B,** CT scan from the same patient after 7 months of chemotherapy. The tumor has markedly decreased in size and no vessel involvement is apparent.

RESULTS

Chemotherapy

All of the initial 38 patients were evaluable for response, toxicity, and survival. There were 14 partial responses and one complete response, for an overall response rate of 39% (15 of 38). Eleven (50%) of 22 females and 4 (25%) of 16 males responded to the treatment regimen. A representative CT scan of a patient with a partial response is shown in Fig. 1.

The median time to observe an objective response to chemotherapy was 3 months. The median time to progression of disease in all patients was 8 months (range 1 to 42 months); it was 13 months in those patients who initially had responded to treatment (range 7 to 42 months). The median length of chemotherapy treatment prior to the second operation was 6.5 months. The toxicity of chemotherapy in all patients is shown in Table I. The toxicity of chemotherapy in the six patients undergoing a second operation was no different from the toxicity seen in all patients.

The median survival for all 38 patients was 15.5 months (range 1.5 to 53 months), and the 1-year survival rate was 70%. The median survival for the 32 patients who only received the drugs (i.e., exclusive of those 6 patients who underwent reexploration) was 13 months (range 1 to 50 months), and the 1-year survival was 67%. The median survival of the 15 responding patients was 23 months (range 9 to 53 months), and the 1-year survival was 86%. Six of the

Table I. Toxicity of chemotherapy—All 38 patients (graded according to the Southwest Oncology Group)

Toxic side effect	Mild	Moderate	Severe	Life-threatening
Anorexia	14	11	0	0
Nausea/vomiting	20	4	3	0
Stomatitis	18	16	10	0
Diarrhea	18	10	0	0
Neutropenia	15	15	1	0
Thrombocytopenia	5	10	1	0
Hand-foot syndrome	10	3	13	0
Hemolytic-uremic syndrome	1	2	0	1
Headaches	6	26	0	0

15 responding patients had sufficient tumor regression to justify reexploration.

Surgical Considerations

The reasons for unresectability at the initial laparotomy included superior mesenteric vein involvement (n = 3), superior mesenteric artery involvement (n = 2), portal vein involvement (n = 3), and hepatic artery involvement (n = 1) by tumor. In all cases the primary surgeon believed that the tumor was unresectable because of vascular involvement. A variety of procedures were performed at the first exploration and they are listed in Table II.

Four of the six patients who underwent reoperation had a curative resection; two patients were still unresectable. One of these patients still had tumor invasion of the superior mesenteric vein; one patient still had invasion of both the superior mesenteric and portal veins. Resectable patients had fibrous scar in the areas where vascular invasion by tumor had been present at the first operation. One patient had no microscopic evidence of malignancy in the resected specimen, that is, there was one complete pathologic response. All of the others had gross and/or microscopic evidence of tumor still present (Fig. 2). There were no operative deaths or postoperative complications.

The mean operative time for patients undergoing pancreatic resection was 8 hours (range 7.5 to 8.9 hours). This was significantly longer than that for patients undergoing resection without any previous operations (7.1 hours; $P < 0.05$). (This and the following comparisons were made for the single surgeon [H.A.R.] who performed all of the resections.) The mean blood loss for patients undergoing resection at the second operation was 380 ml, and the mean hospital stay was 13.4 days. Blood loss and length of hospital stay were similar to that of patients undergoing resection without previous explorations or attempts at resection (402 ml and 12.4 days, respectively). The procedures performed during the second operation included the following: standard Whipple (n = 3), Whipple with extended soft tissue and lymph node dissection (n = 1), pylorus-preserving Whipple and portal vein reconstruction (n = 1), and exploratory laparotomy (n = 2).

The median survival of the six responding patients who underwent a second operation was 28 months (range 11 to 53 months) from the time of diagnosis (Table III). One-, 2-, and 3-year survivals were 83%, 71%, and 29%, respectively. One patient is alive and

Table II. Procedures performed at first operation

Type of operation	No. of patients
Cholecystojejunostomy	1
Cholecystojejunostomy + gastrojejunostomy	3
Choledochoduodenostomy	1
Gastrojejunostomy	1
Exploratory laparotomy only	1

Table III. Survival of six patients undergoing second operation*

Patient	Resection	Adjuvant treatment	Survival (mo)
1	No	No	11
2	No	No	24
3	Yes	Yes	24
4	Yes	Yes	35
5	Yes	Yes	50
6	Yes	Yes	53+

*Four patients were resected and, after recovery, continued to receive the same four-drug chemotherapy regimen for an additional 4 months.

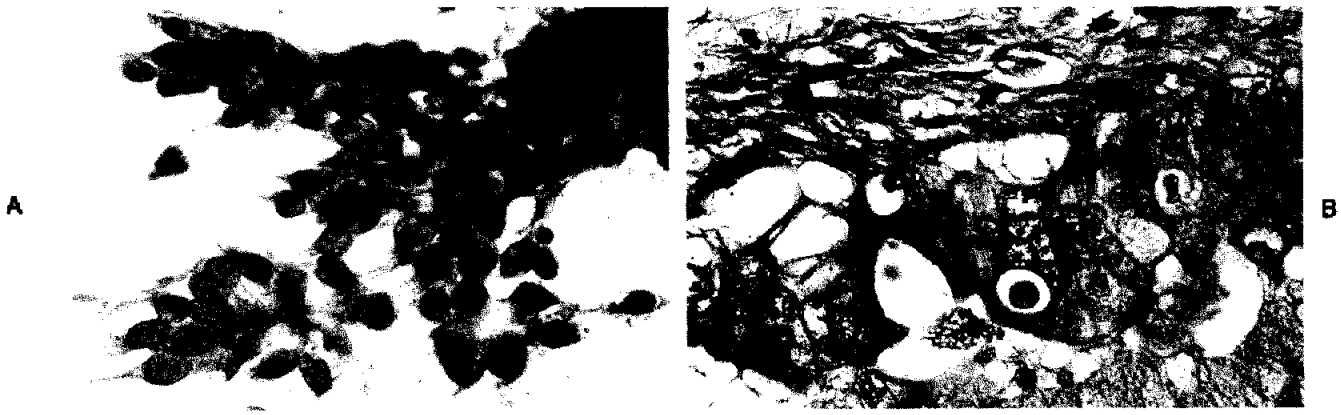

Fig. 2. A, Fine-needle aspiration from a pancreatic mass found at initial exploration. The tumor was unresectable because of vascular involvement. **B,** Section from the pancreas of the same patient after a Whipple procedure was performed. Residual tumor cells are present and the changes are consistent with chemotherapy treatment (vacuolated cytoplasm).

free of disease 53 months after the initial diagnosis. One patient recently died at 50 months after diagnosis. The patterns of recurrence of tumor in the six patients undergoing a second operation include liver metastases (n = 4), intra-abdominal carcinomatosis (n = 1), and lung metastases (n = 1). One patient had no evidence of recurrence. The median survival for the four patients undergoing resection was 41 months.

Off-Study Treatment Regimen

Of the 32 additional patients (20 males, 12 females) evaluated through April 1997, there were 12 partial responders (6 males, 6 females) (37% response rate). Three of these 12 patients had sufficient tumor regression to allow reoperation and resection of the neoplasm (Whipple procedure). Therefore, to date, nine patients have undergone a second operation and seven of nine patients have been resectable.

DISCUSSION

Rationale for Drug Regimen

Advanced gastrointestinal cancers usually grow slowly with long doubling times and low proliferative indices. Since 5-FU is a cell cycle–specific antimetabolite with a plasma half-life of only 20 minutes,[8] intermittent intravenous bolus doses would be expected to treat a relatively small population of proliferating cancer cells for short intervals of time. In contrast, prolonged continuous administration of 5-FU would expose a greater population of proliferating cancer cells to effective concentrations of drug for longer periods. Indeed there is evidence from clinical trials with colorectal cancer that response rates are higher and the incidence of significant complications (e.g., leukopenia, sepsis) is lower with the infusional approach.[9]

Once within the cell, 5-FU is metabolized to fluorodeoxyuridine monophosphate (FdUMP), which binds to its target enzyme thymidylate synthase (TS). This prevents the conversion of deoxyuridine (dUMP) to thymidylate (dTMP), the precursor to thymidine triphosphate (dTTP), which is one of the four essential nucleotides required for DNA synthesis and repair.[10] When the endogenous pool of reduced folates is increased, the binding of FdUMP to TS is enhanced. This provides the rationale for the use of LV, which has been shown both in vivo and in vitro, to expand the folate pool.[11,12] In an attempt to overcome the intracellular depletion of dTMP, cells not only increase the synthesis of TS but also that of thymidine kinase. This enzyme converts intracellular thymidine to TdMP, allowing for the continued synthesis of DNA, in spite of the effective binding of TS by FdUMP.[13,14] Dipyridamole inhibits nucleoside transport and thereby prevents the replenishment of the intracellular pool of thymidine. This may enhance the cytotoxic effects of 5-FU by interfering with the cell's ability to utilize this salvage pathway.[15,16]

Mitomycin-C is an antitumor antibiotic that has activity against a variety of gastrointestinal malignancies. In vitro, a synergistic effect on cell kill was seen when cells were exposed to mitomycin-C for 4 hours, followed by continuous exposure to 5-FU for 7 days.[17]

The response rate of this drug regimen was higher in these patients with locally advanced disease than that which has been reported with more conventional treatment. Indeed the 32 additional patients who were

treated subsequently experienced a response rate (37%) that was similar to that of the study group. The effect of the drugs on survival is less clear. In the entire group of 38 treated patients, the median survival was 15.5 months. This appears to compare favorably with the approximately 10-month survival in the unresected patients reported in the Gastrointestinal Tumor Study Group trial of radiation and 5-FU.[3] However, if one excludes the six patients who underwent reexploration, the remaining 32 patients had a median survival of 13 months. Selected subgroups clearly fared better. In those 15 patients who responded to the chemotherapy, the median survival was 23 months. The six patients who underwent reoperation had a median survival of 28 months. And in those who underwent resection, the median survival was 41 months. One patient is alive 53 months after diagnosis and is clinically free of disease. However, while it is tempting to conclude that these patients benefited from the chemotherapy and subsequent surgery, the design of the study does not allow even this conclusion.

It was interesting that the one patient who underwent resection, and who had no microscopic evidence of tumor in the specimen, died of lung metastases 25 months after that operation. She had been treated after the resection with the same drugs for an additional 21 months. Thus it is clear that even a complete pathologic response did not mean that all of the tumor had been eradicated.

We anticipated that the operation would take longer in the patients who were undergoing reexploration after the chemotherapy treatment than in those who are resectable at the first attempt. Obvious reasons include the inevitable adhesions that must be taken down and the various bypass operations that were done at the time of initial exploration. The latter usually complicated the assessment of resectability, which was a time-consuming part of the second procedure. In each patient the original operation had involved dissection of tumor in the area of the major vessels. At the second operation these areas had considerable scar, the distinction between scar and persistent tumor was not readily apparent, and a meticulous dissection was required to avoid vascular injury and major blood loss. One patient underwent resection and reconstruction of the superior mesenteric-portal vein, because of concern about vascular involvement on the lateral aspect of the vein after the pancreas had been divided. The pathology report indicated that this was scar, not tumor. The final result for all of the patients was the addition of approximately 1 hour to the operative procedure. However, the mean operative blood loss, duration of hospital stay, and type and frequency of postoperative complications were not increased.

There were more female than male responders to the chemotherapy (50% vs. 25%). This suggests that gender may be of prognostic significance. If this is true, one could postulate that the presence of specific hormone receptors may have influenced the response to treatment and the outcome. We are investigating this possibility.

Reports about downstaging of pancreatic cancer and the conversion of unresectable to resectable disease are uncommon. Pilepich and Miller[18] evaluated 17 patients with locally advanced pancreatic carcinoma and reported their results in 1980. None of their patients had undergone exploration to confirm unresectability, and all received radiation therapy (4000 to 5000 rad) over a 5-week period. After 6 weeks, 11 patients had exploratory operations, and six underwent resection. Two of these patients were free of disease after 5 years, at the time the experience was published. More recently Yeung et al.[19] assessed the efficacy of preoperative infusional 5-FU, mitomycin-C, and radiation therapy in 31 patients with biopsy-proved carcinoma of the pancreas or duodenum. The resectability rate was 38% for the 26 patients with pancreatic carcinoma. The clinical response rate in that study was 20%, and the majority of the patients who had a resection were nonresponders. Thus the value of the neoadjuvant treatment in those patients remains obscure.

CONCLUSION

We have reported the ability of this four-drug chemotherapy regimen to downstage locally extensive unresectable stage II and III pancreatic cancer. The drugs were well tolerated, and the overall response rate of approximately 40% appears to be higher than that for other commonly used approaches. A small fraction of the total patient population was able to undergo resection of the tumor at a second operation, which may have conferred a survival advantage. These early pilot results from one center would seem to justify a multi-institutional study in which this regimen is compared prospectively with more conventional therapy.

REFERENCES

1. Gold EB. Epidemiology of and risk factors for pancreatic cancer. Surg Clin North Am 1995;75:819-843.
2. Moertel CG, Childs DS, Reitemeier RJ, Colby MY, Holbrook MA. Combined 5-FU and supervoltage radiation therapy of locally unresectable gastrointestinal cancer. Lancet 1969;2: 865-867.

3. Gastrointestinal Tumor Study Group. Moertel CG, Frytak S, Hahn RG, O'Connell MJ, Reitemeier RJ, Rubin J, Schutt AJ, Weiland LH, Childs DS, Holbrook MA. Therapy of locally unresectable pancreatic carcinoma: A randomized comparison of high dose (6,000 rads) radiation alone, moderate dose radiation (4,000 rads) plus 5-fluorouracil, and high dose radiation plus 5-fluorouracil. Cancer 1981;48:1705-1710.
4. Grem JL, Hoth DF, Hamilton JM, King SA, Leyland-Jones B. Overview of current status and future direction of clinical trial with 5-fluorouracil in combination with folinic acid. Cancer Treat Rep 1987;71:1249-1264.
5. Advanced Colorectal Cancer Meta-analysis Project. Modulation of fluorouracil by leucovorin in patients with advanced colorectal cancer: Evidence in terms of response rate. J Clin Oncol 1992;10:896-903.
6. DeCaprio JA, Mayer RJ, Gonin R, Arbuc SG. Fluorouracil and high dose leucovorin in previously untreated patients with advanced adenocarcinoma of the pancreas: Results of a phase II trial. J Clin Oncol 1991;9:2128-2133.
7. Isacoff WH, Eilber FR, Kuckenbecker SL, Jacobs AD, Taylor O. Continuous infusion 5-fluorouracil given with calcium leucovorin, dipyridamole, and mitomycin-C in patients with advanced colorectal carcinoma: A phase II trial. J Infusional Chemotherapy 1994;10:107-111.
8. MacMillan WE, Woberg WH, Welling PG. Pharmacokinetics of 5-fluorouracil in humans. Cancer Res 1978;38:3479-3482.
9. Lokich JJ, Ahlgren JD, Gullo JJ, Philips JA, Fryer JG. A prospective randomized comparison of continuous infusion fluorouracil with conventional bolus schedule in metastatic colorectal carcinoma: A Mid-Atlantic Oncology Program Study. J Clin Oncol 1989;7:425-432.
10. Chabner BA. Pyrimidine antagonists. In Chabner BA, ed. Pharmacologic Principles of Cancer Treatment. Philadelphia: WB Saunders, 1982, pp 183-212.
11. Santi DV, McHenry C, Sommer H. Mechanism of interaction of thymidylate synthetase with 5-fluorodeoxyuridylate. Biochemistry 1978;17:4018-4024.
12. Evans MR, Laskin JD, Hakala MT. Effect of excess folates and deoxyinosine on the activity and site of action of 5-fluorouracil. Cancer Res 1981;41:3288-3295.
13. Breitman TR. The feedback inhibition of thymidine kinase. Biochim Biophys Acta 1963;67:153-158.
14. Ives DH, Morse PA, Potter VR. Feedback inhibition of thymidine kinase by thymidine triphosphate. J Biol Chem 1963;238:1467-1474.
15. Van Mouwerik TJ, Pangallo CA, Willson JK, Fischer PH. Augmentation of methotrexate cytotoxicity in human colon cancer cells achieved through inhibition of thymidine salvage by dipyridamole. Biochem Pharmacol 1987;36:809-814.
16. Grem JL. Biochemical modulation of fluorouracil by dipyridamole: Principal and clinical experience. Semin Oncol 1992;19:56-65.
17. Rusello O, Romanini A, Sivallen D, Rosso R, Nicolin A, Sobrero A. Time-dependent interaction between 5-FU and mitomycin-C on a human colon carcinoma cell line, HCP-8, in vitro. Eur J Cancer Clin Oncol 1989;25:571-572.
18. Pilepich MV, Miller HH. Preoperative irradiation in carcinoma of the pancreas. Cancer 1980;46:1945-1949.
19. Yeung RS, Weese JL, Hoffman JP, Solin LJ, Paul AR, Engstrom PF, Litwin S, Kowalyshyn MJ, Eisenberg BL. Neoadjuvant chemoradiation in pancreatic and duodenal carcinoma. A phase II study. Cancer 1993;72:2124-2133.

Discussion

Dr. H. Pitt (Baltimore, Md.). I think we have all been looking for a regimen of adjuvant therapy that would have a higher response rate than we have seen in the past, and this certainly looks like something that is promising. It does need to be confirmed. We have seen two patients who have been treated with this regimen and it seems their disease was downstaged. They were resected at our institution. So this is not just a single-institution experience, to date. The majority of these patients have not been resected, and therefore it can be very difficult to know for sure whether these were pancreatic cancers as opposed to distal bile duct or ampullary duodenal cancers, both of which have a potentially better prognosis. My first question is how do you really know these are pancreatic cancers? How many patients were explored by the surgeon who subsequently performed the resection? As we all know, it can be very difficult to separate the tumor from the vein, but some surgeons are willing to go further than others.

What was the status of the margins and lymph nodes and did these patients receive any adjuvant therapy? Do you have any clue as to why the females responded better than the males?

Dr. K. Todd. With regard to your first question about whether or not these were pancreatic cancers, 33 of the 44 patients underwent exploratory laparotomy so we were able to actually look at their tumors and they were found to have tumors of the pancreas. Only 14 of the patients underwent CT scans with needle biopsy. Five of the seven patients underwent exploration at UCLA and three of the five were performed by the senior author (H.A.R.), two by other surgeons with whom he was able to interact. The other two were done at outside hospitals. We were able to call those surgeons and find out what their criteria were for unresectability.

In terms of your third question about margin status and lymph node involvement, four of the seven patients did have one positive lymph node. There were two poorly differentiated tumors; two moderately well-differentiated and four moderately differentiated tumors. The data did not seem to be skewed. Some patients did receive adjuvant treatment, the thought being that if the bulk of the tumor was removed in the patients who were responding, perhaps they would continue to respond to this therapy in case there were some tumor cells still present. In terms of why the fe-

males might respond better than the males, it is thought that perhaps there may be some different hormone receptors on these tumors.

Dr. L. Warshaw (Boston, Mass.). Do you have any information about the margins since that appears to be a major determinant of survival in these patients.

Dr. Todd. The margins were all clear.

Dr. R.H. Bell (Seattle Wash.). I wanted to ask you about the specific group with vascular invasion. At an earlier presentation Dr. Reber showed us a good scoring system that was developed by radiologists at your institution for determining the extent of vascular involvement. I wonder if you could tell us how many of those patients with vascular involvement had objective downscoring of their radiologic score for vascular involvement after therapy?

Dr. Todd. They all did. When we examined the CT scans from the seven patients who underwent reexploration, there was no evidence of vascular involvement. We use spiral CT scans and are able to very accurately determine whether or not there is tumor involvement of the major vascular structures.

Dr. Bell. I am not just asking about the patients who were resected; I am asking about the whole group.

Dr. Todd. I believe all of them were treated after the era of the spiral CT had begun, but all of the patients who are listed as responders were downgraded from having vascular involvement to not having vascular involvement or having a lesser degree of vascular involvement than they did initially.

Dr. Bell. But were there some who had vascular involvement as the cause of unresectability who did not respond as well? What is that percentage if you just look at the group who were initially declared unresectable purely on the basis of vascular involvement.

Dr. Reber. I do not know that number.

Dr. Bell. Since some of the apparent vascular involvement shown on CT scan or the apparent vascular involvement based on surgical considerations at the first exploration may not have been cancer but pancreatitis, do you have biopsy reports on those responders to show that they really did have cancer?

Dr. Reber. In addition to the patients for whom I was the original surgeon, there are actually two more patients that have been added to this total who were not part of the presentation, so we really are talking about seven resections out of a total of nine patients who have undergone reexploration. Of those seven, I think I was the original surgeon in five. In every case where I was the original surgeon and considered the patient to be unresectable because of vascular involvement, tissue samples were taken from where we thought the vessels were involved and, in fact, there was histologic evidence of tumor. The second time around in those patients who were resectable, when we biopsied those same areas, there was only scar.

Somatostatin Analogue Predisposes Enterocytes to Apoptosis

Jon S. Thompson, M.D.

The somatostatin analogue octreotide impairs intestinal regeneration and the adaptive response to intestinal resection and other stimuli. These effects are mediated in part by inhibition of enterocyte migration and proliferation. The aim of this study was to determine whether octreotide promotes enterocyte apoptosis. Twenty-four New Zealand white rabbits were studied including 18 animals that underwent patch enteroplasty in the distal ileum to stimulate the mucosa and six unoperated controls. The patched animals either received 100 μg or 1000 μg of subcutaneous octreotide daily or served as operated control subjects. Normal ileal mucosa adjacent to the patch was evaluated at 7 days for villus height, crypt depth, crypt cell production rate (CCPR), and in situ end labeling of DNA fragmentation. Mean DNA fragmentation was significantly greater in octreotide-treated animals ($P < 0.05$ Mann-Whitney rank test). Fragmentation scores ranged from 1.0 to 1.5 in controls and 1.1 to 2.65 in treated animals. Staining of enterocytes was quite heterogenous, however, among the villi of individual treated animals. Staining was greater and cells with chromatin condensation were more prevalent near the tip of the villus. Octreotide increased apoptosis at the villus tip, lateral villus, and crypt. The two control groups had similar villus height, crypt depth, and CCPR. The two octreotide-treated groups had similar villus height and CCPR compared to control animals. However, crypt depth was significantly less in the octreotide-treated animals (100 ± 9 μm, and 90 ± 6 μm, 100 μg, and 1000 μg) compared to controls (121 ± 10 μm and 117 ± 10 μm, unoperated and operated; $P < 0.05$). Crypt depth but not villus height correlated with DNA fragmentation. Neither correlated with CCPR. The following conclusions were reached: (1) Octreotide treatment is associated with increased DNA fragmentation in enterocytes; (2) octreotide promotes apoptosis in both villus and crypt compartments; (3) predisposition to apoptosis may play a role in octreotides effects on intestinal regeneration and adaptation; and (4) the role of proliferation and apoptosis in determining the size of the enterocyte compartments remains unclear. (J GASTROINTEST SURG 1998;2:167-173.)

The somatostatin analogue octreotide impairs intestinal regeneration and the adaptive response of the small intestine to intestinal resection and other stimuli.[1-3] These effects are mediated in part by inhibition of enterocyte migration and proliferation. However, intestinal growth is also regulated by the rate of cell death. Apoptosis (programmed cell death) can be induced by a variety of signaling pathways including growth factor withdrawal and other metabolic perturbations.[4] Since somatostatin inhibits release of a variety of regulatory gastrointestinal polypeptides and has direct effects on enterocytes, it might influence the rate of apoptosis in the intestinal epithelium.[5] Given the generally inhibitory effects of somatostatin on intestinal growth, an increase in the rate of apoptosis might be expected in response to somatostatin and its analogue. The aim of the present study was to determine whether octreotide promotes enterocyte apoptosis in stimulated intestinal epithelium.

MATERIAL AND METHODS

Twenty-four male new Zealand white rabbits (3 to 4 kg) were included in the study. Six unoperated animals served as the control group (group 1). All other animals had 2 × 5 cm ileal defects patched with adjacent colon serosal surface. Group 2 consisted of six animals undergoing patch enteroplasty alone. Groups 3 (n = 6) and 4 (n = 6) received octreotide, 100 μg/day and 1000 μg/day subcutaneously, in two divided equal

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doses, respectively. Animals were killed at 7 days and the ileum was studied 5 cm proximal to the patch. Intestinal structure was assessed by morphometric measurements on histologic sections. Proliferative activity of the mucosa was evaluated by measuring the mucosal crypt cell production rate (CCPR). Apoptosis was estimated by in situ end labeling of DNA fragmentation and morphologic assessment.

Operative Procedure

Operations were performed after an overnight fast using sterile technique. Anesthesia was achieved with intramuscular ketamine (35 mg/kg) and xylazine (7 mg/kg) and maintained with halothane by inhalation. Intestinal patching was performed by making a 5 cm incision on the antimesenteric border of the ileum, beginning 20 cm proximal to the ileocecal junction. The serosal surface of adjacent colon was apposed to the cut edge of the defect with a continuous inverting 4-0 silk suture, thus creating a 2 × 5 cm serosal patch exposed to the lumen of the small intestine. The animals received perioperative ampicillin and supplemental subcutaneous fluid until they resumed oral intake on postoperative day 2. The rabbits were active, ate normally, and maintained their body weight during the study.

Morphologic and Biochemical Measurements

Ileal segments were excised 5 cm proximal to the patched intestinal defect at sacrifice. Samples were processed histologically, and transverse sections were stained with hematoxylin and eosin. Villus height and crypt depth were measured at 10 sites around the circumference with the aid of an eyepiece micrometer.

CCPR was determined using the metaphase arrest technique with vincristine sulfate.[6] One milligram of vincristine sulfate was injected intraperitoneally 2 hours prior to sacrifice. The mucosal samples were fixed in Carnoy's fixative, hydrolyzed in an acid at 60° C for 5 minutes, and stained with Schiff's reagent. The crypts were dissected free using a dissecting microscope. Samples of crypts were transferred to a glass slide in 15% glacial acetic acid and squashed for determination of the number of metaphases per crypt in a minimum of 10 crypts. CCPR was calculated assuming a linear accumulation for 2 hours.

In Situ End Labeling

In situ end labeling was performed using Klenow polymerase with detection of biotinylated nucleotides using a streptavidin-horseradish peroxidase conjugate (Frag EL, Oncogene Research Products, Cambridge, Mass.).[7] Paraffin sections (3 μm) were dewaxed with xylene and taken through alcohol (100% to 70%). The sections were permeabilized with 40 μg/ml proteinase K in 10 mol/L Tris (pH 8) for 60 minutes at room temperature and rinsed with Tris-buffered saline solution. Endogenous peroxidases were inactivated by incubation with 3% H_2O_2 for 5 minutes. Labeling was carried out after a 30-minute incubation in diluted Klenow equilibration buffer (0.5 mol/L Tris [pH 8], 0.5 mol/L NaCl; and 0.1 mol/L $MgC1_2$). Sixty microliters of labeling reaction mix (58.4 μl labeling reaction mix with 1.6 μl Klenow enzyme) was applied to each specimen and the slides were incubated in a humidified chamber for 1.5 hours at 37° C. The reaction was terminated by incubation with 0.5 mol/L EDTA (pH 8) for 5 minutes. Sections were covered with 4% bovine serum albumin in phosphate-buffered saline solution for 10 minutes. Peroxidase streptavidin conjugate was applied for visualization of the avidin-biotin complex and the section was incubated at room temperature for 30 minutes. Slides were rinsed and developed in 3.3 diaminobenzidine. Sections were lightly counterstained with methyl green. Positive and negative controls were evaluated for each sample. Positive controls were generated by incubation with Klenow DNAase for 20 minutes. Negative controls had the polymerase omitted from the labeling mixture.

Quantitation of Apoptosis

Apoptosis was evaluated by a blinded observer grading DNA fragmentation on the in situ end-labeled sections and quantitating cells with morphologic characteristics of apoptosis. Fragmentation was graded on a scale of 1 to 3 as follows: 1 = minimal staining and two or fewer densely stained nuclei/villus; 2 = diffuse light staining and two or fewer densely stained nuclei/villus; and 3 = diffuse staining with more than five densely stained nuclei/villus (Fig. 1). Twenty consecutive villi were graded on each section. Under high-power microscopy, cell morphology was studied to determine the presence of chromatin condensation in the nucleus, separation of the cell from adjacent enterocytes, and the presence of apoptotic bodies. Intraepithelial lymphocytes were excluded based on location and size. Enterocytes were counted in 10 consecutive axially oriented villi and apoptotic cells expressed as the number per 100 apical, lateral, and total villus cells. Ten crypt cross sections were also examined to determine the number of apoptotic cells.

Statistical Analysis

Data are expressed as mean ± standard deviation. Analysis of variance with the Bonferonni correction

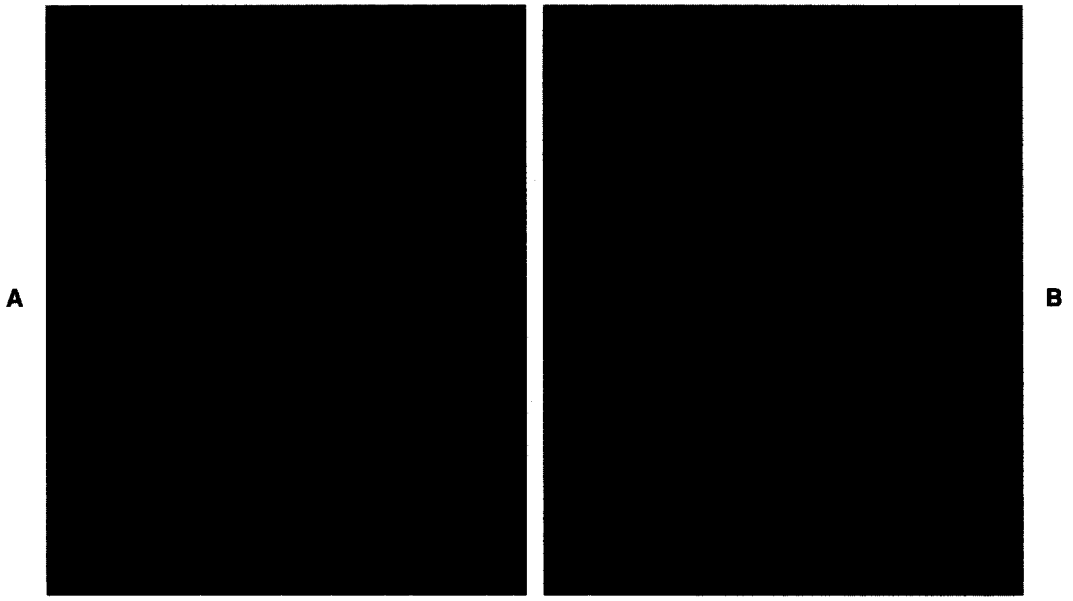

Fig. 1. DNA fragmentation scores ranged from 1 **(A)** to 3 **(B)** based on differences in overall staining and number of intensely stained cells at the villus tip. Staining was greater and cells with chromatin condensation were more prevalent near the tip of the villus.

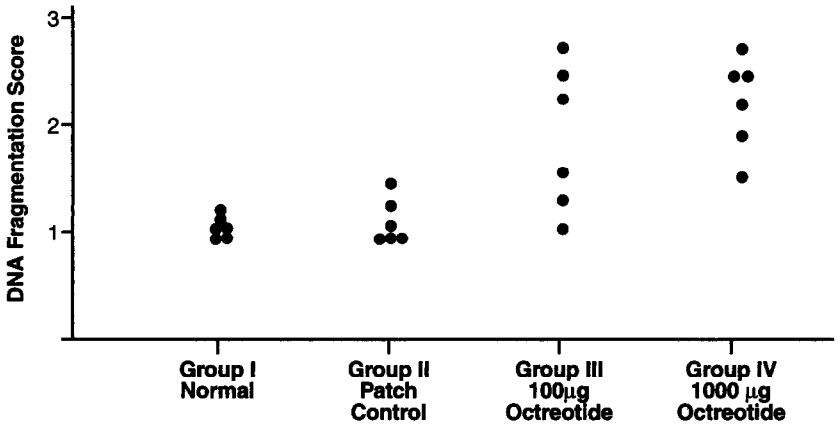

Fig. 2. DNA fragmentation scores were significantly higher in the two octreotide-treated groups compared to normal ileum and ileum of patched control animals ($P < 0.05$).

and the Mann-Whitney rank test were used for comparisons. Correlations were evaluated by linear regression analysis. Statistical significance was ascribed to P values <0.05.

RESULTS

DNA fragmentation scores were significantly greater in the octreotide-treated animals compared to the unoperated and patched controls ($P < 0.05$) (Fig. 2). Fragmentation scores ranged from 1.0 to 1.5 in normal tissue and tissue from patched control animals. There was no significant difference between the two octreotide-treated groups. Staining of enterocytes was quite heterogenous, however, among the villi of the individual treated animals and scores ranged from 1.1 to 2.7. There was at least one grade 3 villus in five (84%) and six (100%) of the octreotide-treated animals compared to one (16%) patched control and none of the unoperated control animals ($P < 0.05$). In general, staining was greater and cells with chromatin condensation were more prevalent near the tip of the villus in all animals.

The number of cells with chromatin condensation

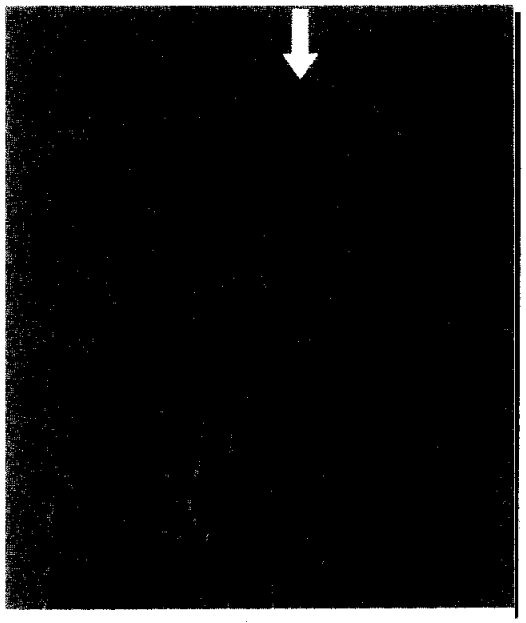

Fig. 3. Apoptotic cells at the villus tip in octreotide-treated animal. One is being extruded into the lumen (arrow).

Table I. Comparison of apoptosis by site

Site	Cell index (apoptotic cells per 100 cells)			
	Normal ileum	Patch control	100 μg octreotide	1000 μg octreotide
Villus tip	19 ± 7*	18 ± 7*	45 ± 8*†	38 ± 9*†
Lateral villus	2 ± 1	3 ± 2	9 ± 4†	14 ± 3†
Total villus	6 ± 3	6 ± 3	16 ± 7†	18 ± 4†
Crypt	10 ± 4	10 ± 3	17 ± 5†	15 ± 5

*P <0.05 vs. lateral villus.
†P <0.05 vs. normal and patch control.

Table II. Comparison of intestinal mucosa

	Normal ileum	Patch control	100 μg octreotide	1000 μg octreotide
Villus height (μm)	382 ± 51	382 ± 43	338 ± 49	342 ± 30
Crypt depth (μm)	121 ± 10	117 ± 10	100 ± 9*	90 ± 6*
CCPR (cells/hr)	5.8 ± 1.2	7.3 ± 2.1	8.7 ± 1.4	8.1 ± 1.6

CCPR = crypt cell production rate.
*P <0.05 vs. normal ileum and patch control.

was greatest at the villus tips compared to the lateral aspect of the villus in all of the groups (Table I and Fig. 3). Apoptotic cells were more prevalent among the 10 apical villus cells and enterocytes in the lateral villus in the octreotide-treated animals compared to the control groups but were similar at both doses of octreotide. These cells were also found more frequently in crypt cross sections in octreotide-treated animals (Fig. 4).

Mean villus height and CCPR in the normal ileum of unoperated animals and adjacent to a serosal patch in the control and octreotide-treated groups were similar (Table II). Crypt depth was significantly less in the two octreotide-treated groups compared to the control groups. Crypt depth correlated with the DNA fragmentation score ($r = -0.698$, P = 0.002) but not the CCPR ($r = 0.144$, $P = 0.616$). There was no cor-

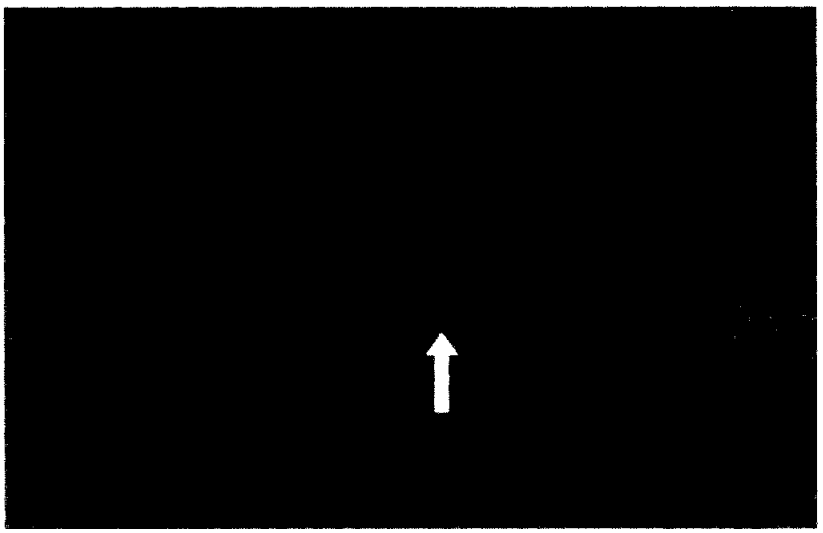

Fig. 4. Apoptotic cell in crypt cross section (arrow).

relation between either the CCPR ($r = 0.165$, $P = 0.651$) or the DNA fragmentation score ($r = 0.456$, $P = 0.133$) and mean villus height.

DISCUSSION

Somatostatin has a variety of effects on the intestinal epithelium. In addition to its known antiproliferative effect, somatostatin has been demonstrated to impair enterocyte migration and differentiation.[1,8] In the present study the somatostatin analogue octreotide predisposed enterocytes to apoptosis. Whereas we found previously that enterocyte migration and proliferation in regenerating epithelium was inhibited by 1000 μg but not 100 μg/day of octreotide, both doses had similar effects on apoptosis in the present study. Thus somatostatin appears to influence intestinal growth and structure via several different mechanisms.

The effect of octreotide on enterocyte apoptosis was studied in a model of stimulated epithelium (adjacent serosal patch) because previous studies have demonstrated that the antiproliferative effects of somatostatin occur only in stimulated epithelium. Whereas somatostatin has no effect on proliferation of normal mucosa, it inhibits the increased proliferation induced by intestinal resection and growth factors including epidermal growth factor, growth hormone, and gastrin.[1-3,5,9-10] Creation of a serosal patch results in increased proliferation in the immediately adjacent intestinal epithelium.[11] However, villus height and crypt depth and the CCPR were similar in the normal ileum and ileum adjacent to the intestinal patch in the present study so stimulation of mucosa was not as marked as with these other stimuli. Whether or not the somatostatin analogue would promote enterocyte apoptosis in normal intestinal epithelium remains unclear.

Apoptosis (programmed cell death) is a complex process that is still incompletely understood.[12] However, it appears to be a highly regulated, genetically controlled process of cell deletion without any signs of inflammation or disruption of tissue architecture. Proliferation and programmed cell death are the important regulators of the cell pool in tissue homeostasis. Although there appears to be a persistent low rate of spontaneous apoptosis in the intestine, there are a variety of positive and negative triggers of induced programmed cell death.[12] Growth factor deprivation appears to be an important initiating factor that results in downregulation of survival genes and perhaps new gene expression leading to endonuclease activation and ultimately cell death.[13] However, several mechanisms of injury, for example, irradiation and various other agents, may also trigger this process. Once initiated, the transduction of the cell death signal also follows several different pathways. In the present study there were both increased DNA fragmentation and morphologic changes of apoptosis in the somatostatin-treated animals.

The role of apoptosis in regulation of normal intestinal homeostasis is unclear. Certainly apoptosis is triggered by normal physiologic stimuli. In the rat, apoptosis has a circadian rhythm and is increased by fasting.[14] However, the potential regulatory sites of apoptosis within the enterocyte compartment are con-

troversial. Although some investigators suggest that apoptosis occurs at the villus tip with cells being extruded into the lumen, others only find DNA fragmentation in the crypts.[15,16] Using a technique similar to that employed in the present study, Hall et al.[16] found that apoptotic cells could be found in most villi and crypts, being more frequent at the top of the villus. Similarly, in the present study, apoptotic cells were more frequent at the tip of the villus compared to the lateral surface in the various control and experimental groups. Furthermore, in both operated and unoperated control animals there was a detectable rate of apoptosis on the lateral villus and in the crypts. A recent study in humans does suggest that apoptosis is an important mechanism for cell loss at the villus tip.[17]

The findings in the present study suggest that octreotide enhances apoptosis diffusely throughout the enterocyte compartment. A similar observation has been made in patients with celiac sprue where apoptosis is not only increased but also more diffusely distributed rather than occurring predominantly at the villus tip.[18] However, in an in vitro study, Stange et al.[8] found no increase in DNA in shed medium from cell cultures exposed to somatostatin, which suggests that there is no enhanced cellular loss in the presence of somatostatin.

The mechanism of somatostatin-induced apoptosis in the intestinal epithelium might be related to inhibition of growth factor activity or release or via a direct stimulatory effect. Both direct and indirect actions have a role in the antiproliferative effects of somatostatin.[5,8-10] Either explanation is plausible given existing knowledge about apoptosis. Furthermore, somatostatin exerts its effects on tissue via a variety of different postreceptor signal transduction mechanisms, suggesting that it might also influence apoptosis via more than one mechanism.[19] Thus further studies would be necessary to elucidate the mechanism of somatostatin-induced apoptosis.

In the present study, mean crypt death but not villus height correlated with apoptosis in the individual animals. Neither parameter correlated with the CCPR. Thus it is difficult to support a hypothesis that either cell proliferation or death is the dominant factor determining the size of the villus compartment. However, there was a tendency toward reduced villus height in the octreotide-treated animals despite a comparable or greater CCPR. The diminished crypt depth in the octreotide-treated animals may be related to enhanced apoptosis in the stem cell compartment, which would be expected to diminish the villus enterocyte compartment unless cell survival on the villus was prolonged. There was marked heterogenicity in DNA fragmentation, even among villi from the same histologic section, so that sampling error may be a problem in interpretation.

In summary, octreotide treatment is associated with increased DNA fragmentation in enterocytes, suggesting endonuclease activation. Enterocytes with morphologic characteristics of apoptosis were more prevalent in both villi and crypts. Thus predisposition to apoptosis may play a role in octreotide's effects on intestinal regeneration and adaptation. Since there was only a correlation with crypt depth and apoptosis but not between villus height and either CCPR or apoptosis, the role of these factors in determining the size of the enterocyte compartment remains unclear.

REFERENCES

1. Thompson JS, Nguyen BLT, Harty RF. Somatostatin analogue inhibits intestinal regeneration. Arch Surg 1993;128: 385-389.
2. Holmes SJK, Jaspan JB, Moosa AR. The effect of somatostatin on postresectional ileal hyperplasia. Endocrinology 1982;111: 1397-1399.
3. Bass BL, Fischer BA, Richardson C, Harmon JW. Somatostatin analogue treatment inhibits postresectional adaptation of the small bowel in rats. Am J Surg 1991;161:107-112.
4. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. Science 1995;267:1456-1462.
5. Conteas CN, Nandimajumdar AP. The effects of gastrin, epidermal growth factor, and somatostatin on DNA synthesis in a small intestinal crypt cell line (IEC-6). Proc Soc Exp Biol Med 1987;184:307-311.
6. Wright N, Watson A, Morely A, et al. The measurement of cell production rates in the crypts of Lieberkuhn. Virchows Arch 1974;364:311-323.
7. Gavrieli Y, Sherman Y, Ben-Sassin SA. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 1992;119:493-501.
8. Stange EF, Schneider A, Schuselziarra V, Ditschuneit H. Inhibitory effects of somatostatin on growth and differentiation in cultured intestinal mucosa. Horm Metab Res 1987;16:74-78.
9. Lehy T, Dubrasquet M, Bonfils S. Effect of somatostatin on normal and gastrin-stimulated cell proliferation in the gastric and intestinal mucosa of the rat. Digestion 1979;19:99-109.
10. Konturek SJ, Brzozowski T, Dembinski A, Warzecha Z, Konturek PK, Yanaihara N. Interaction of growth hormone releasing factors and somatostatin on ulcer healing and mucosal growth in rats: Role of gastrin and epidermal growth factor. Digestion 1988;41:121-128.
11. Saxena SK, Thompson JS, Joshi SS, Sharp JG. Extent and role of urogastrone in the adaptive response on the rat intestine to patching or a surgical defect in the ileum. J Invest Surg 1993;6:485-492.
12. Binder C, Hiddemann W. Programmed cell death—Many questions still to be answered. Ann Hematol 1994;69:45-55.
13. Collins MKL, Perkins GR, Rodriquez-Tarduchy G, Nieto MA, Lopez-Rivas A. Growth factors as survival factors: Regulation of apoptosis. Bioessays 1994;16:133-138.
14. Iwakirii R, Aw TY. Apoptosis in rat small intestine: Circadian rhythm and effect of feeding and fasting [abstr]. Gastroenterology 1995;108:A292.

15. Watson AJM. Manipulation of cell death—The development of novel strategies for the treatment of gastrointestinal disease. Aliment Pharmacol Ther 1995;9:215-226.
16. Hall PA, Coates PJ, Ansari B, Hopwood D. Regulation of cell number in the mammalian gastrointestinal tract: The importance of apoptosis. J Cell Sci 1994;107:3569-3577.
17. Shibahara T, Nakahara A, Fukutomi H. The fate of effete enterocytes at the villus tips of human small intestine with special reference to their exfoliating process and apoptosis. Gastroenterology 1996;110:A838.
18. Attia AL, Moss RF, Walters JRF, Wang S, Holt PR. Increased small bowel epithelial apoptosis reflects celiac sprue activity [abstr]. Gastroenterology 1995;108:A271.
19. Patel PC, Barrie R, Hill N, Laudeck S, Kurozawa D, Woltering EA. Postreceptor signal transduction mechanisms involved in octreotide-induced inhibition of angiogenesis. Surgery 1994;116:1148-1152.

Role of the Ileocecal Junction in the Motor Response to Intestinal Resection

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Extensive resections of the distal small intestine are associated with motor disruption in the proximal remnant. Luminal contents such as bacteria and short-chain fatty acids may play a role. We evaluated the effect of bypass of the ileocecal junction (ICJ) on the motor response to a 50% distal resection. Thirty-five dogs were divided into three groups: transection control (TC, n = 11); 50% distal resection with intact ICJ (DR, n = 12), and 50% distal resection with jejunocolostomy to bypass the ICJ (DRBP, n = 12). Motor activity, intestinal transit, nutrition, absorption, and motor active hormones were studied over a 3-month period. Caloric intake was reduced and nutritional status similarly impaired in both resected groups. Steatorrhea, however, was significantly greater after DRBP. Intestinal structural adaptation was similar in both resected groups at 12 weeks. Animals in the bypass group demonstrated elevated intraluminal short-chain fatty acid and anaerobic bacterial counts. Migrating motor complex frequency was similar in the three groups; distal starts, however, were more frequent in both resected groups. Clustered contractile activity was prominent in the remnant after both DR and DRBP (50% and 32% recording time occupied by clusters, respectively [not significant]). Basal levels of peptide YY were increased following resection and this increase was unaffected by ICJ bypass. Postprandial neurotensin concentrations were transiently increased after distal bowel resection. In contrast, the postprandial neurotensin response was abolished following resection with bypass of the ICJ. Basal motilin levels were reduced following resection alone but not after resection with ICJ bypass. The motor response to resection does not appear to be related to alterations in circulating levels of hormones localized to the distal ileum; neither does it seem to be influenced by luminal bacteria and short-chain fatty acids or retention of a sphincteric mechanism at the ICJ. These findings also raise questions about the role of short-chain fatty acids and bacteria in the generation of the various distinctive motor patterns of the distal ileum. Resection of the distal ileum through loss of the receptor site for either retarding reflexes or bile salt absorption may be of greater importance in determining the motor response to resection. (J GASTROINTEST SURG 1998;2:174-185.)

The motor response of the intestinal remnant to intestinal resection has recently been investigated. During early recovery after extensive intestinal resection, there is marked disruption of motor activity.[1] Repetitive clusters of contractions and prolonged propagated contractions, features of normal motility in the terminal ileum, predominate in the jejunal remnant. This disruptive stage is followed by an adaptive phase where motor activity returns to relatively normal patterns.[2] These observations in the canine model have been corroborated by studies in humans.[3,4]

The regulation of this motor response to resection is unknown. Certainly bacteria and other luminal contents, such as short-chain fatty acids, may play a role.[5,6] Short-chain fatty acids, indeed, have been shown to induce both clusters and prolonged propogated contractions in the intact ileum.[6] Enteric peptides, which have been implicated in structural adaptation, are also potential regulatory factors for the adaptive motor responses.[7] There may also be resection-related neural and myogenic alterations, which could influence motor activity.[8,9] Our previous observation that the distal

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remnant takes on motor properties characteristic of the terminal ileum suggests that factors regulating motor function in this region may play a role in postresection motor adaptation.[1]

Resection of the distal ileum and ileocecal junction (ICJ) has a more deleterious effect on intestinal absorption and nutritional status than more proximal resection.[10] The ICJ is thought to play an important role in optimizing intestinal function both in the intact animal and following resection.[10-12] This is presumably due, at least in part, to a sphincteric mechanism at the ileocecal valve, which in turn may alter motor patterns in the more proximal intestine and thus slow intestinal transit and promote absorption.[10-14] However, this beneficial effect might also be due to a barrier function of the ICJ in preventing reflux of colonic contents such as bacteria and short-chain fatty acids.[11,12] The terminal ileum itself has unique motor properties and possesses the exclusive sites for bile salt and vitamin B_{12} absorption. Receptors in the distal ileum also appear to mediate reflexes that retard intestinal transit and gastric emptying in response to increased concentrations of intraluminal lipid—the ileal brake.[15] These reflexes may assume a homeostatic function in the patient with steatorrhea and serve to minimize caloric losses.[13]

It is reasonable to speculate, then, that alterations in the luminal concentrations of either bacteria or short-chain fatty acids may play a role in the motor response to intestinal resection. Thus the aim of the present study was to evaluate the effect of bypass of the ICJ, a procedure that should promote bacterial colonization of the remnant and reflux of short-chain fatty acids, on the motor response to a 50% distal resection.

METHODS

Thirty-five mongrel dogs (14 to 26 kg) were included in the study; these dogs were divided into three groups. The first group (n = 11) underwent transection and reanastomosis and served as control animals (TC). The other two groups (n = 12) underwent 50% resection of the distal intestine with preservation of the ICJ in one group (DR) and distal resection with bypass of the ICJ by jejunocolostomy in the other (DRBP). Serosal strain gauges and intraluminal catheters were placed in all animals. Approximately half of the animals in each group were killed at 4 weeks and the remainder at 12 weeks after operation. Thus from 0 to 4 weeks 11, 12, and 12 animals were studied in the TC, DR, and DRBP groups, respectively, and from 8 to 12 weeks six animals were studied in each group.

All animals were fed standard dog chow and caloric intake was recorded daily. Nutritional status was determined at 4-week intervals by measuring body weight and serum albumin levels. Intestinal absorption was evaluated by determination of stool weight, moisture, and fat content every 4 weeks. Luminal content of short-chain fatty acids and bacteria was measured via intraluminal cannulas. Intestinal motility was assessed by recordings of motor activity and transit time. The adaptive response to intestinal resection was determined by comparing measurements of intestinal remnant length and circumference and mucosal thickness at the initial operation with those made at the time the animals were killed. The hormonal response to the procedures was assessed by measuring fasting and postprandial plasma levels of the motor-active peptides YY, motilin, and neurotensin.

Operative Procedures

After an overnight fast, the animals were premedicated with acepromazine (20 mg) and anesthetized with intravenous thiamylal sodium and halothane by inhalation. Through a midline incision, the small intestine was measured twice along the antimesenteric border from the duodenojejunal junction to the ICJ. The average of those two values was used for the intestinal length. In the TC group, the intestine was transected at the midpoint and anastomosed. In the DR and DRBP animals, 50% of the measured intestinal length was resected from the midpoint to 5 cm proximal to the ICJ. In the DR group, intestinal continuity was restored by a two-layer end-to-end anastomosis. In the DRBP group, the distal ileum was stapled closed and imbricated to minimize the stump, and a two-layer end-to-side ileocolostomy was created 5 cm distal to the ICJ. Segments of bowel were taken from the margins of resection for histologic examination. Serosal strain gauges (n = 10) were placed on the small intestine 10, 20, 30, 40, 50, and 75 cm proximal to the anastomosis, on the antrum, on the duodenum 5 and 25 cm distal to the pylorus, and at a point midway between the distal duodenal and the most proximal jejunal strain gauge. Plastic cannulas (outside diameter 5 mm) were placed intraluminally 45 cm proximal to the anastomosis. Leads from the strain gauges and the sampling cannula were brought out of the lateral abdominal wall via a metal cannula.

Cefazolin (250 mg) was administered by intramuscular injection, once preoperatively and twice postoperatively. The animals received intravenous fluid during the operation and took only water by mouth for

48 hours after the procedure. On the third postoperative day the dogs were offered dog chow (Wayne Dog Food, Pet Products Plus, St. Charles, Mo.) consisting of 25% protein, 8% fat, and 50% carbohydrate. The animals were killed by means of intravenous sodium pentobarbital.

Analytic Procedures

Tissue specimens were placed in formalin and processed for histologic examination with a light microscope. Transverse sections were stained with hematoxylin and eosin. The thickness of the underlying mucosa was quantitated at 10 sites around the circumference of the section with the aid of an ocular micrometer. Serum albumin values were measured by means of standard laboratory techniques. Stool was collected for 48 hours. Stool wet weight was measured and stool dry weight was determined by freeze-drying to estimate stool moisture. Fecal fat was determined by the ether extraction technique.[16]

Assessment of intraluminal contents was performed on a separate day from other studies. Using sterile technique, the cannula was opened and the intestinal contents allowed to drain into a sterile container. Samples of this drainage fluid were then submitted for bacteriologic analysis and short-chain fatty acid estimation.

Bacteriologic samples were cultured both aerobically and anaerobically. Following appropriate incubation periods, total colony counts were performed on plates containing 30 to 300 colonies. Each colony type was described and subcultured to a blood agar plate for aerobic and anaerobic incubation to ensure its purity. Each colony type was examined for its tinctorial properties with a Gram stain. Bacterial counts were expressed as the number per milliliter of aspirate.

Short-chain fatty acids (acetic acid, propionic acid, butyric acid, isobutyric acid, isovaleric acid, and valeric acid) were measured using gas-liquid chromatography as described by Jouany.[17] Samples were filtered through mesh gauze and mixed with a 1% (volume/volume [v/v]) solution of mercuric chloride and 5% (v/v) orthophosphoric acid in distilled water and then stored at −20° C. Thawed samples were centrifuged at 5000 *g* for 5 minutes. Ten microliters of a 1% solution of 4-methylvaleric acid was added to 100 μl of sample to serve as an internal standard; 0.5 μl fractions were injected for chromatographic analysis (Hewlett-Packard 5890 series II gas chromatograph, Hewlett-Packard Co., Wilmington, Del.). Each short-chain fatty acid was identified by the appropriate retention time and quantitated by integrating the peak area. Concentrations were expressed as micrograms per milliliter of aspirate.

Hormone Studies

Following an 18-hour overnight fast, meal studies were performed in all dogs. For blood sampling, a plastic intravenous catheter (Critikon, Inc., Tampa, Fla.) was placed in a front leg vein and kept patent with heparinized saline solution (10 units/ml; 0.15 mol/L NaCl). Two fasting blood samples were drawn 15 minutes apart after clearing the dead space in the catheter. The animals were then fed a meal of beef-flavored dog food (constituents as percentages of total weight: protein 8%, fat 1.5%, fiber 2%, and moisture 78%; Sir Johns Choice, Bern Extrusion Inc., Bern, Kan.). The meal was consumed promptly and in a similar fashion in all three groups. Additional blood samples were drawn at 15, 30, 60, and 120 minutes after the beginning of the meal. Blood samples were drawn into chilled tubes containing ethylenediaminetetraacetic acid (1.5 mg/ml) and aprotinin (400 KIU/ml). Tubes were centrifuged at 1000 *g* for 10 minutes at 4° C, and the plasma was decanted and frozen at −80° C for subsequent extraction.

Hormones (peptide YY, motilin, and neurotensin) were measured using established specific and sensitive radioimmunoassays that had been previously standardized and optimized for measurement of dog regulatory peptides.[18] The mean basal, incremental integrated response, and total integrated response were calculated in individual animals, for each hormone, using the trapezoidal method.[19] Concentrations were expressed as picomoles per liter.

Motility Recordings

For measurement of motor activity, the output from all strain gauges was transferred to a Sensormedics R711 recorder (Sensormedics Corp., Anaheim, Calif.) using alternating-current amplifiers and displayed at a paper speed of 0.5 mm/sec. Recordings were begun 2 weeks following the surgical procedure. Each animal was then studied at 2-week intervals over 4 to 12 weeks. Dogs were fasted for 36 hours before each study and rested quietly in a Pavlov sling during recordings. Recordings were first performed with the dogs in the fasted state until at least two complete cycles of the migrating motor complexes (MMCs) had been recorded. The animals were then fed a meal consisting of 400 g of meat and recordings were continued for at least another hour.

Recordings of motor activity were analyzed visually. MMCs were identified, and the periodicity, propagation velocity, and duration of each phase of the MMC at each strain gauge site were analyzed using methods described by Code and Marlett[20] and presented previously.[1]

In view of the prominence of clustered motor activity following extensive resection,[1] particular attention was given to the identification of clusters of contractile activity, defined as uninterrupted bursts of rhythmic contractions other than phase 3 of the MMC, of greater than 30 seconds' duration, and separated by a quiescent period of at least 30 seconds' duration. The incidence, propagation, and duration of all such clustered activity were documented. For each recording, the percentage of the total recording time occupied by clusters was also calculated.

Individual recordings from the fasted state were also classified according to the overall organization of motor activity. The following patterns were recognized: *MMC*, normal migrating motor complex cycles featuring phases 1 through 3 in sequence; *MMC with clusters*, regularly occurring phase 3 fronts separated by bursts of clustered contractions; and *clusters alone*, no evidence of MMC or phase 3 activity, the motor activity consisting of recurrent clusters. Tracings were also inspected for the presence of prolonged propagated contractions, defined as rapidly propagating, large-amplitude, prolonged (>6 seconds' duration) waves.[13] We also inspected tracings for the presence of other abnormal patterns.

Small intestinal transit time was assessed by timing the movement of the head of a column of barium in conscious dogs sedated with ketamine. By means of an orogastric tube, 100 ml barium was instilled in the stomach. The animal was then placed in the right lateral decubitus position to empty the stomach. With the use of intermittent fluoroscopy, the transit time was measured by determining when the head of the barium column had passed from the pylorus to the respective junction of the small and large intestine.

Statistical Analysis

All data are expressed as mean ± standard error of the mean (SEM). Comparisons between groups were performed using analysis of variance with the Bonferroni correction as appropriate. Statistical significance was ascribed to P values <0.05.

RESULTS

Nutritional and Absorptive Status

Caloric intake was significantly reduced in the two resected groups during the second week after operation (Table I). Caloric intake was also decreased at 8

Table I. Comparison of nutritional parameters

Study group	Nutritional parameters		
	Transection	Resection	Resection with bypass
Caloric intake (cal/kg/day)			
Preop	85 ± 4	78 ± 6	78 ± 6
Week 2	76 ± 6	56 ± 8*	60 ± 8*
Week 4	79 ± 8	70 ± 5	72 ± 8
Week 8	77 ± 9	72 ± 5	62 ± 6*
Week 12	78 ± 8	68 ± 4	59 ± 8*
Body weight (kg)			
Preop	18.9 ± 0.9	19.1 ± 1.6	20.6 ± 1.4
Week 4	17.8 ± 0.6*	17.0 ± 1.5*	17.1 ± 1.2
Week 8	16.8 ± 0.7*	16.5 ± 1.6*	15.8 ± 1.4
Week 12	16.2 ± 0.9	15.6 ± 1.5*†	15.2 ± 1.5*†
Serum albumin (g/dl)			
Preop	3.8 ± 0.9	3.3 ± 0.1	3.6 ± 0.2
Week 4	3.3 ± 0.6	2.6 ± 0.2*	2.8 ± 0.2
Week 8	2.9 ± 0.1	2.5 ± 0.3	2.3 ± 0.2*
Week 12	2.6 ± 0.2*	1.9 ± 0.1*	2.1 ± 0.2*†

*P <0.05 vs. 0 weeks.
†P <0.05 vs. 4 weeks.

Table II. Comparison of absorption studies

Study group	Absorption studies		
	Transection	Resection	Resection with bypass
Stool weight (g/24 hr)			
Preop	135 ± 20	181 ± 12	182 ± 19
Week 4	181 ± 24	139 ± 18	158 ± 17
Week 8	135 ± 20	110 ± 16*	118 ± 14*
Week 12	141 ± 23	136 ± 27	160 ± 29
Stool moisture (%)			
Preop	57 ± 4	62 ± 2	63 ± 2
Week 4	62 ± 2	62 ± 3	72 ± 2*†‡
Week 8	60 ± 1	60 ± 4	70 ± 3*†‡
Week 12	63 ± 2	62 ± 2	66 ± 3
Stool fat (%)			
Preop	2.0 ± 0.2	2.1 ± 0.5	2.3 ± 0.3
Week 4	2.5 ± 0.4	8.6 ± 2.2*†	15.6 ± 2.0*†‡
Week 8	1.9 ± 0.4	8.2 ± 2.0*†	13.9 ± 2.3*†‡
Week 12	2.4 ± 0.4	6.7 ± 0.6*†	13.6 ± 2.2*†

*P <0.05 vs. 0 weeks.
†P <0.05 vs. transection.
‡P <0.05 vs. resection.

and 12 weeks in the animals undergoing resection and bypass. Overall the average caloric intake was lower in the two groups undergoing resection compared to those undergoing transection (P <0.05).

Nutritional status was impaired in the resected animals (Table I). Body weight decreased in all three groups, but weight loss was greater and more sustained in the resected animals (Table I). By 12 weeks body weight was 81% of initial weight following resection alone and 74% of initial weight following resection and bypass. Serum albumin levels decreased in all three groups to approximately 60% of initial values (Table I).

Clinically, diarrhea occurred during the first few weeks after resection but resolved promptly. However, stool weight did not increase significantly in any of the animal groups (Table II). Stool moisture increased significantly only in the animals undergoing resection and bypass but had returned to normal by 12 weeks. Persistent steatorrhea occurred in both resected groups but stool fat was significantly greater after resection and bypass (Table II).

Structural Adaptation

Intestinal adaptation occurred to a similar extent in both resected groups. Intestinal remnant length proximal to the anastomosis increased by approximately 10% in both resection groups in comparison to transection animals but was similar in the two resected groups (174 ± 10 cm and 180 ± 10 cm vs. 156 ± 7 cm, resection and resection with bypass vs. transection, respectively). Intestinal diameter increased significantly throughout the remnant in resected animals (proximal jejunum initial vs. final, 2.3 ± 0.1 vs. 2.7 ± 0.1 cm, 2.4 ± 0.0 vs. 2.6 ± 0.0 cm, and 2.4 ± 0.0 ± 2.4 ± 0.0 cm, resection, resection with bypass, and transection, respectively). Intestinal diameter increased in all three groups at the midpoint just proximal to the anastomosis (2.2 ± 0.1 vs. 2.6 ± 0.2 cm, 2.2 ± 0.1 vs. 2.6 ± 0.1 cm, and 2.1 ± 0.1 vs. 2.6 ± 0.1 cm, resection, resection with bypass, and transection, respectively). Mucosal thickness at the intestinal midpoint increased significantly in both resected groups (1254 ± 124 vs. 1939 ± 162 μm and 1319 ± 125 vs. 1662 ± 162 μm, resection and resection with bypass, respectively; P <0.05). Mucosal thickness at the midpoint in the transection group at the time the animals were killed was 1537 ± 140 μm.

Luminal Short-Chain Fatty Acids and Bacteria

Luminal concentrations of short-chain fatty acids were significantly greater in animals undergoing resection and bypass compared to the other two groups (3126 ± 1094 vs. 1791 ± 538 μg and 1600 ± 446 μg/ml, resection with bypass, resection alone, and transection, respectively; P <0.05). Although all short-chain fatty acids tended to increase in the ani-

Table III. Characteristics of migrating motor complex (MMC)

	Transection	Resection	Resection with bypass
MMC origin (%)			
Proximal remnant	100*	66	65
Midpoint of remnant	0	8	12
Distal remnant	0	26	22
Extent of MMC propagation (% of remnant)			
100%	100*	50	54
50%-100%	0	25	17
<50%	0	25	29

*P <0.05 vs. resection and resection with bypass.

mals undergoing bypass, the most significant increase occurred in the less common branched-chain fatty acids, that is, isobutyric, valeric, and isovaleric acids (403 ± 266 μg/ml, 74 ± 71 μg/ml, and 64 ± 76 μg/ml, resection with bypass, resection, and transection, respectively; P <0.05). As has been previously reported,[21] however, there were no significant differences in the proportions of the different short-chain fatty acids between the various groups and short-chain fatty acid content did not differ at 4, 8, and 12 weeks after operation.

Aerobic bacterial cultures demonstrated greater than 10^5 bacteria/ml in all animals. *E. coli* (present in 100% of all samples) and *Proteus* (48%) were the most prominent aerobic organisms. Anaerobic bacteria were present in concentrations in excess of 10^5 in 92% (24 of 26) cultures of the bypassed animals at each time point. Only 45% (9 of 20) of the cultures from transected and 62% (13 of 21) of the cultures from animals undergoing resection alone had greater than 10^5 anaerobes present. This difference was maintained throughout the study period and was highly significant (P <0.05). *Bacteroides* (100%) and *Clostridium* (59%) were the most common anaerobic organisms.

Motility

The frequency and characteristics of the MMCs, as well as clustered contractions and prolonged propagated contractions, were compared. MMC frequency was similar in all three groups: mean (range) 0.54 (0.40 to 0.78), 0.39 (0.15 to 0.62), 0.52 (0.31 to 0.68) cycles/hr for transection, resection, and resection plus bypass, respectively. In transected animals all MMCs originated in the antrum or proximal duodenum and propagated through the recorded segment. In both resected groups, distal starts were more frequent and the extent of MMC propagation was reduced (P <0.001), but there were no differences between these two groups (Table III).

In transected animals, fasting motor activity was organized into recurring cycles of the MMC (Fig. 1). In both resection groups, MMC with clusters was the dominant pattern of fasting motor activity in the jejunal remnant (Fig. 2). Cluster activity was present throughout the remnant in 50% of recordings in animals undergoing resection alone and in 32% of those undergoing resection plus bypass (not significant). Prolonged propagated contractions were identified only in the bypass group, being observed in 7% of recordings, and were seen to extend to the proximal remnant (Fig. 3).

Intestinal transit time was significantly shorter 4 weeks after resection compared to transection but was not different between the two resected groups (TC vs. DR vs. DRBP: 37 ± 9 minutes vs. 14 ± 3 and 19 ± 8 minutes; P <0.05).

Hormones

Basal and postprandial hormone levels are shown in Tables IV to VI. Basal plasma concentrations of peptide YY were increased to a similar degree in both resection groups (Table IV). There were no significant differences between all three groups in the incremental integrated response of peptide YY to a meal. The total integrated response was increased in both resection groups. Basal neurotensin levels were similar in all three groups (Table V). There was a small and transient increase in the neurotensin response to a meal after resection alone. In contrast, the postmeal response of neurotensin was abolished after resection with bypass. Basal motilin levels fell in the resection group and transiently in the transection control group, but not in the bypass group (Table VI).

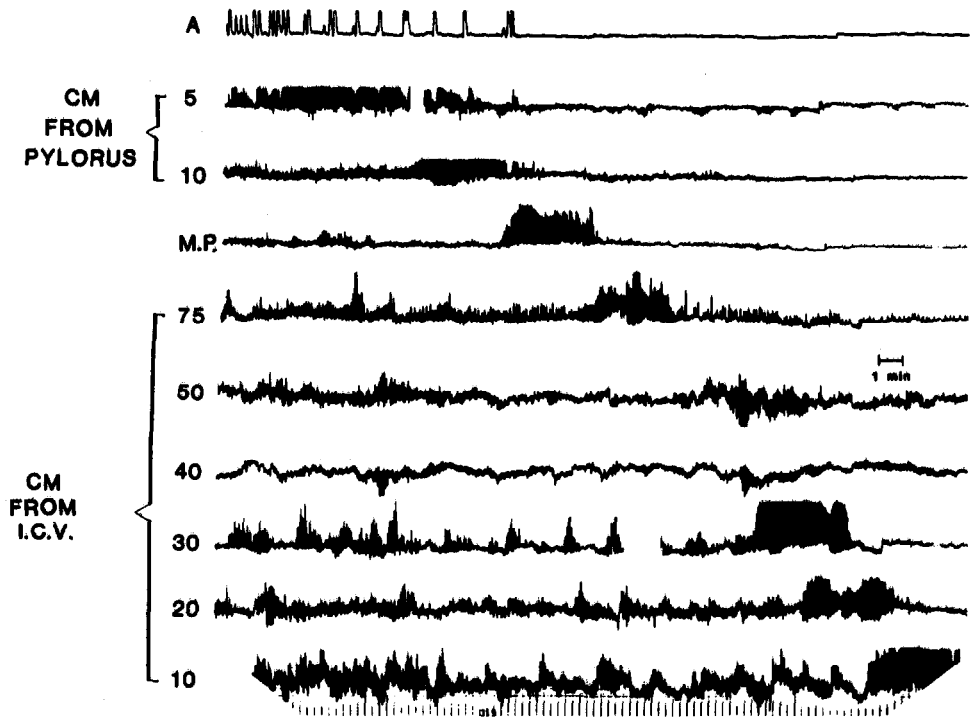

Fig. 1. Intestinal motility in transection control animal. Recording of fasting motor activity from several strain gauges on the antrum *(A)* and along the small intestine. Note phase 3 of migrating motor complex migrating from antrum to strain gauge 10 cm from the ileocecal valve *(ICV)*. Note prominent clustered contractions in recordings from strain gauges within 30 cm of ICV. MP = midpoint of small intestine.

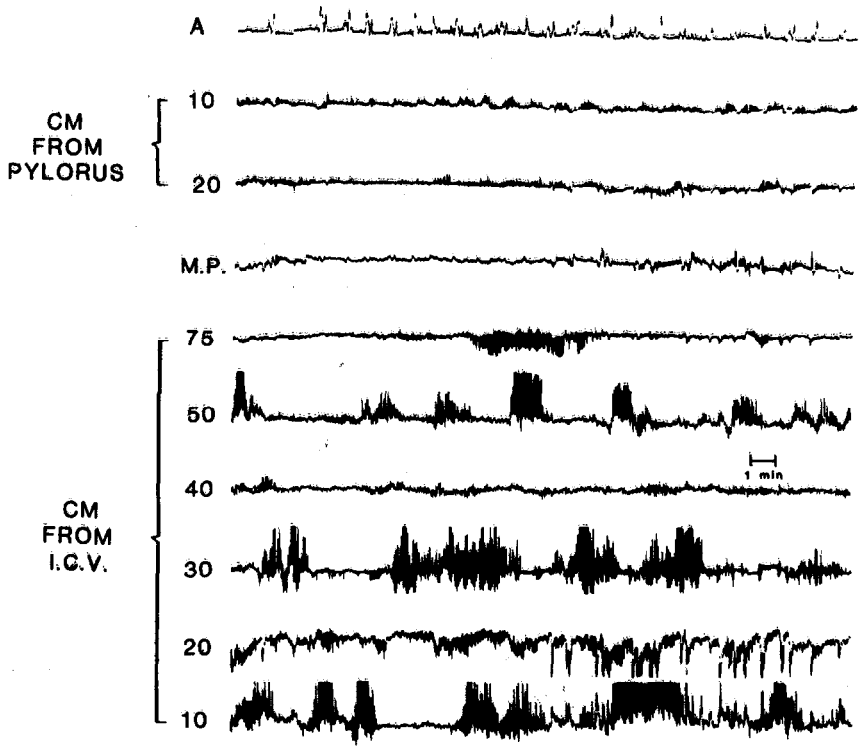

Fig. 2. Intestinal motility following 50% resection of distal small intestine. Recordings of fasting motor activity from serosal strain gauges on the antrum *(A)* and along the intestinal remnant. Anastomosis located 5 cm proximal from the ileocecal valve *(ICV)*; distal recording sites are located therefore at 5, 15, 25, 35, 45, and 70 cm from the distal end of the jejunal remnant. Note disruption of jejunal motor activity in the distal remnant with prominent propagating clusters. MP = midpoint of intestinal remnant.

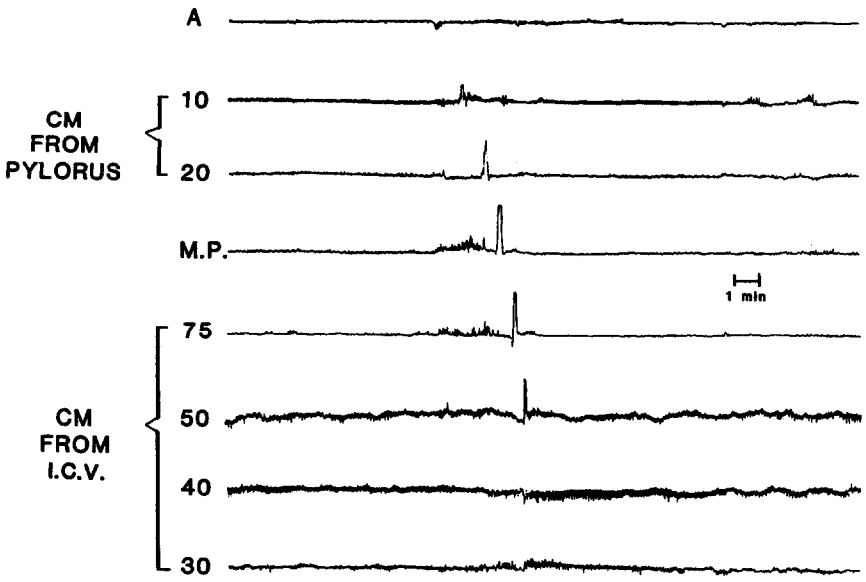

Fig. 3. Prolonged propagating contraction. Recording of fasting motor activity from serosal strain gauges on the antrum *(A)* and along the intestinal remnant from an animal that had undergone a 50% distal resection and ileocolonic bypass. Note distinctive high-amplitude, prolonged-duration wave migrating rapidly along the length of the intestinal remnant. MP = midpoint of intestinal remnant.

Table IV. Plasma peptide YY concentrations

	Transection	Resection	Resection with bypass
Fasting (pmol/L)			
Preop	77 ± 4	81 ± 7	78 ± 5
Week 4	99 ± 6*	137 ± 17*	129 ± 14†
Week 8	84 ± 6	117 ± 12	112 ± 12
Week 12	76 ± 11	115 ± 11	104 ± 8
Total integrated response (pmol/L/180 min)			
Preop	12,641 ± 704	14,441 ± 1340	12,605 ± 1067
Week 4	14,904 ± 625	21,239 ± 2226*	20,504 ± 2000†
Week 8	14,138 ± 1637	19,513 ± 1070	19,024 ± 876*
Week 12	12,184 ± 1193	18,467 ± 1502	18,336 ± 595
Incremental integrated response (pmol/L/180 min)			
Preop	3481 ± 599	4685 ± 1078	3220 ± 882
Week 4	2954 ± 866	4859 ± 1101	5077 ± 612
Week 8	4070 ± 1112	5548 ± 2431	5594 ± 1538
Week 12	3064 ± 262	4667 ± 1195	5841 ± 360

*P <0.05 vs. preoperative value.
†P <0.01 vs. preoperative value.

Table V. Plasma neurotensin concentrations

	Transection	Resection	Resection and bypass
Fasting (pmol/L)			
Preop	4.1 ± 0.6	4.0 ± 0.4	3.7 ± 0.4
Week 4	3.8 ± 0.5	4.8 ± 0.7	3.6 ± 0.3
Week 8	3.8 ± 0.5	3.7 ± +0.5	3.6 ± 0.3
Week 12	4.4 ± 0.6	3.7 ± +0.2	3.6 ± 0.5
Total integrated response (pmol/L/180 min)			
Preop	1121 ± 45	1123 ± 66	1157 ± 224
Week 4	1248 ± 33	1447 ± 53*	444 ± 62†
Week 8	1206 ± 97	1076 ± 52	435 ± 29†
Week 12	1149 ± 35	1105 ± 47	396 ± 43†
Incremental integrated response (pmol/L/180 min)			
Preop	629 ± 88	649 ± 20	712 ± 189
Week 4	790 ± 50	874 ± 98	18 ± 44†
Week 8	746 ± 126	638 ± 18	11 ± 21†
Week 12	668 ± 52	661 ± 51	54 ± 31†

*P <0.005 vs. preoperative value.
†P <0.001 vs. preoperative value.

Table VI. Plasma motilin concentrations

	Transection	Resection	Resection and bypass
Fasting (pmol/L)			
Preop	71 ± 16	75 ± 12	70 ± 8
Week 4	48 ± 6*	46 ± 8*	67 ± 5
Week 8	73 ± 12	36 ± 12*	88 ± 19
Week 12	58 ± 15	46 ± 6*	89 ± 6
Total integrated response (pmol/L/180 min)			
Preop	6310 ± 895	5535 ± 599	6411 ± 414
Week 4	5425 ± 326	5123 ± 94	6720 ± 318
Week 8	6008 ± 278	3394 ± 79	7543 ± 895
Week 12	5880 ± 729	4267 ± 822	7585 ± 243
Incremental integrated response (pmol/L/180 min)			
Preop	−2150 ± 996	−3445 ± 1201	−2004 ± 612
Week 4	−255 ± 249	−457 ± 965	−1320 ± 544
Week 8	−2772 ± 1248	−866 ± 1301	−3046 ± 1522
Week 12	−1040 ± 1106	−1192 ± 328	−3135 ± 524

*P <0.05 vs. preoperative value.

These were no differences in the normal postprandial decrements in motilin levels.

DISCUSSION

In the present study, marked disruption of motor activity was again noted after 50% distal resection with an intact ICJ. MMCs demonstrated more distal starts and a shortened length of propagation and clustered contractions were prominent. These animals had transient diarrhea, malnutrition, and persistent mild steatorrhea, consistent with previous observations.[1] As we have previously reported in detail, structural adaptation was not striking.[21] Luminal concentrations of anaerobic bacteria and short-chain fatty acids were similar in these animals compared to those that had undergone transection alone. These results suggest that the marked motor disruption that occurs early after intestinal resection is not related to changes in luminal bacteria and short-chain fatty acids.

The abnormal motor patterns observed after distal resection alone were not altered by bypass of the ICJ. Intestinal transit time was also similar in the two groups. Bypassed animals had transient diarrhea and malnutrition, similar to the group undergoing resection alone, but they developed more severe steatorrhea. Structural adaptation was not influenced by bypass of the ICJ. However, in comparison to the animals undergoing resection alone, these animals had more anaerobic bacteria and a significantly increased luminal content of short-chain fatty acids. Given the similarity of the motor activity of the jejunal remnant in the two resected groups, these findings again fail to support a relationship between altered motor activity and luminal content of bacteria and short-chain fatty acids.

The failure of bypass of the ICJ to alter the motor response to intestinal resection was surprising. In the dog, the ICJ features a physiologically defined sphincter that could slow intestinal transit.[11,13,14,22] Such a sphincter might influence the motor activity of the jejunal remnant. However, although we demonstrated that an artificially constructed sphincter altered motility in the intact intestine, this effect was not observed following subtotal intestinal resection.[23,24] It is of interest that Fich et al.[25] also failed to note any alteration in small intestinal or ileocolonic transit following a right hemicolectomy.

Bypass of the ICJ permitted reflux of bacteria and short-chain fatty acids into the small intestine. Although all of the animals that had undergone bypasses in the present study had greater than 10^5 anaerobic bacteria present in the jejunal remnant, only 40% to 50% of resection only and transection control animals had significant anaerobic growth. Myrvold et al.[26] similarly demonstrated diffuse overgrowth of anaerobic bacteria in the jejunal remnant after resection and ileocecal bypass. Construction of an artificial valve reduced this growth of anaerobic bacteria to more normal levels. Chardaroyne et al.[27] also demonstrated the efficacy of an artificial valve in reducing bacterial growth in the small intestine after resection and bypass. Using *S. marcescens* as a marker bacterium, this group demonstrated that this was related to reduced reflux of colonic bacteria. Both bacteria and short-chain fatty acids stimulate intestinal motility.[5,6,28] Luminal microflora appear to play an important role in maintaining physiologic cycling and abnormal propagation of the MMC.[5] However, bypass of the ileocecal region did not modify the motor response to 50% distal resection in the present study, despite the induction of higher remnant bacterial and short-chain fatty acid concentrations by this procedure; this suggests that, at least in this model, neither bacteria nor short-chain fatty acids are mediators of the motor response to resection. This finding also questions the role of bacteria and short-chain fatty acids in the mediation of the clustered contractions and prolonged propagated contractions normally seen in the distal ileum. Prolonged propagated contractions were observed solely in the bypass group and were seen to originate in the proximal small intestine, supporting a possible role of short-chain fatty acids in their mediation. Neither the ileocolonic sphincter nor alterations in luminal bacteria and short-chain fatty acid content appear, therefore, to influence the motor response to resection.

In both resection groups the distal ileum was lost. This segment of the intestine possesses some unique absorptive and motor properties. This is the sole location of the absorptive transport mechanisms for bile salts and the vitamin B_{12} intrinsic factor complex. Bile salts have been shown to influence MMC activity.[29] Whether their malabsorption and loss following ileal resection may contribute to the motor effects observed remains to be examined. It is of interest to note that we did not observe any significant change in MMC cycling, as has been observed by Ozeki et al.[30] following biliary diversion; the role of bile salts in the regulation of motor activity remains unclear.[31] Of interest, Scott[32] did not report a significant effect of biliary diversion on duodenal MMC activity. The terminal ileum also appears to possess the receptor(s) site(s) for the afferent arc of the intestinointestinal and intestinogastric reflexes that generate the ileal brake effect. Whether this homeostatic reflex is mediated by a neural or hormonal effect remains uncertain, al-

though recent evidence points to a major role for peptide YY.[33] In any event, the instillation of fat into the distal ileum has been shown to retard both gastric emptying and small bowel transit. Resection of the terminal ileum could abolish this reflex. The motor correlates of this mechanism have not been completely explored.[34]

Our observation that bypass of the ICJ impairs absorption after resection is consistent with other reports. Singleton et al.[12] found that resection of the ICJ in dogs after 50% distal small bowel resection resulted in greater weight loss, fat malabsorption, and diarrhea than resection alone. Stahlgren et al.[35] reported that ileocecal bypass reduced transit time and increased fecal losses of fat and water after 85% resection in dogs. This impaired absorption was most likely related to disruption of luminal digestive and absorptive mechanisms because of the increased luminal content of anaerobic bacteria and short-chain fatty acids.[36] Interestingly, we demonstrated previously that creation of an artificial sphincter proximal to an intact ICJ after resection reduced steatorrhea compared to resection alone.[24]

The present study also provided some limited observations regarding the possible role of hormones in motor adaptation. There were modest differences in the plasma concentrations of the peptides studied. Peptide YY, neurotensin, and motilin were studied because of their potential involvement in motor activity. Basal peptide YY was increased in both resected groups. However, postprandial peptide YY responses were not significantly increased, as has been shown previously.[7] Distal resection caused a small but significant increase in postprandial neurotensin concentrations 4 weeks after surgery, although at 8 and 12 weeks the postprandial neurotensin responses were not significantly different from the preoperative response. In contrast to resection alone, distal resection with bypass of the ICJ abolished the normal postprandial response of neurotensin. Neurotensin is localized to the terminal ileum and thus the diminished levels may reflect bypass of the terminal 5 cm of this region where neurotensin concentrations are highest. Resection caused a reduction in basal plasma motilin concentrations throughout the 12-week study period compared with transection, which only transiently decreased motilin levels at 4 weeks. In contrast, there was no decrease in motilin levels in the group with resection and bypass of the ICJ. No significant differences in the postprandial decreases in motilin levels were seen between either of the resection groups and control animals. Unlike peptide YY and neurotensin, motilin is found primarily in the upper small intestine.[37] Changes in luminal microflora can alter endocrine cell number and the release of biologically active peptides from the intestine.[38] Thus the increased anaerobic bacteria in the bypassed group may alter these responses. Since motor patterns in the two resected groups were similar, changes in neurotensin and motilin do not appear to play an important role in the motor response to resection. In particular, the reduction in basal motilin levels was not associated with any difference in MMC frequency.

Increased luminal short-chain fatty acids might also influence the hormonal response to resection. Small bowel resection is associated with marked changes in neuropeptide and hormone release in response to luminal short-chain fatty acids and fat.[39] There is a striking blunting of the responses of vasoactive intestinal polypeptide, substance P, peptide YY, and neurotensin to luminal fat and short-chain fatty acids. However, bypass of the ICJ did not alter this change in hormone release after resection. Since peptide YY is also released from the colonic epithelium in response to short-chain fatty acid infusion, colonic peptide YY may make an important contribution to the stimulated plasma levels observed with or without ICJ bypass.[40] Although changes in release of the regulatory peptides may be involved in motor and secretory adaptation to resection, they are unlikely to account for the malabsorption seen after loss of the ICJ.

Bypass of the ICJ exacerbates malabsorption and increases luminal concentrations of both anaerobic bacteria and short-chain fatty acids after 50% distal intestinal resection but does not influence the motor response to resection. Thus the motor response to resection does not appear to be influenced by luminal bacteria and short-chain fatty acids or retention of a sphincter mechanism at the ICJ or associated hormonal changes. We would speculate, then, that resection of the terminal ileum, through loss of the receptor site for retarding reflexes or perhaps bile salt malabsorption, may be of primary importance in determining the motor response to resection.

REFERENCES

1. Quigley EMM, Thompson JS. The motor response to intestinal resection: Motor activity in the canine small intestine following distal resection. Gastroenterology 1993;105:791-798.
2. Quigley EMM, Thompson JS, Adrian TE. Motor adaptation following extensive resection of small intestine [abstr]. Gastroenterology 1995;108:A673.
3. Remington M, Malagelada JR, Zinsmeiser A, Fleming CR. Abnormalities in gastrointestinal motor activity in patients with short bowels: Effect of a synthetic opiate. Gastroenterology 1983;85:29-36.
4. Pigot F, Messing B, Chaussade S, Pfeiffer A, Pouliquen X, Jian R. Severe short bowel syndrome with a surgically reversed small bowel segment. Dig Dis Sci 1990;35:137-144.

5. Husebye E, Hellstrom PM, Midtvedt T. Intestinal microflora stimulates myoelectric activity of the rat small intestine by promoting cyclic initiation and aboral propagation of migrating myoelectric complex. Dig Dis Sci 1994;39:946-956.
6. Kamath PS, Phillips SF, Zinsmeister AR. Short-chain fatty acids stimulate ileal motility in humans. Gastroenterology 1988;95:1496-1502.
7. Adrian TE, Thompson JS, Quigley EMM. Time course of adaptive regulatory peptide changes following massive small bowel resection in the dog. Dig Dis Sci 1996;41:1194-1203.
8. Thompson JS, Quigley EMM, Lassiter D, Adrian TE. Smooth muscle contractility after intestinal resection. J Surg Res 1996;60:379-384.
9. Chin BC, Tan DTM, Scott RB. The adaptive contractile response of jejunal circular muscle following massive intestinal resection [abstr]. Gastroenterology 1994;106:A479.
10. Cosnes J, Gendre JP, LeQuintrec Y. Role of the ileocecal valve and site of intestinal resection in malabsorption after extensive small bowel resection. Digestion 1978;18:329-336.
11. Gazet JC, Kopp J. The surgical significance of the ileocecal junction. Surgery 1964;56:565-573.
12. Singleton AO, Redmond DC, McMurray JE. Ileocecal resection and small bowel transit and absorption. Ann Surg 1964;159:690-694.
13. Quigley EMM, Phillips SF, Dent J. Distinctive patterns of interdigestive motility of the canine ileocolonic junction. Gastroenterology 1984;87:836-866.
14. Quigley EMM, Phillips SF, Dent J, Taylor BM. Myoelectric activity and intraluminal pressure of the canine ileocolonic sphincter. Gastroenterology 1983;85:1054-1062.
15. Spiller RC, Trotman IF, Higgins BE, et al. The ileal brake—Inhibition of jejunal motility after fat perfusion in man. Gut 1986;25:365-374.
16. Van De Kamer JH, Huiniuk TB, Weyers HB. Rapid method for the determination of fat in feces. J Biol Chem 1949;177: 347-355.
17. Jouany JP. Volatile fatty acid and alcohol determination in digestive contents, silage juices, bacterial cultures and anaerobic fermenter contents. Sciences Aliments 1982;2:131-144.
18. Adrian TE, Quigley EMM, Rose SG, Johnson TJ, Thompson JS. Effects of jejunoileal autotransplantation on gastrointestinal regulatory peptides. Dig Dis Sci 1994;39:2457-2466.
19. Perdikis G, Wilson P, Hinder RA, et al. Gastroesophageal reflux disease is associated with enteric hormone abnormalities. Am J Surg 1994;167:187-192.
20. Code CF, Marlett JA. The interdigestive myoelectric complex of the stomach and small bowel in dogs. J Physiol 1974;296: 289-309.
21. Thompson JS, Quigley EMM, Palmer JM, West WW, Adrian TE. Luminal short chain fatty acids and postresection intestinal adaptation. JPEN 1996;20:338-343.
22. Phillips SF, Quigley EMM, Kumar D, Kamath PS. Motility of the ileocolonic junction. Gut 1988;29:390-406.
23. Quigley EMM, Thompson JS, Lof J. Disruption of jejunal interdigestive myoelectrical activity by an artificial ileocecal sphincter. Studies of the intestinal motor response to a surgically fashioned sphincter substitute. Dig Dis Sci 1989;34: 1434-1442.
24. Quigley EMM, Thompson JS. Effects of artificial ileocolonic sphincter on motility in intestinal remnant following subtotal small intestinal resection in the dog. Dig Dis Sci 1994;39: 1222-1228.
25. Fich A, Steadman CJ, Phillips SF, et al. Ileocolonic transit does not change after right hemicolectomy. Gastroenterology 1992;103:794-799.
26. Myrvold H, Tindel MS, Isenerg HD, et al. The nipple valve as a sphincter substitute for the ileocecal valve: Prevention of bacterial overgrowth in the small bowel. Surgery 1984;96:42-47.
27. Chardaroyne R, Isenberg HD, Tindel M, et al. Microbiologic efficacy of a surgically constructed nipple valve. Am J Surg 1984;147:230-233.
28. Mashah C, Cherbut C, Bruley des Varannes S, et al. Short chain fatty acids do not alter jejunal motility in man. Dig Dis Sci 1992;37:193-197.
29. Kruis W, Haddad A, Phillips SF. Chenodeoxycholic and ursodeoxycholic acids alter motility and fluid transit in the canine ileum. Digestion 1986;34:185-194.
30. Ozeki K, Sarna SK, Condon RE, Chey WY, Koch TR. Enterohepatic circulation is essential for regular cycling of duodenal migrating motor complexes in dogs. Gastroenterology 1992;103:759-767.
31. Hellstrom PM, Nilsson I, Svenberg T. Role of bile in regulation of gut motility. J Intern Med 1995;237:395-402.
32. Scott RB. Effect of duodenal bile acid delivery on fasting intestinal motor activity. Am J Physiol 1987;250:G836-G841.
33. Lin HC, Zhao XT, Wang L, Wong H. Fat-induced ileal brake in the dog depends on peptide YY. Gastroenterology 1996; 110:1491-1495.
34. Dreznik Z, Meininger TA, Barteau JA, Brocksmith D, Soper NJ. Effect of ileal oleate on interdigestive intestinal motility of the dog. Dig Dis Sci 1994;39:1511-1518.
35. Stahlgren LH, Umana G, Roy R, Donnelly J. A study of intestinal absorption in dogs following massive small intestinal resection and insertion of an antiperistaltic segment. Ann Surg 1962;156:483-492.
36. King CE, Toskes PP. Small intestinal bacterial overgrowth. Gastroenterology 1979;76:1035-1055.
37. Poitras P. Motilin is a digestive hormone in the dog. Gastroenterology 1984;87:909-913.
38. Uribe A, Alam M, Johansson O, Midtvedt T, Theodorsson E. Microflora modulates endocrine cells in the gastrointestinal mucosa of the rat. Gastroenterology 1994;107:1259-1269.
39. Adrian TE, Thompson JS, Quigley EMM, Staab P. The release of enteric neuropeptides following small bowel resection with and without bypass of the ileocecal junction [abstr]. Gastroenterology 1995;108:A268.
40. Longo WE, Ballantyne GH, Savoca PE, Adrian TE, Bilchik AJ, Modlin IM. Short chain fatty acid release of peptide YY in the isolated rabbit distal colon. Scand J Gastroenterol 1991;26:442-448.

A Hospital's Annual Rate of Esophagectomy Influences the Operative Mortality Rate

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The reported operative mortality rate for esophagectomy for malignancy ranges from 2% to 30%. The goal of this retrospective study was to evaluate the relationship between a hospital's annual rate of esophagectomy for esophageal cancer and the clinical outcome of the operation. Discharge abstracts of 1561 patients who had undergone esophagectomy for malignancy at acute care hospitals in California from 1990 through 1994 were obtained from the Office of Statewide Health Planning and Development. The hospitals were grouped according to the number of esophagectomies performed during the 5-year period, and a mortality rate was calculated for each group. Logistic regression analysis was used to determine the relationship between a hospital's rate of esophagectomy and the mortality rate. Esophageal resections were performed in 273 hospitals. An average of two or fewer resections were performed annually in 88% of hospitals, which accounted for 50% of all patients treated. The mortality rate in hospitals with more than 30 esophagectomies for the 5-year period was 4.8%, compared with 16% for hospitals with fewer than 30 esophagectomies. This could not be accounted for by other health variables affecting the patients' risk for surgery. There was a striking correlation between a hospital's frequency of esophagectomy and the outcome of this operation. The results support the proposition that high-risk general surgical procedures, such as esophagectomy for malignancy, should be restricted to hospitals that can exceed a yearly minimum experience. (J GASTROINTEST SURG 1998;2:186-192.)

Esophageal resection for malignancy is a complex, uncommon operation with high morbidity and mortality rates.[1-3] Besides being technically demanding, esophagectomy is stressful to the patient, who is often elderly and afflicted with cardiovascular and respiratory disease. The outcome of this procedure is related to the experience of the surgeon and other members of the team involved in the patient's care including anesthesiologists, intensivists, cardiologists, and interventional radiologists.

With approximately 300 esophageal resections being performed each year in California, the average surgeon and the rest of the team are unable to gain much experience. This study evaluated the relationship between a hospital's annual rate of esophagectomy and the postoperative outcome of this operation in patients with esophageal malignancy. We found, as hypothesized, that the mortality rate was lower in hospitals where the operative experience was greater.

PATIENTS AND METHODS

We used hospital discharge data obtained from the California Office of Statewide Health Planning and Development (OSHPD) from 1990 through 1994. This database contains discharge data for every hospitalization from each acute care hospital in the state. In addition to the hospital's identification, the abstracts contain the following data: the patient's age, sex, race, admission year, primary payer source, diagnoses (principal diagnosis and up to 16 secondary diagnoses), principal procedure, length of hospitalization, discharge disposition (home, other facility, or death), and total hospital charges. Secondary diagnoses available included comorbid medical conditions not related to the malignancy (e.g., diabetes mellitus, coronary artery disease, hypertension, chronic obstructive pulmonary disease) and operative complications (e.g., hemorrhage, infection). The OSHPD database uses diagnostic and procedural codes from

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Table I. Hospital groups

Group	No. of operations/ 5 yr	No. of hospitals	% of hospitals	No. of patients	% of patients
I	1-5	196	72	453	29
II	6-10	43	16	320	21
III	11-20	20	7	291	19
IV	21-30	9	3	224	14
V	>30	5	2	273	17
TOTALS		273	100	1561	100

the International Classification of Diseases, Clinical Modification, Ninth Revision, Fourth Edition (ICD-9), issued by the United States Department of Health and Human Services.[4] Our inquiry obtained data on patients who had undergone excision of the esophagus (ICD-9 code 42.4-42.6). From this group we selected patients who had a concomitant diagnosis of esophageal malignancy (ICD-9 code 150.0-150.9).

Operative mortality rate was defined as the percentage of patients who died before being discharged from the hospital. We did not obtain information on patients who died after discharge from the hospital, even if death occurred within 30 days from the date of the operation. The role of individual patient characteristics in influencing the operative mortality rate was assessed using univariate analysis, with statistical significance determined by the chi-square test. The following individual patient characteristics were analyzed: age, sex, race, payer source, location of tumor, and number of secondary diagnoses. A logistic regression analysis was performed to determine the relationship between the number of esophagectomies performed in a hospital and the operative mortality rate, while controlling for differences in individual patient characteristics. The patient characteristics were treated as covariates, whereas the operative mortality rate was the dependent variable. No data are available regarding the type of surgeon who performed the operation (general vs. thoracic surgeon).

Hospitals were divided into five groups based on the number of esophageal resections for malignancy performed within the 5-year period (Table I). This ensured that the number of patients in each group was large enough for statistical analysis. A crude mortality rate, discharge disposition (home, other facility, or death), length of hospital stay, and total hospital charges were calculated for each group. The frequency and outcome of the major operative complications (hemorrhage and infection) were also determined.

Differences between these measures of outcome were analyzed by analysis of variance. Chi-square analysis of contingency tables, linear regression analysis, and Wilcoxon/Kruskal-Wallis sum test were used where appropriate. A P value of less than 0.05 was considered to be statistically significant. Regression analysis, which included only patient characteristics, was performed to calculate a predicted mortality rate. This information was used to obtain a standardized or risk-adjusted mortality rate for the individual hospitals, which would control for differences in patient characteristics at the time of admission.

Table II. Mortality rates following esophagectomy by admission year

Year	No. of hospitals	No. of patients	No. of deaths
1990	149	312	55 (18%)
1991	139	294	50 (17%)
1992	133	328	50 (15%)
1993	114	313	30 (10%)
1994	131	314	35 (11%)
	133*	1561	220 (14%)

*An average of 133 hospitals reported performing esophagectomies during each year.

RESULTS

From 1990 to 1994, 1561 patients underwent esophageal resections for malignancy in California. A total of 273 hospitals reported this operation to the database, but an average of only 133 hospitals performed esophageal resections during any given year of the study. The operative mortality rate decreased from 1990 to 1994, with an overall average during the study period of 14% (Table II).

Patient Demographics

Patient demographic information included age, sex, race, number of secondary diagnoses, and source of

payment (Table III). Fifty-five percent of the patients were older than 64 years. The operative mortality rate was directly related to advancing age ($P < 0.0001$). Seventy-eight percent of the patients were men, and there was no difference in the mortality rate between men and women ($P = 0.72$). The majority of patients were white (79%), followed by Hispanic (8%), Asian (6%), black (6%), and others (1%). The operative mortality rate did not differ according to race.

The presence of more secondary diagnoses correlated with a higher mortality rate (see Table III). The rate was 4% for patients with four or fewer secondary diagnoses, and it rose with more than four. The overall incidence of postoperative hemorrhage was 5%, and 23% of these patients died. Postoperative infections developed in 8% of patients, of whom 27% died. The incidence of each of these complications correlated directly with the mortality rate.

The location of the tumor was specified in the database in 1404 (90%) of the 1561 cases. Seventy-nine percent of tumors were located in the distal esophagus, 12% in the middle esophagus, and 9% in the proximal esophagus (Table III). The operative mortality rate did not differ according to tumor location (Table IV). The method of reconstruction was listed in 638 (41%) of the 1561 patients. The stomach was used in 568 (89%) patients and the colon in the remaining 70 (11%) patients. The death rate for these two was 11% and 9%, respectively (not significant). In 80% of cases where the colon was used, the procedure was performed in a high-volume hospital.

Table III. Patient characteristics and associated operative mortality rate

Patient characteristics	No. of patients	Operative mortality rate	*P* value*
Age (yr)			<0.000
0-34	14	0%	1
35-54	279	5%	
55-64	412	10%	
65-75	587	16%	
>75	269	26%	
Sex			NS
Male	1213	14%	
Female	348	14%	
Race			NS
White	1235	13%	
Hispanic	120	18%	
Asian	93	13%	
Black	89	23%	
Native American	2	0%	
Other	14	7%	
Unknown	8	25%	
No. of secondary diagnoses			<0.0001
0-4	436	4%	
5-8	569	11%	
9-12	344	22%	
13-16	212	30%	
Tumor site			NS
Upper	126	18%	
Middle	163	14%	
Lower	1115	13%	
Other†	157	20%	
Payer source			<0.0001
Private	666	8%	
Government	895	19%	

NS = not significant.

*By chi-square testing.

†ICD-9 code 150.8. Other specified part: Malignant neoplasm of contiguous or overlapping sites of esophagus whose point of origin cannot be determined.

The source of payment was associated with differences in mortality rates. Patients whose hospitalization was paid for by government programs (Medicare or Medi-Cal) had higher rates than did patients covered by private insurers (Blue Cross/Blue Shield, health maintenance organizations, preferred health plans, and private insurance companies). This effect was examined for possible confounding factors (e.g., age, comorbidity) and remained an independent predictor of mortality. The payer mix was similar in high- and low-volume hospitals.

Patient characteristics were further analyzed in a logistic regression model. When the hospital's annual rate of esophagectomy was excluded, year of operation, age, payer source, and increasing number of secondary diagnoses independently affected the operative mortality rate (Table IV). When the hospital's rate of esophagectomy was included, these variables remained significant.

Relationship Between Hospital Volume and Outcome

Hospitals were divided into five groups based on the number of esophageal resections for malignancy performed between 1990 and 1994 (Table I). An average of two or fewer resections per year were performed in 88% of hospitals, which accounted for 50% of all operations. The hospitals performing the most operations (i.e., group V) treated more than 30 patients each during the study period, but this accounted for only 1.8% of the reporting hospitals (5 of 273 hospitals) and 17.5% of the treated patients (273 of 1561 patients). A number of measures of outcome were evaluated and these are presented in Table V.

Hospital Stay. Length of hospital stay, which averaged about 3 weeks, did not vary with the frequency of the operation.

Discharge Disposition. The percentage of patients who were sent home without being transferred to an intermediate-care facility was higher in centers performing more operations ($P < 0.001$), and this difference persisted after patients who died were excluded.

Hospital Charges. Average total hospital charges increased from the hospitals with the lowest to those with the highest rate of esophagectomy ($P < 0.05$).

Mortality Rate. The overall mortality rate for the study population was 14.1%. Crude operative mortality rates decreased with increasing rates of surgery, from 17% to 19% in the hospitals performing the fewest operations to 4.8% in the hospitals performing the most ($P < 0.0001$ by linear regression analysis and chi-square test). When crude mortality rates were risk adjusted to account for differences in patient characteristics (Table IV), the relationship between surgery rate and mortality rate persisted (Table V and Fig. 1).

Postoperative Complications. The incidence of infection and hemorrhage was similar in all groups (Table VI). The mortality rate associated with infection, however, was 32% in hospitals with few cases and 4% in those with the most ($P = 0.008$). The mortality rate for hemorrhage was lowest in the high-volume centers, but this trend was not quite significant ($P = 0.08$).

Table IV. Factors affecting surgical mortality rate

Independent variable*	*P* Value
Year of operation	<0.05
Age	<0.05
Sex	NS
Race	NS
Payer source	<0.05
Location of tumor	NS
Increasing no. of secondary diagnoses	<0.0001
Decreasing hospital volume	<0.0001

NS = not significant.
*Dependent variable is *operative* mortality rate.

Table V. Outcome measures

Group	No of operations/ 5 yr	Length of hospital stay (days)*	% of patients discharged home†	Total charges‡	Crude mortality rate (%)	Risk-adjusted mortality rate (%)
I	1-5	22	70	$94,781	18	17
II	6-10	21	70	$87,887	19	19
III	11-20	24	75	$107,545	11	10
IV	21-30	20	79	$100,788	15	16
V	>30	22	88	$118,500	5	6

*P = NS.
†Home vs. no home ($P < 0.001$).
‡$P < 0.05$.

Table VI. Incidence of postoperative complications and outcome

	Group						
	I	II	III	IV	V	*P* value	Overall
Infection							
Incidence	8%	9%	9%	7%	9%	0.8	8%
Mortality rate	32%	39%	19%	40%	4%	0.008	27%
Hemorrhage							
Incidence	4%	5%	3%	4%	7%	0.4	5%
Mortality	25%	35%	11%	44%	6%	0.08	23%

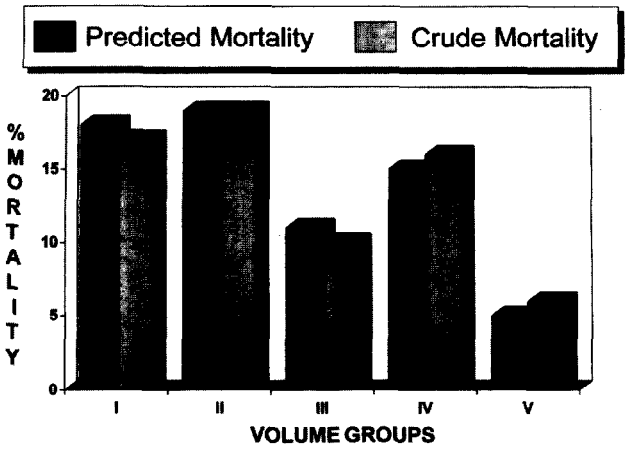

Fig. 1. Esophageal resection for cancer in California: 1990 to 1994. Relationship between operative mortality rates (crude and risk adjusted) and hospital volume.

DISCUSSION

Several studies have examined how the frequency with which a specific operation is performed affects the outcome.[5-9] All of these reports, as well as ours, have limitations. The functional status of the patient at the time of admission was unknown, and adjustment for comorbid conditions was based entirely on the number of secondary diagnoses. In-hospital death was the only measure of outcome; there was no information concerning the patient's functional status at the time of discharge, quality of life, and need for further medical intervention because of late sequelae. Nonetheless, the conclusions are clearcut (i.e., statistically significant), and one's principal reaction when examining the findings is not so much a concern about their validity as a desire for more detail.

Grumbach et al.,[6] for example, measured the relationship between a hospital's annual rate of coronary artery bypass surgery and outcome of the operation in Canada, California, and New York between 1987 and 1989. Approximately 60% of coronary artery bypass operations in Canada and New York were performed in hospitals doing many such procedures (>500 coronary artery bypass operations/yr; 10 [31%] of a total of 32 hospitals) where the mortality rate was 2.5%; in contrast, approximately one third of coronary artery bypass operations in California were done in hospitals that performed fewer than 200 of these procedures per year (72 [65%] of 111 hospitals), where the mortality rate was 3.8% to 4.7% ($P < 0.01$). Our study is similar. Eighty percent of hospitals doing esophageal resections between 1990 and 1994 performed only two or fewer procedures annually. Fifty percent of patients were treated at low-volume centers, and the mortality rate in these least experienced centers was three- to fourfold higher than the rate in the five high-volume centers (1.8% of the 273 hospitals). The authors of the coronary artery bypass surgery study recommended that this operation be regionalized in California to eliminate low-frequency teams, which would allow the results in California to match those in Canada and New York.

Studying the same question in pancreatic resection for malignancy, Gordon et al.[9] showed that the mortality rate for pancreaticoduodenectomy was 19% in hospitals performing few procedures, whereas it was only 2% in those doing the most. Similarly, we recently published data showing that the results of operations for iatrogenic biliary stricture depended substantially on the previous experience of the surgeon in treating that problem.[10]

Esophagectomy is the only definitive treatment for esophageal cancer, but it has among the highest mortality rates for elective operations. The reasons are many. Patients with esophageal cancer are at high risk for any major operation, for they are usually elderly, malnourished, and chronic cigarette smokers. Esophageal resection is a complex operation with many opportunities for error. The procedure must be tailored

to the location and stage of the tumor, and the surgeon must be capable of working in the abdomen, chest, and neck. Various organs can be used as esophageal substitutes, and a leak of the esophageal anastomosis may be fatal. Postoperative complications, which occur in approximately two thirds of patients, often require prolonged treatment in the intensive care unit.

The past two decades have seen major improvements in the mortality rate of esophageal resection. The rate was around 40% forty years ago,[1,11] but it ranges from 0% to 10% in current reports.[3,12-18] These results are not obtained in every hospital, however, but are limited to those with considerable experience in the treatment of this disease. For example, the mortality rate decreased from 20% in the 1960s to 7% in the 1970s at the Cleveland Clinic,[12] and from 10% in the 1970s to 3% in the 1980s at the University of California, Los Angeles.[18]

Interestingly, the incidence of postoperative complications seems to have remained about the same over this period,[15-18] but in contrast with the past, these complications can now be treated successfully in most instances.[19,20] The mortality rate associated with hemorrhage was lower in the high-volume centers which suggests that these centers were just better prepared to treat such complications. In addition to the surgeon, the patient is attended to by anesthesiologists, intensivists, interventional radiologists, cardiologists, and nurses. In another context, we reported that the improved results following pancreaticoduodenectomy noted in recent decades were partly attributable to the effectiveness of interventional radiologists in the treatment of postoperative pancreatic leaks and peripancreatic infections.[21] As in the present study, the major complications of this operation were still being seen, but they were much less often fatal. The expertise of specialists such as these can develop only in centers where complex operations are performed frequently.

Since reports on this subject have consistently demonstrated the same phenomenon, it is now reasonable to assume that as a rule, the results of a complex operation will depend substantially on the experience of the surgeon and the other professionals who are directly involved. Furthermore, the differences are great enough to be important to the public. So, although specific data have not yet been reported on operations such as hepatic lobectomy, ileoanal pullthrough, and total gastrectomy, one could reasonably expect to find the same results with these procedures as with the ones that have already been studied.

There are no data that scientifically explain the reasons for these findings, but they conform to what common sense would predict and what has been shown in other human endeavors (e.g., the military, sports, music, and so forth) involving particularly difficult tasks. In some respects it is surprising that medical practice has been reluctant to accept these concepts and to make organizational changes to deal with them. The resistance may stem from excessive faith in the time-honored credentialing system (e.g., specialty boards), which has done so much in the past to raise the standards of surgery, and the natural inclination to resist further encroachment on the autonomy of individual practitioners. Nevertheless, outcome data concerning medical practice will become increasingly available in the future, and the profession, as well as the payers and the public, will be called on to react in ways that maximize the quality of care, particularly when major additional expenditures can be avoided. This has already occurred to some extent in cardiac surgery, where payers and governmental agencies require a minimum number of cases yearly by the surgeon or the team in order to qualify for support.

CONCLUSION

Evidence is accumulating to indicate that the results of complex operations are considerably better in facilities where they are performed more frequently. In addition to the skill and experience of the surgeon, the entire system in which the operation is carried out is also of major importance. Although contributions of the system have not received much attention in the past, in regard to surgical results, modern concepts of how high-risk activities succeed or fail[22] demand that the narrow focus on the surgeon alone be expanded to take into account the environment in which the operation is performed. In the present context, one could reasonably conclude that esophagectomy for malignancy should be restricted to facilities where this operation is performed at a minimum of six times per year. Whether even better results could be obtained with a higher minimum requires more study. Analyses such as these are shifting the definition of *tertiary care* from an intuitive to a firmer data-supported foundation.

REFERENCES

1. Earlam R, Cunha-Melo JR. Oesophageal squamous cell carcinoma: I. A critical review of surgery. Br J Surg 1980;67:381-390.
2. Lund O, Kimose HH, Aagaard MT, Hasenkam JM, Erlandsen M. Risk stratification and long-term results after surgical treatment of carcinomas of the thoracic esophagus and cardia. A 25-year retrospective study. J Thorac Cardiovasc Surg 1990;99:200-209.
3. Law SYK, Fok M, Wong J. Risk analysis in resection of squamous cell carcinoma of the esophagus. World J Surg 1994; 18:339-346.

4. Jones M, Brouch K, Hall D, Aaron W. St. Anthony's Compact ICD-9-CM: Code Book for Physician Payment, vol. 1 and 2. Reston: St. Anthony Publishing, Inc., 1995.
5. Showstack JA, Rosenfeld KE, Garnick DW, Luft HS, Schaffarzick RW, Fowles J. Association of volume with outcome of coronary artery bypass graft surgery. Scheduled vs. nonscheduled operations. JAMA 1987;257:785-789.
6. Grumbach K, Anderson GM, Luft HS, Roos LL, Brook R. Regionalization of cardiac surgery in the United States and Canada. Geographic access, choice and outcomes. JAMA 1995;274:1282-1288.
7. Laffel GL, Barnett AI, Finkelstein S, Kaye MP. The relation between experience and outcome in heart transplantation. N Engl J Med 1992;327:1220-1225.
8. Lieberman MD, Kilburn H, Lindsey M, Brennan MF. Relation of perioperative deaths to hospital volume among patients undergoing pancreatic resection for malignancy. Ann Surg 1995;222:638-645.
9. Gordon TA, Burleyson GP, Tielsch JM, Cameron JL. The effects of regionalization on cost and outcome for one general high-risk surgical procedure. Ann Surg 1995;221:43-49.
10. Stewart L, Way LW. Bile duct injuries during laparoscopic cholecystectomy. Factors that influence the results of treatment. Arch Surg 1995;130:1123-1128.
11. Ong GB, Lam KH, Wong J, Lim TK. Factors influencing morbidity and mortality in esophageal carcinoma. J Thorac Cardiovasc Surg 1978;76:745-754.
12. Galandiuk S, Hermann RE, Gassman JJ, Cosgrove DM. Cancer of the esophagus. The Cleveland Clinic experience. Ann Surg 1986;203:101-108.
13. DeMeester TR, Zaninotto G, Johansson K-E. Selective therapeutic approach to cancer of the lower esophagus and cardia. J Thorac Cardiovasc Surg 1988;95:42-54.
14. Ellis FH. Treatment of carcinoma of the esophagus or cardia. Mayo Clin Proc 1989;64:945-955.
15. Gertsch P, Vauthey J-N, Lustenberger AA, Friedlander-Klar H. Long-term results of transhiatal esophagectomy for esophageal carcinoma. A multivariate analysis of prognostic factors. Cancer 1993;72:2312-2319.
16. Orringer MB, Marshall B, Stirling MC. Transhiatal esophagectomy for benign and malignant disease. J Thorac Cardiovasc Surg 1993;105:265-277.
17. Bolton JS, Ochsner JL, Abdoh AA. Surgical management of esophageal cancer. A decade of change. Ann Surg 1994;219: 475-480.
18. Swisher SG, Hunt KK, Holmes EC, Zinner MJ, McFadden DW. Changes in the surgical management of esophageal cancer from 1970 to 1993. Am J Surg 1995;169:609-614.
19. Patti MG, Wiener-Kronish JP, Way LW, Pellegrini CA. Impact of transhiatal esophagectomy on cardiac respiratory function. Am J Surg 1991;162:563-567.
20. Nishi M, Hiramatsu Y, Hioki K, et al. Risk factors in relation to postoperative complications in patients undergoing esophagectomy or gastrectomy for cancer. Ann Surg 1988; 207:148-154.
21. Pellegrini CA, Heck CF, Raper S, Way LW. An analysis of the reduced morbidity and mortality rates after pancreaticoduodenectomy. Arch Surg 1989;124:778-781.
22. Cook RI, Woods DD. Operating at the sharp end: The complexity of human error. In Bogner MS, ed. Human Error in Medicine. Mahwah, N.J.: Lawrence Erlbaum Associates, 1994, pp 255-310.

Parahiatal Hernia With Volvulus and Incarceration: Laparoscopic Repair of a Rare Defect

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A rare case of parahiatal hernia with gastric volvulus and incarceration is reported. An anatomically distinct diaphragmatic defect was present adjacent to a structurally normal esophageal hiatus. Laparoscopic repair was performed with excellent results. (J GASTROINTEST SURG 1998;2:193-197.)

Parahiatal hernia is an exceedingly rare condition in which a diaphragmatic muscular defect is present adjacent to a structurally normal esophageal hiatus.[1] Its existence has been debated.[2,3] We report a case in which gastric volvulus and incarceration occurred through an anatomically separate diaphragmatic defect lateral to the left crus of a structurally normal, although enlarged, esophageal hiatus. This report photographically documents parahiatal hernia and is one of the first to describe its repair laparoscopically.

CASE REPORT

A 64-year-old woman with a 10-year history of heartburn and regurgitation presented with an exacerbation of her symptoms over the preceding 6 months. She complained primarily of postprandial epigastric pain, intermittent substernal pressure, and occasional regurgitation. She lost 36 pounds in weight over this time interval. Esophagogastroduodenoscopy demonstrated esophageal erosions, and herniation of the gastric cardia and fundus above the diaphragm. Histologic findings on esophageal biopsy were consistent with erosive esophagitis. A provisional diagnosis of type III (combined sliding and paraesophageal) hiatal hernia was made and she was referred for surgical evaluation.

Outpatient esophageal manometric studies demonstrated normal proximal esophageal peristalsis. The sphincter was 3 cm in length and was located below the diaphragm. The body of the esophagus exhibited normal peristaltic contractions with a few poorly formed, low-amplitude waves seen in the proximal lead (20 to 30 mm Hg). Contractions were well propagated. The lower esophageal sphincter pressure was decreased (5 to 7 mm Hg). A Bernstein acid perfusion test was positive, reproducing upper abdominal pain and substernal burning discomfort consistent with the patient's reflux symptoms. These symptoms could be precipitated and alleviated by switching between acid and antacid.

At the time of surgical evaluation, the patient appeared severely dehydrated and her symptoms had progressed to include malaise, worsened upper abdominal pain, left shoulder pain, persistent chest tightness, and daily postprandial vomiting. Current medications included cisapride (20 mg twice daily), lansoprazole (30 mg once daily), and hormonal replacement therapy. She had no history of tobacco or alcohol abuse.

On admission an electrolyte panel revealed a profound hypochloremic, hypokalemic metabolic alkalosis (sodium 137 mg/dl, potassium 1.9 mg/dl, chloride 62 mg/dl, bicarbonate 54 mg/dl). On physical examination the chest was clear and the abdomen was soft, flat, and nontender. A chest roentgenogram demonstrated a retrocardiac air/fluid level. A contrast roentgenogram of the upper gastrointestinal tract demonstrated a large hiatal hernia with mesenteroaxial volvulus and incarceration of the majority of the gastric fundus and cardia above the diaphragm with high-grade partial gastric outlet obstruction (Fig. 1).

Nasogastric suction was instituted. After an initial period of rapid intravenous crystalloid hydration, total parenteral nutrition was begun. The electrolyte abnormalities gradually normalized over the next several days and she was brought into positive nitrogen balance. Her symptoms resolved and serial abdominal examinations remained benign. On the sixth day after admission, she was taken to the operating room for laparoscopic repair of the hiatal defect.

Operative Repair

A standard approach for laparoscopic repair of paraesophageal hernia was used. A total of five ports were placed in the upper abdomen. Initial visualization of the

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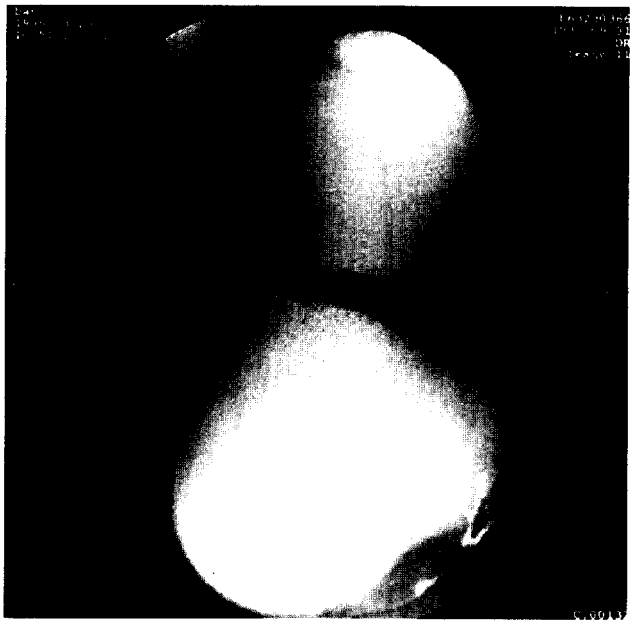

Fig. 1. Preoperative contrast study showing an apparent paraesophageal hiatal hernia with mesenteroaxial volvulus and incarceration of the stomach.

esophageal hiatus clearly showed gastric incarceration. The stomach was grasped with Babcock forceps and reduced into the abdomen using a hand-over-hand technique. In so doing, a small full-thickness tear of the gastric wall occurred, which was repaired with interrupted absorbable suture in two layers. The potion of the gastric fundus that had been incarcerated was edematous and friable, but there was no evidence of necrosis. An edematous hemorrhagic ring on the stomach wall clearly demarcated the neck of the hernia sac.

Dissection near the esophageal hiatus revealed a discrete 5 cm diameter extrahiatal defect immediately adjacent to the left crus of the diaphragm (Fig. 2). A well-developed hernia sac was present within this defect, which ascended into the retrocardiac space adjacent to the esophagus. Dissection was undertaken circumferentially at the margin of the defect and the hernia sac was completely excised. The hernia margin was composed of a well-developed musculofascial rim.

The esophagus was mobilized circumferentially, with careful identification of the anterior and posterior vagus nerves. The left crus was approximately 1 cm thick and moderately attenuated. Its lateral border formed the medial margin of the parahiatal diaphragmatic defect. The right

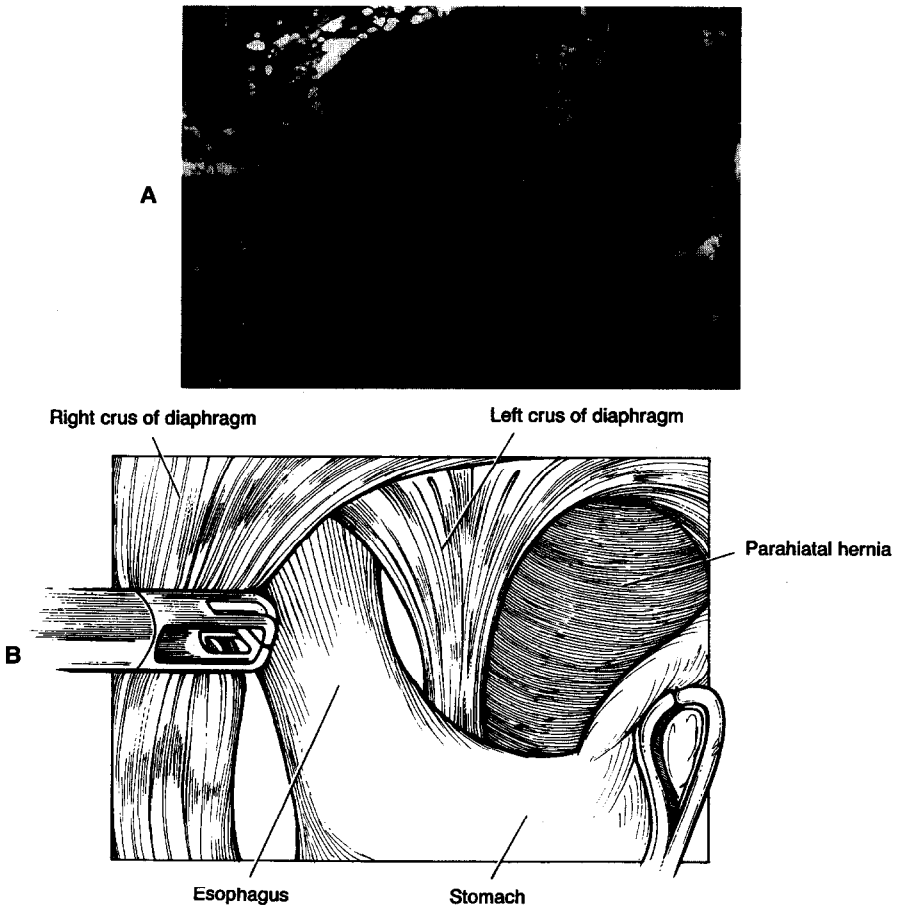

Fig. 2. Operative view after initial reduction of the stomach from the chest into the abdomen. **A,** Frame taken from the videotaped procedure. **B,** Corresponding schematic of the operative view. A parahiatal hernia is present lateral to the left crus of the diaphragm.

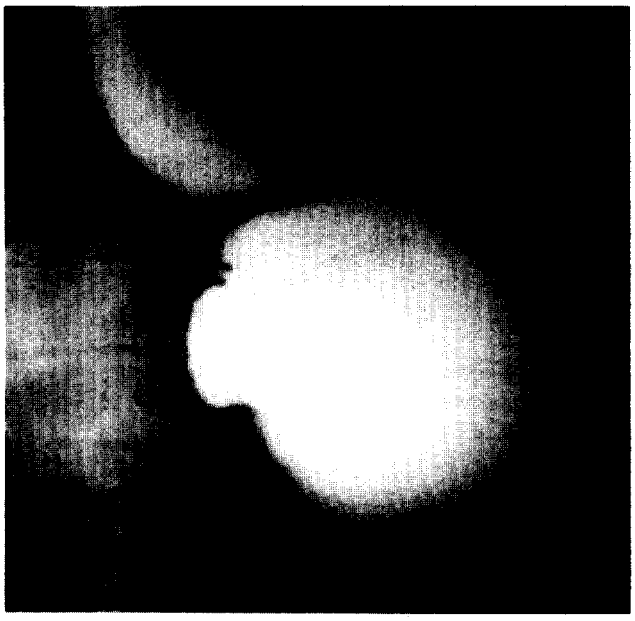

Fig. 3. Barium swallow at 2-month postoperative follow-up. There is no evidence of reflux, the presence of the fundoplication is apparent, and the stomach is below the diaphragm.

and left crural muscle fibers were dissected circumferentially exposing the takeoff of the left crus from the right crural leaflet, thereby demonstrating a structurally normal, although enlarged, esophageal hiatus.

Closure of the parahiatal defect was performed in continuity with the hiatal repair. Edges were approximated without tension using interrupted nonabsorbable suture. The hiatus was closed posterior to the esophagus. Several additional sutures were required anterior to the esophagus in order to completely close the hernia defect.

Following repair of the hiatal defect, the short gastric vessels were divided and a standard Nissen fundoplication employing a "short, floppy" 2 cm fundic wrap over a 60 F dilator was performed. The operation was completed with an anterior gastropexy using percutaneously placed T-fasteners (Ross Laboratories, Columbus, Ohio). In our opinion, gastropexy is a useful adjunct in that it may help to reduce the risk of gastric volvulus if the hernia recurs. Total operative time was 240 minutes, which is only slightly longer than the time required for laparoscopic repair of paraesophageal hiatal hernias in our early experience.

Other than mild subcutaneous emphysema, which quickly resolved, the postoperative course was uncomplicated and notable for complete, immediate resolution of the patient's preoperative symptoms. After a contrast roentgenographic study to make sure there was no leakage from the gastric perforation, the patient was started on a clear liquid diet on the first postoperative day. She was discharged on the third postoperative morning tolerating a mechanical soft diet.

At 2-month follow-up examination, she was asymptomatic. Roentgenographic examination of the upper gastrointestinal tract revealed an intact parahiatal hernia repair and Nissen fundoplication without evidence of gastroesophageal reflux (Fig. 3). The patient was very satisfied with her postoperative result and remained asymptomatic at subsequent 15-month postoperative follow-up.

DISCUSSION

Parahiatal hernia, which is exceedingly rare, is characterized by the presence of a diaphragmatic hernia defect immediately adjacent to an anatomically normal esophageal hiatus.[1] Because of its rarity, the existence of parahiatal hernia has been debated. Many authors have doubted the occurrence of the defect, primarily because proof of its existence has never been conclusively documented.[2,3] Case reports are few in number, and in no case is photographic documentation provided. Confusion surrounding terminology of parahiatal hernia has existed in the past. This most likely stems from variable assignment of terminology to various anatomic configurations of hiatal hernia in older major texts.[4,5] Parahiatal hernia has been described and illustrated in several of these sources, but in recent times the defect has been only rarely mentioned.

The most generally accepted nomenclature of hiatal hernia includes four principal categories (Fig. 4, *A* to *D*). Type I, or sliding hiatal hernia, accounts for approximately 95% of all hiatal hernias. The esophagogastric junction is displaced through the hiatus into the mediastinum secondary to circumferential weakening of the phrenoesophageal ligament. Type II, or paraesophageal hernia, accounts for roughly 5% of hiatal hernias. The phrenoesophageal membrane is focally rather than diffusely weakened, typically anterior, and lateral to the esophagus. The esophagogastric junction remains fixed below the diaphragm, and the gastric fundus herniates through the defect into the mediastinum. Type III is a combination of both types I and II in which sliding and paraesophageal hernia components are present. A fourth category, or type IV, is also gaining more widespread acceptance in the surgical literature. This category comprises cases of anatomically complex hiatal hernia.

Parahiatal hernia is differentiated from these three types by the presence of a separate extrahiatal diaphragmatic defect in which intervening normal crural muscle tissue is present (see Fig. 4, *D*). The hiatus is structurally normal and both crura are intact. In the case described in this report, a sliding hiatal hernia was present in combination with the parahiatal defect.

The etiology of parahiatal hernia has been postulated to be secondary to failure of closure of the pleuroperitoneal canal in embryonic life resulting in a persistent pneumoenteric recess.[6] Parahiatal hernia arising from both right and left pneumoenteric recesses has been described, but conclusive anatomic detail is

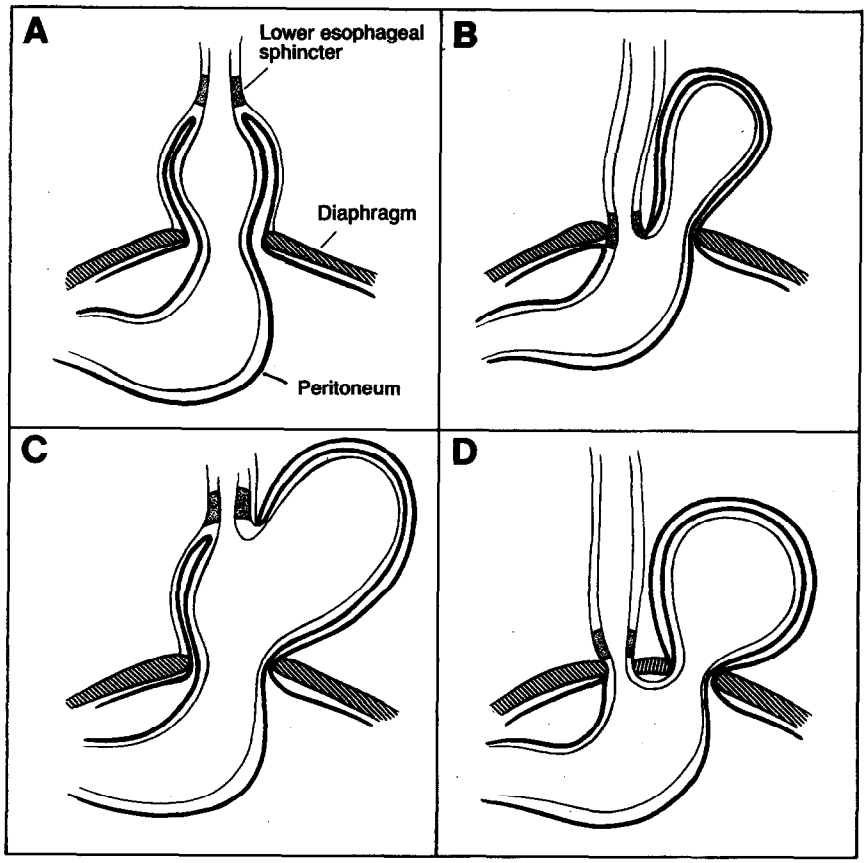

Fig. 4. Schematic illustration of hiatal hernias. **A,** Type I. Sliding hiatal hernia with upward displacement of the lower esophageal sphincter complex. **B,** Type II. Paraesophageal hiatal hernia. The lower esophageal sphincter remains normally positioned at the diaphragmatic hiatus. **C,** Type III. Mixed sliding and paraesophageal hernia. **D,** Parahiatal hernia.

lacking in these studies making it difficult to draw firm conclusions. In the case described in this report, the presence of a well-developed hernia sac suggests that the parahiatal defect was not acute in nature but rather had been present for some time. Conversely, the presentation at a late age argues against this defect being congenital in origin. Perhaps there was a localized congenital diaphragmatic weakness with later acquired development of the frank defect.

In recently published large series of paraesophageal hernia spanning 15- to 20-year experiences, extrahiatal defects were not reported.[7,8] Naturally occurring parahiatal hernia has been reported in the recent literature. One presented with gastric strangulation; this was repaired through a transthoracic approach.[9] Another was repaired laparoscopically.[10] A case of parahiatal hernia has been described after previous thoracic operative repair of a hiatal hernia in which the diaphragm was not incised, although in this situation the etiology of the defect may have been related to the antecedent operative intervention.[11]

Laparoscopic repair of paraesophageal hernia has been described in several reports.[12-18] These have included laparoscopic reduction and repair of incarceration[15] and volvulus[16] associated with paraesophageal hernia. In these cases a standard sutured approximation of the crura was used with good results. The use of mesh for closure of paraesophageal hernia defects has also been described but is controversial.[17,18]

CONCLUSION

Increasing use of the laparoscopic approach for repair of sliding and paraesophageal hernia may lead to more frequent documentation of parahiatal hernia. Visualization and video documentation of the hiatal structures and anatomy is generally excellent using the laparoscopic approach. In the case presented herein, laparoscopic repair was readily performed with excellent results at 15-month follow-up.

REFERENCES

1. Ellis FH. Diaphragmatic hiatal hernias. Recognizing and treating the major types. Postgrad Med 1990;88:113-124.
2. Hill LD, Tobias JA. Paraesophageal hernia. Arch Surg 1968;96:735-744.
3. Naunheim KS, Baue AE. Paraesophageal hiatal hernia. In Shields TW, ed. General Thoracic Surgery, 4th ed. Baltimore: Williams & Wilkins, 1994, p 645.
4. Shackelford RT. Surgery of the Alimentary Tract, 1st ed. Philadelphia: WB Saunders, 1955, pp 2403-2405.
5. Shackelford RT. Surgery of the Alimentary Tract, 2nd ed. Philadelphia: WB Saunders, 1978, pp 489-491.
6. Macdougall JT, Abbott AC, Goodhand TK. Herniation through congenital diaphragmatic defects in adults. Can J Surg 1963;6:301-315.
7. Ellis FH, Crozier RE, Shea JA. Paraesophageal hiatus hernia. Arch Surg 1986;121:416-420.
8. Pearson FG, Cooper JD, Ilves R, Todd TRJ, Jamieson WRE. Massive hiatal hernia with incarceration: A report of 53 cases. Ann Thorac Surg 1983;35:45-51.
9. Demmy TL, Boley TM, Curtis JJ. Strangulated parahiatal hernia: Not just another paraesophageal hernia. Ann Thorac Surg 1994;58:226-227.
10. Trus TL, Bax T, Richardson WS, Branum GD, Mauren SJ, Swanstrom LL, Hunter JG. Complications of laparoscopic paraesophageal hernia repair. J GASTROINTEST SURG 1997;1:221-228.
11. Vallieres E, Waters PF. Incarcerated parahiatal hernia with gastric necrosis. Ann Thorac Surg 1987;44:82-83.
12. Congreve DP. Laparoscopic paraesophageal hernia repair. J Laparoendosc Surg 1992;2:45-48.
13. Pitcher DE, Curet MJ, Martin DT, Vogt DM, Mason J, Zucker KA. Successful laparoscopic repair of paraesophageal hernia. Arch Surg 1995;130:590-596.
14. Oddsdottir M, Franco AL, Laycock WS, Waring JP, Hunter JG. Laparoscopic repair of paraesophageal hernia. New access, old technique. Surg Endosc 1995;9:164-168.
15. Cloyd DW. Laparoscopic repair of incarcerated paraesophageal hernias. Surg Endosc 1995;8:893-897.
16. Koger KE, Stone JM. Laparoscopic reduction of acute gastric volvulus. Am Surg 1993;59:325-328.
17. Kuster GGR, Gilroy S. Laparoscopic technique for repair of paraesophageal hiatal hernias. J Laparoendosc Surg 1993; 3:331-338.
18. Edelman DS. Laparoscopic paraesophageal hernia repair with mesh. Surg Laparosc Endosc 1995;5:32-37.

Effects of Cholecystokinin on Gastric Injury and Gastric Mucosal Blood Flow

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Cholecystokinin (CCK) is a vasodilator and prevents gastric injury from ethanol. Its effects against other irritants are unknown. This study was conducted to (1) assess whether CCK or oleate, a CCK secretagogue, could prevent gastric injury from other damaging agents and (2) examine the role of blood flow in CCK-induced gastroprotection. Conscious rats were pretreated for 10 minutes with intravenous saline solution or CCK (5 nmol/kg) or were given 1 ml of orogastric water or oleate (100 mmol/L) 30 minutes before a 1 ml orogastric bolus of acidified ethanol (150 mmol/L hydrochloric acid/50% ethanol), 0.75N hydrochloric acid, or 0.2N sodium hydroxide. Rats were killed 5 minutes after receiving an irritant and the total area (mm^2) of macroscopic injury was quantified. The duration of CCK-induced gastroprotection against acidified ethanol was examined at 5, 10, 30, and 60 minutes after its administration. Other rats had gastric mucosal blood flow determined (fluorescent microspheres) at identical time points. CCK and oleate decreased gastric injury from all three luminal irritants. CCK-induced gastroprotection was present for 30 minutes but only enhanced gastric mucosal blood flow at 5 and 10 minutes. These data suggest that endogenous CCK may play a role in gastric mucosal defense and that blood flow alone does not fully explain CCK gastroprotection. (J GASTROINTEST SURG 1998;2:198-206.)

Cholecystokinin (CCK) is an endogenous gut peptide produced by "I" cells located within the proximal gastrointestinal tract.[1] Following dietary intestinal stimuli, secretory granules containing CCK are released from the basolateral surface of these cells into the circulation, allowing this peptide to exert its biologic actions.[2] Although multiple molecular forms of CCK exist, biologic activity is retained by the carboxyl terminal octapeptide[3] and is the peptide form most frequently used to examine both the in vitro and in vivo actions of exogenous CCK.

CCK has been demonstrated to not only coordinate digestion through its actions on pancreatic secretion and gallbladder contractility but to also regulate several important gastric functions.[3,4] This gut peptide has been shown to inhibit gastric emptying,[5] inhibit pentagastrin as well as meal-stimulated gastric acid secretion,[6,7] and vasodilate the gastric microcirculation.[8] Recent experience with exogenous CCK octapeptide also indicates that it is a potent protective agent against gastric injury from acidified ethanol[9,10] and concentrated ethanol[11-14] when given in high doses. However, despite attenuating ethanol-induced gastric injury, CCK did not prevent the damaging effects of acidified aspirin to gastric mucosa.[9,14] Furthermore, the protective actions of CCK with doses closer to physiologic significance are unclear, as are its effects against other luminal irritants. Moreover, the protective mechanism responsible for CCK-induced gastroprotection remains to be fully elucidated.

This study was undertaken in conscious rats to ascertain whether exogenous CCK had gastroprotective actions against injury caused by other luminal irritants and to determine whether orogastric administration of oleate, a CCK secretagogue,[15] could prevent gastric injury with the same degree of efficiency as exogenous CCK. Additionally, since CCK is a vasodilator[8] and blood flow is known to play an important role in gastric mucosal defense,[16] further studies were conducted to examine the role of gastric mucosal blood flow in CCK-induced gastroprotection using fluorescent microspheres.[17] Portions of this work have been published in abstract form.[18]

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MATERIAL AND METHODS

Chemicals

CCK-octapeptide and oleic acid were obtained from Sigma Chemical (St. Louis, Mo.). CCK was dissolved in 0.9% saline solution for intravenous administration. Fluorescent microspheres with a diameter of 15.5 ± 0.5 μm were used for blood flow experiments and were obtained from Molecular Probes (Eugene, Ore.). MK-329 was the generous gift of Dr. Roger Friedinger from Merck Research Laboratories, West Point, Pennsylvania. This antagonist was dissolved in 1:1 dimethyl sulfoxide–Tween 80. This solution was subsequently diluted with 0.9% saline solution to a final concentration of 10% dimethyl sulfoxide and Tween 80.

Animals

Female Sprague-Dawley rats weighing approximately 200 g were used in all studies and were housed at constant room temperature with 12-hour light/dark cycles. All experiments were performed in conscious rats deprived of food for 18 to 24 hours but allowed free access to water. On the day of experimentation, all animals were randomly assigned to one of several groups. All experimental protocols had been previously approved by the University of Texas at Houston Animal Welfare Committee before any studies were begun.

Assessment of Protective Actions of CCK and Oleate Against Gastric Injury From Luminal Irritants

Exogenous CCK Experiments. The first set of experiments was designed to ascertain whether a low dose of CCK could prevent gastric injury caused by either concentrated acid or concentrated base. On the day prior to experimentation, rats were anesthetized with an intraperitoneal injection of 6 mg/kg of xylazine and 70 mg/kg of ketamine, and a transverse cervical incision was made. Through this incision, the right jugular vein was isolated and cannulated with silicone tubing (Silastic, inside diameter 0.020 inches, Dow Corning Corp., Midland, Mich.). Catheters were tunneled subcutaneously to an exit point behind the head, ligated, and secured with adhesive tape such that the animals were able to move freely about their cages. After placement of intravenous catheters, all animals were put into individual wire mesh–bottom cages, allowed to recover, and fasted (free access to water) for 18 to 24 hours prior to initiation of experiments. On the day of experimentation, conscious rats were given 0.5 ml of CCK (5 nmol/kg), while control rats received a comparable volume of saline solution. This dose of CCK has been shown to be protective within 10 minutes following its administration.[19] Thus, following a 10-minute pretreatment, rats were randomized to receive a 1 ml orogastric bolus of either concentrated acid (0.75N hydrochloric acid) or concentrated base (2N sodium hydroxide). Since these luminal irritants have been shown to cause macroscopic gastric injury 5 minutes after their administration,[20] rats were killed 5 minutes after receiving a damaging agent. Immediately following sacrifice, stomachs were removed, opened, gently rinsed with water, and the total area of gastric mucosa involved with macroscopic damage was determined as previously reported.[4] A sample size of five or more animals per groups was used.

Oleic Acid Experiments. In a separate set of experiments, the effect of oleate, a CCK secretagogue,[15] against gastric injury from the identical damaging agents, as well as acidified ethanol (150 mmol/L hydrochloric acid/50% ethanol), was assessed. Using a similar protocol, conscious rats were randomized to receive a 1 ml orogastric bolus of oleate (100 mmol/L), while control rats received a comparable volume of water. Since CCK levels are elevated 30 minutes after administration of orogastric CCK secretagogues,[21] gastric injury was induced with 1 ml of an orogastric luminal irritant 30 minutes after the rats were given oleate or water. Five minutes after administration of acidified ethanol, concentrated acid, or concentrated base, rats were killed and the total area of macroscopic gastric injury was determined as previously described. A sample size of five or more animals per group was used.

Since orogastric oleic acid had gastroprotective actions (see Results), additional studies were performed using the type "A" CCK receptor antagonist, MK-329, which was previously shown to dose dependently inhibit CCK-induced gastroprotection.[9] In this set of experiments MK-329 (1 mg/kg) was given intraperitoneally 30 minutes prior to pretreatment with orogastric oleate or water, while control rats received a comparable volume of vehicle (dimethyl sulfoxide, Tween 80, and saline solution 1:1:8 vol/vol/vol). Following the 30-minute pretreatment with oleate or water, gastric injury was induced with orogastric acidified ethanol and macroscopic damage assessed.

Examination of the Relationship Between CCK-Induced Gastroprotection and Gastric Mucosal Blood Flow

These experiments were designed to examine the role of gastric mucosal blood flow as a potential protective mechanism for CCK-induced gastroprotection utilizing the fluorescent microsphere technique[17]

to measure blood flow as previously published.[10] The first set of experiments assessed the time course of CCK-induced gastroprotection against acidified ethanol. For these experiments, CCK (5 nmol/kg) was given intravenously for 0, 5, 10, 30, or 60 minutes before administration of 1 ml of orogastric acidified ethanol. Five minutes after receiving the damaging agent, rats were killed and macroscopic injury was determined as previously cited.

After completion of the CCK gastroprotective time-course experiments, additional experiments were performed to assess the effect of CCK on gastric mucosal blood flow, both in the presence and in the absence of the luminal irritant acidified ethanol. On the day prior to experimentation, rats were anesthetized with an intraperitoneal injection of 6 mg/kg xylazine and 70 mg/kg ketamine, and the femoral artery was cannulated with a silicone tube containing a polyethylene (PE-50) tip. A PE-50 tube was also inserted into the right carotid artery and introduced into the left ventricle through a transverse cervical incision. The correct catheter position was confirmed by obtaining the typical left ventricular tracing. All catheters were tunneled subcutaneously to an exit point behind the head as previously described, placed in a spring coil apparatus, secured to a harness, and connected so that the animals could move freely about their cages. Animals were allowed to recover and fasted overnight with free access to water until the time of experimentation the following day. As with the gastroprotection studies, all experiments were performed in conscious animals to obviate any effects that a general anesthetic might have on blood flow.[22] Blood flow determinations followed the recommendations of Tuma et al.[23]

In the first set of blood flow experiments, animals were randomized to receive either intravenous saline solution or CCK (5 nmol/kg) for 10 minutes followed by administration of orogastric acidified ethanol for 5 minutes as previously cited. Different labeled microspheres were injected at four time points so that determination of blood flow was established for baseline, mid-pretreatment (5 minutes), at the end of pretreatment (10 minutes), and after injury. Blood flow was quantified by injecting into the carotid artery 0.2 ml of the microsphere solution (0.9% saline solution + 0.02% Tween 20), which contains approximately 200,000 microspheres (15.5 ± 0.5 μm), at a rate of 0.2 ml/min followed by a 0.3 ml flush of saline solution. Blood reference samples were withdrawn from the femoral catheter beginning 10 seconds before microsphere injection and continued for 190 seconds at a rate of 0.2 ml/min. Blood volume losses were replaced with an equal volume of saline solution (0.9%) during each withdrawal. Precalibrated micropumps (Instech Laboratories, Inc., Plymouth Meeting, Pa.) were used for all injections and withdrawal. The amount of microspheres injected ensured recovery of more than 400 microspheres in each organ and reference blood sample, the minimum necessary to reduce random variation.[24] Recovery of fluorescence for microspheres extracted from tissues was roughly 100% with the fluorospheres filtration devices used in our study.[17] In this set of experiments, the damaging agent was present within the stomach during the last blood flow measurement. In additional animals, intravenous saline solution of CCK (5 nmol/kg) was given and the duration of CCK-induced hyperemia examined without exposing the stomach to acidified ethanol. In these animals, gastric mucosal blood flow was determined at baseline and 10, 30, and 60 minutes after injection of saline solution or CCK by injecting microspheres at these time points. For all of these protocols, a sample size of five or more animals per group was used.

After completion of all protocols, animals were killed and the mucosa and submucosa of the gastric fundus were stripped from the muscularis externa or serosa. The mucosa and submucosa were measured together as one compartment because the mucosal vascular bed behaves as a bed serially connected to the submucosal vasculature.[25,26] In addition, both kidneys were removed to assess equal microsphere distribution of our blood flow measurements. Tissue samples were weighed and digested with digestion solution (4 mol/L potassium hydroxide + 2% Tween 80) for 12 hours in a water bath (45° to 50° C). After digestion, tissue samples were centrifuged at 1800 rpm for 10 minutes (model TJ-6, Beckman Instruments, Inc., Fullerton, Calif.). The upper phase was rinsed with 2% Tween 80 and centrifuged again, followed by the addition of 5 ml isopropyl alcohol and another centrifugation, after which the remaining alcohol was allowed to evaporate. Sphere solvent was subsequently added to the upper phase and centrifuged, and the fluorescence of the solvent was determined on a luminescence spectrometer (model LS50B, Perkin-Elmer Corp., Norwalk, Conn.). Similarly the fluorescence was determined in the blood reference sample with the exception of digestion solution, which was 16 mol/L potassium hydroxide + 2% Tween 80. Organ blood flow was calculated using the following equation: $V_0 = V_R \times S_0/S_R$, where V_R is the velocity of the reference blood sample (0.2 ml/min), S_R is the fluorescent signal of the reference blood sample, and S_0 is the fluorescent signal of the organ.[17] Blood flow to the stomach and kidney was divided by the respective weights of these organs and recorded as blood flow per 100 g of tissue. For gastric mucosal blood flow de-

terminations, the weight of the mucosa and submucosa was used and not the weight of the full-thickness gastric wall.

Statistics

All data were expressed as mean ± standard error. Five or more animals were used in all experimental groups. For all experiments, differences among the various groups were determined using analysis of variance followed by a Scheffe post hoc test. Changes in blood flow within groups and between groups were compared with one-way analysis of variance followed by a Dunnett post hoc test. Differences in mean values were considered significant at a P value <0.05. Simple regression analysis was performed to compare blood flow in the left and right kidneys.

RESULTS

CCK and Oleate Prevent Gastric Injury

As shown in Fig. 1, control animals pretreated with saline solution followed by administration of a luminal irritant had extensive damage to the gastric mucosa. Damage was confined to the acid-secreting portion of the stomach and was characterized by the presence of hemorrhagic lesions oriented parallel to the gastric folds. In contrast, administration of CCK almost completely prevented the development of lesions caused by concentrated acid and concentrated base. In a similar fashion, the CCK secretagogue was also found to be efficacious for attenuating the extent of macroscopic damage caused by exposure to luminal irritants. As shown in Fig. 2, there was significantly more gastric mucosal injury from acidified ethanol, concentrated acid, and concentrated base in animals pretreated with orogastric water than in animals receiving orogastric oleate for 30 minutes. In the type "A" CCK–receptor blockade studies, which used a dose of antagonist previously shown to negate exogenous CCK-induced gastroprotection, it was found that MK-329 partially reversed the gastroprotective actions of oleate against acidified ethanol-induced injury when compared to vehicle/oleate-pretreated rats (78 ± 12 mm^2 vs. 33 ± 10 mm^2; $P<0.05$).

CCK Augments Gastric Mucosal Blood Flow

The studies designed to examine the relationship between CCK-induced gastroprotection and gastric mucosal blood flow are illustrated in Figs. 3 to 5. As shown in Fig. 3, the gastroprotective actions of CCK were present as early as 5 minutes after its administration. However, this effect was noted to diminish over time. Although gastroprotection was still present at 30 minutes after injection of CCK, no protective effects were apparent 60 minutes after its administration.

The results of the studies measuring gastric mucosal blood flow after pretreatment with saline solution or CCK for 10 minutes followed by orogastric administration of acidified ethanol for 5 minutes are shown in Fig. 4. As depicted, pretreatment with CCK

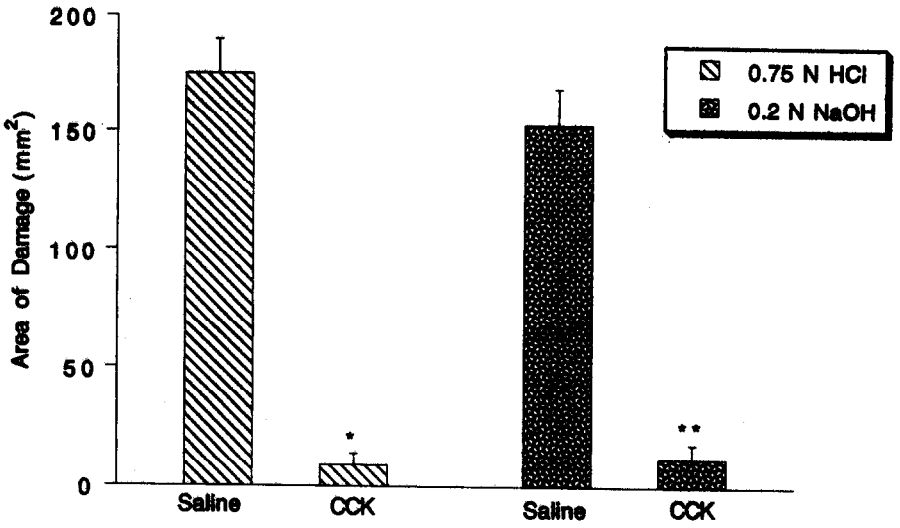

Fig. 1. Effect of intravenous cholecystokinin (*CCK*; 5 nmol/kg) given 10 minutes before exposing the stomach to concentrated acid or concentrated base for 5 minutes on total area of macroscopic injury to rat gastric mucosa expressed as mean ± standard of the mean; N ≥5 for all groups. *P <0.001 vs. saline counterpart; **P <0.001 vs. saline counterpart.

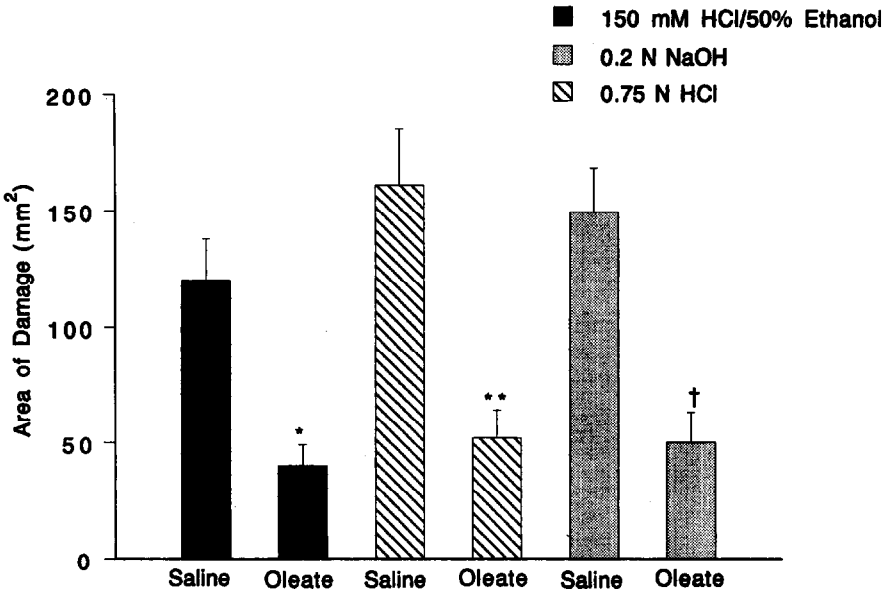

Fig. 2. Effect of orogastric oleate (150 mM) given 30 minutes before exposing the stomach to acidified ethanol, concentrated acid, or concentrated base for 5 minutes on total area of macroscopic injury to rat gastric mucosa expressed as mean ± standard error of the mean; N ≥5 for all groups. **P* = 0.002 vs. saline counterpart; ***P* = 0.005 vs. saline counterpart; †*P* = 0.008 vs. saline counterpart.

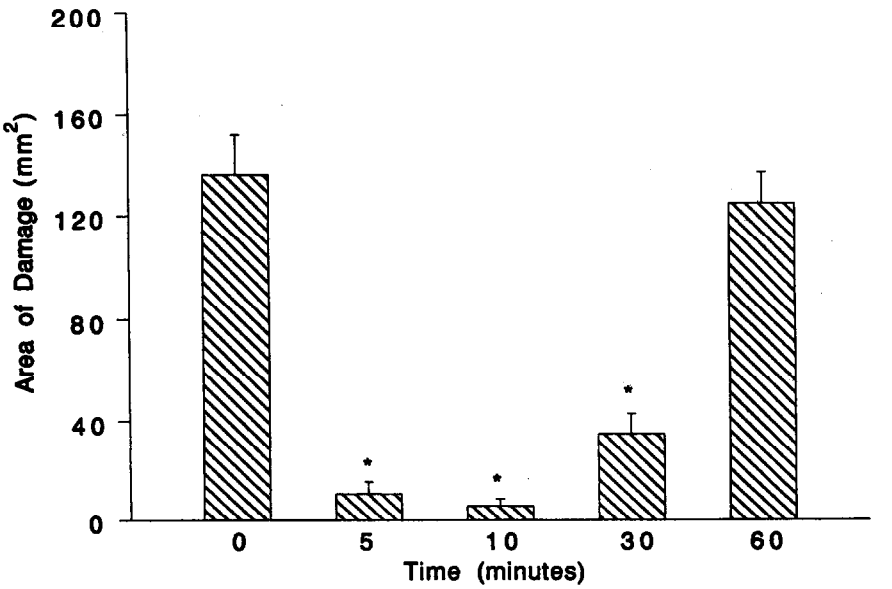

Fig. 3. Effect of intravenous cholecystokinin (5 nmol/kg) on macroscopic injury induced by acidified ethanol at indicated time points. Macroscopic injury is expressed as mean ± standard error of the mean; N ≥5 for all groups. **P* ≤0.001 vs. time point 0 minutes.

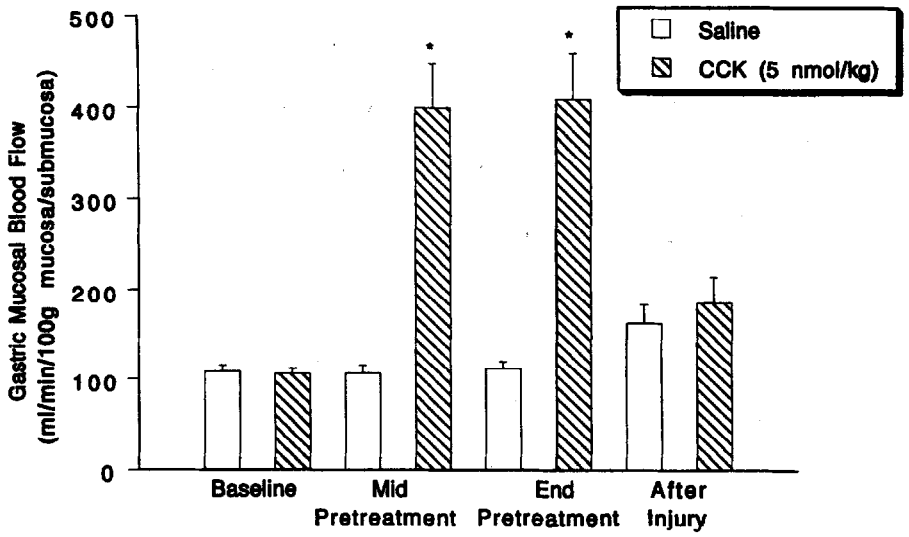

Fig. 4. Fluorescent microsphere (15.5 μm) determined blood flow responses in stomachs of conscious, unrestrained Sprague-Dawley rats. Blood flow is measured at baseline, mid-pretreatment (5 minutes), and at the end of pretreatment (10 minutes) after intravenous administration of either saline solution or cholecystokinin (*CCK;* 5 nmol/kg). Acidified ethanol was given as a 1 ml orogastric bolus 10 minutes after pretreatment with saline solution or CCK and blood flow determined 5 minutes later. Values are means ± standard error of the mean; N ≥5 for all groups. **P* <0.05 vs. corresponding saline-treated animals.

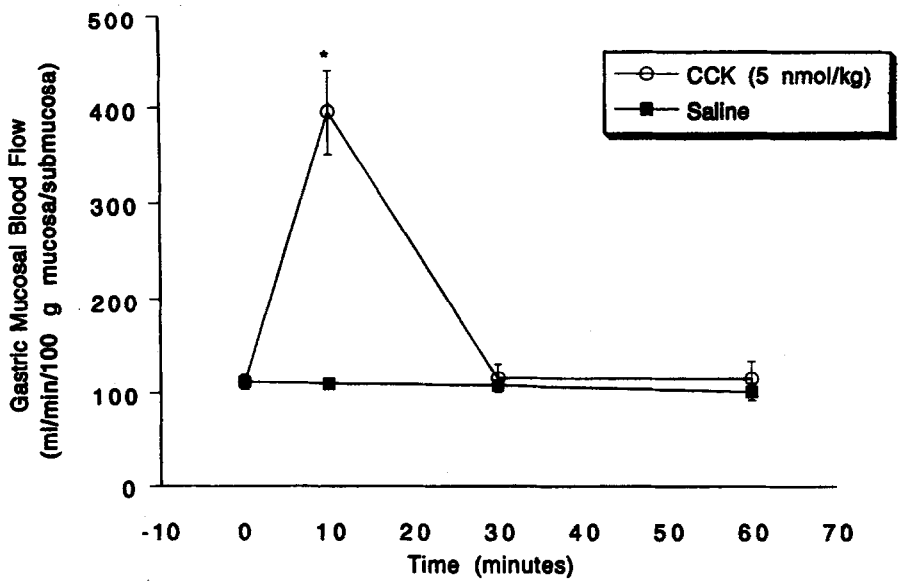

Fig. 5. Effect of intravenous cholecystokinin (*CCK;* 5 nmol/kg) on gastric mucosal blood flow over time in the absence of the luminal irritant acidified ethanol. Blood flow was determined at baseline and 10, 30, and 60 minutes after administration of saline solution or CCK; N ≥5 for all groups. Values are means ± standard error of the mean. **P* ≤0.001 vs. saline and baseline determination.

significantly increased gastric mucosal blood flow at both 5 and 10 minutes after its administration in comparison to saline-pretreated animals. The augmentation in mucosal blood flow after CCK pretreatment represented a threefold increase in blood flow to the stomach from baseline determinations. However, after the 5-minute exposure of the stomach to acidified ethanol, no significant difference could be detected between animals receiving CCK or saline solution. In these experiments the equal distribution of our blood flow measurements with microspheres was also confirmed by comparing the left and right kidneys (data not shown). Regression analysis revealed that $R = 0.946$ ($P < 0.001$).

The studies characterizing the effect of CCK on gastric mucosal blood flow over time in the absence of a luminal irritant are displayed in Fig. 5. As shown, CCK enhanced gastric mucosal blood flow from baseline determinations at 10 minutes but not at 30 or 60 minutes. Taken together, these data demonstrated that although CCK-induced gastroprotection was still present at 30 minutes, the ability of CCK to augment gastric mucosal blood flow was of even shorter duration inasmuch as blood flow had already returned to baseline 30 minutes after its administration.

DISCUSSION

This study demonstrated that a low dose of CCK was capable of preventing gastric injury caused by acidified ethanol, concentrated acid, and concentrated base. The time-course studies characterizing the rapidity and duration of CCK-induced gastroprotection clearly demonstrated that this response is present as early as 5 minutes after intravenous injection and that CCK remains protective for at least 30 minutes. Furthermore, oleate, an agent known to release endogenous stores of CCK, also significantly attenuated macroscopic gastric injury from all three luminal irritants. The gastroprotective effect of oleate was, in turn, partially reversed by type "A" CCK receptor blockade. Taken together, these findings indicate that the gastroprotective actions of exogenous CCK are not limited to ethanol-induced gastric injury and suggest that endogenous CCK may play a role in gastric mucosal defense. However, they also suggest that orogastric oleate has gastroprotective actions independent of its ability to release CCK, as its protective action was not completely abolished during type "A" CCK receptor blockade.

Intravenous CCK also significantly enhanced gastric mucosal blood flow at 5 and 10 minutes after its administration. However, after exposure of the stomach to acidified ethanol for 5 minutes, no significant difference was observed in gastric mucosal blood flow measurements between saline- and CCK-pretreated animals. Moreover, in the absence of an irritant, gastric mucosal blood flow had returned to baseline 30 minutes after CCK pretreatment, a time point when significant gastroprotection was still present. These findings suggest that although an augmentation in gastric mucosal blood flow may prepare the gastric mucosa to withstand damage, it is quite likely that the mechanism responsible for CCK-induced gastroprotection involves contributory factors.

Our findings with exogenous CCK confirm those reported by others. Stroff et al.[13] demonstrated that low doses of CCK reduce gastric injury caused by ethanol given orogastrically to conscious rats. Konturek et al.[14] found that low doses of exogenous CCK decrease gastric injury from ethanol but not aspirin. Since aspirin-induced gastric mucosal injury is acid dependent,[27] whereas ethanol is not,[28] the latter study speculated that CCK may only prevent gastric injury from luminal irritants that caused injury via an acid-independent mechanism.[14] However, molecular acid is known to play an important role in acidified ethanol-induced gastric injury,[28] and CCK significantly reduced gastric injury from this luminal irritant. In addition, our study found that CCK attenuated the damaging effects of concentrated acid to gastric mucosa, suggesting that factors other than acid explain the ability of CCK to prevent aspirin-induced gastric mucosal injury. Moreover, our study demonstrated that CCK-induced gastric mucosal protection is not limited to damage caused by ethanol.

Our findings with orogastric oleate are in agreement with the observations made by Konturek et al.[14] In that study, transduodenal oleate reduced gastric injury from ethanol and these protective actions were negated by administration of the type "A" CCK receptor antagonist MK-329.[14] The selectivity of MK-329 for CCK-induced actions has been previously reported[29] and is the identical antagonist that we[9] and others[12,14] have shown prevents CCK-induced gastroprotection. Thus these findings would suggest that release of endogenous CCK is primarily responsible for oleate-induced gastroprotection from ethanol. Nevertheless, it is noteworthy that Konturek et al.[14] also found that oleate prevents aspirin-induced gastric injury, whereas exogenous CCK does not. This would suggest that oleate also elicits a gastroprotective response against aspirin that functions independently from CCK release. Alternatively it might be argued that orogastric oleate or reflux of transduodenal oleate into the stomach acted as mild irritants and thereby had an adaptive cytoprotective action.[30] Apropos of this possibility, that argument would not explain the ability of the type "A" CCK receptor antagonist to diminish the protective actions of transduo-

denal oleate found by Konturek et al.,[14] because we found that type "A" CCK receptor blockade did not reduce the magnitude of adaptive cytoprotection against acidified ethanol.[31] As a result, the available data indicate that release of endogenous CCK may indeed play a role in gastric mucosal defense against some luminal irritants.

Vasodilation of the gastric microcirculation by CCK as measured by in vivo microscopy was first reported by Guth and Smith.[8] Utilizing fluorescent microspheres to directly measure gastric mucosal blood flow, we previously reported that high doses (micromoles) of CCK augmented blood flow to the stomach after its administration.[10] Konturek et al.,[14] as well as Heinemann et al.,[32] later found CCK to cause gastric hyperemia when given in a physiologic dose range. However, careful gastroprotective time-course experiments and gastric mucosal blood flow responses in the presence and in the absence of a luminal irritant have not been previously reported. Our data from this study indicate that both CCK-induced gastroprotection and hyperemia are rapid in onset but of limited duration. Nonetheless, it is our contention that other mechanisms explain CCK-induced gastroprotection because blood flow is at baseline (30 minutes) when gastroprotective actions still exist. This is not to imply that gastric mucosal blood flow is inconsequential, because maintenance of blood flow to the gastric mucosa is important in ensuring a healthy gastric epithelium and enables the stomach to withstand damage when exposed to a potentially injurious luminal insult.[16] However, blood flow by itself probably does not fully explain protection. Instead, it could simply ensure that those biochemical intracellular processes that underlie mucosal resistance to injury can proceed unabated.

CONCLUSION

Exogenous CCK and the CCK secretegogue oleate prevented gastric injury from acidified ethanol, concentrated acid, and concentrated base. These findings indicate that the gastroprotective actions of CCK are not limited to ethanol. Exogenous CCK was also a potent vasodilator and augmented gastric mucosal blood flow, although this effect was shorter lived than CCK-induced gastroprotection. These findings suggest that other contributing factors are likely responsible for the ability of CCK to maintain the integrity of the gastric mucosa in the face of a damaging luminal insult. Nevertheless, the fact that a low dose of CCK and a substance known to release endogenous CCK could prevent gastric injury from luminal irritants suggests that this peptide may play a role in gastric mucosal defense.

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REFERENCES

1. Buffa R, Solcia E, Go VLW. Immunohistochemical identification of the cholecystokinin cell in the intestinal mucosa. Gastroenterology 1976;70:528-532.
2. Buchan A, Polak J, Solcia E, Capella C, Hudson D, Pearse A. Electron immunohistochemical evidence for the human intestinal I cell as the source of CCK. Gut 1978;9:403-407.
3. Liddle RA. Cholecystokinin. In Walsh JH, Dockray GJ, eds. Gut Peptides Biochemistry and Physiology. New York: Raven, 1994, pp 175-216.
4. Walsh JH. Gastrointestinal hormones. In Johnson LR, ed. Physiology of the Gastrointestinal Tract, vol I. New York: Raven, 1987, pp 181-253.
5. Debas HT, Farooq O, Grossman MI. Inhibition of gastric emptying is a physiological action of cholecystokinin. Gastroenterology 1975;68:1211-1217.
6. Lloyd K, Kent C, Raybould HE, Walsh JH. Cholecystokinin inhibits gastric acid secretion through type "A" cholecystokinin receptors and somatostatin in rats. Am J Physiol 1992;263:G287-G292.
7. Lloyd KCK, Maxwell V, Kovacs TOG, Miller J, Walsh JH. Cholecystokinin receptor antagonist MK-329 blocks intestinal fat-induced inhibition of meal-stimulated gastric acid secretion. Gastroenterology 1992;102:131-138.
8. Guth PH, Smith E. The effect of gastrointestinal hormones on the gastric microcirculation. Gastroenterology 1976; 71:435-438.
9. Mercer DW, Cross JM, Barreto JC, Strobel NHP, Russell DH, Miller TA. Cholecystokinin is a potent protective agent against alcohol-induced gastric injury in the rat. Dig Dis Sci 1995;40:651-660.
10. Mercer DW, Klemm K, Cross JM, Smith GS, Cashman M, Miller TA. Cholecystokinin-induced protection against gastric injury is independent of endogenous somatostatin. Am J Physiol 1996;271:G692-G700.
11. Evangelista S, Maggi CA, Meli A. Influence of peripherally administered peptides on ethanol-induced gastric ulcers in the rat. Gen Pharmacol 1987;18:647-649.
12. Evangelista S, Maggi CA. Protection induced by cholecystokinin-8 (CCK-8) in ethanol-induced gastric lesions is mediated via vagal capsaicin-sensitive fibres and CCK_A receptors. Br J Pharmacol 1991;102:119-122.
13. Stroff T, Lambrecht N, Peskar BM. Nitric oxide as mediator of the gastroprotection by cholecystokinin-8 and pentagastrin. Eur J Pharmacol 1994;260:R1-R2.
14. Konturek SJ, Brzozowski T, Pytko-Polonczyk J, Drozdowicz D. Exogenous and endogenous cholecystokinin protects gastric mucosa against the damage caused by ethanol in rats. Eur J Pharmacol 1995;273:57-62.
15. Lewis LD, Williams JA. Regulation of cholecystokinin secretion by food, hormones, and neural pathways in the rat. Am J Physiol 1990;248:G512-G518.
16. Ritchie WP, Mercer DW. Mediators of bile acid-induced alterations in gastric mucosal blood flow. Am J Surg 1991;161: 126-130.
17. Glenny RW, Bernard S, Rinkley M. Validation of fluorescent-labeled microspheres for measurement of regional organ perfusion. J Appl Physiol 1993;74:2585-2597.
18. Cross JM, Chang L, Mercer DW. Effects of cholecystokinin (CCK) on gastric injury and gastric mucosal blood flow (GMBF). Gastroenterology 1997;112:A1436.

19. Mercer DW, Smith GS, Cross JM, Myers SI, Miller TA. Cholecystokinin-induced protection against gastric injury is a physiologic effect: Role of eicosanoid intermediaries. Gastroenterology 1995;108:A1233.
20. Smith GS, Barreto JC, Schmidt KL, Tornwall MS, Miller TA. Protective effect of dimethylthiourea against mucosal injury in rat stomach. Dig Dis Sci 1992;37:1345-1355.
21. Liddle RA, Goldfine ID, Williams JA. Bioassay of plasma cholecystokinin in rats: Effects of food, trypsin inhibitor, and alcohol. Gastroenterology 1984;87:542-549.
22. Kvietys PR, Barrowman JA, Granger DN. Effects of anesthetics and other experimental conditions on splanchnic blood flow. In Granger DN, Bulkley GB, eds. Measurement of Blood Flow: Applications to the Splanchnic Circulation. Baltimore: Williams & Wilkins, 1982, pp 56-65.
23. Tuma RF, Vasthare US, Irion GL, Wiedeman MP. Considerations in use of microspheres for flow measurements in the anesthetized rat. Am J Physiol 1986;250:H137-H143.
24. Buckberg GD, Luck JC, Payne B, Hoffman JI, Archie JP, Fixler DE. Some sources of error in measuring regional blood flow with radioactive microspheres. J Appl Physiol 1971; 32:598-604.
25. Greenway CV, Murthy VS. Effects of vasopressin and isoprenaline infusion on the distribution of blood flow in the intestine: Criteria for the validity of microsphere studies. Br J Pharmacol 1972;46:177-188.
26. Archibald LH, Moody FG, Simons MA. Measurement of gastric blood flow with radioactive microspheres. J Appl Physiol 1975;38:1051-1056.
27. Carmichael HA, Nelson IM, Russell RI. Cimetidine and prostaglandin: Evidence for different modes of action on the rat gastric mucosa. Gastroenterology 1978;74:1229-1232.
28. Barreto JC, Smith GS, Russell DH, Miller TA. Gastric damage caused by acidified ethanol: Role of molecular HCl. Am J Physiol 1993;265:G133-G137.
29. Chang RSL, Lotti BJ. Biochemical and pharmacological characterization of an extremely potent and selective non-peptide cholecystokinin antagonist. Proc Natl Acad Sci USA 1986; 83:4923-4926.
30. Smith GS, Myers SI, Bartula LL, Miller TA. Adaptive cytoprotection against alcohol injury in the rat stomach is not due to increased prostanoid synthesis. Prostaglandins 1991;41: 207-223.
31. Mercer DW, Cross JM, Smith GS, Miller TA. Protective action of gastrin-17 against alcohol-induced gastric injury in the rat: Role in mucosal defense. Am J Physiol 1997;273:G365-G373.
32. Heinemann A, Jocic M, Peskar BM, Holzer P. CCK-evoked hyperemia in rat gastric mucosa involves neural mechanisms and nitric oxide. Am J Physiol 1996;270:G253-G258.