Ulcerative Colitis and Colon Carcinoma: Epidemiology, Surveillance, Diagnosis, and Treatment

*The SSAT, AGA, ASLD, ASGE, AHPBA Consensus Panel**

Although a small fraction (<1%) of all cases of colorectal cancer arise in the setting of chronic ulcerative colitis, the development of gastrointestinal neoplasia presents a formidable problem for both the patient with chronic ulcerative colitis and the physician. This consensus provides a summary of the magnitude of the problem of colorectal cancer in patients with ulcerative colitis, current surveillance recommendations, improved diagnostic modalities, and treatment. The panel was asked to discuss and draw conclusions regarding the following questions:

1. Is there a risk of developing colon cancer in patients with chronic ulcerative colitis and, if so, which patients are at greatest risk?
2. Is dysplasia a reliable and valid histologic marker in the identification of patients at risk for developing colon cancer in the face of ulcerative colitis?
3. Is colonoscopic surveillance of benefit in reducing cancer mortality in patients with chronic ulcerative colitis?
4. Are there other biochemical or immunologic markers of value in identifying patients with ulcerative colitis at risk for the development of colorectal cancer currently available or to be anticipated in the near future?
5. Is there a role for prophylactic colectomy in patients with dysplasia-associated lesions or masses (DALM), high-grade dysplasia, low-grade dysplasia, and in patients with long duration of disease or early onset of disease?

Summary

1. Is there a risk of developing colon cancer in patients with chronic ulcerative colitis?

There is a clear relationship between chronic ulcerative colitis and the risk of developing colorectal carcinoma. The overall incidence of ulcerative colitis may be as much as 10-fold higher than what other epidemiologic studies in the Western World have reported previously. The survival decrease in patients with ulcerative colitis is clearly related to colon cancer and not to other medical conditions. The two best established risk factors for developing colorectal cancer in chronic ulcerative colitis are duration of disease and anatomic extent of disease. Colorectal cancer is only rarely encountered when the total duration of colitis is less than 8 to 10 years, but thereafter the risk of cancer rises at the rate of approximately 0.5% to 2% per year. Patients with extensive colitis, that is, those with disease proximal to the splenic flexure, are at the greatest risk of developing cancer, whereas those with only proctitis have very little risk for colorectal cancer. Persons with left-sided colitis are also at risk, although this appears to be lower than in those with pancolitis. The age of onset as a risk factor remains controversial. Although some studies report that onset of colitis at a young age is a risk factor for subsequent colorectal cancer, the bulk of evidence suggests that childhood onset of colitis does not confer an added risk independent of total disease duration and anatomic extent. Nevertheless, there were disturbing data presented by the panel suggesting that in patients diagnosed with colitis before the age of 15 years and who are then followed up to the age of 50 years, there is up to a 50% risk of developing colorectal carcinoma. In addition, several studies have indicated that the small subset of patients with primary sclerosing cholangitis may be at high risk for developing colorectal carcinoma. A family history of colon cancer is associated with a two- to threefold greater risk for colon cancer in persons with ulcerative colitis as opposed to those with colorectal carcinoma unrelated to colitis. It is likely that other genetic subgroups will be identified that are also at increased risk.

2. Is dysplasia a reliable or valid means of identifying at-risk patients?

In the absence of randomized prospective trials, it is not possible to determine the full impact of dysplasia surveillance in ulcerative colitis. Thus the answer to this question remains "yes and no." There is a definite positive predic-

Correspondence: James M. Becker, M.D., James Utley Professor and Chairman of Surgery, Boston University School of Medicine, Surgeon-in-Chief, Boston Medical Center, One Boston Medical Center Place, Boston, MA 02118-2393.

*The Society for Surgery of the Alimentary Tract, American Gastroenterological Association, American Society for Liver Diseases, American Society for Gastrointestinal Endoscopy, and American Hepato-Pancreato-Biliary Association.

tive value in identifying cancer in patients with DALM, high-grade dysplasia, and perhaps low-grade dysplasia. These dysplastic markers, however, have a poor negative predictive value and are inadequate if the overall goal is to reduce the risk of developing cancer to zero. In patients with DALM there is a 40% to 50% chance that they will develop invasive carcinoma, in patients with high-grade dysplasia the chance is greater than 40%, in patients with low-grade dysplasia there may be up to a 20% chance of them having invasive carcinoma, and in patients with indefinite dysplasia the risk may be as high as 5% to 10%. There is remaining concern about the significant variability among pathologic experts in accurately differentiating low-grade from high-grade dysplasia.

3. Is colonoscopic surveillance of benefit in reducing cancer in patients with chronic ulcerative colitis?

To date, no prospective randomized trials have shown that surveillance reduces cancer-related mortality. Two retrospective studies indicate that mortality is reduced by a surveillance program. The panel would recommend that in an effort to enhance the value of dysplasia surveillance in universal ulcerative colitis, colonoscopy should be performed every 1 to 2 years starting from the eighth year and yearly after the fifteenth year of disease. Flexible sigmoidoscopy to 60 cm with biopsy should be performed in the alternate year when colonoscopy is done every other year. The standardization of surveillance is important for gathering prospective data. Biopsies must be taken at the time of each colonoscopy. The panel would recommend that three to four biopsies be taken each 10 cm resulting in a total of at least 25 to 30 biopsies. This raises significant questions regarding logistical and financial realities that may limit patient or physician compliance. It is also important to address further the issue of patients who are not on surveillance programs and present for the first time to the physician or those who have dropped out of surveillance programs or who are not compliant. This may be of greater importance than further standardization of any currently accepted surveillance program.

4. Are there other biochemical, immunologic, or molecular markers of value in identifying at-risk patients?

As of 1998, no molecular markers of clinical value are available. Whether there will be some in the near future is unclear. The evidence for mutations of p53 suppressor genes, aneuploidy, and abnormal mucin-associated antigens (STn) as markers is encouraging, although clinically unproved. Further identification of genetic subgroups at greater risk for developing carcinoma may be a promising strategy.

5. Is there a role for prophylactic colectomy in ulcerative colitis?

In part, this question depends on what is meant by "prophylactic" colectomy. If the colectomy is done in the face of a positive premalignant histologic marker and cancer is indeed found, then this no longer represents prophylaxis. The panel would conclude that when DALM or high-grade dysplasia are discovered, this interpretation should be confirmed by a second experienced pathologist. If reconfirmed, then colectomy should be performed. When low-grade dysplasia is discovered, this interpretation should also be confirmed by a second experienced pathologist. If confirmed, low-grade dysplasia in multiple areas of the colon is probably an indication for colectomy. Some of the panel would suggest that even if low-grade dysplasia has been demonstrated and confirmed in only one area of the colon, strong consideration should be given to colectomy. The alternative of repeating the colonoscopy in 6 months to reconfirm the presence of low-grade dysplasia may fail to uncover additional evidence of dysplasia simply on the basis of a sampling error. In some circumstances, truly "prophylactic" colectomy may be recommended in the absence of dysplasia of any type; however, very seldom is this the only indication for colectomy. The statistical risk of cancer often will be compounded by other indications including lifestyle, persistent steroid requirement, drug intolerance, or the presence of extensive pseudopolyps. Some experts continue to recommend strong consideration of colectomy at 10 years after the diagnosis of the disease, particularly in patients with anxiety. After 20 years, the case becomes much stronger for colectomy, even in patients without dysplasia or other positive markers.

Several surgical alternatives are available for patients undergoing colectomy. These include proctocolectomy with Brook ileostomy or continent ileostomy, subtotal colectomy with ileorectal anastomosis, which is currently recommended very seldom throughout the world, and finally colectomy with ileal pouch–anal anastomosis. In patients undergoing ileoanal anastomosis, consideration must be given to performing mucosectomy. Most experts would recommend mucosectomy beginning at the dentate line for patients undergoing colectomy in the presence of dysplasia, particularly in the rectum. Others have suggested that with severe rectal dysplasia, a standard proctocolectomy may be the best option.

Although a number of questions remain, the panel would conclude that the overall relative risk of developing cancer, the fear among patients of this dreaded complication, and the excess mortality among patients with ulcerative colitis are sufficiently high to warrant careful clinical vigilance. Cancer surveillance in ulcerative colitis is worthwhile, although to date the cost-benefit ratios in surveillance programs have not been established. There are also no prospective data that support a reduction in cancer-related mortality with these surveillance programs. The risk of cancer in ulcerative colitis is clear. To reduce the risk of developing cancer in a disease that is surgically curable, it will be important in the future to maximize clinical judgment, improve epidemiologic data, and utilize newer and better pathologic and molecular markers.

Biology of Colorectal Cancer in Ulcerative Colitis

Bret A. Lashner, M.D., M.P.H., Bradley D. Shapiro, M.D.

Patients with chronic ulcerative colitis have an increased risk of developing colorectal cancer (CRC) that rises with increasing duration and extent of disease.[1-4] Periodic surveillance colonoscopy with multiple biopsies is recommended in an attempt to identify patients at high risk of cancer-related mortality. Patients who have low-grade dysplasia (LGD), high-grade dysplasia (HGD), or asymptomatic cancer detected at colonoscopy are advised to undergo proctocolectomy. In general, surveillance programs have failed to markedly reduce cancer-related mortality.[1,5-9] Factors cited to explain this shortcoming have included poor patient compliance and the poor performance of dysplasia as a criterion for a positive test. Better premalignant markers are needed.

DYSPLASIA

Dysplasia, first proposed as a premalignant lesion in ulcerative colitis patients in 1967, has been used in surveillance programs for more than 20 years.[10,11] Initially HGD and cancer were the only lesions for which colectomy was recommended. Once HGD has been detected at colonoscopy, the risk of finding cancer at colectomy is 42%.[1] Furthermore, another one third of patients with HGD will eventually be found to have developed cancer during a subsequent examination. More recently, LGD or worse has been the proposed criterion for a positive test, but this recommendation lacks widespread acceptance.[1,5,12-16]

Use of dysplasia as the criterion for a positive test in cancer surveillance programs is problematic for several reasons. First, there is a high degree of inter- as well as intraobserver variability. Even among experts, agreement as to the diagnosis and the degree of dysplasia ranges from as low as 42% to 65%.[11,17] Second, dysplasia frequently occurs in flat mucosa and can easily be missed because of sampling errors, thereby decreasing sensitivity. The current standard of practice is to obtain two to four biopsies every 10 cm, a practice that samples less than 0.2% of the total colonic surface area.[18,19] Third, dysplasia can be misdiagnosed in areas of active inflammation (poor specificity). All biopsies read as being definite for LGD or HGD should be confirmed by a second pathologist experienced in inflammatory bowel disease pathology, since the penalty for an error is an unnecessary colectomy. Fourth, dysplasia defines benign adenomatous polyps, lesions that may occur coincidentally in patients with ulcerative colitis.

ALTERNATIVE MARKERS OF MALIGNANCY

Cancer evolves as a result of a multistep process that is influenced by both inherited factors and environmental agents. Identifying specific genetic alterations has helped further the understanding of ulcerative colitis–associated neoplasia (UCAN) biology and has uncovered tumor markers potentially suitable for use in cancer surveillance.[20] An ideal marker is objective and not subject to much inter- or intraobserver variability, is sensitive by being present often in patients who develop cancer, is specific by being absent in patients who do not develop cancer, and is inexpensive. Of the candidate premalignant markers (global DNA hypomethylation, DNA aneuploidy, abnormal mucin expression, proto-oncogene mutations, suppressor gene mutations and loss of heterozygosity, and microsatellite instability), aneuploidy, abnormal mucins, and suppressor gene mutations hold the most promise for use as a complementary marker for dysplasia.

DNA ANEUPLOIDY

In the presence of environmental toxins, normal diploid cells can develop an abnormal DNA content. Clonal expansion of aneuploid cell populations is objectively detected by flow cytometry and found in approximately 50% to 70% of sporadic CRCs. Aneuploidy was initially recognized as a premalignant

From the Center for Inflammatory Bowel Disease, Department of Gastroenterology, The Cleveland Clinic Foundation, Cleveland, Ohio. Correspondence: Bret A. Lashner, M.D., The Cleveland Clinic Foundation, 9500 Euclid Ave., Desk S40, Cleveland, OH 44195.

marker in ulcerative colitis when aneuploid cell lines were detected within dysplastic mucosa in a 46-year-old man.[21] After 1 year, a large adenocarcinoma developed in the corresponding area of a colon. Several subsequent reports have confirmed the high prevalence of aneuploidy in UCAN.[22-26] The frequency of aneuploidy increases with worsening grades of dysplasia and cancer. DNA aneuploidy has been reported in 39% of dysplastic specimens and 74% of ulcerative colitis–associated CRC.[26] Only 5% of nondysplastic tissue had changes of aneuploidy. Other investigators also have found a higher rate of aneuploidy in dysplasia (21%) and cancer (29%) compared to nondysplastic tissue (15%).[27] Mapping studies of colectomy specimens with UCAN have demonstrated DNA aneuploidy in cancerous areas as well as in distant flat nondysplastic tissue.[25,28] Also, the finding of multiple areas of aneuploidy concentrated in areas of cancer or dysplasia further supports the hypothesis of clonal expansion. Aneuploidy by itself does not necessarily represent a specific genetic event that is essential for the development of cancer but represents genomic instability, which characterizes "premalignant mucosa."[29]

ABNORMAL MUCINS

Mucins are large-molecular-weight glycoproteins secreted by goblet cells and, to a lesser extent, by columnar cells. There are qualitative differences between colorectal mucins from healthy subjects and patients with ulcerative colitis.[30-32] Furthermore, abnormal mucins in the colon are exposed in areas of either neoplasia or inflammation. Several staining methods have been used on biopsy specimens including mucin histochemistry, lectin binding, and immunohistochemistry with monoclonal antibodies targeted at specific antigens. Mucin histochemistry involves staining colonic mucosa with Alcian blue and high-iron diamine. Staining patterns are based on the charge and type of mucin. Mucins are either neutral mucins (no staining), sialomucins (blue staining), or sulphomucins (brown staining). Most goblet cells of the colon stain brown because of a predominance of sulphomucins.[33] There are increased amounts of sialomucins in the colonic mucosa of patients with ulcerative colitis and an even greater proportion in dysplastic tissue.[34,35] These changes are also present prior to the development of dysplasia or cancer.[36] Because colonic sialomucin predominance occurs in non-neo-plastic disease states such as active inflammation and regenerative mucosa in ulcerative colitis, it is not sufficiently specific as a surveillance tool for cancer.[33,37-39]

A new development involving mucins utilizes monoclonal antibodies and immunohistochemical staining. There are many antigens present in colonic tissue, including blood group antigens A, B, H, Lewis^a, and Lewis^b, as well as sialosyl-Tn (STn). The most promising of these for cancer screening in ulcerative colitis appears to be STn, an antigen that is seen in up to 90% of sporadic colorectal cancers.[40-42] STn also is expressed in approximately half of adenomatous polyps but not in hyperplastic polyps. In patients with ulcerative colitis, up to 85% of cells with HGD stain with STn. STn as a premalignant marker for UCAN has been examined in several case-control studies.[43] Six of seven ulcerative colitis patients (86%) who developed cancer or dysplasia expressed STn in at least one prior nondysplastic biopsy specimen from the same site. Furthermore, STn staining was positive in 82% of patients with noninflamed, nondysplastic mucosa remote from the resected cancer or dysplasia, but in none of the normal mucosa from 16 patients with sporadic colon cancer. Unfortunately, the false positive rate of staining was high—that is, four of six dysplasia-free control subjects with active ulcerative colitis expressed STn. STn also has been observed within goblet cells of the crypts in normal proximal colon and in mucosa at a distance from colon cancer.[44] Another case-control study compared STn staining of biopsy specimens from ulcerative colitis patients who developed dysplasia or cancer with specimens from control subjects who were free of neoplasia.[45] STn staining occurred in 44% of ulcerative colitis biopsy specimens vs. only 11% of control biopsies. In addition, STn antigen preceded the detection of neoplasia by up to 7 years.

TUMOR SUPPRESSOR GENES

Tumor suppressor genes encode for nuclear proteins that delay DNA transcription in cells with damaged DNA until repair can occur or, if the DNA damage is excessive, programmed cell death (apoptosis) can occur. This family of suppressor genes includes the p53 gene, adenomatous polyposis coli (APC) gene, mutated in colon cancer (MCC) gene, deleted in colon cancer (DCC) gene, and retinoblastoma (Rb) gene. The p53 suppressor gene has been termed the "guardian of the genome."[46] It is located on the short arm of chromosome 17 and encodes for a 53 kD phosphoprotein. Both a mutation and loss of an allele (loss of heterozygosity [LOH]) need to occur before the function of this gene is lost. p53 suppressor gene mutations and LOH are present in at least half of all malignant tumors including up to 85% of CRCs. These abnormalities also are commonly found in both dysplastic lesions and CRCs in patients with ulcerative colitis.[47-56]

Mapping studies suggest that p53 mutations occur

early in the sequence of carcinogenic events leading to the development of UCAN.[57] Regions that contain the p53 suppressor gene mutation in resected neoplastic colons were not only more extensive than dysplasia but also were found in colonic mucosa indefinite for dysplasia and in normal mucosa adjacent to regions of cancer or dysplasia.[57] Also, there is a high correlation between the presence of p53 suppressor gene mutations and aneuploidy. Therefore p53 mutations occur early and most likely proceed to the development of aneuploidy or dysplasia. In two separate studies p53 LOH was detected in biopsy specimens classified as indefinite for dysplasia, once again implying that significant genetic abnormalities may exist prior to the development of morphologic dysplasia.[58] p53 LOH also occurs in early lesions such as LGD or dysplasia-associated lesions or masses but not in histologically normal colonic tissue.[56] These findings are the opposite of those seen in sporadic CRC where p53 suppressor gene mutations occur relatively late in the adenoma-carcinoma sequence and are detected in only 8% to 24% of adenomas.[51,59-64]

Several recent studies have employed immunohistochemistry to better characterize the frequency and timing of p53 mutations in UCAN.[47,55,62] A series of 10 patients with UCAN demonstrated the presence of p53 suppressor gene mutations in two of seven specimens with LGD obtained 2 years before the development of carcinoma or HGD.[62] Our own cohort study of 95 patients showed that p53 mutations occurred in half of all ulcerative colitis–associated CRCs and in morphologically normal colonic tissue up to 7 years prior to the development of dysplasia or cancer.[64] In addition, patients with p53-positive cancers were diagnosed at a later Dukes' stage than those with p52-negative cancers.

FUTURE DIRECTIONS

The current standard of practice is for patients with panulcerative colitis to begin cancer surveillance after 7 years of disease. Two to four biopsies should be obtained every 10 cm throughout the entire colon. In addition, any suspicious areas or raised plaques should be biopsied as well. The interval between screening examinations can vary from 1 to 3 years based on a patient's individual risk.[65] Shortening the interval will increase the sensitivity of surveillance but will also increase the cost, both monetarily and in terms of potential morbidity, as well as decrease specificity.[12,65] Any specimen interpreted as LGD, HGD, or cancer should prompt the recommendation of colectomy.[1] Prior to colectomy, confirmation of LGD or HGD by a second experienced pathologist is advised.

The practice of performing colectomy for the finding of LGD has not become universally accepted. Up to 19% of patients with LGD will have evidence of cancer at colectomy and another one third will have HGD or cancer detected at subsequent colonoscopy, but a significant proportion may never develop a malignancy.[6] Possibly, in the future, p53 staining of surveillance biopsies will become routine and improve our management strategies. The presence of p53 mutations in the setting of LGD may signify the aggressive nature of a lesion that may best be treated with colectomy. However, LGD without p53 mutations might in the future be managed conservatively by increasing the intensity of surveillance, since this dysplastic lesion may be less aggressive. The finding of p53 mutations in normal tissue without concomitant dysplasia may prompt more frequent surveillance or even consideration of prophylactic colectomy. Continued research in this area will allow surveillance programs to be altered so that cancer-related mortality in ulcerative colitis can be substantially reduced.

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Risk of Cancer in Ulcerative Colitis

Anders Ekbom, M.D.

Among any given population in the Western World, 20% to 30% will be diagnosed with cancer during their lifetime. With this in mind, it is not surprising that an association between different cancer forms and ulcerative colitis has been reported implicating almost every conceivable site. In most instances these studies have emanated from either small case series or follow-up studies of patient groups where selection bias could be a concern. Thus, to study the risk of cancer among patients with ulcerative colitis, there is a need for a comparison group and ideally to follow unselected patient groups over time. Moreover, when assessing the risk of cancer among patients with ulcerative colitis, surveillance bias is an additional concern as these patients will have more regular contact with any health care system than the normal population. Focusing on mortality instead of cancer morbidity is one way to avoid this problem.

In the classical report from 1971, a long-term follow-up of all pediatric cases of ulcerative colitis treated at the Mayo Clinic demonstrated decreased long-term survival compared to the general population.[1] The excess mortality was confined to the patient group left with an intact colon, and death resulting from colorectal cancer was the main reason for the decreased survival in this patient group. Similar findings have been reported from Sweden where a somewhat decreased long-term relative survival among patients with ulcerative colitis was demonstrated.[2] In a population-based study from Sweden, we were able to confirm an overall increased risk for cancer among patients with ulcerative colitis,[3] 202 observed cases compared to 142.1 expected (standard incidence ratio [SIR] = 1.6; 95% confidence interval [CI] = 1.4 to 1.8). The standardized mortality ratio (SMR) was also increased, although to a somewhat lesser extent (SMR = 1.3; 95% CI = 1.1 to 1.6). After excluding colorectal cancer, the risk for cancer did not differ from that of the general population (SIR =1.0; 95% CI = 0.9 to 1.2) nor did the mortality rate (SMR = 1.0; 95% CI = 0.7 to 1.5). Colorectal cancer, therefore, seems to be the major cause of the increased morbidity and mortality in patients with ulcerative colitis. Consistent with other studies we also found an increased risk of cancer of the bile ducts,[4,5] but the number of cases was small and this increase was offset by a decreased risk for cancers of the respiratory tract and breast. The former finding may be explained by the lower incidence of smoking among patients with ulcerative colitis. The latter finding has been shown in other studies, although the underlying reason for this remains unknown.[4,6]

The risk of colorectal cancer in patients with ulcerative colitis varies substantially in different studies. However, with the exception of studies from Copenhagen,[7,8] an increased risk has been found in all studies. The cumulative incidence 25 to 35 years after diagnosis ranges from 8%[9] to 43%,[11] and standardized incidence ratios between 2[10] and 30[11] have been reported. In the Danish studies, in which no increased risk was shown, this may in part be explained by the fact that left-sided colitis and pancolitis were analyzed together and coupled with a high rate of colectomy. It is important to note that there is an increased risk of colorectal cancer among Danish patients with ulcerative colitis outside of Copenhagen.[12]

Besides duration of disease, extent of disease at the time of diagnosis appears to be the most important factor for the risk of colorectal cancer in patients with ulcerative colitis.[13] Patients with proctitis do not differ in risk compared with the normal population, even after analyzing rectal cancer as a single entity. This finding implies that it is not the inflammation alone, but some additional exposure is needed for a malignant transformation in patients with ulcerative colitis. There is an increased risk of colorectal cancer among patients with left-sided colitis at diagnosis,[13] but this risk is substantially lower compared to the risk among patients with pancolitis at the time of diagnosis. Moreover, the latency period is 10 to 15 years longer compared to pancolitis before patients with left-sided colitis are at increased risk of colorectal can-

From the Department of Epidemiology, University Hospital, Uppsala, Sweden.
Correspondence: Department of Medical Epidemiology, Karolinska Institute, Box 281, S-171 77, Stockholm, Sweden. E-mail: Anders.Ekbom@mep.ki.se.

cer. Whether this increased risk is because patients initially diagnosed as having left-sided colitis progress to pancolitis or the risk is present among patients with disease confined to the left colon remains unknown. It is important to recognize that the extent of disease in most studies published to date was assessed by barium enema. This is a crude measurement, perhaps not accurate enough to clearly define patient groups at risk. In the future, assessment will require endoscopic evaluation. It remains to be seen to what extent that will affect risk estimates.

To what extent age of onset is an independent risk factor for colorectal cancer among patients with ulcerative colitis remains controversial. In our study from Sweden, we found that there was a cumulative risk of 40% by 35 years after diagnosis in patients with a young age of onset[13]; this risk is very close to that reported from the Mayo Clinic in patients less than 15 years of age at the time of diagnosis. Moreover, further follow-up of patients in the Uppsala study revealed that 50% of all patients with pancolitis diagnosed before the age of 15 years were diagnosed with colorectal cancer before the age of 50. These findings suggest that prophylactic proctocolectomy in this patient group might be appropriate. It has also been proposed that patients with ulcerative colitis are at maximum risk for development of colorectal cancer at approximately 50 years of age regardless of the age of onset or duration of disease.[14] In the only other study in which this was analyzed, there was, however, an increased risk persisting in all age groups, even among patients with ulcerative colitis after age of 70 years, which seems to refute that hypothesis.[13]

In conclusion, colorectal cancer in patients with ulcerative colitis is the only malignancy that has any impact on overall cancer-related morbidity or mortality. Duration and extent of disease and perhaps age of onset are the only risk factors that have been clearly identified.

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How Reliable/Valid Is Dysplasia in Identifying At-Risk Patients With Ulcerative Colitis?

Robert H. Riddell, M.D., F.R.C.Path, F.R.C.P.C.

Patients with ulcerative colitis at greatest risk of developing carcinoma are those with the greatest anatomic extent of disease—especially disease extending proximally to the splenic flexure. In these patients the risk of carcinoma increases in conjunction with the total duration of disease, with the risk beginning to increase after approximately 7 to 10 years of disease. Within this group, patients whose disease began in childhood are at greatest risk.[1] It has been estimated that this risk is greatest in patients with pancolitis in whom the cumulative incidence has been estimated at 25%.[1] Other factors associated with an increased risk include primary sclerosing cholangitis and possibly low serum folate levels. Recent data have suggested that patients who have had a cholecystectomy are also at increased risk.[2]

This report examines the reliability and validity of histologically demonstrated dysplasia in identifying at-risk patients with ulcerative colitis. Factors affecting the reliability of dysplasia in ulcerative colitis include the following:

1. The definition of dysplasia
2. Problems with the diagnosis of dysplasia, particularly its diagnosis in the presence of inflammation, the need for full-thickness mucosal involvement for the diagnosis of dysplasia, and the distinction between adenomas and dysplasia-associated lesions or masses (DALM)
3. Interobserver variability
4. The sampling problem
5. Absence of dysplasia in some carcinomas
6. The need for an alternative "gold standard" for dysplasia (e.g., molecular biology or increased ability to detect dysplasia at colonoscopy)
7. Analyses of combined series from the literature (pseudometa-analysis) as a basis for therapy
8. Differing philosophies concerning the timing of colectomy

Definition of Dysplasia

Dysplasia is an unequivocally neoplasic lesion and excludes anything that might be considered reparative; it may also represent the superficial part of an infiltrating adenocarcinoma.[3] Although "an unequivocally neoplastic lesion" is fine in theory, this reasoning is circuitous because neoplastic lesions, whether invasive or not, are also by definition dysplastic. In practice, dysplasia is a morphologic diagnosis that is based on a largely subjective interpretation of the similarity of nuclear and cytologic features to adenomas and their morphologic variants. While there are objective descriptors for the distinction between high-grade and low-grade dysplasia, there is also a subjective component that is, to a large extent, dependent on individual experience.

Problems With the Diagnosis of Dysplasia

Although the diagnosis of dysplasia is seemingly straightforward, problems remain including those described below.

Diagnosis in the Presence of Inflammation. Usually inflammation does not involve dysplastic mucosa, so that glands in which acute inflammation is present, or in which there is active regeneration in the same biopsy, should be treated conservatively unless overtly dysplastic.

Need for Full-Thickness Mucosal Involvement for the Diagnosis of Dysplasia. Although evidence of full-thickness mucosal involvement is usually present, in some biopsies dysplasia can only be diagnosed with certainty because it is maximal at the surface. Sometimes dysplasia is maximal at the base of the crypt with apparent surface maturation; this feature is therefore not completely reliable as a marker of reactive changes.

Adenoma vs. DALM. This can be a problem from both a diagnostic and a management viewpoint.

From the Department of Pathology, McMaster University Medical Center, Hamilton, Ontario, Canada.

Schneider and Stolte[4] attempted to distinguish between adenomas and dysplasia using architectural distortion in dysplasia to distinguish between them, with some success. Patients with ulcerative colitis are not immune to adenomas and in the adenoma age-bearing range (e.g., >45 years old) they are quite acceptable. A practical approach to this is that if the lesion in question can be completely excised endoscopically and biopsies around the base are negative, there are currently no data suggesting that these patients are at increased risk for dysplasia or carcinoma, and they can likely be treated similarly to patients with a simple adenoma. However, should this occur in a young patient (e.g., <40 years), an argument can be made that the lesion is likely the result of the colitic process, and possibly a DALM. However, such events are currently anecdotal.

Interobserver Variability

Variability among pathologists in the diagnosis of dysplasia, especially low-grade dysplasia (LGD), which is a critical node in the management algorithm, is considerable.[5-8] Fortunately, high-grade dysplasia (HGD) has less interobserver variability than LGD, and colectomy for indefinite dysplasia seems to be less of an issue if it really does have the same potential for current and subsequent risk of neoplasia as LGD.

Any interobserver variability has the potential to cause problems. Whenever there is a grading system that has a subjective component, a typical distribution curve can be expected around a mean, and the question is how far out the tails go. For example, if the mean is exactly HGD, then the tail may not extend beyond LGD, in which case there will be high interobserver agreement. Alternatively if LGD, it could extend into the indefinite for dysplasia category (or lower) at one end or the HGD category at the other. Disagreement means that some features are present that have not been seen by the pathologist of record or have been interpreted differently. In dysplasia both may apply. At the ends of any subjective spectrum, in this case no dysplasia or HGD, higher agreement would be expected as disagreement can only go in one direction for each—that is, higher in the case of no dysplasia and lower in HGD. Anything higher than HGD becomes invasive carcinoma because carcinoma in situ is included in that category.

The Sampling Problem

Dysplasia may be invisible or visible colonoscopically, and if visible may be the superficial part of an invasive carcinoma, or a DALM if not obviously recognizable as a carcinoma. However, if the dysplasia is invisible, detection relies on the presence of random biopsies. In contrast to Barrett's esophagus, where a relatively short length of esophagus is sampled intensively, this is not the case in ulcerative colitis. In ulcerative colitis an invisible patch of dysplasia 2 cm in diameter has a surface area of 3.14 cm^2. Usually two biopsies are taken every 10 cm of bowel (100 cm^2). To reasonably guarantee that the dysplasia will be detected would require 100/3.14 (=32) evenly spaced biopsies every 10 cm. In a 100 cm length of bowel (a short colon), this would require 320 biopsies. The implications are far reaching:

1. The more biopsies the better (e.g., 10 to 20 in four containers)
2. If unequivocal dysplasia is found in one biopsy, the chances of the endoscopist performing a repeat biopsy are remote. Repeating the biopsies "to confirm the diagnosis" is likely to be futile unless the dysplasia is widespread or multifocal.
3. It can be expected that dysplasia "comes and goes" or appears to be reversible (present at one colonoscopy but not the next).

The problem of cost for large numbers of biopsies remains despite a reduction in the fees for colonoscopy. Increasingly, a compromise is to put large numbers of biopsies (e.g., 10 to 20) into four or five containers (or cassettes), one each from the right colon, transverse colon, left colon, and rectosigmoid. In addition, any endoscopic abnormality that could represent dysplasia, adenoma, or carcinoma is biopsied.

Absence of Dysplasia in Some Carcinomas

Studies have suggested that approximately 20% to 25% of clinical carcinomas (as opposed to those found incidentially at colonoscopy or in resection specimens) are associated with the absence of dysplasia. Reasons for this include the possibility that the invasive carcinoma may destroy a preceding lesion or may not be sampled histologically, even if present (the entire tumor would need to be sampled to determine this). The 20% figure may be lower for smaller preclinical cancers, where incidental dysplasia is more likely to be found, analogous to the likelihood of finding an adenomatous edge in small noncolitic carcinomas. However, the possibility that there is a nondysplastic alternative pathway must be considered and appears likely. In the stomach, "diffuse" types of carcinoma including signet ring carcinoma have a very subtle type of dysplasia that is probably not diagnosable on biopsy. The same applies to endocrine carcinomas. Both of these tumor types occur in colitis. These carcinomas can be very fast growing, infiltrative, and plaquelike and could well account for ap-

proximately 10% of all colitic cancers. This implies that such tumors will always be a threat, although not necessarily a lethal one, and raises the issue of the need for an alternative "gold standard" for the detection of dysplasia.

Need for Increased Ability to Detect Dysplasia or an Alternative "Gold Standard" for Dysplasia

Molecular markers are always a potential gold standard to replace histology but have not yet become a reality. The colitic pathway seems to be in the replication error rather than oncogene/loss of suppressor gene pathway. However, replication error is common in nondysplastic mucosa in ulcerative colitis but so far has not been correlated with cancer risk, although it seems highly likely that it will be implicated. No specific oncogene/gene product yet predicts carcinoma better than dysplasia.

Making the invisible visible endoscopically is a challenge. Targeted endoscopic biopsies have the highest yields. Endoscopic targets include plaques, villous areas, and odd polyps. Potential methods for increasing visibility include the use of porphyrins, for example, which can potentially be combined with photodynamic therapy. Magnification endoscopy may visualize mucosal irregularities that may be dysplastic or carcinomatous. Atypical areae colonicae may be a marker of dysplasia. All of these may be potentiated with dye spray techniques either at endoscopy or in oral electrolyte solutions such as polyethylene glycol. The potential of cytologic studies, particularly as a source of DNA for molecular markers from colonic washes, seems great but this has yet to be realized.

Validity of Dysplasia as a Marker: Analyses of Combined Series From the Literature (Pseudometa-Analysis) as a Basis for Therapy

There have been two analyses of combined series from the literature.[9,10] The conclusions were that LGD is associated with approximately a 10% chance of an invasive carcinoma (as was epithelium indefinite for dysplasia, suggesting that the significance of this lesion has been underestimated). HGD is associated with approximately a 40% chance. Although many of these series were not intended to be used in this manner, they are the best that we have. Furthermore, LGD may progress to HGD or invasive cancer in 54% of patients,[9] whereas approximately 40% of patients with HGD have cancer on resection. This has led to a distinct shift in management so that colectomy is seriously considered once LGD has developed (diagnosed by a pathologist with an interest in this field) but also assumes that this can be accurately diagnosed. The figures suggested by Bernstein et al.[9] are as follows:

	Probability of Carcinoma	
Diagnosis	Immediate colectomy	Subsequent colectomy
DALM	43% (17/40)	NA
HGD	42% (10/24)	32% (15/47)
LGD	19% (3/16)	8% (17/204)
Indefinite	NA	9% (9/95)
No dysplasia	NA	2% (11/595)

Philosophies Concerning the Timing of Colectomy: Can Surveillance Work?

In all studies there are flat cancers that are highly infiltrative and advanced when discovered, and these are rarely preceded by detectable dysplasia (e.g., signet ring, "diffuse-type carcinoma," endocrine carcinoma). This implies that unless they are detected early, some patients will die of carcinoma. The best results in terms of the lowest risk of death from colorectal cancer, but tempered by refraining from performing unnecessary colectomies, are achieved by subscribing to the philosophy that the risk of a patient developing/dying from carcinoma is minimal if surveillance is carried out regularly, every 1 to 2 years, provided that none of the biopsies demonstrate dysplasia. Colectomy is performed when dysplasia of any grade develops. Such a policy is currently being followed with success in Scandinavia. A summary of four such studies is revealing.[11-14] Together these articles followed 423 patients over 12 to 15 years. Of eight cancers that developed, two were detected outside the program, two were found at the initial (screening) colonoscopy (Dukes' B and C), and four were detected while patients were under surveillance. One of these was stated to have no associated dysplasia and was classified as Dukes' A carcinoma, two were found incidentally in resections for dysplasia, both of which were classified as Dukes' A, and one patient initially refused resection for dysplasia but a Dukes' B carcinoma was found during a resection eventually performed for severe colitis. These types of studies suggest that with the use of current modes of follow-up, death from colorectal carcinoma in patients at risk is indeed minimized.

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How Do We Assess the Value of Surveillance Techniques in Ulcerative Colitis?

Charles N. Bernstein, M.D.

This article addresses the following issues regarding endoscopic surveillance in ulcerative colitis: (1) endoscopic surveillance techniques; (2) outcomes from endoscopic surveillance based on the available literature; and (3) use of prophylactic colectomy as an alternative approach. Finally, some recommendations for the endoscopist will be made.

ENDOSCOPIC SURVEILLANCE TECHNIQUES

Unfortunately, there is no standard approach to the process as exists for other screening techniques used in medicine. Screening mammography, for instance, is associated with relatively standard radiologic procedures. In a questionnaire administered to 89 practicing gastroenterologists in the Seattle and Los Angles communities, as well as to senior gastroenterology trainees from across the United States, practitioners were specifically asked to document their techniques.[1] It was found that 79% of physicians take two to four biopsies per site, 54% biopsy five to nine sites, and 36% biopsy at least 10 sites in the colon.[1] However, some take one biopsy per site and some take only a single biopsy at one to four sites. A review of the literature reveals that a number of studies have used 10 cm intervals.[2-9] For some patients with ulcerative colitis, this may represent only six sites. When reporting approach by sites, investigators have varied from 15[10] to 12[11] to 10[12] to nine[13] to six sites.[14] Some investigators have simply reported taking 20 biopsies,[15] whereas others take two biopsies per site,[8,13,14] three to four biopsies per site,[7] or even five to eight biopsies per site.[5] Unfortunately for endoscopists with tight endoscopy schedules, performing surveillance biopsies is tedious. Furthermore, a long and arduous biopsy process is especially taxing after the rush to reach the cecum. Nonetheless, it has been estimated that 64 biopsies are required to have a 95% certainty of finding the highest grade of neoplastic lesion when dysplasia is present.[16] Thus if endoscopic surveillance is to be pursued, endoscopists will have to recognize that more (biopsies) is better. After all, it has been estimated that a set of 10 surveillance biopsies covers only 0.05% of the surface area of the colon.[11]

Endoscopists typically submit biopsies for dysplasia assessment to their local pathologists. The literature informs us that interobserver agreement among pathologists considered experts at detecting dysplasia in ulcerative colitis can vary from 45% to 77%.[17,18] The agreement is better for high-grade dysplasia vs. low-grade or indeterminate dysplasia but is far from perfect even for high-grade dysplasia. The primary issue then is not that we cannot trust our local pathologists to be correct in their morphologic diagnoses but rather that we may simply be expecting too much of them. Thus, there is "safety in numbers" and despite a general understanding that agreement between two pathologists on a diagnosis of dysplasia is desirable, only 43% of physicians ask for a second opinion regarding morphologic findings.[1]

Outcomes From Endoscopic Dysplasia Surveillance Studies

Although no randomized controlled study of endoscopic surveillance vs. no surveillance has been or will likely ever be done, there are a number of studies that allow us to understand the implications of making the diagnosis of dysplasia. One study presented a synthesis analysis of the literature published by early 1994,[19] and two later studies corroborated the findings in this literature review.[13,18] At initial endoscopy for dysplasia/cancer surveillance, dysplasia or cancer

From the University of Manitoba Inflammatory Bowel Disease Clinical and Research Centre, Winnipeg, Manitoba, Canada.
Correspondence: Charles N. Bernstein, M.D., Section of Gastroenterology, University of Manitoba, GB445-Health Sciences Centre, 820 Sherbrook St., Winnipeg, Manitoba, Canada R3A-1R9. E-mail: chernst@cc.umanitoba.ca.

will be found in approximately 10% of subjects.[18,19] This removes a large burden of the ultimate load of patients in any one practice who may ultimately develop neoplasia. If the initial endoscopy findings are negative for dysplasia, then only approximately 3% of subjects will ultimately develop neoplasia when followed over time.[18,19] Hence, endoscopists may note that in any one clinical practice of dysplasia surveillance, dysplasia is infrequently diagnosed.

Unfortunately, the literature reports that if low-grade dysplasia is the indication for colectomy, a cancer may already be present in 19% of cases.[19] Subjects with low-grade dysplasia who undergo further surveillance endoscopy have a high likelihood of ultimately progressing to a highly neoplastic lesion (high-grade dysplasia, dysplasia-associated lesion or mass [DALM], or cancer) 16%,[19] 35%,[13] or up to 54%[18] of the time. If high-grade dysplasia or DALM is the indication for colectomy, a cancer may already be present in 40% to 45% of cases, and of these cancers, 50% are Dukes' stage C or D.[19] Others have estimated an even higher rate of cancer progression from these lesions.[18] The finding of dysplasia may come too late. This is not widely known as only 54% of questionnaire respondents suspected that more than 20% of cases of high-grade dysplasia would be associated with concurrent cancer.[1] For indefinite dysplasia, 20% to 30% of subjects will ultimately have an ominous lesion found (high-grade dysplasia, DALM, or cancer),[19] and thus subjects with this diagnosis must be rigorously followed up because of their indefinite status.

Important Caveats to Bear in Mind. Although it can be reassuring that if the initial dysplasia surveillance endoscopy is negative that the risk of progression to dysplasia or cancer when followed over time may be as low as 3%, there are special circumstances that clinicians must bear in mind. First, there is a paucity of hard data in the literature specific to patients in the higher risk groups (disease duration >25 years). Since approximately 10% of patients have dysplasia or cancer at their first surveillance colonoscopy, [18,19] this implies that the neoplasm has been evolving prior to the typical surveillance initiation date of at least 8 years. Furthermore, it has been reported that some patients presented with cancer within 1 to 2 years of their last negative dysplasia surveillance colonoscopy.[18] Thus for some patients progression of the neoplasm does not follow as slow a course as is typically expounded. In approximately 25% of colorectal cancers in ulcerative colitis, dysplasia was not found in the colectomy resection specimens.[18,20,21] Although the true sensitivity of the process is unknown, these caveats underscore the notion that it is simplistic to view the ulcerative colitis–cancer progression as one that only begins after 8 to 10 years of disease, that in flat mucosa random biopsies will likely find it, and finally that dysplasia evolving to cancer always develops slowly enough that annual or biennial colonoscopies are frequent enough.

Prophylactic Colectomy

In the 1960s, an era prior to the establishment of dysplasia surveillance, prophylactic colectomy was viewed as an approach to solving the ulcerative colitis–cancer evolution problem. Twenty to 30 years later, despite the limited success of dysplasia surveillance and the confusion that surrounds even the definition of dysplasia, physicians are very reluctant to consider prophylactic colectomy as an option.[1] According to one report, only 26% were willing to consider it.[1] For some cases of absolutely asymptomatic patients with disease durations of 20 years or more, it is easy to see how a laparotomy and the possibility of an ileostomy could be "a difficult sell." As long as the physician has discussed all of the pitfalls of dysplasia surveillance with these patients and involved them in the decision-making process, the physician can then share the burden of decision with these patients. Patients who have chronic niggling symptoms or intermittent moderate-to-severe flare-ups of disease, or who are increasingly frustrated by the cost or nuisance of taking regular 5-aminosalicylates, may be ideal candidates for a "prophylactic" colectomy. Their colitis symptoms alone may not be absolute indications for colectomy, but the discussion of the rising cancer risk and the fallibility of the dysplasia surveillance process may tip the balance in favor of surgery. A recently published Markov model of life expectancy found that prophylactic colectomy would add 2 to 10 months of life per patient compared with any surveillance scheme and 1.1 to 1.4 years compared with no surveillance.[22] The life expectancy benefit of prophylactic colectomy compared very favorably with that of other accepted practices (e.g., annual Pap smears for cervical cancer beginning at age 20 years adds 3 months of life per patient).

As more hard data are accrued on patients who have had ulcerative colitis for more than 25 years and their true risk of developing cancer, physicians will be able to make more informed decisions as to the absolute necessity for prophylactic colectomy as opposed to its being used simply because of the fallibility of the current alternative. After all, we insist that at some point patients with familial adenomatous polyposis have colectomies and we at times have seen patients who have mastectomies because of indeterminate mammograms, particularly in the setting of widespread fibrocystic diseases. Perhaps the risk of

colorectal cancer in long-standing ulcerative colitis will fall somewhere in between these two examples.

Recommendations for Endoscopic Dysplasia Surveillance

At the present time, the assumption is that dysplasia surveillance colonoscopy is a rational choice, at least until some of the newer markers are proved to be easily accessible through blood or stool tests, or are more easily identifiable via endoscopy, without or despite the blind random sampling approach currently being used. Despite the caveats discussed earlier in this report, it is incumbent that we choose some approach that is rational for the majority of patients. The following recommendations are made with this in mind.

Technique

1. If a patient is undergoing a colonoscopy for any reason prior to 8 years of disease, multiple biopsies should be obtained from multiple sites. These should be sent to the pathologist for assessment of dysplasia. This may be most applicable to patients whose history suggests the presence of disease that has smoldered for years prior to the actual diagnosis. If these biopsies are negative for dysplasia, standard surveillance should be instituted as usual (after 8 years from diagnosis).
2. At 8 years of disease, the surveillance approach should be initiated after a detailed discussion with the patient of the pitfalls of the process. In particular, the patient should be informed that there is a possibility that if dysplasia is found, cancer may already be present. Furthermore, for the patient with chronic active symptoms, colectomy might be given stronger consideration since active inflammation may compromise the accuracy of dysplasia surveillance.
3. As many biopsies as possible should be obtained. The landmarks of the colon can be followed if it is still intact or, if not, the 10 cm rule can be applied, but at least eight sites should be biopsied with at least four large-cup biopsies per site. There is no point in using the commonly used 2.8 mm channel forceps if the point of the exercise is to randomly obtain tissue. When the sigmoid colon and rectum are reached, an increased number of biopsies should be obtained since these are sites with a higher incidence of colorectal cancer in ulcerative colitis.
4. If the pathologist reports finding indefinite, low-grade, or high-grade dysplasia, a second opinion should be requested from a pathologist with extensive experience with dysplasia in ulcerative colitis.

Postendoscopy Surveillance Decisions

1. If the initial endoscopy findings are negative, after 8 years surveillance should be performed every 2 or 3 years until 20 years of disease. At 20 years, the frequency of surveillance should be increased to annually.
2. If low-grade or high-grade dysplasia or DALM is diagnosed, then colectomy is recommended. The patient should understand that there is a high likelihood that cancer is already present at that point. Perhaps endoscopists and patients would be better served by the following grading scheme: dysplasia present, dysplasia absent, and indefinite for dysplasia. Although this is an issue for pathologists to consider, endoscopists need to be aware that any grade of definite dysplasia can be associated with concurrent cancer.
3. If the result is "indefinite for dysplasia," then endoscopy should be repeated within 2 months and an increased number of biopsies should be obtained compared with the previous endoscopy. If the indefinite diagnosis arose in the face of active inflammation, then the inflammation should be treated more aggressively and the surveillance endoscopy repeated within 6 months.
4. At 20 years of disease, when the risk of cancer rises exponentially, the pitfalls of the surveillance process should again be discussed with the patient. The option of prophylactic colectomy should also be presented. It is important that the patient be involved in the decision as to how to proceed.

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Management of Dysplasia in Ulcerative Colitis: Is Prophylactic Colectomy the Preferred Strategy?

Tony Axon, M.D., F.R.C.P.

It is well recognized that there is an increased incidence of colorectal cancer in patients who have had total or extensive colitis. The risk begins to rise after approximately 10 years and increases in the years subsequently. As a result of this, in the early 1960s patients with extensive ulcerative colitis were often advised to undergo colectomy 10 years after their initial attack. When it was recognized that in many cases cancer is preceded by a dysplastic change in the rectal mucosa, this policy was changed. With the introduction of colonoscopy, which enabled the entire colon to be viewed and biopsied, the practice of surveillance colonoscopy was instituted as the logical means of managing patients with long-standing extensive colitis. Over the past 5 to 10 years, a number of studies have been reported indicating that this approach may reduce the number of patients who are likely to die of cancer; however, no controlled study has been carried out and when the published works are reviewed in detail, it is apparent that, in fact, few lives are saved by employing this approach.[1] The question of early colectomy therefore needs to be reconsidered.

PROPHYLACTIC COLECTOMY AND ULCERATIVE COLITIS

At present, colectomy is undertaken for high-grade dysplasia or dysplasia-associated lesions or masses. Few would argue with this approach because the likelihood of cancer in high-grade dysplasia is 32% and in dysplasia-associated lesions or masses it rises to 43%.[2] However, the problem with this approach is that it relies on surveillance colonoscopy to detect the dysplastic change and this has been shown to be less than satisfactory.

As a result of this Bernstein et al.,[2] after reviewing the literature, commented that "immediate colectomy is essential for all patients diagnosed with high-grade or low-grade dysplasia." This raises doubts as to the value of the diagnosis of low-grade dysplasia and the following questions need to be asked: Is this condition predictive of cancer, can it be diagnosed reliably, and what is the likelihood that an individual patient with ulcerative colitis will develop low-grade dysplasia? The data reviewed by Bernstein et al.[2] from the 10 studies when analyzed independently suggest that patients who developed low-grade dysplasia had an 8% likelihood of developing cancer, those who were indefinite for dysplasia had a 9% likelihood, and in all patients the likelihood was 5.5%. These figures do not support the view that low-grade dysplasia is a particularly good predictor of cancer. Indeed, even when the diagnostic criteria are tightened up and biopsies are carefully reviewed by experienced pathologists, interobserver agreement is poor. Connell et al.[3] reported agreement in 82% of cases where no dysplasia was present; however, agreement for indefinite dysplasia was only 19%; for low-grade dysplasia, 43%; and for high-grade dysplasia, 42%. In all cases of dysplasia there was disagreement more often than agreement.

Another difficulty with low-grade dysplasia is that if patients are followed for a long period of time, virtually all of them will in the end develop low-grade dysplasia.[4] Again the diagnosis of low-grade dysplasia is dependent on patients being prepared to undergo surveillance colonoscopy and the weaknesses inherent in this approach. Indeed in the study referred to earlier,[1] where 12 surveillance studies were combined to include 1916 patients, although 92 cancers were described, only 39 were discovered as a result of clinical follow-up. If the success rate is limited just to those who underwent colonoscopic screening, only 11 (12%) were successfully identified by colonoscopy preoperatively. This represents a success rate of only 1 per 476 colonoscopies.

COLECTOMY FOR ALL PATIENTS WITH ULCERATIVE COLITIS AT 10 YEARS

An alternative approach is not to wait until the dysplasia is discovered but to routinely perform colec-

From the Centre for Digestive Diseases, The General Infirmary at Leeds, Leeds, U.K.
Reprint requests: Centre for Digestive Diseases, The General Infirmary at Leeds, Great George Street, Leeds LS1 / 3EX, U.K.

tomy in all patients with ulcerative colitis 10 years after the onset of extensive colitis. Under these circumstances, we would have to ask the following questions: (1) If this policy were adopted, how many cancers would be prevented; (2) what would be the quality of life; and (3) what would be the benefit in terms of cost? No study has been undertaken to assess how many cancers would be prevented by this approach, and we must therefore rely on the published data that are currently available. One logical approach would be to analyze studies in which colonoscopic surveillance was undertaken and to see what would have happened had the patients been operated on rather than submitted to surveillance. Unfortunately, there are no controlled studies to address this issue. However, the study by Choi et al.[5] is particularly valuable because it included not just those patients who had undergone colonoscopic surveillance but also those patients who presented to the clinic with cancer who had not been admitted to the surveillance protocol; that is, it included the whole range of patients who developed cancer in a single service. Altogether 41 cases of cancer were diagnosed over the study period, 19 in the surveillance group and 22 found retrospectively. If we are to determine the success of colectomy at 10 years, we should include as successes all those still alive following surgery and all those who have since died but in whom surgery could have been undertaken earlier. If one analyzes the 41 cases of ulcerative colitis cancer in the series of Choi et al.,[5] 11 would not have been operated on anyway because these patients either had only left-sided colitis or developed their cancer less than 10 years after the onset of colitis. An additional eight patients died following the first colonoscopy in which the cancer was identified. It follows that surgery would not have been undertaken any earlier in these patients because they had not been submitted for surveillance earlier. Overall, of the 41 cases of ulcerative colitis–related cancer, there would have been 22 successes out of 41 cancers. This is not a high success rate and, in fact, when the data are examined, once again it must be noted that only one additional cancer patient would have been saved by early colectomy rather than surveillance. It is difficult to know how many operations would have been performed; since 213 patients were submitted for surveillance, that would be the lowest number.

QUALITY OF LIFE AFTER SURGERY

Only one study comparing patients with ulcerative colitis treated with medical as opposed to surgical therapy has been undertaken.[6] This study compared 95 patients who were attending a joint medicosurgical colitis clinic who had not undergone surgery with 103 patients in whom restorative proctocolectomy had been carried out (five of whom had had familial adenomatous polyposis). Mean ages in the two groups differed, with restorative proctocolectomy patients having a mean age of 34 years and medically treated patients a mean age of 42 years. A questionnaire was distributed to these two groups with varying results. Frequency of bowel action was statistically greater in those who had had surgery compared with those who were receiving medical treatment. Similarly, nocturnal defecation was also more common in the surgically treated group. Conversely, urgency was worse in those treated medically compared to the surgically treated group, and the overall functional scores were better in those undergoing restorative proctocolectomy. More patients complained of sexual dysfunction in the medical than in the surgical group, realizing that the medically treated patients were older. The amount of time lost from work was greater in those who had surgery than in those who received medical treatment. Although more people were taking medication in the surgical group compared to the medical group, more of the latter were being treated with steroids. Overall quality of life was better in those who had surgery than in those who continued on medical treatment.

COST BENEFIT

It is difficult to assess the cost benefit. Provenzale et al.[7] attempted to assess the effectiveness of different approaches using a mathematical model. They found that prophylactic colectomy would increase life expectancy by 1.25 years and surveillance by 9.5 months overall. Those findings have been criticized on a number of grounds, but particularly because great reliance was placed on low-grade dysplasia and thus 90% of patients in the surveillance group would eventually require operation.

IS EITHER COLECTOMY OR SURVEILLANCE COLONOSCOPY NECESSARY?

Langholtz et al.,[8] reported an inception cohort of 1161 patients with ulcerative colitis. These patients had been treated with aggressive medical therapy, but with early resort to colectomy if this seemed appropriate on clinical grounds. The colectomy rate was 32.4% at 25 years and the cumulative cancer incidence was 3.1% at 25 years. Over the entire cohort, the lifetime risk of colorectal cancer was not increased above the norm and of the six cases of cancer that did occur, three developed in patients with proctitis who would not have undergone early colectomy anyway.

This may be the most appropriate way to manage ulcerative colitis.

CONCLUSION

Surveillance followed by colectomy is of limited benefit. A policy of colectomy at 10 years or more, surprisingly, may not prevent all cancers. The quality of life after restorative proctocolectomy is acceptable. Workload and cost benefit are difficult to predict inasmuch as little work has been done in those areas.

Rigorous medical treatment with close follow-up and early colectomy in those in whom this seems clinically appropriate may be the best approach to this condition. Ulcerative colitis is a variable condition and patients themselves differ in terms of their hopes, aspirations, and expectations. An individual and flexible approach therefore is probably more desirable than a rigid protocol.

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Characterization of Allograft Rejection in an Experimental Model of Small Intestinal Transplantation

Michihiro Hayashi, M.D., Olivia M. Martinez, Ph.D., Sheri M. Krams, Ph.D., Washington Burns, M.D., Carlos O. Esquivel, M.D., Ph.D.

Graft rejection continues to be a major barrier to the success of clinical small intestinal transplantation. The objective of this study was to characterize histopathologic and immune parameters of allograft rejection in an experimental model of small intestinal transplantation. Heterotopic intestinal transplants were performed in allogeneic and isogeneic rat strain combinations. An additional group of allogeneic recipients was treated with tacrolimus (1 mg/kg/day) for 7 days beginning on posttransplant day 1. Recipients of allografts and isografts were killed on days 1 to 7 following transplantation, and tacrolimus-treated allograft recipients were killed on days 4 and 7. Grafts and native intestines were examined for histopathology and cytokine gene expression. Very early rejection was observed on posttransplant day 3 and severe rejection was apparent by day 7. The key histopathologic features of acute graft rejection including apoptosis, crypt epithelial cell injury, and an inflammatory infiltrate were uniformly identifiable on day 4 and progressed in severity through day 7. Interleukin (IL)-2, IL-4, IL-5, IL-6, interferon-γ (IFN-γ), and tumor necrosis factor-α mRNA were readily detectable in allografts on days 1 to 7. However, only IFN-γ mRNA showed a significant early and sustained increase in allografts as compared to isografts and native intestine. Treatment of allograft recipients with tacrolimus abrogated the major histopathologic features of rejection and markedly inhibited IFN-γ gene expression. These results indicate that graft rejection in small intestinal transplantation is characterized by a local and specific immune response marked by IFN-γ production that results in crypt epithelial cell injury and apoptosis. Tacrolimus abrogates the histopathologic features of rejection in association with a marked inhibition of IFN-γ gene expression. (J GASTROINTEST SURG 1998;2:325-332.)

The clinical success of small intestine transplantation (SIT) has been hampered by the high incidences of acute rejection and posttransplant lymphoproliferative disorder.[1] The enhanced immunogenicity of small intestine grafts has been attributed to massive stimulation by the abundance of lymphocytes and antigen-presenting cells within the graft. Treatment with tacrolimus has improved the outcome of SIT; however, the need to use aggressive immunosuppressive regimens to ablate graft rejection has resulted in a high incidence of posttransplant lymphoproliferative disorder in SIT recipients.[2] Furthermore, the diagnosis of rejection is difficult since there is no specific clinical picture and no serologic indicator.[3] Histopathologic examination of mucosal biopsy specimens from intestinal allografts is the primary method for assessing mucosal integrity and has been verified as a useful means of monitoring for the onset of rejection.[4]

The cellular and molecular events leading to rejection of SIT allografts have not been clearly established. McDiarmid et al.[5] demonstrated, by reverse transcription-polymerase chain reaction (RT-PCR) analyses, that the cytokines interleukin (IL)-2, interferon-γ (IFN-γ), IL-6, and tumor necrosis factor-α (TNF-α) are specifically upregulated in allografts taken from Lewis rat recipients of Lewis x Brown-Norway F_1 intestine. However, Toogood et al.,[6] using similar techniques in a D-A to Lewis rat model of SIT, found that IFN-γ was the only cytokine that was

From the Department of Surgery, Transplant Immunobiology Laboratory (M.H., O.M.M., S.M.K., and C.O.E.), and Lucile Packard Children's Hospital, Stanford University School of Medicine, Stanford, Calif.; and the Department of Pathology, California Pacific Medical Center (W.B.), San Francisco, Calif.

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Reprint requests: Dr. Olivia M. Martinez, Stanford University School of Medicine, MSLS, 3rd floor, MC: 5492, Stanford, CA 94305.

specifically upregulated in the gut wall of allografts. The latter study suggests that the molecular mechanisms of SIT allograft rejection may differ from that of other vascularized organs.

Since histologic examination of biopsy specimens is critical to the clinical management of SIT recipients, we sought to directly correlate the pathologic changes observed during allograft rejection with the expression of intragraft cytokines in an experimental model of rat SIT transplantation. Furthermore, we analyzed the effect of tacrolimus, the major immunosuppressive agent for SIT recipients, on the expression of intragraft cytokines.

MATERIAL AND METHODS

Animals

Six- to 8-week-old male inbred Lewis rats ($RT1^l$) and male inbred August Copenhagen Irish (ACI) rats ($RT1^a$), weighing 200 to 240 g, were purchased from B&K Universal Inc. (Fremont, Calif.). All animals had access to water and standard laboratory chow ad libitum and were housed in accordance with institutional animal care policies. All experiments were performed with prior approval and in accordance with the guidelines of the Administrative Panel on Laboratory Animal Care of Stanford University.

Small Intestinal Transplantation

Donor and recipient surgery was performed aseptically under anesthesia induced with 50 mg/kg intraperitoneally of pentobarbital (Nembutal, Abbott Laboratories, North Chicago, Ill.). The allogeneic combination consisted of ACI ($RT1^a$) donors and Lewis ($RT1^l$) recipients, whereas Lewis rats were both donors and recipients in the isogeneic transplants. An additional group of allogeneic recipients was treated with tacrolimus (1.0 mg/kg/day, Fujisawa Pharmaceutical, Osaka, Japan) on days 1 to 7 following transplant. Preliminary experiments determined that this regimen was effective in abrogating rejection as determined by histopathologic criteria. Food was withheld from both donor and recipient animals for 24 hours prior to surgery. For the donor operation, the entire length of small intestine from the ligament of Treitz to the ileocecal valve was harvested and flushed intraluminally with cold (4° C) lactated Ringer's solution containing 0.5% neomycin sulfate. The small intestine was preserved in cold (4° C) lactated Ringer's solution for 60 minutes. Small intestinal grafts were transplanted heterotopically with end-to-side aortoaortic and portocaval anastomoses using the technique described by Monchik and Russell.[7] The proximal and distal ends of the graft were exteriorized as stomata to the right flank. There was no difference in duration of cold and warm ischemia time between the isogeneic and allogeneic transplant groups. After transplantation, all animals received 1.0 mg of gentamicin subcutaneously. SIT recipients were examined daily for general condition and changes in body weight.

Specimens

Groups of three to five animals, in both the allogeneic and isogeneic groups, were killed daily (days 1 to 7). Groups of tacrolimus-treated animals were killed on postoperative days 4 and 7. Macroscopic examination revealed no evidence of infection in recipient animals. Specimens from the proximal, mid, and distal portions of both the small intestinal graft and the native intestine were obtained. A portion of each section was fixed in 10% neutral buffered formalin for histologic examination, and the remainder of each section was snap-frozen and subsequently stored at −70° C for molecular analyses.

Histology

Tissue samples for histologic examination were embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin. All samples were subjected to blinded histopathologic examination by a single pathologist using previously established criteria.[8] Briefly, the histopathologic features that were examined included (1) villous changes including height, blunting, mucin depletion, hemorrhage, and necrosis, (2) crypt epithelial injury including apoptosis, (3) cryptitis, (4) inflammatory infiltrate including eosinophilia, and (5) vasculitis. Graft rejection in this model was defined as the presence of the characteristic lymphoid infiltrate, crypt epithelial cell injury, and apoptosis.

RNA, cDNA, and Polymerase Chain Reaction

The midportions of both the graft and the native intestine were used for molecular analyses. Total RNA was prepared as previously described.[9] Briefly, 50 to 100 mg of frozen tissue was minced in 1.0 ml of denaturing solution (TRIzol reagent, GIBCO Laboratories and Life Technologies, Inc., Gaithersburg, Md.) and homogenized at room temperature in a ground-glass tissue grinder. After a 5-minute incubation, 0.2 ml of chloroform was added, and the samples were centrifuged at 12,000 × g for 15 minutes at 4° C. RNA in the aqueous phase was precipitated by isopropanol, washed, air dried, and dissolved in 15 μl of RNase-free water. The RNA was quantitated by

spectrophotometry and the integrity of the RNA confirmed by detection of the 28S and 18S RNA bands following agarose gel electrophoresis. cDNA was prepared by reverse transcription of 1.0 μg of total cellular RNA using avian myeloblastosis virus transcriptase as previously described.[10] Amplification of transcripts for IL-2, IFN-γ, IL-4, IL-5, IL-6, and TNF-α was accomplished by RT-PCR essentially as previously described.[11] Amplification of rat β-actin was used as an internal control. Samples were amplified in a thermal cycler (MJ Research, Watertown, Mass.) with an initial denaturation at 94° C for 3 minutes followed by 35 to 50 cycles of a 30-second 94° C denaturation, a 30-second 55° C annealing of primers, and a 60-second extension of primers at 72° C. Negative controls consisted of PCR mixtures without cDNA subjected to the same amplification program as experimental samples. PCR products were identified by electrophoresis in 2% agarose gel in 1 × Tris-Borate-EDTA buffer with 0.5 μg/ml ethidium bromide and ultraviolet illumination. The DNA ladder obtained by the HaeIII digest of øx174 was used for molecular weight markers. Arbitrary units were determined for each cytokine by comparison to β-actin as a housekeeping gene.

Statistics

Results are expressed as a mean value of at least three animals at each time point. Data analysis was performed by Student's *t* test. *P* values <0.05 were considered statistically significant.

RESULTS

Histology of Small Intestinal Grafts and Native Intestine

Microscopic examination of graft tissue obtained on days 1 and 2 following transplantation showed nonspecific changes associated with surgical inflammation and preservation injury and were not included in the histopathologic review. Table I summarizes the major histopathologic features observed in allografts as compared to isografts and native intestine on days 3 to 7 post transplantation. Histopathologic signs characteristic of early rejection, including mild infiltration of the villi, crypt cell apoptosis, and lymphoblast-like cells around the base of crypts, were initially detected on day 3 after allogeneic SIT. On day 4 these manifestations of early rejection, in addition to cryptitis and more characteristic infiltration in the villi, crypts, and lamina propria, were evident in all animals (Fig. 1, *A*). In contrast, allograft recipients treated with tacrolimus showed no or slight mixed infiltrate in the crypts and lamina propria and no epithelial injury (Fig. 1, *B*).

Apoptosis was a major feature in early rejection and, as rejection proceeded, resulted in dissolution of crypt epithelium. By day 5, changes associated with mild rejection were established histologically. Histopathologic features progressed to moderate-to-severe rejection on day 6 and included hemorrhagic necrosis of villi, crypt necrosis, apoptosis, epithelial damage with a marked infiltrate, and occasional serosal fat involvement. On day 7 severe rejection changes characterized by blunted villi, epithelial necrosis with hemorrhage and focal ulceration, and a severe inflammatory infiltrate were apparent (Fig. 2, *A* and *B*). The characteristic cellular infiltrate was composed predominantly of mononuclear cells with large numbers of blast-like or activated lymphocytes. The infiltrate appeared early, at or near the base of the crypts. As rejection progressed, the infiltrate became more widely dispersed and included the lamina propria and serosal fat. Variable numbers of neutrophils and eosinophils were also detected in the infiltrate.

Tacrolimus treatment ameliorated the histopathologic features of rejection. Villi were intact but showed some blunting (Fig. 2, *C*). Crypts had no necrosis and the infiltrate was minimal or absent (Fig. 2, *D*). Isografts showed no changes, except mild preservation injury up to day 3, with gradual recovery by day 7 (data not shown). Likewise, native intestine from rats that underwent allogeneic or syngeneic SIT showed no remarkable changes histopathologically. The characteristic manifestations of graft-versus-host disease, such as 20% of greater weight loss of preoperative body weight, skin rash, and cachexia, were not observed in any group.[12] Thus in a high-responder allogeneic SIT model, specific histopathologic changes compatible with acute rejection were apparent by day 4, with progression to severe rejection occurring by day 7.

Cytokine Expression After SIT

To determine the functional characteristics of graft infiltrating cells during allogeneic SIT, cytokine gene expression was examined. On day 1 post transplant, allografts and isografts had comparable levels of IL-2, IL-4, IL-5, IL-6, and TNF-α transcripts, suggesting that nonspecific inflammatory events elicited very early cytokine expression. By day 2 or 3 post transplant the expression of IL-2 mRNA was slightly elevated in the intestine of allograft recipients, as compared to native intestine and isograft recipients, but the increase was not significant (Fig. 3, *A*). In contrast, message for IFN-γ was significantly and specifically increased in allografts by day 3 ($P = 0.038$) and remained elevated through posttransplant day 7 ($P = 0.041$) (Fig. 3, *B*).

Table I. Histopathologic features of small intestine transplantation

	Isograft	Allograft	Tacrolimus-treated allograft	Native intestine
Day 3				
Villi	Mild shortening—normal	1+ infiltrate	ND	Mild shortening
Crypts	No epithelial injury	1+ apoptosis	ND	No epithelial damage
Infiltrate	No infiltrate	1+ focal, base of cypts	ND	No infiltrate
Diagnosis	No rejection	Very early rejection	ND	No rejection
Day 4				
Villi	Mild shortening—normal	1+ infiltrate, intact	Intact	Normal
Crypts	1+ apoptosis	2+ apoptosis, 1+ cryptitis	No epithelial injury	No epithelial damage
Infiltrate	No infiltrate	2+ focal, lamina propria, serosa	0 to 1+ lamina propria, fat	No infiltrate
Diagnosis	No rejection	Early rejection	No rejection	No rejection
Day 5				
Villi	Normal	Focal hemorrhage	ND	Normal
Crypts	No epithelial injury	2+ apoptosis, focal necrosis	ND	No epithelial damage
Infiltrate	No infiltrate	2+ lamina propria, serosa	ND	No infiltrate
Diagnosis	No rejection	Mild rejection	ND	No rejection
Day 6				
Villi	Normal—recovered PI	Focal hemorrhage, necrosis	ND	Normal
Crypts	No epithelial injury	2+ apoptosis, focal necrosis	ND	No epithelial damage
Infiltrate	No infiltrate	3+ lamina propria, serosa	ND	No infiltrate
Diagnosis	No rejection	Moderate-severe rejection	ND	No rejection
Day 7				
Villi	Normal	Necrosis, ulceration, hemorrhage	Intact, focal hemorrhage	Normal
Crypts	No epithelial injury	2+ apoptosis, epithelial necrosis	0 to 1+ apoptosis	No epithelial damage
Infiltrate	No infiltrate	3+ throughout intestine	0 to 1+ infiltrate	No infiltrate
Diagnosis	No rejection	Severe rejection	No rejection	No rejection

0 = None; 1+ = mild; 2+ = moderate; 3+ = severe; PI = preservation injury; ND = not done.

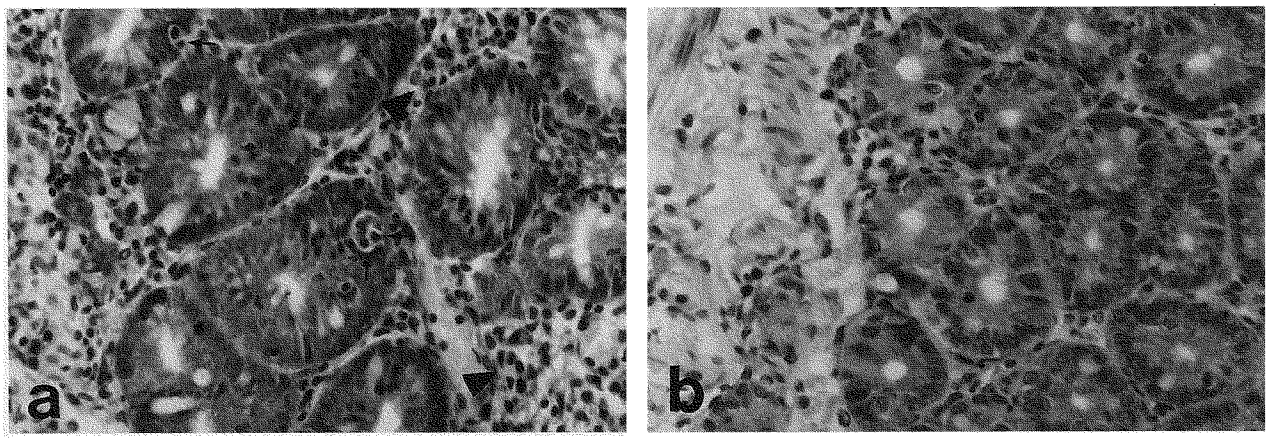

Fig. 1. Sections of allografts from untreated **(A)** and tacrolimus-treated **(B)** recipients on day 4 following small intestine transplantation. Allografts show features of rejection including an inflammatory infiltrate in **A** and cryptitis. Note apoptotic cells in **A** within the crypts. In contrast, tacrolimus-treated grafts show no epithelial injury and minimal inflammatory infiltrate. (Hematoxylin and eosin stain; original magnification ×400.)

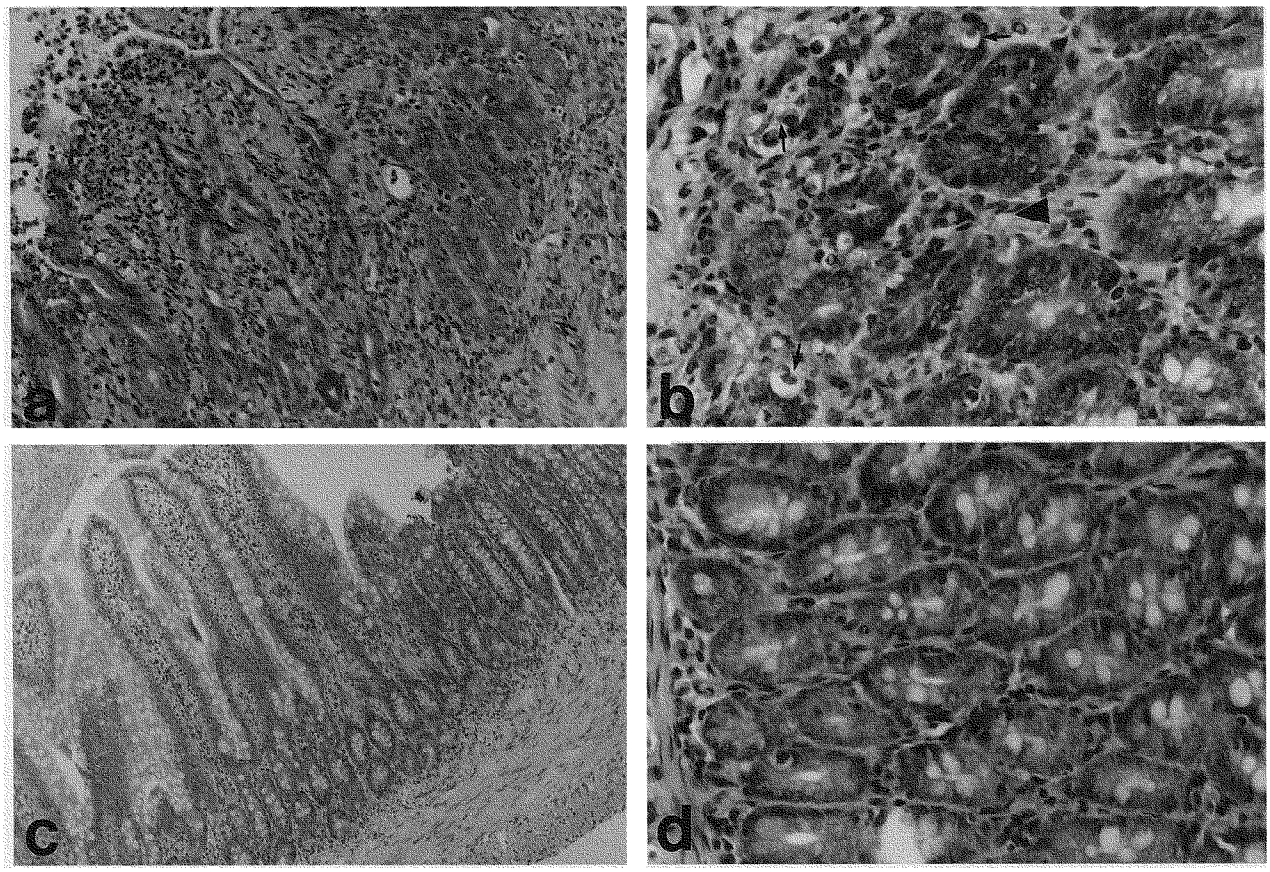

Fig. 2. Sections of untreated (**A** and **B**) and tacrolimus-treated (**C** and **D**) allografts on day 7 following small intestine transplantation. An inflammatory infiltrate distributed throughout the intestine and extensive necrosis and ulceration are seen in allografts **(A)**. Crypts within the allograft show epithelial necrosis, apoptosis (small arrows), and a marked infiltrate (large arrowhead) **(B)**. Allografts from tacrolimus-treated recipients have intact villi **(C)**. Only a minimal infiltrate is seen within the crypts **(D)**. (Hematoxylin and eosin stain; original magnification: **A** and **B**, ×200; **C**, ×100; **D**, ×400.)

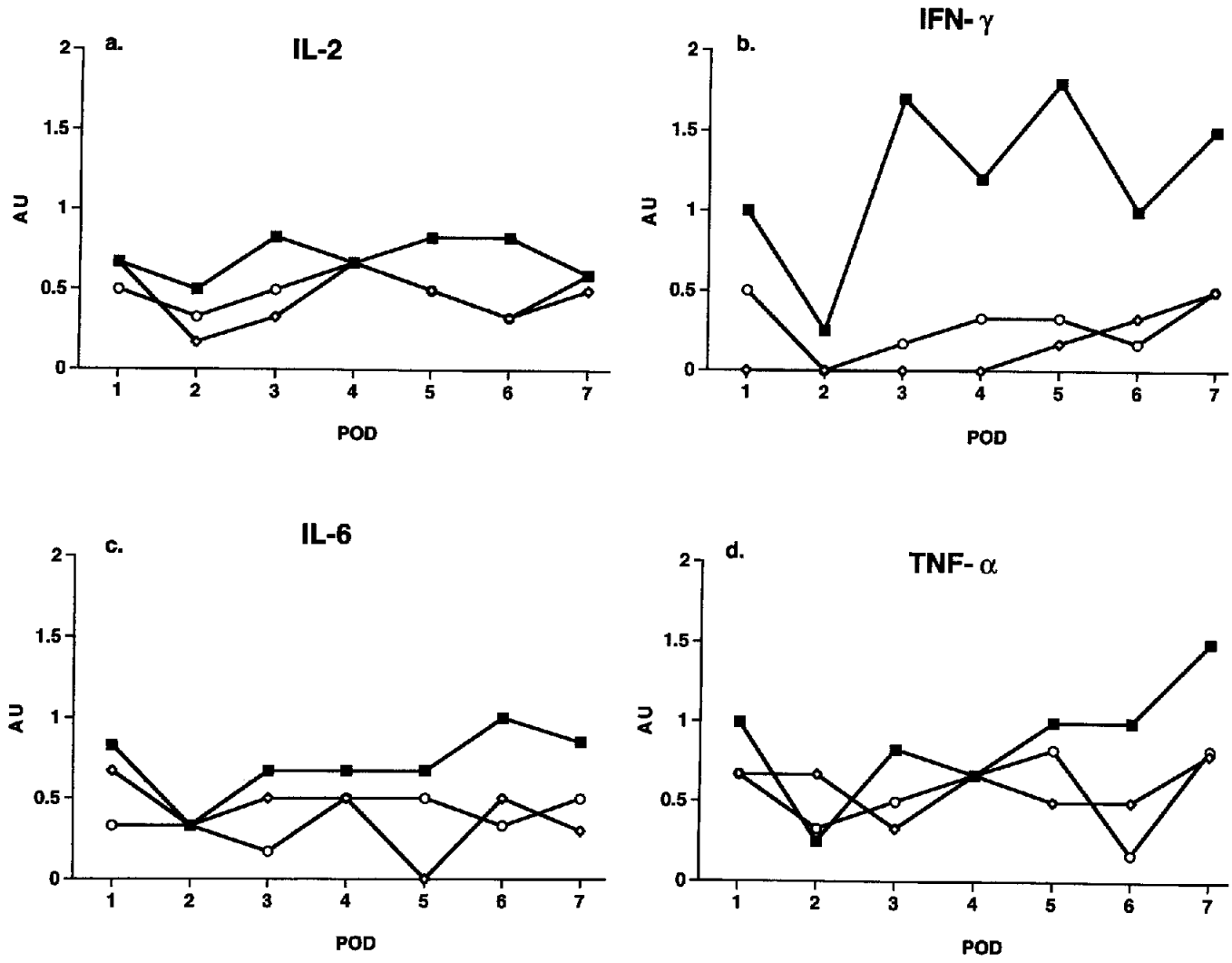

Fig. 3. Temporal expression of intestinal IL-2 **(A)**, IFN-γ **(B)**, IL-6 **(C)**, and TNF-α **(D)** mRNA after small intestine transplantation in allografts (■), isografts (○), and native intestine from allograft recipients (◇). Data are expressed in relative units and represent the mean (n = 3 to 5) of all samples within each group. POD = postoperative day; AU = arbitrary units.

Transcripts for the Th2 cytokines IL-4, IL-5, and IL-6 were detected in all small intestinal tissues. There was no difference in IL-4 gene expression in allografts, isografts, or native intestine (data not shown). A trend toward enhanced IL-5 and IL-6 gene expression was noted in allografts as compared to isografts; however, the difference was not significant (Fig. 3, *C*). There was persistent and increasing expression of TNF-α on days 3 to 7 following transplantation in allografts (Fig. 3, *D*). During the later phases of the rejection response (day 7), the expression of TNF-α was significantly elevated in allografts compared to isografts ($P = 0.046$).

Effects of Tacrolimus on Intragraft Cytokine Expression

The effects of tacrolimus on day 7 intragraft cytokine expression were evaluated by RT-PCR (Fig. 4). Allograft recipients that were treated with 1 mg/kg/day of tacrolimus on days 1 to 7 following transplantation had decreased IFN-γ transcripts in the graft compared to allografts without tacrolimus treatment ($P < 0.01$). IL-2 gene expression was also inhibited by tacrolimus. Indeed, IL-2 and IFN-γ transcripts in tacrolimus-treated allografts were decreased to levels comparable to those in isografts receiving no tacrolimus (data not shown). Tacrolimus

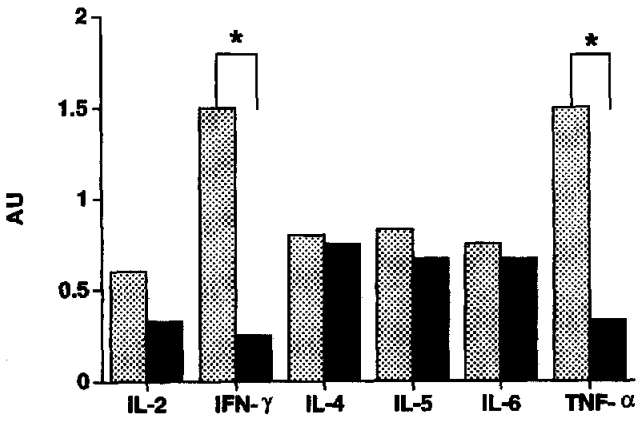

Fig. 4. Effects of tacrolimus on intragraft cytokine mRNA expression in untreated allografts (stippled bar) and tacrolimus-treated allograft recipients (solid bar) on postoperative day 7. Data are expressed in relative units and represent the mean of all samples (n = 3 to 5) within each group. AU = arbitrary units; * = $P \leq 0.05$.

treatment also inhibited TNF-α gene expression in day 7 allografts ($P = 0.01$). In contrast, tacrolimus treatment had minimal effect on expression of IL-4, IL-5, and IL-6 mRNA in allograft recipients.

DISCUSSION

In this study the relationship between histopathologic changes and cytokine gene expression was analyzed in a rat experimental model of allogeneic heterotopic SIT. Histologic examination revealed that Lewis rats engrafted with fully allogeneic ACI rat intestine vigorously reject the graft within 7 days of transplantation. The first histopathologic changes characteristic of rejection, including apoptosis, mild infiltration of the villi, and focal infiltration at the base of the crypts, can be identified by the posttransplant day 3. These early changes were associated with marked expression of IFN-γ mRNA within the allograft. While IFN-γ gene expression persisted at high levels after day 3, the histopathologic changes progressed rapidly until severe rejection was observed on day 7. During the later stages of rejection (days 6 to 7), notable increases in the expression of TNF-α transcripts were also observed. In agreement with other reports,[4,13] the features most indicative of rejection, when present together, were the characteristic lymphoid infiltrate, apoptosis, and crypt epithelial injury.

In addition, we examined the effect of tacrolimus on the characteristic histopathologic features of rejection in relation to cytokine gene expression. We found that tacrolimus abrogated the histopathologic changes associated with rejection while dramatically inhibiting the expression of IFN-γ mRNA in allograft recipients. IL-2 and TNF-α allograft gene expression were also inhibited by tacrolimus. Interestingly, the T-helper type 2 (Th2) cytokines, IL-4, IL-5, and IL-6 mRNA were relatively unaffected by tacrolimus treatment.

Our findings suggest that IFN-γ may be an important immune mediator of graft rejection in SIT. In our study the expression of IFN-γ in allografts appears to be specific because isografts and native intestine have minimal expression of IFN-γ mRNA after day 2. These findings are in agreement with previous reports in experimental rat models of SIT.[5,6] Furthermore, preliminary evidence from our laboratory indicates that the levels of immunoreactive IFN-γ in the circulation are elevated in allograft recipients as compared to isograft recipients (unpublished results). IFN-γ is also specifically upregulated during chronic rejection of intestinal allografts.[14] Inasmuch as increased levels of IFN-γ were detected in an experimental model of Crohn's disease, but IFN-γ levels were normal in an animal model of ulcerative colitis,[15] IFN-γ is not merely a marker of chronic intestinal inflammation. In addition to the conventional immune mechanisms by which IFN-γ could exacerbate graft rejection, such as upregulation of MHC antigen expression and enhancement of effector cell function,[16] there are other potential mechanisms that may be particularly pertinent to the intestine. Madara and Stafford[17] demonstrated that IFN-γ increases interepithelial tight junction permeability in vitro. Alterations in intestinal epithelial cell barrier function could have important consequences in SIT. The local production of IFN-γ during rejection could promote bacterial translocation thereby enhancing infectious complications. Furthermore, IFN-γ has direct deleterious effects on gut epithelium. High systemic doses of IFN-γ produced epithelial necrosis on villous tips[18] and Liesenfield et al.[19] suggested that IFN-γ mediates necrosis in the ileum of mice following infection with *Toxoplasma gondii*. IFN-γ also inhibits proliferation of jejunal smooth muscle cells.[14] Thus the persistent elevation of IFN-γ observed after SIT may cause significant injury and dysfunction in the epithelial integrity.

Although the increases in the IFN-γ message were most notable in our study, we also observed a slight increase of IL-6 and TNF-α transcripts in allografts after day 5. These observations are consistent with recent reports on intragraft cytokine expression in SIT in mice[20] and in rats.[14] TNF-α causes tissue damage and promotes the infiltration of macrophages and neutrophils. Intestinal mucosal production of TNF-α is increased in both Crohn's disease and ulcerative colitis.[21] Similarly there is increased mucosal production of IL-6 in Crohn's disease and ulcerative colitis.[22]

In clinical SIT, serum levels of IL-6 were reported to correlate well with episodes of acute rejection.[23] On histologic examination, necrosis was present in allografts, especially on day 7, indicating that at the late stage of rejection, necrosis can accompany apoptotic cell death. Proinflammatory cytokines, such as TNF-α and IL-6, produced at the site of rejection may contribute to inflammatory changes, which culminate in necrosis within the allograft.

Recent studies in murine SIT suggested that a shift toward Th1 predominance may occur with ongoing rejection, whereas both Th1 and Th2 subsets, or Th0 cells, are involved in the earlier stage.[20] Our findings indicate that Th1 cytokines predominate in SIT rejection and are important in the pathologic changes seen during graft rejection since both graft destruction and Th1 cytokines are abrogated with tacrolimus treatment.

The specific mechanisms by which IFN-γ may participate in the histopathologic changes during intestinal allograft rejection are not known. Our results suggest that IFN-γ may have utility as a marker of graft rejection, and the development of therapeutics that specifically target this cytokine may improve the clinical outcome of SIT.

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Bile Reflux in Benign and Malignant Barrett's Esophagus: Effect of Medical Acid Suppression and Nissen Fundoplication

H. J. Stein, M.D., W. K. H. Kauer, M.D., H. Feussner, M.D., J. R. Siewert, M.D., F.A.C.S.

Bile reflux has been implicated in the pathogenesis and malignant degeneration of Barrett's esophagus, but clinical studies in patients with adenocarcinoma arising in Barrett's esophagus are lacking. Ambulatory esophageal measurement of acid and bile reflux was performed with the previously validated fiberoptic bilirubin monitoring system (Bilitec) combined with a pH probe in 20 asymptomatic volunteers, 19 patients with gastroesophageal reflux disease (GERD) but no mucosal injury, 45 patients with GERD and erosive esophagitis, 33 patients with GERD and Barrett's esophagus, and 14 patients with early adenocarcinoma arising in Barrett's esophagus. Repeat studies were done in 15 patients under medical acid suppression and 16 patients after laparoscopic Nissen fundoplication. The mean esophageal bile exposure time showed an exponential increase from GERD patients without esophagitis to those with erosive esophagitis and benign Barrett's esophagus and was highest in patients with early carcinoma in Barrett's esophagus ($P < 0.01$). Pathologic esophageal bile exposure was documented in 18 (54.5%) of 33 patients with benign Barrett's esophagus and 11 (78.6%) of 14 patients with early adenocarcinoma in Barrett's esophagus. Nissen fundoplication but not medical acid suppression resulted in complete suppression of bile reflux. Bile reflux into the esophagus is particularly prevalent in patients with Barrett's esophagus and early cancer. Bile reflux into the esophagus can be completely suppressed by Nissen fundoplication but not medical acid suppression alone. (J GASTROINTEST SURG 1998;2:333-341.)

Barrett's esophagus, that is, columnar epithelial metaplasia in the distal esophagus, develops as the consequence of chronic gastroesophageal reflux in up to 20% of patients with documented gastroesophageal reflux disease (GERD).[1] Compared to reflux patients without columnar metaplasia, gastroesophageal reflux in patients with Barrett's esophagus is characterized by a combination of acid and bile reflux, and frequently results in complications such as ulcers and stenosis.[1-6] In addition, columnar metaplasia is associated with a clearly increased risk of malignant degeneration.[1] Experimental data indicate a possible pathogenetic role of bile reflux in the process of malignant degeneration of Barrett's esophagus.[7] Reliable clinical data supporting this concept are so far lacking since methods to directly measure bile reflux into the esophagus have been inaccurate or cumbersome. For the same reasons the effect of medical acid suppression or Nissen fundoplication on bile reflux into the esophagus has been poorly studied.

A fiberoptic system for circadian monitoring of biliary duodenogastric and duodenogastroesophageal reflux (Bilitec, Synectics Medical, Stockholm, Sweden) was recently developed and clinically introduced by Becchi et al.[8] The principle of the measurement is based on the light absorption characteristics of bilirubin, the major pigment of bile. A series of subsequent validation studies has confirmed that, despite some shortcomings inherent to the system, the combined esophageal pH and Bilitec measurements allow for a reliable assessment of acid and bile reflux into the esophagus in the clinical setting.[9-12] We used this new technology to evaluate the prevalence, severity, and circadian pattern of bile reflux into the esophagus in a

From the Chirurgische Klinik und Poliklinik, Klinikum rechts der Isar der TU München, München, Germany.
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Reprint requests: Dr. med Hubert J. Stein, Chirurgische Klinik und Poliklinik, Klinikum rechts der Isar der TU München, Ismaninger Str. 22, D-81675 München, Germany.

series of patients with GERD without Barrett's esophagus, patients with Barrett's esophagus but no evidence of carcinoma, and patients with high-grade dysplasia or early adenocarcinoma arising in Barrett's mucosa. We also studied the effect of standard medical acid suppression and Nissen fundoplication on esophageal bile exposure time.

MATERIAL AND METHODS

Validation Studies

To identify an optimal threshold for detection of bilirubin with the Bilitec system in the clinical setting, 114 samples of gastric and duodenal aspirates were obtained from 42 patients who had nasogastric or nasoduodenal tubes placed for a variety of medical reasons. In each sample the bilirubin concentration was determined with a standard spectrophotometer and expressed as milligrams per deciliter (mg/dl). Each sample was then placed in a light-protected container, and the absorption value obtained with the Bilitec system was recorded and correlated to the respective bilirubin concentration determined spectrophotometrically.

Combined Ambulatory Esophageal pH and Bilitec Monitoring

Esophageal Bilitec monitoring was performed simultaneously with pH monitoring as described in detail previously.[9,11] Briefly, a glass pH and a fiberoptic bilirubin probe were placed 5 cm above the manometrically determined upper border of the lower esophageal sphincter and connected to the respective data recorders (Digitrapper Mark IV and Bilitec 2000, Synectics Medical, Stockholm, Sweden). Recordings were performed over a 24-hour period and under ambulatory conditions. Diet was restricted to three meals and food with a pH between 4 and 7. Calibration of the pH and fiberoptic bilirubin probes was performed before and after each recording. Studies with a drift of more than 0.2 pH units or 0.15 absorbance units over 24 hours were discarded. Analysis of the pH and Bilitec record was performed with commercially available software (Synectics Medical). The pH records were analyzed for the percentage of total monitoring time with a pH <4. An esophageal acid exposure time (pH <4) greater than 4.5% of the total monitoring period was considered indicative of increased esophageal acid exposure.[13] Based on the in vitro validation studies, a value exceeding 0.25 absorbance units on the Bilitec record was considered indicative of bile reflux. The percentage of time with an absorbance value greater than 0.25 on the Bilitec record was calculated separately for the total monitoring time (excluding the meal periods), the interdigestive upright period, the postprandial period, and the sleep period. Patients were considered to have abnormal esophageal exposure to bilirubin if the percentage of time with an absorbance greater than 0.25 on Bilitec monitoring exceeded the ninety-fifth percentile level of the data obtained in the 20 normal volunteers.

Patient Population and Studies Performed

Combined ambulatory esophageal pH and Bilitec monitoring was performed in 20 asymptomatic volunteers (11 males and 9 females; mean age 35.6 years) and a total of 97 patients with symptoms of GERD (heartburn, regurgitation, and/or dysphagia) and increased esophageal acid exposure documented by ambulatory 24-hour esophageal pH monitoring. On endoscopy, 19 of the GERD patients (9 males and 10 females; mean age 46.3 years) had no mucosal injury, 45 patients with GERD (23 males and 22 females; mean age 47.9 years) had erosive esophagitis, 33 patients (25 males and 8 females; mean age 49.0 years) had Barrett's esophagus without evidence of carcinoma or high-grade dysplasia on multiple biopsies. Combined ambulatory 24-hour esophageal pH and Bilitec monitoring was also performed in an additional 12 patients (10 males and 2 females; mean age 59.1 years) with early adenocarcinoma arising in Barrett's esophagus (T1 tumor stage[14] on endoscopic ultrasonography) and in two patients (both males, 48 and 62 years of age, respectively) with high-grade dysplasia in Barrett's esophagus but no evidence of invasive carcinoma on multiple biopsies. Barrett's esophagus was defined by the endoscopic documentation of a columnar epithelial lining of the distal esophagus over at least 3 cm and histologic confirmation of specialized columnar epithelium.[1]

Prior to the initial combined ambulatory esophageal pH and Bilitec studies, all acid-suppressing and prokinetic medications were discontinued for at least 7 days in all patients. Fifteen of the patients with GERD volunteered for repeat studies under a standard dose of medical acid suppression with a proton pump inhibitor (omeprazole, 20 mg twice a day), and 16 patients had repeat studies performed between 3 months and 1 year after laparoscopic Nissen fundoplication. Patients with early adenocarcinoma in Barrett's esophagus underwent resection.

All studies in normal volunteers and patients with early carcinoma were approved by the local human ethics committee.

Statistical Analysis

Data are expressed as means unless otherwise stated. The degree of association between variables was assessed with Pearson's correlation coefficient. Fisher's exact test was used to compare proportions between two groups. Standard statistical analysis for paired and unpaired data sets was used to compare data between and within groups as appropriate. A P value <0.05 was considered significant.

RESULTS

In Vitro Validation Studies

Fig. 1 illustrates the relationship between Bilitec measurements and spectrophotometrically determined bilirubin concentrations in gastric and duodenal aspirates. There is a linear correlation between Bilitec measurements and spectrophotometric bilirubin concentrations above a threshold of 0.25 absorbance units on the Bilitec record, with a tapering of the correlation curve at the upper limit of sensitivity of the Bilitec device at around 0.8 to 1.0 absorption units. Below the threshold of 0.25 absorption units, there is a grey area with no significant correlation between Bilitec readings and true bilirubin concentrations. A threshold of 0.25 absorbance units on the Bilitec record was therefore chosen to calculate esophageal exposure time to bile in the ambulatory 24-hour recordings.

Clinical Studies in GERD Patients and Patients with Benign and Malignant Barrett's Esophagus

Fig. 2 shows a typical combined esophageal pH and Bilitec record in a patient with Barrett's esophagus with severe acid and bile reflux into the esophagus. The mean esophageal acid (expressed as percentage of time with pH <4 on pH monitoring) and bilirubin exposure time (expressed as percentage of time with absorbance greater than 0.25 on Bilitec monitoring) showed a marked increase from normal volunteers, to GERD patients without esophagitis, GERD patients with erosive esophagitis, and patients with Barrett's esophagus (P <0.01; Fig. 3). Esophageal bilirubin exposure time was highest in the group of patients with high-grade dysplasia or early carcinoma in Barrett's esophagus. Analysis of the circadian pattern of esophageal bilirubin exposure showed that bile reflux occurred primarily during the postprandial and supine monitoring periods (Fig. 4).

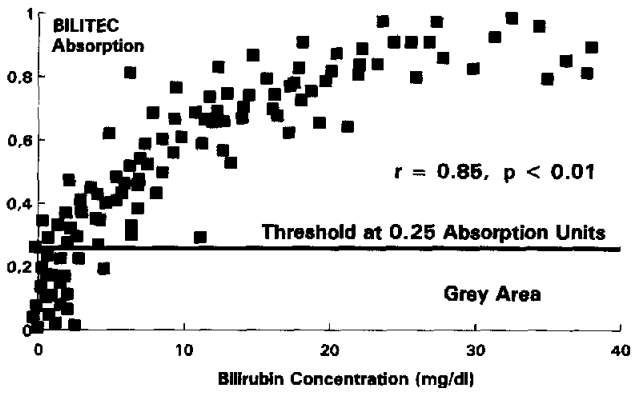

Fig. 1. Correlation between Bilitec measurements and bilirubin concentrations determined by spectrophotometry in gastric and duodenal aspirates.

The esophageal exposure time to bilirubin on ambulatory 24-hour esophageal Bilitec monitoring for the individual subjects in the various study groups is shown in Fig. 5. Pathologic esophageal bilirubin exposure, defined as an esophageal bilirubin exposure exceeding the ninety-fifth percentile of the normal volunteers (i.e., 6.9% of the total monitoring time with absorption >0.25), was observed in 2 (10.5%) of 19 GERD patients without esophagitis, 10 (22.2%) of 45 GERD patients with erosive esophagitis, 18 (54.5%) of 33 patients with benign Barrett's esophagus, and 11 (78.6%) of 14 patients with early adenocarcinoma or high-grade dysplasia in Barrett's esophagus. Both patients with high-grade dysplasia had an abnormal esophageal bilirubin exposure time (see Fig. 5).

Assessment of Treatment Effects

Repeat Bilitec measurements under 20 mg of omeprazole twice a day in 15 patients showed an almost complete abolishment of acid reflux (mean 19.7% of total monitoring time without acid suppression, 2.9% with acid suppression; P <0.01) and a marked reduction in esophageal bile exposure (mean 16.2% of total monitoring time without acid suppression, 8.9% with acid suppression; P <0.05). Medical therapy with omeprazole, although highly effective in terms of suppressing acid reflux, did not reduce esophageal bile exposure time into the normal range (Fig. 6). In contrast, laparoscopic Nissen fundoplication normalized esophageal acid and bile exposure in all but 1 of 16 patients who volunteered for follow-up studies after antireflux surgery (mean preoperative esophageal bilirubin exposure time 16.6%, mean postoperative esophageal bilirubin exposure time 2.4%; P <0.01) (Fig. 7). Fig. 8 illustrates how laparoscopic Nissen fundoplication resulted in complete suppression of bile reflux in a patient with Barrett's esophagus.

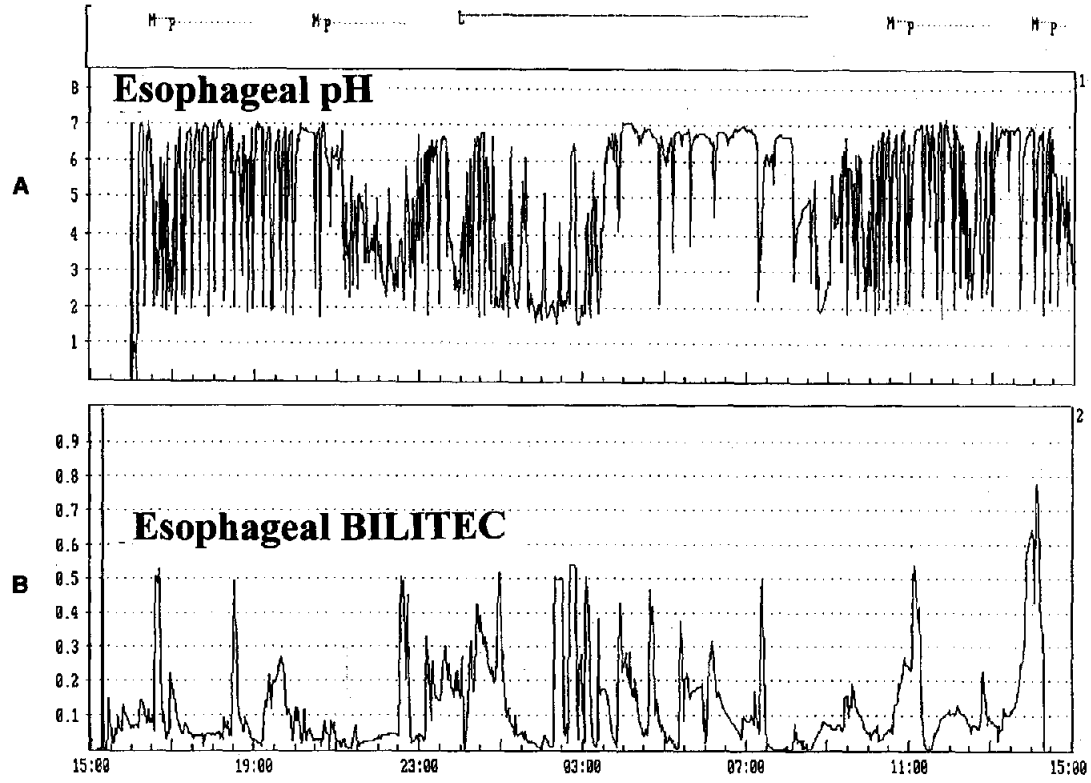

Fig. 2. Typical combined ambulatory 24-hour esophageal pH **(A)** and Bilitec **(B)** record in a patient with Barrett's esophagus showing severe acid and bile reflux. The time is indicated on the x-axis. The y-axis on the pH record shows pH units. Esophageal acid exposure is calculated as the percentage of time with a pH <4. The y-axis on the Bilitec record shows absorption units. Esophageal bilirubin exposure is calculated as the percentage of time with absorption greater than 0.25. M = meal period; P = postprandial period; L = supine period.

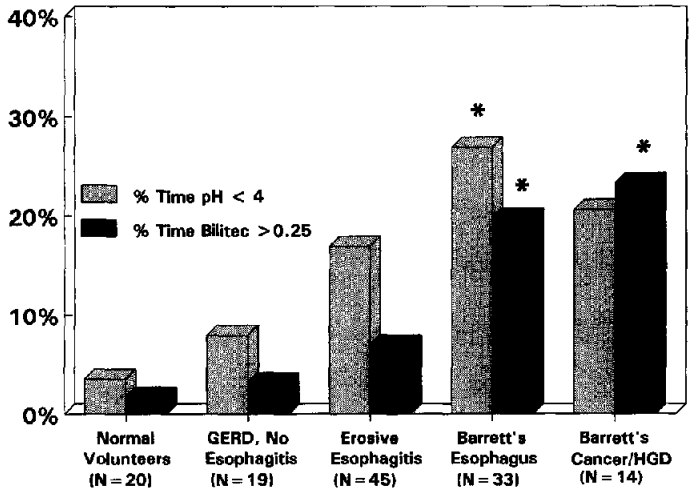

Fig. 3. Mean overall esophageal acid and bile exposure time on combined ambulatory 24-hour esophageal pH and Bilitec monitoring in normal volunteers, patients with gastroesophageal reflux disease *(GERD)* with and without esophagitis, patients with Barrett's esophagus, and patients with high-grade dysplasia *(HGD)* or early carcinoma in Barrett's esophagus. * = $P < 0.01$ vs. other groups.

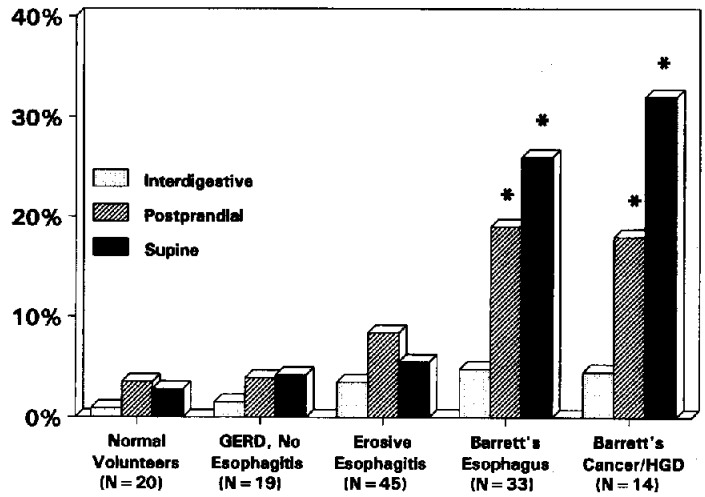

Fig. 4. Circadian pattern of intestinoesophageal bile reflux in normal volunteers, patients with gastroesophageal reflux disease *(GERD)* with and without esophagitis, patients with Barrett's esophagus, and patients with high-grade dysplasia *(HGD)* or early carcinoma in Barrett's esophagus as measured by ambulatory 24-hour esophageal Bilitec mitoring (% time with absorption >0.25). * = $P < 0.01$ vs. interdigestive period and other groups.

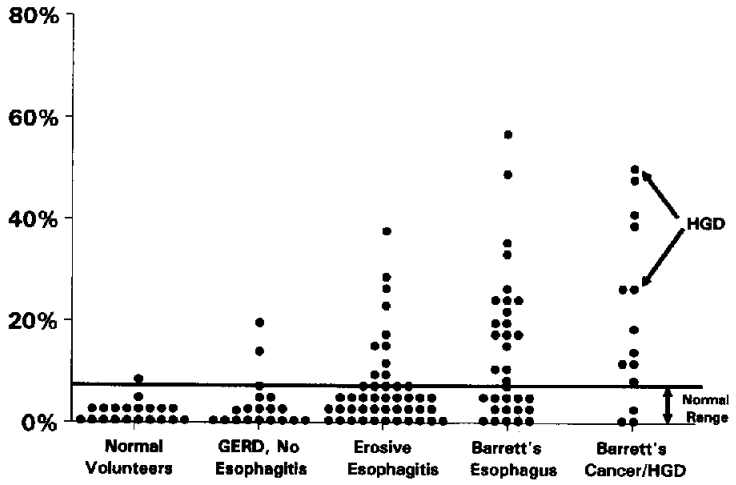

Fig. 5. Individual overall esophageal exposure time to bilirubin on ambulatory 24-hour esophageal Bilitec monitoring (% time with absorption >0.25) in normal volunteers, patients with gastroesophageal reflux disease *(GERD)* with and without esophagitis, patients with Barrett's esophagus, and patients with high-grade dysplasia *(HGD)* or early carcinoma in Barrett's esophagus.

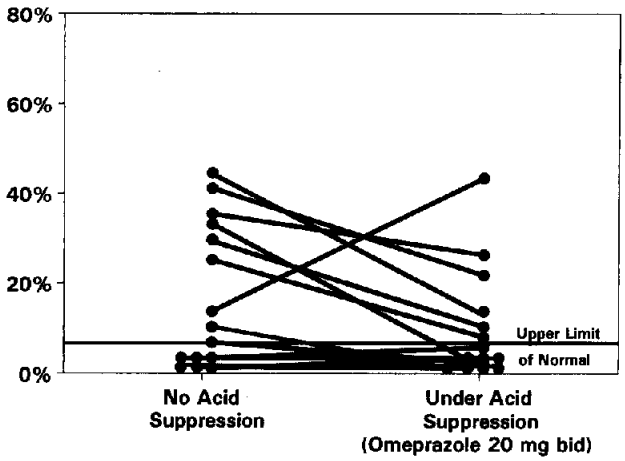

Fig. 6. Individual overall esophageal bilirubin exposure time on ambulatory 24-hour esophageal Bilitec monitoring (% time with absorption >0.25) in reflux patients and without medical acid suppression (20 mg omeprazole twice a day).

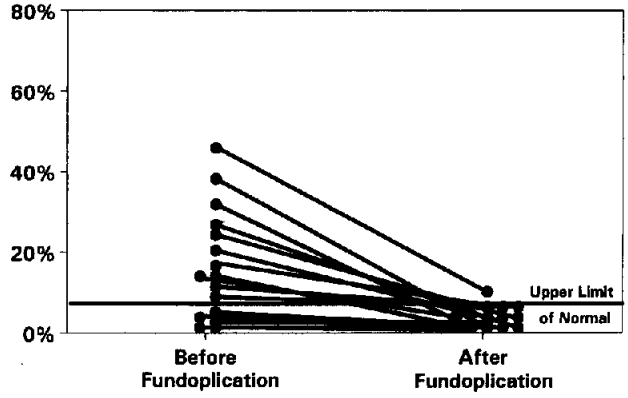

Fig. 7. Individual overall esophageal bilirubin exposure time on ambulatory 24-hour esophageal Bilitec monitoring (% time with absorption >0.25) in reflux patients before and after laparoscopic Nissen fundoplication.

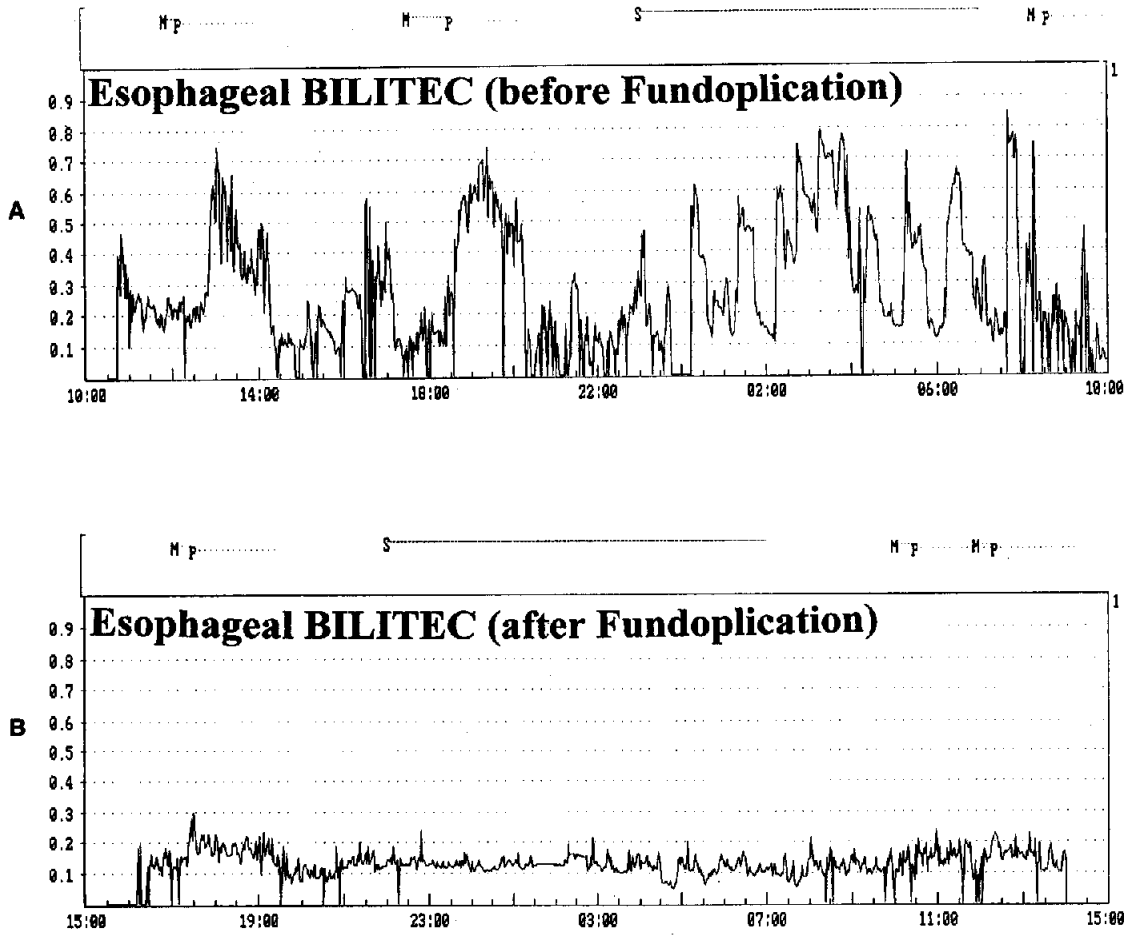

Fig. 8. Representative 24-hour esophageal Bilitec record in a patient with Barrett's esophagus before (A) and after (B) Nissen fundoplication. The time is indicated on the x-axis. The y-axis on the Bilitec record shows absorption units. Esophageal bilirubin exposure is calculated as the percentage of time with absorption greater than 0.25. M = meal period; P = postprandial period; S = supine period.

Neither medical therapy nor Nissen fundoplication resulted in a change in the extent of columnar epithelium. Erosive esophagitis healed in all but three patients (two patients with medical acid suppression and one patient with Nissen fundoplication). All three of these patients had either persistent acid or acid and bile reflux.

DISCUSSION

The concept of bile reflux as an initiator or promoter of benign and malignant foregut disorders has been a topic of controversy for several decades.[2,7,15] This was due to the lack of objective and clinically applicable tests to quantitate reflux of duodenal contents over prolonged periods of time and the poor understanding thus far of the interactions between bile acids, pancreatic enzymes, and gastric acid in vivo. The recent introduction of the Bilitec system has renewed the debate as to the prevalence, pathophysiologic mechanisms, consequences, and clinical relevance of duodenogastric and duodenogastroesophageal bile reflux. In a series of clinical studies with the Bilitec unit, excessive reflux of bile in patients with Barrett's esophagus has been reported by several groups.[2-4,16] The present study is the first to demonstrate a high prevalence of bile reflux into the esophagus in patients with early adenocarcinoma arising in Barrett's esophagus and to show a direct protective effect of Nissen fundoplication.

Measurement of duodenogastroesophageal bile reflux with the Bilitec fiberoptic bilirubin sensor is based on the hypothesis that bilirubin, which by itself causes no harm to intestinal mucosa, is a suitable tracer for potentially noxious duodenal contents (i.e., bile acids and pancreatic enzymes).[8] Bilirubin itself has no apparent physiologic function and is a waste product of heme catabolism which, after conjugation, is secreted via the biliary tract into the duodenum. In humans, conjugated bilirubin constitutes the major pigment of bile. We and others have evaluated the correlation between Bilitec readings and the concentration of bile acids in gastric and duodenal aspirates.[8-12] Overall there was a good correlation between bilirubin concentrations and the presence of bile acids in these studies, thereby supporting the clinical usefulness of the Bilitec device to detect bile reflux. Controversy persists, however, concerning the threshold required to reliably detect bile reflux with the Bilitec unit. Furthermore, because the Bilitec device indiscriminately records any absorbance at 450 to 470 nm, a variety of food products may result in false positive readings.[10] Based on the results of the validation studies presented, we have chosen to set the threshold for detection and measurement of bile reflux at 0.25 absorption units on Bilitec monitoring. This detection threshold is higher and thus more specific than the threshold used by other investigators. In addition, we have excluded meal periods from the analysis. This may explain the lower esophageal bilirubin exposure times in GERD patients with erosive esophagitis or benign Barrett's esophagus observed in the present study as compared to the data reported by other investigators.[2-4,16] Conversely, the prevalence and severity of excessive bile reflux in patients with early cancer in the present study would have been even higher if the lower detection thresholds used by other investigators had been applied.

The exact mechanism by which bile reflux might initiate or promote tissue injury and carcinogenesis in humans is unclear at the present time. Bile salts alone do not seem to be mutagenic but promote mutagenicity of aromatic amines. Alternatively, bile acids may also facilitate the action of other endoluminal carcinogenic agents by mucosal barrier disruption and exposure of the proliferating epithelial compartment.[7] In vitro studies and in vivo animal models have shown that soluble bile acids can enter mucosal cells when in their nonionized lipophilic form and accumulate there to up to eight times the luminal concentration.[17] Such excessive intracellular concentrations of bile acids result in increased mucosal permeability by dissolution of cell membranes and tight junctions. Entering of mucosal cells and accumulation is pH driven and dependent on the conjugation status of the bile acids and their solubility. Thus the potential injurious effect of bile reflux is not only related to their luminal concentration but is also dependent on the environmental pH, with a pH between 3 and 6 providing the optimal environment for bile acid injury.[18]

A reduction of bile reflux into the esophagus by medical acid suppression has been reported by Champion et al.[4] and was also observed in the present study. This effect has been attributed to the marked reduction in both gastric acid and gastric volume with proton pump inhibitors. In both studies, however, medical acid suppression failed to reduce esophageal bile exposure to normal values. In contrast, similar to the well-described effect of Nissen fundoplication on acid gastroesophageal reflux, the antireflux procedure also resulted in normalization of esophageal bile exposure in all but one of the patients assessed in the present study. This observation is not surprising since reconstruction of the defective barrier function of the lower esophageal sphincter in patients with GERD will protect against reflux of any gastric contents (i.e., acid and duodenal juice).[16,19] Consequently antireflux surgery constitutes a more logical therapeutic approach for patients with a combination of acid and bile reflux than medical acid suppression and may of-

fer the chance to prevent the development of Barrett's esophagus with its inherent risk of malignant degeneration.

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Discussion

Dr. J. Peters (Los Angeles, Calif.). Now that you have demonstrated that bile reflux is present in the malignant Barrett's metaplasia, are you inserting bile probes in all candidates for antireflux surgery at your institution? How do you use that as a practical measure in your everyday practice? Is it a risk factor?

Dr. H.J. Stein. We have a study in progress on bile reflux in patients with Barrett's esophagus, and we continue to combine pH and bile reflux monitoring. If we were to find combined bile and acid reflux in a patient, this would certainly support our intention to perform antireflux surgery rather than medical acid suppression in such a patient. Whether bile reflux is a risk factor we do not know for sure at the present time; much more data are needed before that can be determined. The data that we have, and which I have presented in this report, would certainly point in that direction.

Dr. C. Pellegrini (Seattle, Wash.). How can you explain your findings in relation to what Dr. Richter has shown with larger numbers in terms of medical acid suppression being an effective way to decrease bilirubin exposure in the esophagus? You show the number of patients receiving medical therapy, but it does not seem as though you could measure a large number of patients compared to the total number of patients that you studied. Did you select some patients?

Dr. Stein. The data I have presented in no way contradict what Dr. Richter reported a couple of years ago. He has shown that acid suppression reduces bile reflux. We also showed that acid suppression does reduce bile reflux. Dr. Richter did show that acid suppression did not result in a return of bile reflux to the normal range and we have confirmed this, so there is no discrepancy. We both showed that acid suppression can decrease bile reflux but does not suppress it completely. Did we select patients out for the studies with and without medical acid suppression? Yes we did. We tried to select those patients who did have bile reflux. Obviously not every patient wanted to undergo a second study on medical acid suppression, so we have 15 patients who were tested with and without medical acid suppression.

Dr. T. DeMeester. I concur with Dr. Stein in that oftentimes when the medical investigators present their data, they imply that values return to normal, but if you review Dr. Richter's first paper, these values definitely do not return to normal. What you are looking for is the presence of bile.

Dr. K. Fuchs (Würzburg, Germany). Have you noticed any changes in intragastric bile exposure after Nissen fundoplication?

Dr. Stein. We have not performed intragastric studies after Nissen fundoplication. We cannot place three or four probes at a time, so we limited our postoperative studies to monitoring of intraesophageal pH and bile.

Dr. J. Hunter (Atlanta, Ga.). Some of your patients, it appeared, did normalize the amount of bile acid reflux while on proton pump inbibitor therapy. For those of us who do not have Bilitec monitors, are there any ways to predict this group?

Dr. Stein. There is no way to predict, based on the available data, which patients will respond to medical acid suppression as far as bile exposure in the esophagus is concerned. Most patients become asymptomatic with medical acid suppression because it is the acid that causes the symptoms, but bile may continue to reflux, even though the acid has been suppressed.

Postprandial Gastroesophageal Reflux in Normal Volunteers and Symptomatic Patients

Rodney J. Mason, M.D., Stefan Öberg, M.D., Cedric G. Bremner, M.D., Jeffrey H. Peters, M.D., Michael Gadenstätter, M.D., Manfred Ritter, M.D., Tom R. DeMeester, M.D.

A structurally intact and competent lower esophageal sphincter in the experimental model shortens and becomes incompetent during gastric distention. The aim of this study was to evaluate postprandial reflux as an indirect measure of this volume-induced sphincter shortening and incompetency. Reflux (pH <4) in the 2-hour period following a meal was retrospectively analyzed from the 24-hour esophageal pH recordings of 94 healthy volunteers and 609 symptomatic patients. Forty-six percent of patients had pathologic postprandial reflux (>95th percentile of normal). The prevalence was lower in patients with a structurally intact compared to a defective lower esophageal sphincter (32% vs. 58%; $P < 0.001$). Pathologic postprandial reflux was greater in patients with abnormal compared to normal findings on 24-hour pH study (76% vs. 21%; $P < 0.001$). Patients with a normal 24-hour pH study and postprandial reflux had shorter sphincter lengths (2.33 vs. 2.82 cm; $P < 0.001$) and lower pressures (10.78 vs. 14.24 mm Hg; $P < 0.005$). A hiatal hernia increased the prevalence of postprandial reflux ($P < 0.001$) in all patients (67% vs. 38%) and in the subgroup with a structurally intact sphincter (75% vs. 27%, $P < 0.001$). Postprandial reflux is a dynamic indicator of sphincter competency, and increases as the structural sphincter characteristics deteriorate and is augmented by a hiatal hernia. (J GASTROINTEST SURG 1998;2:342-349.)

The "gold standard" for the assessment of increased esophageal acid exposure is 24-hour pH monitoring.[1,2] Esophageal motility is the preferred method for the evaluation of lower esophageal sphincter (LES) integrity.[3] A typical patient with gastroesophageal reflux disease (GERD) will have a structurally defective LES and abnormal findings on 24-hour pH study.[3-5] Patients with a structurally intact LES may have either a normal or abnormal 24-hour pH study. This observation implies a sphincter that dynamically changes throughout the day from being competent to incompetent. The reason for this is unclear. It is well documented, however, that gastric distention results in a transient loss of sphincter competency and gastroesophageal reflux.[6-10] The mechanism explaining these events is controversial and could be controlled either mechanically or neurologically. Recently it has been shown that gastric distention results in a mechanical unfolding of the sphincter, loss of sphincter length, and an increase in the frequency of equalization of gastric and esophageal pressure (common cavity events).[11-14] Th demonstration of this mechanical sphincter shorten ing in humans following gastric distention by foo and swallowed air has not been reported. The con cept, however, suggests that a patient with a short ini tial resting sphincter length would have more gas troesophageal reflux after gastric distention caused b a meal than a patient with a longer sphincter lengtl To test this hypothesis, a retrospective review was per formed of a group of healthy volunteers and patient with symptomatic reflux in whom the relationship be tween gastroesophageal reflux that occurred durin the 2-hour period after a meal and the subject manometric LES parameters was examined.

SUBJECTS AND METHODS

Study Population

The review population consisted of 609 consecu tive patients with foregut symptoms. There were 9 normal volunteers (mean age 34 [range 21 to 71

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

years; male:female ratio 55:39) and 609 symptomatic patients (mean age 51 [range 12 to 89] years; male:female ratio 332:277). All patients underwent both esophageal manometry and 24-hour pH monitoring in the University of Southern California esophageal function laboratory. Patients with achalasia, diffuse esophageal spasm, "nutcracker" esophagus, and hypertensive LES were excluded. Two hundred eighty-seven patients underwent upper gastrointestinal radiologic examination and upper endoscopy with biopsy in addition to manometry and pH testing.

Manometry

Stationary manometry was performed with an eight-channel water-perfused catheter. The pressure characteristics of the LES were measured with a station pull-through technique. All measurements were referenced to the resting intragastric pressure. The lower border of the sphincter was determined when the pressure was consistently elevated 2 mm Hg above the gastric baseline pressure and the upper border when the pressure reached the intraesophageal baseline pressure. The intra-abdominal length was measured from the lower border of the sphincter to the respiratory inversion point. The resting sphincter pressure was measured at the respiratory inversion point as the mean pressure between inspiration and expiration at this point. A mean of the values for each channel was used for the analysis. The three criteria used to determine the structural integrity of the LES were total sphincter length, intra-abdominal segment length, and sphincter pressure. A structurally defective LES by length alone was identified by the presence of an overall length of less than 2 cm and/or an abdominal length of less than 1 cm with a sphincter pressure of greater than 6 mm Hg. A structurally defective LES by pressure alone was identified by a resting sphincter pressure of less than 6 mm Hg, together with an overall length of greater than 2 cm, and an abdominal length of greater than 1 cm.[3] Patients with a structurally defective sphincter by a combination of length and pressure were identified if the total length was less than 2 cm, intra-abdominal length was less than 1 cm, and sphincter pressure was less than 6 mm Hg.

24-Hour Esophageal pH Monitoring

A glass pH probe (Ingold, Urdorf, Switzerland) was positioned 5 cm above the upper border of the LES. Patients were instructed to follow their daily routine as closely as possible and to record in a diary all food ingested, symptoms experienced, and time spent sleeping, as has been previously described.[15,16] Although meals were not standardized for volume or quantity, they were standardized in that all patients were given a list of foods with a known pH >5 and asked to restrict their diets to foods from this list. Patients were instructed to eat two to three meals per 24-hour period, not to eat or drink between meals, not to chew gum and to eat all meals within a 10- to 20-minute period. They were instructed not to drink any carbonated beverages, tea, coffee, or alcohol and to drink only water or milk with meals. Medications were discontinued 3 days before testing was begun (2 weeks for omeprazole). Patients not complying with these instructions were excluded from the study. Analysis was done with a commercially available software program (Synectics Inc, Minneapolis, Minn.). A reflux episode was defined as any drop in pH to <4. The percentage of time the esophageal mucosa was exposed to a pH <4 during the total monitoring period, as well as time spent in the upright and supine positions and during the 2-hour postprandial period, was also recorded. A composite pH score was calculated from selected parameters derived from the total reflux period, upright period, and supine period.[15] A positive score was defined as greater than 14.8 (95th percentile of normal).

The postprandial period was defined as the period 2 hours after a meal. The cumulative exposure time in minutes when the pH was <4 was calculated for the 120-minute period following each meal. This cumulative postprandial time was then expressed as a percentage of the total postprandial period during the 24-hour test. All meals were included. Thus if three meals were eaten, the total postprandial time would be 6 hours and the cumulative reflux time would be a fraction of this total period. The total number of reflux episodes (pH <4), the number greater than 5 minutes, the longest reflux episode, and the fraction of time the pH was <4 for the total cumulative postprandial period were calculated.

Endoscopy

Esophageal injury was defined by the presence of erosive esophagitis, stricture, or Barrett's esophagus. Erosive esophagitis was characterized by the presence of linear or coalescent erosions in the esophageal mucosa. Patients with mucosal erythema alone, or with only histologic signs of reflux esophagitis, were not considered to have esophageal injury. Barrett's esophagus was defined by the presence of specialized intestinal metaplasia on any biopsy taken from the esophagus. The following three endoscopic landmarks were recorded in all patients: the position of the crural impression, the gastroesophageal junction, and the squamocolumnar junction. The gastro-

esophageal junction was defined as the level where the gastric rugal folds ended and the tubular esophagus began. A hiatal hernia was diagnosed when there was a difference of 2 cm or more between the position of the crura and the gastroesophageal junction.

Statistics

Data were reported as means ± standard deviation unless otherwise stated. Analysis of variance (ANOVA) was used to compare differences between groups. Fisher's exact test was used to compare proportions. Significance was determined at the 5% level.

RESULTS

Normal Volunteers

In normal volunteers the esophageal pH was <4 for only 2.26% of the postprandial time. This accounted for 34% of the total time the pH was <4 during the 24-hour test in these subjects. The remaining time occurred predominantly in the interprandial period, as supine reflux was uncommon. Details of postprandial reflux exposure in the normal volunteers and their manometric sphincter characteristics are shown in Table I. Values above the ninety-fifth percentile of the normal volunteers were used to identify increased postprandial exposure of acid in the 609 patients with foregut symptoms.

Symptomatic Patients

An increase in the percentage of postprandial reflux time (>8.4%, 95th percentile of normal) was found in 46% of symptomatic patients. The prevalence of abnormal postprandial reflux in patients with a structurally intact sphincter was 32%. The prevalence in patients with a defective sphincter pressure alone was 48%, defective length alone 56%, and in sphincters defective in both length and pressure 60%. The percentage postprandial time the pH was <4 in these groups is shown in Fig. 1. The prevalence of abnormal postprandial reflux in patients without a hernia was 38% (47/125), which was significantly less than the prevalence of 67% (107/159) in the patients with a hiatal hernia ($P < 0.01$).

In an attempt to further understand the pathophysiology of early disease and to improve the sensitivity of 24-hour pH monitoring, the symptomatic patients were divided into those with normal and abnormal esophageal acid exposure as determined by the 24-hour esophageal pH score.

Patients With Increased Esophageal Acid Exposure (DeMeester Score >14.76)

Two hundred seventy-seven patients had an abnormal 24-hour pH score. The prevalence of abnormal postprandial reflux in these patients was significantly higher than the prevalence in patients who had a normal 24-hour pH score (Fig. 2). These patients had shorter intra-abdominal sphincter lengths with lower pressures compared to normal volunteers and patients with a normal 24-hour pH score (Table II). The overall sphincter lengths and pressures in patients with abnormal postprandial reflux were similar to values in patients with normal postprandial acid reflux (Fig. 3). The presence of a hiatal hernia did not increase the prevalence of abnormal postprandial reflux in this group (77% [86/112] vs. 71% [37/52]; $P = 0.278$). The prevalence of abnormal postprandial reflux in patients with esophagitis was 74% (37/50), which was similar to the prevalence of 67% (36/54) in patients with normal postprandial reflux ($P = 0.119$). The prevalence of abnormal postprandial reflux in patients with Barrett's esophagus was 83% (50/60), which was significantly higher than the prevalence of 70% (73/104) in patients without Barrett's esophagus ($P = 0.044$).

Table I. Two-hour postprandial acid exposure and lower esophageal sphincter characteristics in normal subjects (n = 94)

	Mean	SD	Median	95th Percentile
% Time pH <4 in postprandial period	2.26	2.69	1.20	8.43
No. of pH <4 episodes in postprandial period	3.58	3.83	2.0	11.12
Time pH <4 in postprandial period (min)	2.77	3.39	1.55	10.13
No. of episodes >5 min	0.22	0.59	0	2.0
Longest episode (min)	3.06	5.64	1.3	10.5
Total sphincter length (cm)	3.47	0.80	3.6	4.91
Intra-abdominal length (cm)	1.89	0.68	1.9	3.2
Sphincter pressure (mm Hg)	15.02	6.14	13.15	26.2

SD = standard deviation.

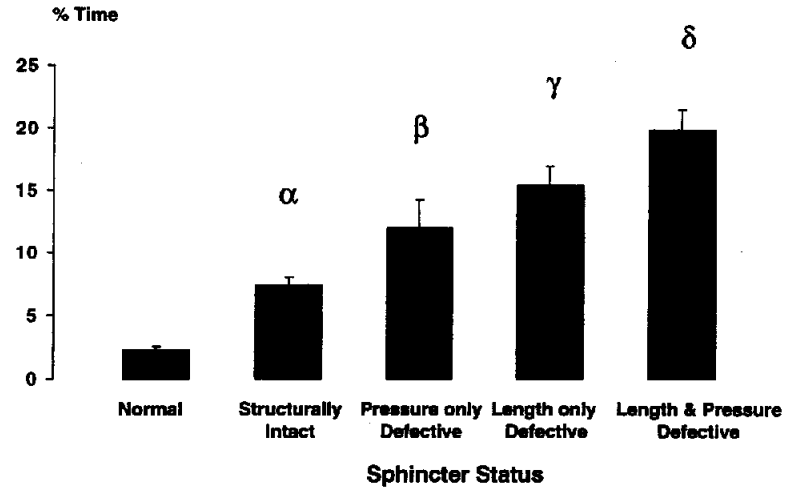

Fig. 1. Percentage postprandial time for normal subjects, patients with a structurally intact sphincter, and patients with a defective sphincter as determined by inadequate sphincter pressure, length, or a combination of inadequate length and pressure ($P < 0.001$ ANOVA; least significant differences: α vs. all other groups, β vs. normal subjects, structurally intact, and length- and pressure-defective groups, γ vs. normal subjects, structurally intact, and length-only and pressure-only groups, δ vs. all other groups).

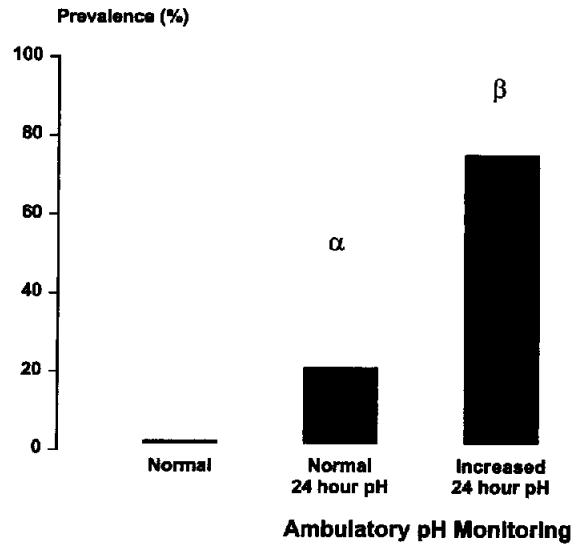

Fig. 2. Prevalence of pathologic postprandial reflux in normal volunteers and symptomatic patients divided into those with normal 24-hour esophageal acid exposure and those with increased 24-hour esophageal acid exposure. Prevalence based on ninety-fifth percentile of the percentage time pH <4 in the postprandial period for normal subjects (8.4%) ($P < 0.001$ ANOVA; least significant differences: α vs. all other groups, β vs. all other groups).

Table II. Postprandial reflux values and manometric sphincter characteristics of the study population

	Normal subjects (n = 94)	Patients with normal 24-hour pH study (n = 331)	Patients with abnormal 24-hour pH study (n = 277)	Significance
% Time pH <4 in postprandial period	2.26 ± 2.69[a]	5.29 ± 8.23[b]	21.26 ± 17.99[c]	All groups significantly different
No. of pH <4 episodes in total postprandial period	7.80 ± 8.56[a]	13.13 ± 18.27[b]	42.12 ± 35.01[c]	a vs. c, b vs. c, c vs. a and b
Total LES length (cm)	3.47 ± 0.80[a]	2.72 ± 1.05[b]	2.18 ± 1.12[c]	All groups significantly different
Intra-abdominal length (cm)	1.89 ± 0.68[a]	1.43 ± 0.87[b]	0.95 ± 0.82[c]	All groups significantly different
Sphincter pressure (mm Hg)	15.02 ± 6.14[a]	13.43 ± 9.07[b]	8.80 ± 7.12[c]	a vs. c, b vs. c, c vs. a and b

LES = lower esophageal sphincter.

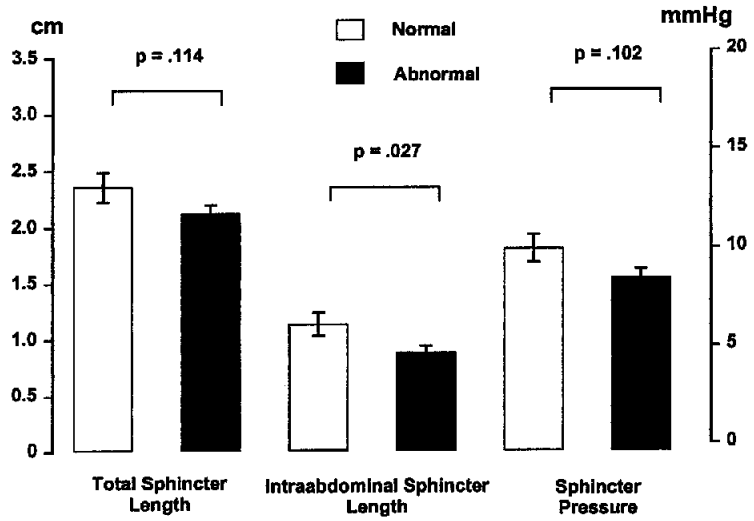

Fig. 3. Lower esophageal sphincter characteristics in patients with an abnormal 24-hour pH study with normal and abnormal postprandial reflux.

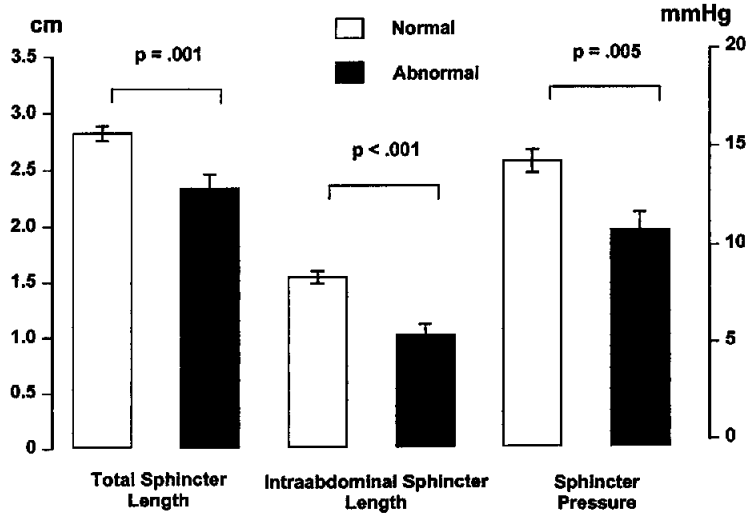

Fig. 4. Lower esophageal sphincter characteristics in patients with a normal 24-hour pH study with normal and abnormal postprandial reflux.

Patients With Normal Esophageal Acid Exposure (DeMeester Score ≤14.76)

Three hundred twenty-one patients had a normal esophageal acid exposure over the 24-hour pH study. Twenty-two percent (70/321) of these patients had an abnormal postprandial esophageal acid exposure, but the time of exposure was not sufficient to make the 24-hour study abnormal. The differences in these patients compared to normal subjects are seen in Table II. Patients with an abnormal postprandial reflux had shorter sphincter lengths and lower sphincter pressures than patients with normal postprandial acid reflux (Fig. 4).

The prevalence of postprandial reflux in the patients with a hiatal hernia was 45%, which was significantly greater ($P < 0.001$) than the prevalence of 14% in the patients without a hernia. To determine the effects of a hernia independent from sphincter integrity, patients with and without a hernia were divided into those with a structurally intact sphincter and those with a structurally defective sphincter (Tables III and IV). A significant increase in postprandial reflux was seen in those patients with a hernia (Fig. 5), irrespective of their sphincter status, demonstrating the independent effect of a hiatal hernia in augmenting postprandial reflux.

Table III. Manometric sphincter characteristics of patients with a normal 24-hour pH score and a structurally intact sphincter, with and without a hiatal hernia

	No hernia (n = 46)	Hernia (n = 12)	Significance
Total length (cm)	3.19 ± 0.57	2.85 ± 0.45	0.054
Intra-abdominal length (cm)	1.89 ± 0.49	1.60 ± 0.34	0.050
Sphincter pressure (mm Hg)	17.31 ± 8.48	12.47 ± 3.82	0.051

Table IV. Manometric sphincter characteristics of patients with a normal 24-hour pH score and a structurally defective sphincter, with and without a hiatal hernia

	No hernia (n = 27)	Hernia (n = 35)	Significance
Total length (cm)	2.25 ± 0.89	2.32 ± 1.05	0.785
Intra-abdominal length (cm)	0.85 ± 0.54	0.65 ± 0.55	0.172
Sphincter pressure (mm Hg)	8.01 ± 4.86	8.88 ± 8.82	0.646

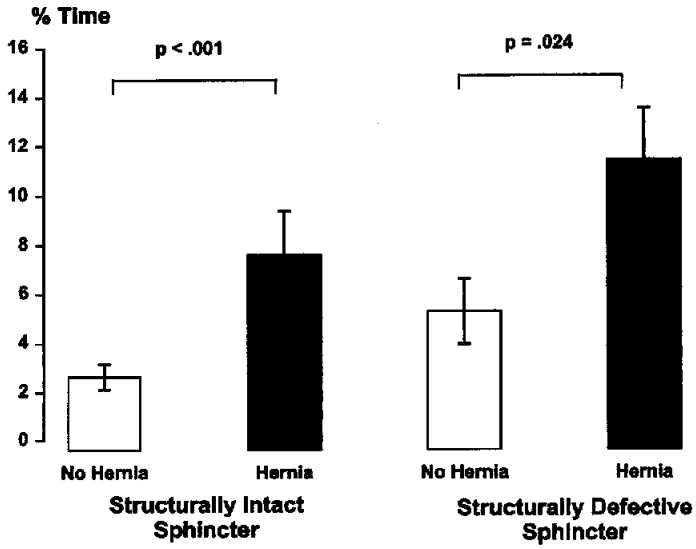

Fig. 5. The effect of a hernia on postprandial reflux time for patients with a normal 24-hour pH study divided into those with a structurally intact LES and those with a structurally defective LES.

The prevalence of postprandial reflux in patients with esophagitis was 58% (7/12), which was significantly higher ($P < 0.001$) than the prevalence of 18% (18/98) in patients without esophagitis. Furthermore, the prevalence of postprandial reflux in the patients with Barrett's esophagus was 60% (6/10), which was significantly higher ($P = 0.018$) than the prevalence of 23% (25/110) in patients without Barrett's esophagus.

DISCUSSION

An important physiologic function of the LES is its ability to vent the stomach of swallowed gas, which has been calculated to be in excess of 3000 ml/day.[17] This venting of air is most common in the postprandial period and is likely to be associated with reflux of gastric contents.[18] The mechanism by which this is achieved is through a progressive unfolding of the LES and mechanical shortening of the sphincter during gastric distention.[19] As structural deficiencies in the sphincter become evident, there is a progressive increase in the percentage of postprandial reflux. Thus postprandial reflux shows a stepwise increase from those patients with only a poor LES pressure, to those patients with only insufficient sphincter length, to those patients demonstrating the greatest amount of postprandial reflux, whose sphincters were defective by a combination of length and pressure.

Structural integrity of the sphincter is therefore not only important in the prevention of positional reflux such as bending, stooping, and lying down, but is also important in the postprandial period. Thus, although a patient may have a structurally normal sphincter, the volume-induced shortening may be excessive and lead to a pathologic increase in reflux only in the postprandial period. The present study demonstrates that this occurs in approximately one third of symptomatic patients with normal LES characteristics. Similarly there is a group of patients (22%) who have normal 24-hour esophageal acid exposure but have abnormal postprandial reflux after meal-induced gastric volume stress. These patients clearly have structurally intact and competent sphincters during most of the interprandial period of the day, but their sphincters become dynamically defective and incompetent in the 2-hour postprandial period.

Occasionally the presence of Barrett's esophagus and esophagitis was seen in patients with negative 24-hour pH studies. In these patients postprandial reflux was significantly greater than normal and serves as a dynamic indicator of volume-induced sphincter incompetency after a meal.

This study also demonstrates the additive influence of a hiatal hernia in volume-induced reflux. It appears that the presence of a hernia results in a more pronounced sphincter unfolding and loss of competency during gastric distention. This was independent of the manometric sphincter characteristics, which have been shown to be inferior in patients with hiatal hernia.[20] Patients with a structurally defective LES and hiatal hernia had significantly greater postprandial reflux than those without a hernia despite similar sphincter characteristics of length and pressure (see Table IV and Fig. 5).

Postprandial reflux is possibly the first step in the genesis of GERD. It has been shown to occur before pathological acid exposure can be demonstrated and before the detection of a structurally defective valve. These results raise the possibility of prevention of volume-induced sphincter shortening in symptomatic patients by means of a valvuloplasty or by dietary manipulation, which involves restricting the meal volume or diet to foods known to empty more rapidly from the stomach.

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Discussion

Dr. M. Patti (San Francisco, Calif.). In patients with postprandial reflux and an incompetent sphincter, what has been your experience with surgery? Should we operate on these patients? What difference do you see in length and pressure between patients who have only upright reflux and those who eventually develop supine reflux?

Dr. R.J. Mason. We have shown experimentally that a Nissen fundoplication maintains sphincter length during gastric distention and that is most probably how it works because it maintains the sphincter length at an adequate level following a meal. If you look at the sphincter length in patients in the present study, although their sphincters were still structurally normal, they were significantly shorter than the sphincters of normal volunteers. A Nissen fundoplication will, in those patients, still prevent the dynamic shortening that occurs with distention after a meal. You can presume that a Nissen fundoplication will be effective in those patients and, as demonstrated in the finding presented earlier, we have a group of patients who we have operated on with structurally normal sphincters.

Dr. T. Gadacz (Augusta, Ga.). Did you look at other factors, such as age, sex, or even volume of meals, that might account for this potential shortening and postprandial reflux?

Dr. Mason. There were no differences relating to age or sex and as far as volume is concerned, we did not control for it, but you raise a good point and we are now controlling for volume in our laboratory. We are trying to control for the meal, size, and volume that patients consume when they go home with the 24-hour pH probe. Also, the amount of air that is swallowed with each meal cannot be controlled, and that will remain a variable factor.

Dr. D. Dempsey (Philadelphia, Pa.). Do you envision finding a subgroup of patients in whom you may be able to avoid 24-hour studies? Perhaps a 2-hour study is sufficient?

Dr. Mason. I think 24-hour studies are still needed. I did not elaborate but there is a significant difference in postprandial reflux between patients with upright reflux and those with supine reflux, which seems to show that those with supine reflux are a different category of patients. I think we will not be able to stop doing 24-hour pH studies just yet because we will not be able to identify that group of patients with supine reflux because supine reflux seems to be different from all other categories and can only be identified on 24-hour pH studies.

Effect of Duodenal Components of the Refluxate on Development of Esophageal Neoplasia in Rats

Yoshinori Yamashita, M.D., Kiichi Homma, M.D., Norio Kako, M.D., Geoffery W.B. Clark, M.D., Thomas C. Smyrk, M.D., Ronald A. Hinder, M.D., Thomas E. Adrian, Ph.D., Tom R. DeMeester, M.D., Sidney S. Mirvish, Ph.D.

When duodenal content is allowed to reflux into the esophagus of nitrosamine-treated rats, esophageal cancer is induced more rapidly and at higher frequency than after carcinogen treatment alone. The purpose of the present study was to identify the components of the duodenal content that are responsible for enhancing esophageal carcinogenesis. Eight-week-old Sprague-Dawley rats underwent one of four operations as follows: diversion of bile alone, pancreatic juice alone, both bile and pancreatic juice into the esophagus, or a control operation with no induced reflux. Two weeks after surgery, rats were treated with the esophageal carcinogen 2,6-dimethylnitrosomorpholine (48 mg/kg [0.1 of LD_{50}] intraperitoneally weekly for 20 weeks). The rats were killed at age 30 weeks. The esophagus was removed and full-length strips were examined under a microscope; separate segments were taken for flow cytometric evaluation. The prevalence of DNA aneuploidy and histologic esophageal papillomas or squamous cancer was increased in carcinogen-treated rats with pancreatic juice reflux ($P < 0.05$ vs. control) and the combination of pancreatic and bile reflux ($P < 0.05$ vs. control) but not in rats with bile reflux alone. We conclude that pancreatic juice is the most potent component of the duodenal refluxate in the promotion of esophageal carcinogenesis in rats. (J GASTROINTEST SURG 1998;2:350-355.)

Acquired columnar-lined lower esophagus, known as Barrett's esophagus, develops as a complication of gastroesophageal reflux. The association between Barrett's esophagus and adenocarcinoma of the esophagus has been convincingly shown. Some recent reports have implicated duodenogastroesophageal reflux as a cause of complications in Barrett's esophagus, including adenocarcinoma.[1-4]

In rats, esophageal papillomas and squamous cell carcinomas can be induced by various nitrosamines. Duodenoesophageal reflux together with gastric juice induces lower esophageal adenocarcinoma in rats treated with 2,6-dimethylnitrosomorpholine (DMNM) or methyl-n-amylnitrosamine (MNAN).[5-7] Duodenal content therefore causes esophageal adenocarcinoma, in contrast to the effect of carcinogen alone, which induces only squamous cell tumors. Duodenal juice contains bile, pancreatic juice, and mucosal secretions. It is not clear which component or combination of these components plays a role in producing Barrett's esophagus, adenocarcinoma, or squamous cell carcinoma. In the present study esophageal neoplasms were induced in a rat esophageal reflux model to investigate the effects of the different components of duodenal juice in the production of esophageal tumors. To simplify the interpretation of results, acid reflux was prevented in all rats.

MATERIAL AND METHODS

Animals and Experimental Groups

One hundred seventy-nine 8-week-old male Sprague-Dawley rats underwent operation. Survivors were kept under standard laboratory conditions and fed a pellet diet and water ad libitum. During DMNM treatment and for 1 week thereafter, rats

From the Departments of Surgery (Y.Y., K.H., N.K., and G.W.B.C.), Pathology (T.C.S.), and Physiology (T.E.A.), Creighton University School of Medicine, Omaha, Neb.; the Department of Surgery, Mayo Clinic (R.A.H.), Jacksonville, Fla.; the Department of Surgery, University of Southern California School of Medicine (T.R.D.), Los Angeles, Calif.; and the Eppley Institute for Research in Cancer (S.S.M.), University of Nebraska Medical Center, Omaha, Neb.
Reprint requests: Ronald A. Hinder, M.D., Department of Surgery, Mayo Clinic, 4500 San Pablo Rd., Jacksonville, FL 32224.

were housed in a laminar flow hood to remove volatile carcinogens. Rats were divided into the following four groups: bile reflux (B, n = 23), pancreatic juice reflux (P, n = 20), both bile and pancreatic juice reflux (B + P, n = 26), and a control group (C, n = 28). Each of the four groups was divided into two subgroups; one was given carcinogen and the other was not.

Surgical Procedures

Operations were carried out under general anesthesia using intramuscular injections of a mixture of xylazine (100 mg/ml) and ketamine (10 mg/ml). An initial dose of 0.4 ml/kg body weight was administered to induce anesthesia and additional doses were given as necessary to maintain adequate anesthesia. In a sterile operation field an upper median laparotomy was made on the shaved abdominal wall. Silk (4-0) and polypropylene (7-0) were used to ligate blood vessels and to complete anastomoses in a single layer. Four types of operation were carried out on randomly selected rats.

Control (Group C) (Fig. 1, *A*). This group had no reflux of duodenal or gastric content into the esophagus. At a point 4 cm distal to the ligament of Treitz, the jejunum was divided. Total gastrectomy was performed, followed by a Roux-en-Y esophagojejunostomy. A jejunojejunostomy was fashioned 25 cm distally from the esophagojejunostomy.

Bile Reflux (Group B) (Fig. 1, *B*). Bile without pancreatic juice was allowed to reflux into the esophagus. The jejunum was divided 4 cm distal to the ligament of Treitz. The esophagus was cut at the esophagogastric junction. The stomach was left intact since pilot experiments had shown a high mortality rate after total gastrectomy in this group. An esophagojejunostomy and jejunojejunostomy were fashioned to create a Roux loop as shown in Fig. 1, *B*, with 25 cm between the two anastomoses. A choledochojejunostomy was fashioned to the tip of the Roux loop over a 22-gauge catheter.

Pancreatic Juice Reflux (Group P) (Fig. 1, *C*). This was designed to produce chronic esophageal reflux of pancreatic juice without bile. After total gastrectomy, an esophagojejunostomy was performed just beyond the ligament of Treitz, and the common bile duct was then anastomosed to the jejunum 25 cm distal to the esophagojejunostomy over a 22-gauge catheter.

Both Bile and Pancreatic Juice Reflux (Group B + P) (Fig. 1, *D*). A total gastrectomy with esophagoduodenostomy to the proximal duodenum was performed just proximal to the entry of the ampulla of Vater.

Carcinogen

2,6-Dimethylnitrosomorpholine was used as the chemical carcinogen to induce carcinoma of the esophagus. It was synthesized by slow addition of HCl to an aqueous solution of 2,6-dimethylmorpholine (Aldrich Chemical Co., Milwaukee, Wis.) and sodium nitrite cooled in ice. The mixture was kept for 1 hour at room temperature and extracted with dichloromethane. The extract was dried over sodium sulfate and the solvent was evaporated to give a mixture of the *syn* and *anti* isomers of almost pure DMNM. This showed an ultraviolet maximum in dichloromethane at 359.5 nm (molar extinction coefficient 107) and a proton nuclear magnetic resonance spectrum in $CDCL_3$ with peaks at 1.25 and 1.36 (CH_3), 2.33 and 3.43 (CH_2N), and 4.65 and 4.94 (CHO) ppm. These properties were not fully described in previous reports.[4,8]

Administration of DMNM commenced 2 weeks after the operation at 10 weeks of age. An intraperi-

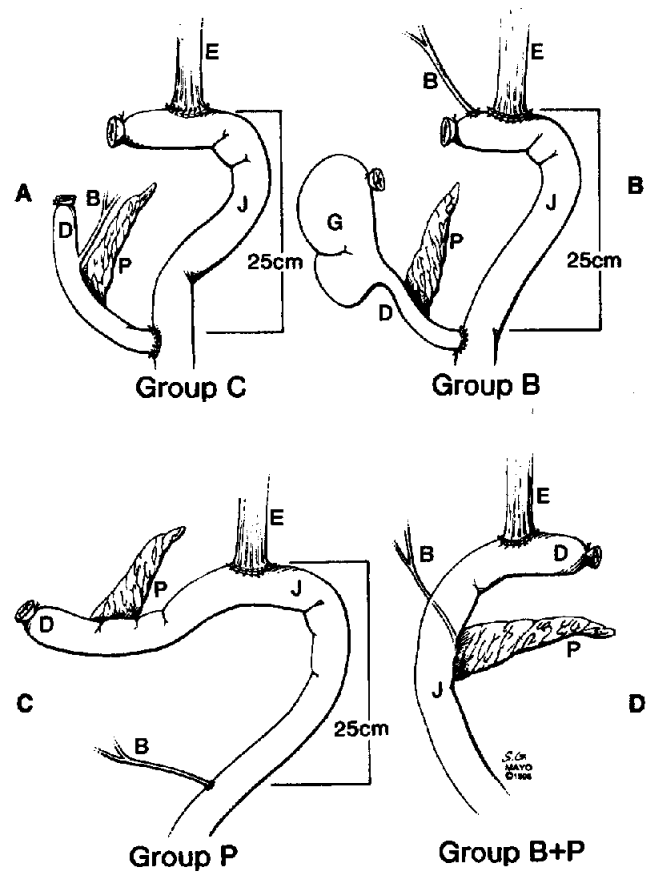

Fig. 1. Operative procedures in the four groups. **A,** Group C (control); **B,** Group B (bile reflux); **C,** Group P (pancreatic juice reflux); **D,** Group B + P (both bile and pancreatic juice reflux). B = choledochus; D = duodenum; E = esophagus; G = stomach; J = jejunum; P = pancreas.

toneal injection of DMNM was given at a dose of 48 mg/kg weekly. Each dose was 10% of the median lethal dose (LD_{50}) (486 mg/kg in male rats; 386 mg/kg in female rats).[9,10] The DMNM was dissolved in 0.5 to 1.0 ml of saline solution without antibiotics. Carcinogen administration was continued weekly for 19 weeks until the rats were 29 weeks old. The total dose of DMNM was 1.88 × LD_{50}.

Evaluation of Specimens

All rats in the carcinogen-treated groups were weighed weekly. Rats that died before 30 weeks of age underwent autopsy. At 30 weeks of age the rats were killed by an overdose of phenobarbital. At the time they were killed, serum gastrin levels of all animals were determined by radioimmunoassay.[11] The esophagus was removed with the proximal esophagus attached to the trachea. The thoracic and abdominal cavities were inspected for tumors. The entire digestive system was inspected and fixed in 10% buffered formalin.

The esophagus was opened longitudinally to inspect the lumen and macroscopic characteristics of lesions in the esophagus were recorded. The esophagus was laid in a single cassette in a "Swiss-roll" fashion so that its full length could be examined en bloc under the microscope; specimens were stained with hematoxylin and eosin. Squamous cell carcinomas were diagnosed as showing penetration beyond the muscularis mucosae to distinguish squamous cell carcinoma from dysplasia. In selected samples, mucicarmine, high-iron diamine for sulfated mucins, and Alcian blue at pH 2.5 were used to identify acid mucin. The tissues were examined by a histopathologist (T.C.S.) who was unaware of the treatment of individual rats. The cellular DNA content of paraffin-embedded histologic material from animals given carcinogen was analyzed. After 50 μ sections were dewaxed with xylene, the samples were prepared for flow cytometry using a Coulter EPICS 541 flow cytometer (Coulter Corp., Miami, Fla.) and the DNA ploidy status was ascertained.[12]

Statistical Analysis

Fisher's exact test was used to determine tumor incidence and aneuploidy rate. Student's *t* test was used for rat weight and gastrin levels. Significance was taken at $P < 0.05$.

RESULTS

Of the 179 original rats in all groups, 25 died of apparent carcinogen toxicity and/or malnutrition and 21 died within 72 hours of the operation. Other cause of death were ileus (n = 11), failed operation (n = 9 peritonitis (n = 7), liver abscess (n = 4), and unknow (n = 5). Only the 97 rats surviving for 30 weeks wer used to constitute the study groups. The rats in a DMNM-treated groups retained their body weigh during the study with no significant changes betwee the starting and ending weights.

Table I. Presence of benign diffuse papillomatosis

Reflux model	Carcinogen	
	Absent	Present
Group C	9/16 (56%)	6/12 (50%)
Group B	4/10 (40%)	5/13 (38%)
Group P	8/10 (80%)	9/10 (90%)*
Group B + P	8/11 (73%)	14/15 (93%)*

*$P < 0.05$ vs. groups B and C.

Gastrin Levels

There was no significant difference in serum gas trin levels among the four groups, even though th distal stomach was not removed in group B. Gastri levels were significantly reduced in the DMNM treated groups compared to those not given DMNM except for group P.

Frequency and Type of Tumor

Benign diffuse papillomatosis was observed in th lower two thirds of the esophagus in 38% to 93% c the rats (Table I). In the DMNM-free groups, this le sion was seen more in groups P and B + P than in th other groups, but this difference was not significan For the DMNM-treated groups, benign diffuse pap illomatosis production in groups P and B + P was sig nificantly higher than in groups B and C.

Squamous cell carcinoma only occurred in th DMNM-treated groups (Table II). No Barrett esophagus or adenocarcinoma was observed in any c the rats. Groups P and B + P had an incidence c 40% for squamous cell carcinoma, which was signifi cantly higher than in control group C in which n carcinomas were seen ($P < 0.05$, see Table II). Fig. shows a representative squamous cell carcinoma. I group B only one rat developed a squamous cell car cinoma (8%). Dysplasia was seen in 31% to 70% c rats treated with DMNM. No carcinomas were foun in the other digestive organs in any of the groups. A high rate of aneuploidy was seen in groups P and B + P (22% and 27%, respectively) (see Table II). Non of the rats in groups B and C had aneuploidy.

Table II. Production rates for squamous cell carcinoma, dysplasia, and aneuploidy in carcinogen-treated rats

Reflux model	Squamous cell carcinoma	Dysplasia	Aneuploidy
Group C	0/12 (0%)	4/12 (33%)	0/10 (0%)
Group B	1/13 (8%)	4/13 (31%)	0/10 (0%)
Group P	4/10 (40%)*	7/10 (70%)	2/9 (22%)
Group B + P	6/15 (40%)*	7/15 (47%)	4/15 (27%)

*P <0.05 vs. control (group C).

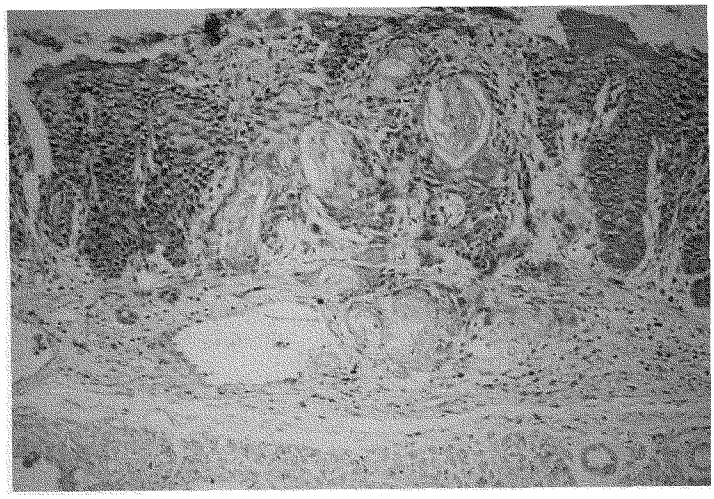

Fig. 2. Squamous cell carcinoma of the esophagus in a rat from group B + P. There is distinct invasion of cancer cells, which penetrate the muscularis mucosae into the submucosa.

DISCUSSION

In this study it was found that with the esophageal reflux of pancreatic juice alone, or together with bile, there was promotion of the appearance of esophageal squamous cell carcinoma and esophageal papillomas but reflux of bile alone did not have this effect. In humans the reflux of duodenal content into the esophagus may be partly responsible for reflux esophagitis. This view is supported by clinical evidence that inhibition of acid and pepsin production by drugs such as H_2 blockers do not consistently cure reflux esophagitis. Bile is present in greater concentration in the stomach and esophagus of patients with severe esophagitis or Barrett's esophagus.[1-3] There is a high incidence of gastric and esophageal cancer in patients after gastrectomy, in which free reflux of duodenal content into the stomach and esophagus occurs.[13,14] In animal studies it has been reported that both bile and pancreatic juice can cause esophageal mucosal damage, whereas gastric juice has less of an effect.[15-18] In our study pancreatic juice reflux produced more benign diffuse papillomatosis and squamous cell carcinoma in carcinogen-treated rats than did bile reflux alone. Kranendonk[15] also found that reflux of pancreatic juice, alone or in combination with bile or gastric juice, produced lesions in the esophagus of rats.

To shorten the tumor induction time, DMNM was used in our study. There are reports of the induction of esophageal squamous cell carcinoma and adenocarcinoma in the rat duodenogastric reflux model with gastric acid present even without carcinogen administration,[19] indicating that duodenal juice can be an initiator as well as a promoter of esophageal cancer. In our study pancreatic juice was a promoter but bile was not. In a previous study we showed that gastric juice reflux-induced by esophagogastroplasty did not produce adenocarcinoma in DMNM-treated rats.[5] Seto et al.[20] reported that total gastrectomy with alkaline reflux and MNAN treatment produced only squamous cell carcinomas but not adenocarcinoma of the esophagus in rats, which is consistent with our results. Hence gastric juice without the influence of duodenal juice induces injury to the esophageal mucosa rather than producing malignancy.[18] We postu-

late that initial or simultaneous exposure to gastric acid is required to produce esophageal adenocarcinoma.

While these experiments were in progress, Pera et al.[21] reported similar experiments in which they also treated rats with DMNM after performing various diversion operations. In their tests both squamous cell carcinomas and adenocarcinomas of the esophagus were induced. Groups with pancreatic, biliary, and pancreatic plus biliary reflux showed esophageal cancer incidences of 14%, 0%, and 33%, respectively. Hence pancreatic reflux appeared more important than bile reflux in inducing esophageal cancer. Our results confirm these findings using somewhat different surgical procedures and different doses of DMNM.

We detected esophageal squamous cell carcinomas but no esophageal adenocarcinomas in any of the rats, even though Pera et al.[6] and our group previously observed esophageal adenocarcinomas in rats with duodenoesophagostomies that were treated with DMNM. The reason for this difference is unclear. The DMNM dose is probably not responsible for the difference since it was the same as that used previously by us but was 10 times the dose used by Pera et al.[21]

We found no carcinomas in group C treated with DMNM alone. In previous reports approximately 20% to 40% of rats receiving the same dose of DMNM would have developed squamous cell carcinoma of the esophagus in the absence of a co-carcinogen. This difference may be due to our strict definition of tissue invasion for the diagnosis of carcinoma. The transition from dysplasia to squamous cell carcinoma is complicated.[22]

The stomach was preserved intact in the bile reflux group because of the surgical stress of total gastrectomy in this group but was removed in the other groups. Despite this difference the serum gastrin level in the bile reflux group was no different than that in the other groups, suggesting that the presence of the isolated stomach did not significantly alter the results in this group.

Pancreatic juice contains trypsin, chymotrypsin, phospholipase A_2, and other digestive enzymes. The proportion of the components of these enzymes in pancreatic juice is not constant. If it were possible to find which ingredient or combination of ingredients plays a role in esophageal carcinogenesis, more specific experiments could be designed to define their role. Antagonists of pancreatic secretions such as the synthetic protease inhibitor camostat mesylate are possibilities for the future.[23,24]

We conclude that pancreatic juice may play a significant role as a co-carcinogen with DMNM for the induction of squamous cell carcinoma of the esophagus in rats. Our studies suggest that esophagitis induced by gastric juice might be simultaneously required to produce Barrett's esophagus or adenocarcinoma, since these lesions were not seen by us when acid reflux was excluded. It remains to be determined whether this result is due to direct genotoxity or to chronic inflammation with damaged mitotic activity during repair.

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Ethanol Inhibits Sphincter of Oddi Motility

Sean Tierney, F.R.C.S.I., Zhiping Qian, M.D., Pamela A. Lipsett, M.D., Henry A. Pitt, M.D., Keith D. Lillemoe, M.D.

Patients with alcohol-induced liver disease are at increased risk for pigment gallstones, which are known to be particularly associated with biliary stasis. Although the effects of ethanol on the sphincter of Oddi are thought to contribute to alcoholic pancreatitis, the precise effects of ethanol on the biliary component of the sphincter of Oddi are unclear. In the prairie dog the common bile and pancreatic ducts enter the duodenum separately, facilitating pressure measurement in the *sphincter choledochus* in isolation. We therefore used this model to test the hypothesis that ethanol administration alters sphincter of Oddi motility. Twenty-six male prairie dogs fed a nonlithogenic diet were studied. With the animals under α-chloralose anesthesia, a side-hole pressure-monitored perfusion catheter was positioned in the sphincter of Oddi and femoral arterial and venous catheters were placed. Sphincter of Oddi phasic wave frequency (F), amplitude (A), and motility index (MI = F × A) and arterial blood pressure were monitored at 10-minute intervals before (baseline), during 20-minute intravenous infusions of 15 mg/kg (n = 9), 150 mg/kg (n = 10), and 1.5 g/kg (n = 7) ethanol and for 20 minutes after ethanol infusion. The 15 mg/kg dose of ethanol had no effect, the 150 mg/kg dose tended to reduce sphincter of Oddi motility, and significant reductions in sphincter of Oddi amplitude and motility index were seen at the 1.5 g/kg dose. These data demonstrate that ethanol infusion inhibits both sphincter of Oddi amplitude and motility index and that this effect persists for at least 20 minutes following ethanol infusion. Ethanol may contribute to gallstone formation by altering biliary sphincter motility. (J GASTROINTEST SURG 1998;2:356-362.)

Chronic alcohol abuse has long been known to be associated with an increased risk of acute and chronic pancreatitis and chronic liver disease. The direct effects of ethanol on sphincter of Oddi motility, as demonstrated in animal models[1-3] and in humans,[4-6] have long been implicated as one of the factors contributing to the etiology of alcohol-induced pancreatitis. However, there is considerable conflict among these reports as to the precise nature of the effects of alcohol on the sphincter of Oddi.

Epidemiologic studies have shown that the risk of developing cholesterol gallstones is reduced in chronic abusers of alcohol. However, these patients are at increased risk of developing pigment stones.[7,8] The association between chronic alcohol abuse and pigment stones[9] may be mediated, in part, by the effects of alcohol on biliary motility and, in particular, on the biliary component of the sphincter of Oddi. Specifically biliary stasis, a key factor in the formation of pigment stones,[10] may occur as a result of altered sphincter of Oddi motility.

Boyden,[11] in a seminal work on the anatomy of the sphincter of Oddi in humans, described the sphincter as having three components: the *sphincter choledochus*, the *sphincter pancreaticus*, and the *sphincter ampullae*. Some studies have suggested that the pancreatic and biliary components of the sphincter of Oddi may respond differently to ethanol.[12] The prairie dog has been widely used as a model for many aspects of human gallstone formation. The extrahepatic biliary anatomy is very similar to that of humans with the exception that the common bile and pancreatic ducts enter the duodenum separately.[13] Thus it is an ideal model in which to test the effects of alcohol on biliary motility in isolation. The present study was designed to test the hypothesis that ethanol promotes biliary stasis through alterations in motility of the biliary component of the sphincter of Oddi.

METHODS

Twenty-six adult male prairie dogs *(Cynomys ludovicianus)*, obtained from Otto Martin Locke (New Braunfels, Tex.), were used in this investigation. The animals were caged in thermoregulated rooms (23° C) with physiologic sleep/wake cycles and were main-

From the Department of Surgery, The Johns Hopkins Medical Institutions, Baltimore, Md.
Supported by National Institutes of Health grants R29-DK41889 (K.D.L.) and R01-DK44279 (H.A.P.).
Reprint requests: Keith D. Lillemoe, M.D., Blalock 603, 600 North Wolfe St., Baltimore MD 21287-4603.

tained on a trace cholesterol, nonlithogenic diet (Purina Laboratory Chow, Ralston-Purina Co., St. Louis, Mo.) for at least 1 month prior to study.

Immediately prior to the experiments, each animal was fasted for 16 hours but allowed water ad libitum. Anesthesia was induced with ketamine (100 mg/kg intramuscularly), and a 24-gauge cannula was inserted into the femoral vein. Anesthesia was maintained thereafter with intermittent intravenous infusion of α-chloralose (2 mg/kg/hr) alternated with lactated Ringer's solution infused at a rate of 0.2 ml/min. A 24-gauge femoral arterial catheter was also inserted to monitor systemic blood pressure during the experiment. Body temperature was maintained between 36.5° and 37.5° C with a warming pad.

The extrahepatic biliary tree, duodenum, and stomach were exposed through an upper midline abdominal incision, and the gallbladder was aspirated with a 19-gauge needle. A 20 cm long polyethylene catheter (0.50 mm inside diameter) was inserted into the gallbladder through the aspiration hole and secured with a 3/0 silk tie. A similar 25 cm long polyethylene catheter was introduced into the common bile duct through a proximal choledochotomy and secured with a 3/0 silk tie to drain gallbladder perfusate and hepatic bile. A second choledochotomy was made more distally, and a 25 cm custom-made triple-lumen perfusion catheter (Arndorfer Medical Specialties, Greendale, Wis.) was inserted through the distal common bile duct into the duodenum.

The side-hole pressure-monitored perfusion catheter measured 1 mm in outer diameter at the tip. The proximal and distal ports were separated by 4 mm and were located at 5 and 1 mm, respectively, from the closed end of the catheter. Using a pull-back technique, the proximal port was positioned in the sphincter of Oddi. The sphincter is identified as a high-pressure zone exhibiting spontaneous phasic wave activity as previously described.[14] The distal port remained in the duodenum, which has a baseline pressure of approximately 0 mm Hg. In addition, an 8 F drainage catheter was placed in the proximal duodenum via a gastrostomy to decompress the stomach and duodenum. Each of the lumens of the triple-lumen catheter and the gallbladder catheter were perfused with degassed water at 0.15 ml/min using a microcapillary infusion system (J.S. Biomedicals, Ventura, Calif.). Pressure changes within the perfusion system were recorded on a Dynograph R611 recorder (Sensormedics Corp., Anaheim, Calif.) using P-23 pressure transducers (Gould, Oxnard, Calif.) to detect changes in pressure in the system.

Gallbladder pressure, systemic blood pressure, and sphincter of Oddi phasic wave amplitude and frequency were monitored continuously during the experiment. A motility index (MI) was calculated for the sphincter of Oddi as the product of the mean amplitude and frequency over each 10-minute period. After 1 hour of stabilization, baseline sphincter of Oddi activity and gallbladder pressures were recorded over a 10-minute period. A single dose of ethanol—15 mg/kg (n = 9), 150 mg/kg (n = 10), or 1.5 g/kg (n = 7) chosen at random—was administered to each animal. Sphincter of Oddi and gallbladder activity during infusion and during the 20 minutes following infusion were compared to baseline (preinfusion) values using the Wilcoxon signed-rank test. Analysis of variance and Fisher's least significant difference were used to compare the effects of the different doses. Both the raw data and the values normalized to the baseline period were evaluated. Results are expressed as mean ± standard error of the mean.

RESULTS

The effects of ethanol infusion on sphincter motility, gallbladder pressure, and mean arterial blood pressure are illustrated in Table I. Compared to baseline values, the amplitude of sphincter of Oddi phasic wave contractions was significantly reduced during the 20 minutes of ethanol infusion at the 1.5 g/kg dose and remained significantly below baseline during the subsequent 20 minutes. No significant effect on amplitude was seen at either of the lower doses. The reduction in sphincter of Oddi amplitude normalized to baseline in response to 1.5 g/kg was statistically significant compared to 15 mg/kg at each of the time points studied (Fig. 1).

Normalized sphincter of Oddi phasic wave frequency was significantly reduced during the first 10-minute period of infusion of the 1.5 g/kg dose and during the first 10 minutes of the recovery period (Fig. 2). Frequency was also reduced during the second 10 minutes of infusion of the 15 mg/kg dose and during the second 10 minutes of infusion of the 150 mg/kg dose. This reduction in frequency was maintained during the recovery period following the 150 mg/kg dose. Although ethanol tended to reduce sphincter of Oddi frequency, there was no consistent dose-effect response and there were no statistically significant differences between the groups.

The lowest dose (15 mg/kg) had no significant effect on overall sphincter of Oddi motility as determined by the motility index (Fig. 3). The 150 mg/kg dose tended to reduce the motility index, but this effect was statistically significant only during the first 10 minutes of ethanol infusion. The 1.5 g/kg dose caused an immediate and sustained reduction in the motility index, which persisted during the entire period of infusion and the recovery period. This reduc-

Table I. Effects of ethanol infusion on sphincter of Oddi frequency, amplitude and motility index, gallbladder pressure, and mean arterial pressure

Dose	Amplitude (mm Hg)	Frequency (No./10 min)	Motility index	Gallbladder pressure (mm Hg)	Mean arterial blood pressure (mm Hg)
Ethanol (15 mg/kg)					
Baseline	7.7 ± 1.2	66 ± 13	500 ± 14	13 ± 3	90 ± 14
Infusion					
0-10 min	7.9 ± 0.9	57 ± 11	455 ± 90	12 ± 3	96 ± 16
10-20 min	8.5 ± 1.1	54 ± 7*	451 ± 66	11 ± 2	96 ± 16
Postinfusion					
0-10 min	8.4 ± 0.9	57 ± 10	471 ± 84	11 ± 2	94 ± 14
10-20 min	8.3 ± 0.9	63 ± 13	521 ± 114	12 ± 3	94 ± 14
Ethanol (150 mg/kg)					
Baseline	9.2 ± 1.3	48 ± 3	458 ± 85	16 ± 2	78 ± 17
Infusion					
0-10 min	7.9 ± 1.1*	46 ± 3	377 ± 67*	17 ± 2	77 ± 17
10-20 min	7.9 ± 1.5	43 ± 4*	352 ± 80	16 ± 2	53 ± 18
Postinfusion					
0-10 min	8.3 ± 1.5	41 ± 3	330 ± 54*	16 ± 2	54 ± 17
10-20 min	8.4 ± 1.4	41 ± 3*	353 ± 64	16 ± 2	54 ± 17
Ethanol (1.5 g/kg)					
Baseline	12.5 ± 2.5	48 ± 3	570 ± 88	15 ± 2	123 ± 14
Infusion					
0-10 min	10.1 ± 2.7*	42 ± 3*	410 ± 92*	16 ± 2	123 ± 12
10-20 min	9.7 ± 2.2*	43 ± 4	399 ± 68*	16 ± 2	90 ± 24
Postinfusion					
0-10 min	8.3 ± 2.1*	38 ± 5	302 ± 62*	15 ± 2	91 ± 24
10-20 min	9.8 ± 2.9	41 ± 5	384 ± 94*	15 ± 2	91 ± 24

*P <0.05 vs. baseline (Wilcoxon).

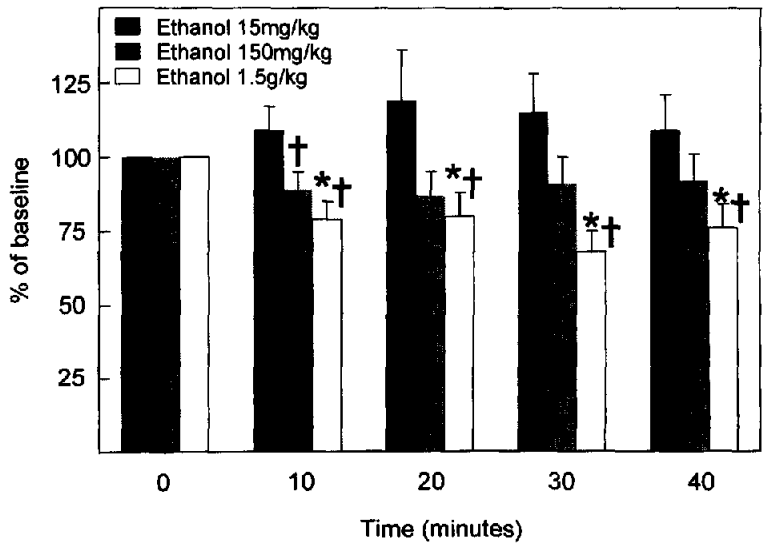

Fig. 1. Percentage change from baseline in sphincter of Oddi phasic wave amplitude in response to infusion of each dose of ethanol (*P <0.05 vs. own baseline; †<0.05 vs. 15 mg/kg dose).

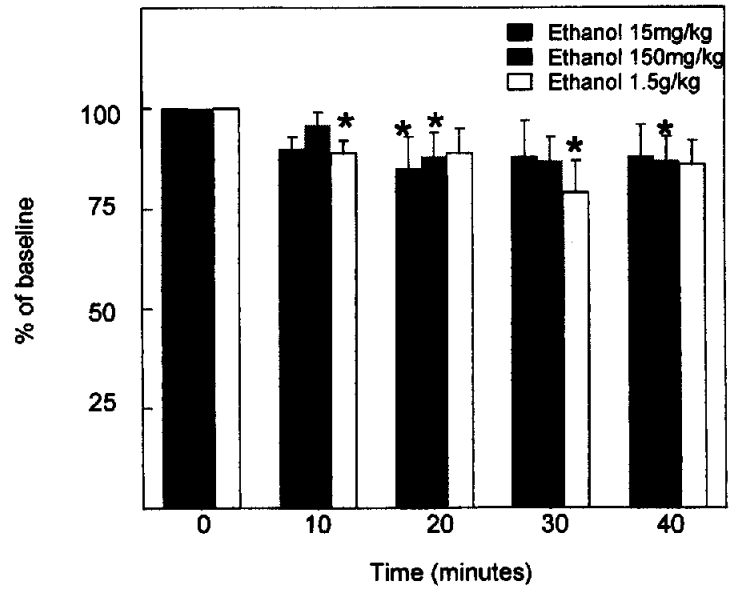

Fig. 2. Percentage change from baseline in sphincter of Oddi phasic wave frequency in response to infusion of each dose of ethanol (*P <0.05 vs. own baseline).

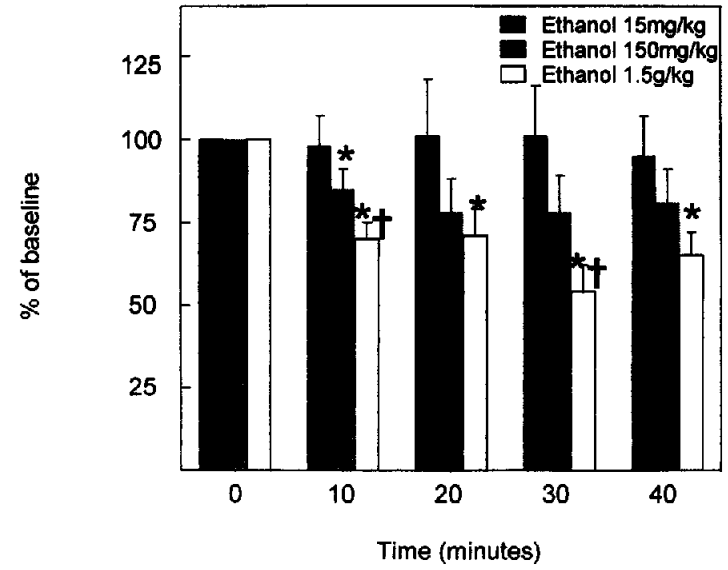

Fig. 3. Percentage change from baseline in sphincter of Oddi phasic wave motility index in response to infusion of each dose of ethanol (*P <0.05 vs. own baseline; †<0.05 vs. 15 mg/kg dose).

tion was also significant when compared to the effects of the 15 mg/kg dose using analysis of variance.

There was no significant change in mean gallbladder pressure during or following infusion of any of the doses of ethanol used in this experiment. Similarly, although ethanol at the 1.5 g/kg dose tended to reduce the mean arterial blood pressure, this effect did not reach statistical significance.

DISCUSSION

This experiment demonstrates that ethanol inhibits biliary sphincter of Oddi motility in a dose-dependent manner. The effects on motility are mediated primarily through a reduction in sphincter phasic wave amplitude, although the frequency of phasic wave contractions was also reduced. Ethanol, at the doses used in this experiment, had no significant effect on the gallbladder or mean arterial blood pressure. In the prairie dog,[15,16] as in the American opossum[17] and the rabbit, sphincter of Oddi phasic wave amplitude and frequency increase in response to cholecystokinin infusion. This phenomenon is thought to represent active propulsion of bile from the common bile duct into the duodenum rather than passive flow through a relaxed sphincter such as occurs in humans. Thus the reduction in sphincter motility by ethanol seen in the present study suggests that ethanol reduces bile flow through the sphincter of Oddi.

The sphincter of Oddi plays a key role in determining bile flow and partitioning between the gallbladder and the duodenum, and thus in determining gallbladder filling and emptying.[18,19] A reduction in activity, as observed in this study, is likely to increase partitioning of bile into the gallbladder and result in biliary stasis. The absence of a significant effect on systemic blood pressure indicates that the effects of ethanol on sphincter motility are not simply due to systemic effects and, similarly, the absence of any alteration in gallbladder pressure indicates that the changes in sphincter of Oddi motility are not secondary to nonspecific effects of ethanol on gastrointestinal smooth muscle.

The effect of alcohol in promoting biliary stasis may be an important factor in explaining the increased risk of pigment stones among chronic alcohol abusers. Epidemiologic studies suggest that alcohol consumption reduces the risk of cholesterol gallstone disease,[20-22] but the overall incidence of biliary stone disease is increased by heavy alcohol consumption because of a much greater prevalence of pigment stones.[7,8] Studies in humans have shown that moderate alcohol intake in humans reduces biliary cholesterol and elevates serum high-density lipoprotein cholesterol.[23] A similar effect of ethanol on biliary cholesterol saturation has been demonstrated in the prairie dog,[24] and ethanol has been shown in this model to protect against cholesterol gallstone formation.[25] This effect may be mediated by a reduction in biliary cholesterol saturation.

Pigment stones are comprised primarily of calcium bilirubinate with lesser amounts of the carbonate, phosphate, and palmitate salts of calcium.[26,27] Biliary stasis produced by ethanol may promote the formation of pigment stones through several pathways. Stasis favors bacterial overgrowth and the β-glucuronidase released by these organisms deconjugates bilirubin to a less soluble form, which will precipitate readily with calcium.[28] Unconjugated bilirubin and its insoluble salts act on the gallbladder mucosa to enhance mucin production.[29] Mucin, in turn, acts as a buffer preventing mucosal acidification of bile in the lumen of the gallbladder. As biliary pH rises, bile becomes supersaturated with calcium carbonate and phosphate, which also precipitates.[28] This insoluble material accumulates as gallbladder sludge[30] which is thought to be a precursor of pigment stones. Biliary stasis prevents adequate clearance of this sludge by the gallbladder, further favoring the formation of pigment stones.[31]

Several previous studies in animal models have indirectly measured the effects of alcohol on biliary motility. Menguy et al.[32] found that ethanol promoted biliary stasis in conscious dogs. Using indwelling catheters, they found that intraduodenal administration of ethanol resulted in increased pressure and increased resistance to flow in both the pancreatic and common bile ducts. Intragastric administration of ethanol in a feline model has also been shown to produce a significant increase in pancreatic ductal pressure, which can be prevented by bypassing the sphincter of Oddi.[1] Becker and Sharp[2] have shown that electrical activity in the sphincter of Oddi in the opposum is increased following ethanol administration and Coelho et al.,[3] in the same model, have demonstrated that although mean pancreatic ductal pressures were not increased, an increase in the frequency of pressure variations was observed.

The dosage of ethanol was chosen so that, on a dose per kilogram basis, the highest dose used would be equivalent to human "physiologic" doses. The amount of alcohol given at the highest dose (1.5 g/kg) corresponds to the total amount of alcohol in eight standard bottles of beer (4% by volume) when consumed by a 70 kg man. Although the effects of this quantity of alcohol in humans are well known, prairie dogs have been reported to tolerate considerably higher doses without ill effect.[25] The dosages used were based on previous work in the same species[25] and therefore serum levels were not measured.

Several studies have investigated the effects of alcohol on sphincter of Oddi motility in humans. A single study in patients with T-tubes in situ after common duct exploration found that intravenous alcohol resulted in a rise in ductal pressure.[6] However, studies that have directly measured changes in sphincter of Oddi pressure in response to ethanol have yielded conflicting results, with some reporting a reduction[17] and others an increase[5] in sphincter of Oddi pressure and phasic wave amplitude.

The conflicts between studies in animal models and human subjects may be partly explained by differences in methodology including the dosage of ethanol used, route of administration, and individual and species variation, and the differential response of the pancreatic and common bile duct components of the sphincter of Oddi. Raddawi et al.[12] have expanded on this final point by demonstrating that alcohol has distinctly different effects on the two components of the sphincter of Oddi. The prairie dog model used in this study is particularly suitable for the investigation of the effects of alcohol on the biliary component of the sphincter of Oddi in isolation because the common bile and pancreatic ducts enter the duodenum separately,[13] thus avoiding any influence of the pancreatic sphincter on the results. Although this anatomic arrangement would also facilitate direct measurement of pancreatic sphincter pressure, neither our group nor others that we know of have yet been successful in cannulating the pancreatic duct in the prairie dog. This was not the objective of the present study but we hope to rise to meet this challenge in future studies.

In summary, intravenous ethanol inhibits biliary sphincter of Oddi motility in the prairie dog in a dose-dependent manner. This effect is primarily due to a reduction in sphincter phasic wave amplitude. Based on our understanding of sphincter of Oddi function in this species, this reduction in motility is likely to result in a reduction in bile flow, thereby promoting biliary stasis. Similar effects of ethanol, if confirmed in humans, may contribute to the greater prevalence of pigment gallstones among patients with chronic liver disease as a consequence of chronic alcohol abuse.

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Role of Extrinsic Innervation in Release of Motilin and Patterns of Upper Gut Canine Motility

Mohammad Siadati, M.D., Michael G. Sarr, M.D.

The need for extrinsic neural input to the upper gut in regulation/control of cyclic interdigestive motility and release of motilin remains a topic of controversy. Our aim was to determine whether extrinsic denervation of the upper gut disrupts cyclic release of motilin in relation to the migrating motor complex. Ten dogs underwent transection of all extrinsic innervation and enteric neural input to the stomach, small intestine, colon, pancreas, and liver while enteric neural continuity within this multivisceral complex was maintained. A cyclic pattern of motility occurred during fasting in all dogs in the small bowel (period = 100 ± 3 min, mean ± standard error of the mean) and in 8 of 10 dogs in the stomach (period = 98 ± 4 min). Gastric cycles were temporally coordinated with small bowel cycles. Plasma motilin concentrations cycled temporally with the motility pattern with the greatest concentrations occurring during gastroduodenal phase III–like activity. Exogenous motilin induced a burst of gastric contractions and a premature migrating motor complex in all dogs. Oral meals disrupted cyclic motility and cyclic changes in plasma motilin. Extrinsic innervation to the upper gut is not necessary for cyclic motor activity, for coordinated cyclic release of motilin, or to initiate a premature migrating motor complex–like response to motilin. Central nervous system input (afferent, efferent) is not necessary for cyclic interdigestive activity or cyclic release of motilin. (J GASTROINTEST SURG 1998;2:363-372.)

During the interdigestive (fasting) period, the upper gut of humans and most nonruminant mammals undergoes a characteristic cyclic pattern of motor activity termed the migrating motor complex (MMC).[1,2] The MMC consists of four phases,[3] the most characteristic of which is phase III or the activity front, which is a burst of high-amplitude propulsive contractions. This band of contractile activity begins in the lower esophageal sphincter and stomach, then appears in the duodenum, and migrates down the length of the small intestine in a sequential orderly fashion propelling intraluminal debris and nondigestible content distally. As the phase III reaches the distal ileum, another phase III of the next cycle begins in the lower esophageal sphincter and stomach; this cycle repeats monotonously during fasting. Feeding disrupts the MMC and induces a noncyclic postprandial "fed" pattern of intermittent contractions that persist for a variable duration that is dependent on the characteristics and caloric content of the meal.[4]

The mechanisms controlling these global motor patterns remain incompletely understood. Although migration of the MMC along the gut is controlled by continuity of the enteric nervous system,[5] the factor(s) that initiate the onset of phase III activity in the stomach and duodenum is (are) not fully understood. Considerable experimental evidence supports both neural and hormonal modulatory roles in both the initiation and inhibition of these motor patterns.[1,6,7]

Our previous work after complete neural isolation of the jejunoileum[8,9] and after neural isolation of the stomach alone[10] showed the persistence of a cyclic motility pattern during fasting that was disrupted by oral meals. Similarly, previous observations in a model of in situ neural isolation of the upper gut suggested preservation of a cyclic motor pattern.[11] Because the

From the Department of Surgery and Gastroenterology Research Unit, Mayo Clinic and Mayo Foundation, Rochester, Minn.
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Correspondence: Michael G. Sarr, M.D., Professor of Surgery, Gastroenterology Research Unit, Mayo Clinic, 200 First Street SW, Rochester, MN 55905.

duodenum is the major source of motilin synthesis and release,[12] and motilin appears to be one important putative modulatory factor in the initiation of the MMC,[13-15] especially in the stomach,[10,16] study of global patterns of upper gut motility and release of motilin after extrinsic denervation of the duodenum is of considerable physiologic interest.

Our hypotheses were that plasma concentrations of motilin are involved in initiating cyclic patterns of motility in the upper gut during fasting and that extrinsic innervation to the stomach and duodenum is not necessary either for the cyclic release of motilin or for the occurrence of cyclic motility patterns. Therefore we developed a novel canine preparation by neurally isolating in situ the upper gut multivisceral complex consisting of the entire stomach, small bowel, proximal colon, liver, and pancreas acutely and permanently from all central neural input while maintaining full enteric neural continuity between these upper gut organs. The aims of this study were to determine the mechanisms by which extrinsic neural innervation of this upper gut complex (1) controls global interdigestive and postprandial motor patterns and (2) regulates plasma motilin concentrations and their temporal relationship to interdigestive patterns of motility.

METHODS

Preparation of Dogs

Surgical procedures and subsequent care and conduct of experiments were performed after approval from and according to criteria set forth by the Animal Care and Use Committee of the Mayo Foundation in accordance with the guidelines of the National Institutes of Health and the United States Public Health Service policy on the humane use and care of laboratory animals.

Ten healthy female mongrel dogs weighing 16 to 23 kg were anesthetized with intravenous methohexital (12.5 mg/kg) and maintained with inhaled halothane. Using a two-staged procedure described previously,[11] these dogs underwent in situ neural isolation of the stomach, small bowel, proximal colon, liver, and pancreas (Fig. 1). The *only* tissue continuity to this upper gut complex was through the wall of the celiac and superior mesenteric arteries and the supra- and infrahepatic vena cava. We did not transect and reanastomose these vessels, completing an *auto*transplantation (and thereby assuring a total in situ neural isolation), because we did not want to introduce the confounding factor of ischemia/reperfusion injury. We have used and validated the completeness of extrinsic denervation in similar models of in situ neural isolation of the stomach alone[10] and the entire jejunoileum.[8]

Next, three intragastric manometric catheters (outside diameter 1.5 mm; inside diameter 0.5 mm) and three adjacent bipolar Ag/AgCl serosal electrodes were implanted in the antrum and nine monopolar serosal electrodes on the small bowel (three on the duodenum and six spaced evenly along the jejunoileum). A Thomas cannula was placed in the proximal stomach. The manometric catheters and wires from the electrodes were cemented in three stainless steel cannulas exteriorized through the anterior abdominal wall. The esophageal hiatus was closed with interrupted sutures, and a pyloromyotomy was performed (in an attempt to prevent gastric stasis based on preliminary studies). The dogs were kept in the fasting state for 3 days and were then fed ad libitum. Because of an impressive anorexia (lack of inter-

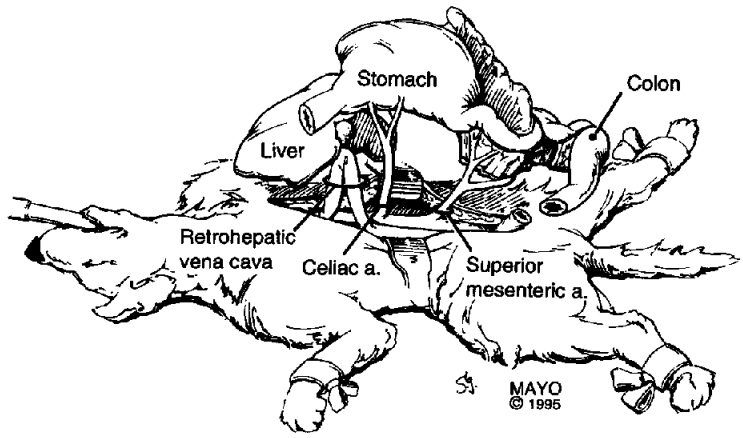

Fig. 1. In situ neural isolation of the upper gut. All tissue connections to the distal 5 cm esophagus, entire stomach and small bowel, proximal 50 cm of colon, pancreas, and liver were transected *except for* the celiac and superior mesenteric arteries and the suprahepatic, retrohepatic, and infrahepatic vena cava, which were completely isolated and stripped of adventitia.

est in eating despite weight loss), gavage via the gastric Thomas cannula was necessary in several dogs in the first several weeks to assure satisfactory nutritional intake. All fasting experiments were conducted within 10 weeks of the denervation operation. In four dogs we measured tissue catecholamine concentrations in the jejunum and proximal colon as described previously[17] at the time the animals were killed; all had decreased to less than 5% of normal values.

Conduct of Experiments

All experiments began after an overnight fast. Dogs were positioned in a Pavlov sling for the duration of each day's experiment. The gastric cannula was opened to allow what content was in the stomach to empty, and the volume was recorded. The gastric cannula was then closed. Myoelectric and manometric activity was recorded continuously both on a Grass model 7D polygraph (Grass Instruments, Quincy, Mass.) using alternating current amplifiers and a time constant of 1 second as well as directly onto a microcomputer as described previously[18] for later analysis.

For fasting experiments (n ≥4 experiments/dog) motility was recorded for up to 12 hours. In eight dogs venous blood samples were collected on ice into vials containing ethylenediaminetetraacetic acid, generally at 20- to 30-minute intervals and additionally when a phase III–like pattern of the MMC was noted in the stomach and duodenum. For experiments involving exogenous motilin administration (n = 3 dogs), 0.1 μg/kg of canine motilin (Peninsula Laboratories, Belmont, Calif.) in 154 mmol/L NaCl containing 0.5% dog plasma was given over a 30-second infusion as before,[19] beginning approximately 30 minutes after a phase III–like burst of activity had appeared in the duodenal electrodes. For the erythromycin experiments (n = 2 dogs) a similar technique was used.

For fed experiments (n = 4 experiments/dog) 50 g and 500 g meals of diced pork liver were offered to seven dogs approximately 20 minutes after a phase III was noted in the duodenal electrode. If the dog did not eat the liver, it was cut into small pieces and administered directly into the stomach through the gastric cannula. Motility was recorded continuously for up to 8 hours postprandially. Blood samples were obtained every 30 minutes as previously described.

Plasma Motilin Determinations

Plasma was separated from the blood samples and stored at −70° C for subsequent batch analysis. A well-characterized motilin radioimmunoassay[15] was used to determine plasma motilin concentrations. Inter- and intra-assay variabilities were 8% and 11%, respectively.

Analysis of Data

Manometric and myoelectric recordings were analyzed by visual inspection to determine the presence or absence of a cyclic pattern of motility. The period of the cycle and the duration of the individual phases were determined separately from the gastric manometry catheters and from the duodenal, midjejunal, and midileal electrodes according to the criteria of Code and Marlett,[3] as we have described previously.[8,10,20] The period was defined as the time between the start of successive bursts of high-amplitude contractions in the stomach (manometry catheters) and migrating phase III activity recorded from the intestinal electrodes. During fasting the mean period of the cyclic pattern and the mean duration of the individual phases were calculated for each day in each dog, means within each dog over the different days were determined, and the grand mean of all dogs was calculated. Similarly the duration of inhibition of the cyclic pattern was determined as the time from the start of the preceding burst of gastric contractions or small bowel phase III prior to feeding until the return of similar phase III–like activity and the occurrence of a cyclic motility pattern thereafter in the four regions (stomach, duodenum, midjejunum, and midileum).

Plasma motilin concentrations were categorized separately according to the phase of the cyclic activity in the stomach and in the duodenum on each day, a mean concentration per phases I, II, III, and IV was determined per dog per day, and then an overall mean of all experiments was determined for each dog; grand means across dogs were also determined. One value of plasma motilin was selected during each MMC cycle usually in early phase I and early phase II and at the start of phase III.

Premature onset of a burst of high-amplitude gastric contractions and phase III activity in the intestinal electrodes after exogenous motilin was defined as the onset of this phase III–like activity in the gastric manometric catheters and in the duodenal electrodes within 10 minutes of the administration of motilin intravenously that then migrated into the jejunum.

Statistical Analysis

No measurements of parameters of interdigestive and postprandial motility were made in the dogs before in situ neural isolation of the upper gut visceral complex because of the complexity of the operative preparation; therefore no formal statistical analyses of

motility parameters before and after in situ neural isolation have been made within dogs. Parameters are summarized and generalized comparisons made in the text to previously reported values from our laboratory.

The plasma concentrations of motilin were compared across the different phases of the cyclic pattern using a one-way analysis of variance. Individual comparisons of phase I vs. phase III–like activity and phase II vs. phase III–like activity were made with Student's *t* test for paired data and employing a Bonferroni correction for the two comparisons. Values summarized in the text will be presented as means ± standard error of the mean (SEM) unless otherwise specified.

RESULTS

Health of Dogs

The dogs lost 15% ± 2% of their body weight postoperatively. Early postoperative care of the dogs was occasionally complicated. Several dogs displayed an impressive anorexia (lack of interest in eating) and required daily gavage feeding for 2 weeks to maintain adequate caloric intake. Intermittent vomiting of ingested food was common in dogs in the first 2 to 3 weeks after the denervation but thereafter became uncommon, and there was rarely any gastric residual volume (>40 ml) after an overnight fast. Stools were watery and loose initially in all dogs but became more solid and were formed by 4 to 8 weeks postoperatively. Six dogs died during the conduct of the study—four from small bowel strangulation obstruction and two from peritonitis of undetermined origin 2 months postoperatively. No measurements of motility were obtained during periods of illness in any dog. Two other dogs developed a small bowel intussusception early in the postoperative period and were treated by simple reduction in one and a short (5 cm) distal ileal resection in the other. No measurements of ileal motility were recorded thereafter distal to the site of ileal resection.

Patterns of Motility

Interdigestive. After an overnight fast, a cyclic pattern of motor and myoelectric activity closely resembling the MMC occurred in the small bowel in all 10 dogs; in 8 of 10 dogs a cyclic pattern of motor and myoelectric activity was evident in the stomach as well, temporarily coordinated with the duodenum as reported previously.[11] The period (in minutes) of this cyclic activity was similar in the stomach, duodenum, midjejunum, and midileum (98 ± 4, 100 ± 3, 102 ± 3, and 97 ± 3, respectively).

There were, however, differences in the fasting motility patterns when compared to those in neurally intact dogs reported from our laboratory.[15,20-23] Intervals of disruption of the cyclic pattern throughout the entire upper gut occurred despite the occurrence of cyclic activity immediately beforehand and a persistent fasting state (Fig. 2). This spontaneous disruption occurred in one of four fasting experiments in three dogs and in one of five fasting experiments in

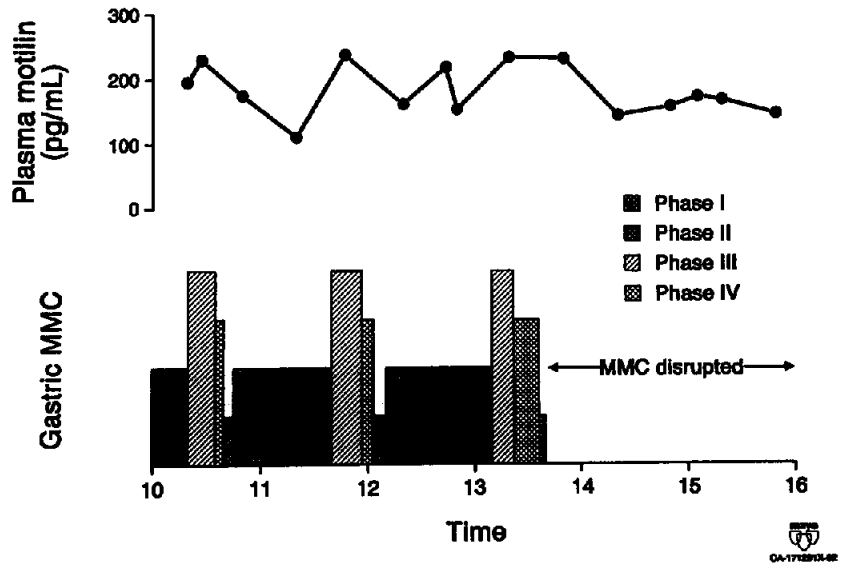

Fig. 2. Temporal relationship of plasma motilin concentrations and motility in a dog after in situ neural isolation of the upper gut. Cyclic activity became disrupted spontaneously despite fasting (arrow), and plasma motilin concentration no longer varied cyclically. X axis denotes hour of the day.

the fourth dog. Such disappearance of cyclic activity persisted for 2 to 6 hours; this disruption of the MMC would be distinctly uncommon from our previous observations in neurally intact dogs.[18,21,23,25]

Postprandial. An oral meal of 50 g of pork liver transiently (~60 minutes) disrupted the cyclic pattern and prolonged the time from the last phase III–like cycle 20 minutes prior to feeding to the onset of the first cyclic pattern after feeding when compared to the period of the fasting cyclic pattern (Fig. 3, *A*). A larger meal of 500 g of liver rapidly disrupted the cyclic pattern and established a noncyclic postprandial pattern of motor and myoelectric activity (Fig. 3, *B*). This noncyclic postprandial motility pattern persisted for 410 ± 21 minutes in the stomach and 298 ± 38 minutes in the ileum until the return of a characteristic cyclic pattern of motility.

Plasma Motilin Concentrations

Fasting. Plasma motilin concentrations varied cyclically during fasting in all dogs (Table I). Peak concentrations ($P < 0.025$) occurred in temporal coordination with the cyclic bursts of gastric contrac-

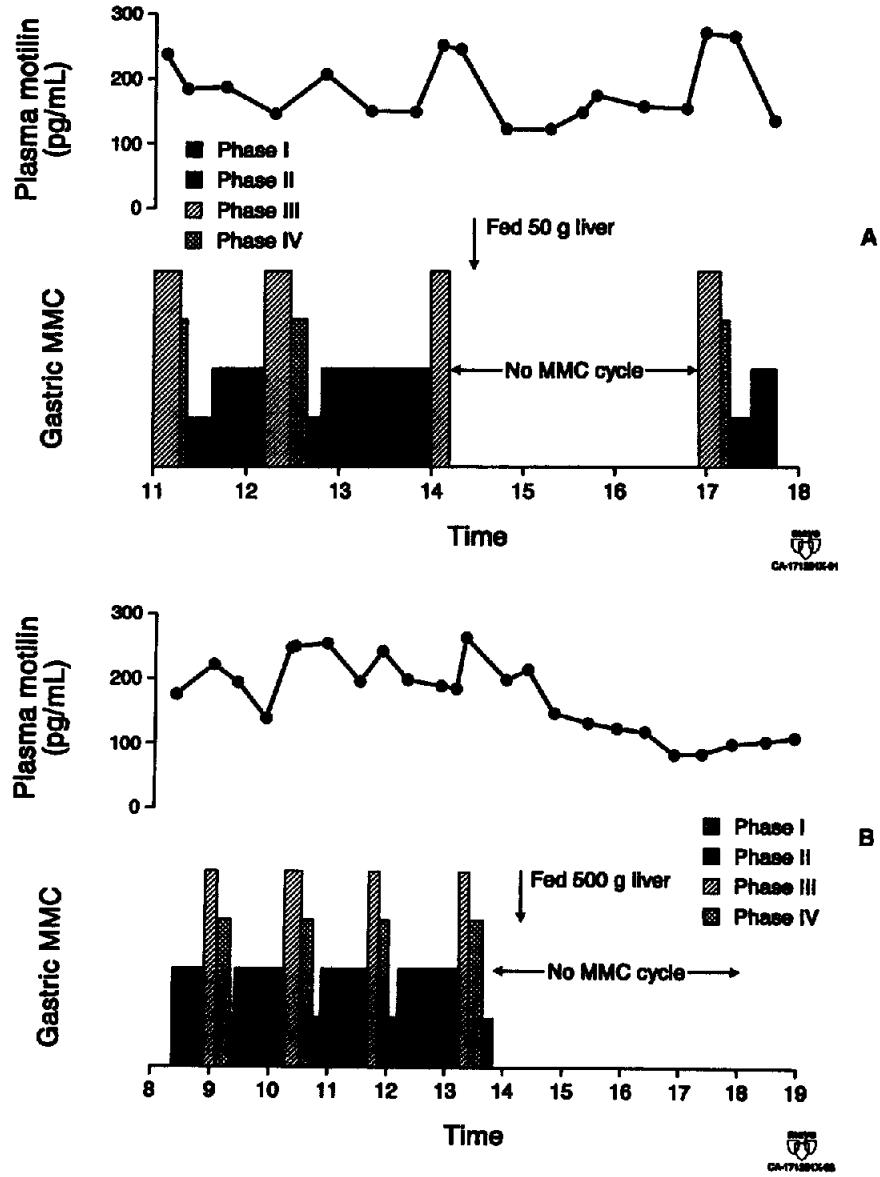

Fig. 3. Effect of an oral meal on motility patterns and on plasma motilin concentrations in a representative dog after in situ neural isolation of the upper gut. X axis denotes hour of the day. **A**, 50 g liver meal; **B**, 500 g liver meal.

Table I. Plasma motilin concentrations during interdigestive and postprandial motility

Motility pattern	Motilin (pg/ml)
Gastric interdigestive (n = 6 dogs)	
Phase I–like activity	178 ± 12*
Phase II–like activity	151 ± 6*
Phase III–like burst of contractile activity	204 ± 13
Phase IV–like activity	203 ± 12
Duodenal interdigestive (n = 8 dogs)	
Phase I	168 ± 12†
Phase II	146 ± 6*
Phase III	202 ± 11
Phase IV	194 ± 10
Postprandial (n = 7 dogs)‡	152 ± 8

Values are means ± standard error of the mean.
*Differs from phase III–like activity; $P < 0.025$.
†Differs from phase III–like activity; $P = 0.04$.
‡Mean value during noncyclic motility.

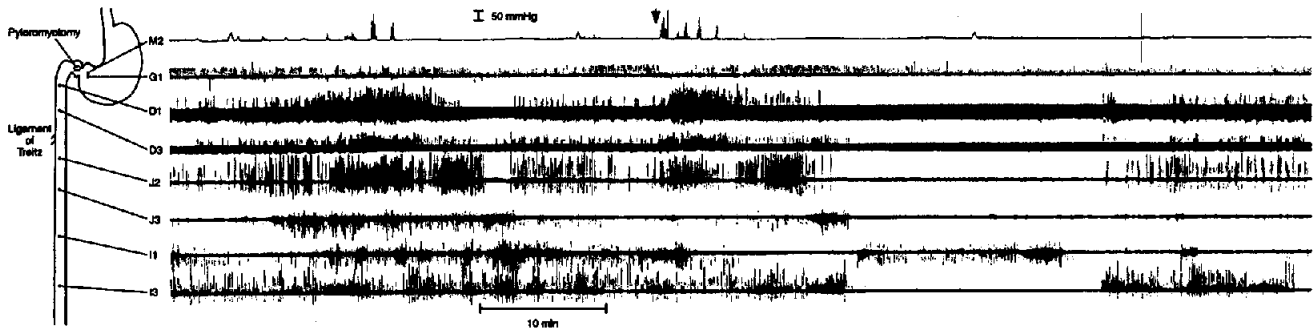

Fig. 4. Effect of exogenous motilin on motility patterns in a representative dog after in situ neural isolation of the upper gut. Motilin (0.1 μg/kg) given intravenously at arrow. Note induction of a burst of high-amplitude gastric contractions and a "premature" phase III in the duodenum.

tions in the stomach and with phase III activity in the duodenum (see Fig. 2). In the two dogs without gastric cycles of motility, plasma motilin concentrations (pg/ml) were also greater during duodenal phase III (dog 1 = 195 ± 2; dog 2 = 158 ± 6) than during phase I (dog 1 = 177 ± 6; dog 2 = 121 ± 11) or phase II (dog 1 = 154 ± 5; dog 2 = 115 ± 4). When cyclic interdigestive activity was disrupted spontaneously despite ongoing fasting, plasma motilin concentrations did not cycle for the duration of the noncyclic motor and myoelectric activity in the stomach and duodenum (see Fig. 2); however, when cyclic activity recurred, plasma motilin peaked and decreased thereafter during phase I.

When motilin was administered exogenously as a 30-second infusion (0.1 μg/kg intravenously in three dogs (all of whom had spontaneous cyclic gastric motility), a burst of high-amplitude contractions occurred within several minutes in the stomach associated with a premature phase III in the duodenum; the duodenal phase III migrated distally along the small intestine in all dogs with the appearance of a typical fasting phase III (Fig. 4). Thereafter cyclic activity resumed as if the "premature" phase III began the next successive cycle of the motility. In two dogs, erythromycin (3 mg/kg) was administered with induction of a similar burst of gastric contractions and a premature MMC in the small intestine. In general, the motilin-induced and erythromycin-induced burst of gastric contractile activity occurred as a single burst of regular high-amplitude contractions that often differed in appearance from the spontaneous phase

III–like activity in that same dog and from phase III activity in neurally intact dogs in our laboratory.[15,20-22] Spontaneous cyclic bursts of gastric contractile activity that preceded a duodenal phase III tended to occur as a burst of three to five clusters of grouped contractions (see Fig. 4). The motilin- and erythromycin-induced gastric contractions usually appeared as a 5- to 8-minute burst of regular high-amplitude contractions; at other times the pattern appeared identical to the spontaneous cyclic pattern of gastric contractions with clusters of grouped contractions (see Fig. 4).

Postprandial. After the 50 g oral meal, plasma motilin concentrations did not cycle during the inhibition of the cyclic motility pattern, but once again cycled in association with resumption of the typical gastric cyclic motor activity seen during fasting (see Fig. 3, *A*). After the 500 g oral meal, cyclic activity was disrupted, and plasma concentrations of motilin decreased and no longer cycled (see Fig. 3, *B*).

DISCUSSION

This study showed that plasma concentrations of motilin cycled in close temporal coordination with characteristic and temporally coordinated interdigestive patterns of cyclic motility in the canine stomach, duodenum, and jejunoileum after complete in situ neural isolation (extrinsic denervation) of the upper gut. Moreover, exogenous infusions of motilin consistently induced a premature burst of contractions in the stomach coordinated with a premature duodenal MMC that migrated along the small intestine. Large meals (500 g of liver), and to a much lesser extent small meals (50 g of liver), inhibited the cyclic pattern of interdigestive motility and disrupted cyclic changes in plasma motilin concentrations. These observations demonstrate that extrinsic innervation to the upper gut (either afferent or efferent) is not necessary for the initiation of cyclic interdigestive motility patterns, coordinated cyclic release of motilin, disruption of interdigestive motility patterns by oral meals, or induction of a premature cycle of interdigestive motor and myoelectric activity in the stomach and small bowel by exogenous motilin.

Our study shows that extrinsic innervation (vagal, sympathetic) to the upper gut is not required for the initiation, coordination, and orderly aborad migration of cyclic interdigestive patterns of motility. Our observations are in agreement with and extend several previous studies.[8-11,20,25-30] Although certain characteristics (duration, timing, presence) of interdigestive motility can be modulated via extrinsic neural pathways to the upper gut,[31-34] the continuity of intact extrinsic innervation is not necessary for cyclic interdigestive motor patterns to be initiated in the denervated segment.

Although our observations differ from some of the implications of a series of previous studies of acute reversible vagal blockade by Diamant et al.[35] and Hall et al.,[36] when taken in context our findings are consistent with theirs in several ways. These investigators showed that transient acute blockade of vagal input by bilateral cervical perivagal cooling to 4° C disrupted cycling of the MMC in the canine upper gut; when the vagal cooling was discontinued, the MMC returned suggesting that vagal input controlled initiation of cyclic changes of upper gut interdigestive motility. When our previous and present findings with chronic denervations[9-11,20,21] are considered with those of Diamant et al.[35] and Hall et al.,[36] our interpretation is that vagal innervation may help to initiate certain characteristic patterns of contractile activity during cyclic interdigestive activity, but vagal input is not requisite for cyclic gastric or small bowel interdigestive patterns to occur.

The primary aim of our study was to determine the relationship between plasma motilin concentrations and the cyclic gastric and duodenal interdigestive pattern of motility after in situ neural isolation of the duodenum, the major site of motilin synthesis and release.[12] We showed that plasma concentrations of motilin cycled in temporal concert with the cyclic interdigestive MMC-like activity in the upper gut. Peak concentrations of motilin coincided with the bursts of contractions in the stomach and the phase III–like activity in the duodenum. In addition, when the cyclic motility was spontaneously disrupted or was disrupted with an oral meal, plasma motilin concentrations did not vary cyclically. Although this close temporal association does not implicate a direct causal effect of increasing plasma concentrations of motilin with induction of phase III of the MMC, it does strongly support the concept of a relationship between motilin and the cyclic interdigestive pattern of motility, as suggested by previous studies.[7,14,15]

Our study does, however, show that extrinsic innervation of the duodenum was not necessary for the cyclic changes in plasma motilin concentrations or for the induction of a premature cycle of interdigestive motility in the gastroduodenal region by exogenous intravenous administration of motilin or the motilin agonist erythromycin. Several of our previous studies and that of Thomas et al.[37] showed that selective extrinsic denervation did prevent the ability of exogenous motilin to induce a premature MMC in the extrinsically denervated jejunum[38,39] but not in the ex-

trinsically denervated stomach.[10] These previous studies raised the question of a role for extrinsic nerves in the hormonal initiation of the MMC in the neurally intact small bowel. Our current study, although suggestive, cannot further or directly prove that spontaneous cycles of interdigestive motility patterns are controlled by changes in plasma motilin concentrations; however, in combination with our previous study of the in situ neurally isolated stomach,[10] the current study provides further support for the concept that plasma motilin concentrations are related to the initiation of cyclic patterns of interdigestive motor activity of the MMC in the stomach and may help to recruit the stomach and possibly the duodenum as well in the temporal coordination of interdigestive patterns of motility. Work by Diamant et al.[35] and Hall et al.[36] suggests that vagal innervation may help to reinforce this recruitment and may alter the pattern of contractions during the phases of cyclic motor activity. Further indirect support for this concept comes from the observation that 4 of the 10 dogs after in situ neural isolation of the upper gut would intermittently have intervals of 3 to 6 hours during which the characteristic MMC would become disrupted spontaneously despite its previous presence and persistent fasting, and plasma motilin concentrations also failed to cycle. This observation would be distinctly uncommon in neurally intact dogs in our laboratory under similar conditions.[17,21,23,24] The cause(s) for this disruption of interdigestive motility patterns is unknown but may be related to the lack of cyclic changes in plasma motilin concentration during this disruption.

Both a small (50 g) and a large (500 g) liver meal disrupted the interdigestive cyclic motility patterns throughout this in situ neurally isolated upper gut multivisceral complex. This finding in conjunction with our other studies[8,10,11,23,24,40] shows that postprandial disruption of upper gut interdigestive patterns of motility does not require an intact extrinsic innervation, as mediated by either afferent or efferent neural pathways. Moreover, after both the small and large meals, plasma motilin concentrations decreased and did not cycle. When cyclic motility patterns returned, plasma motilin again varied in cyclic concert with the gastroduodenal cyclic activity. Because this experimental technique neurally isolated the upper gut from any neural continuity with the central nervous system or with the distal colon, this change to a postprandial pattern of motility must be mediated by a hormonal mechanism and/or by a local neural mechanism arising within the enteric nervous system of the upper gut in response to entry of the meal into the upper gut. Both neural and hormonal mechanisms have been shown to mediate postprandial disruption of the MMC. Exogenous intravenous infusions of regulatory peptides[41] and endogenous enteric release of circulating regulatory hormones[23,40] will disrupt fasting patterns of motility. If this postprandial inhibition of cyclic interdigestive patterns of motility is mediated by hormonal mechanisms, our study suggests that extrinsic innervation to the upper gut is not necessary for release of postprandial regulatory hormones, which inhibit interdigestive motility. In contrast, small liver meals (50 g) inhibited cyclic activity after in situ neural isolation of the upper gut and cyclic changes in plasma motilin concentrations in the plasma for a much shorter duration than our previous work in neurally intact control dogs,[24] suggesting that extrinsic innervation may participate in the release of postprandial regulatory hormones by small meals; large meals can successfully induce the release of enough postprandial hormones to inhibit the interdigestive cyclic motility.

Another potential mechanism of postprandial disruption of interdigestive motility involves nutrient-independent mechanisms. Nonnutrient distention of the innervated proximal stomach by a balloon-volume stimulus[21,42] and intraluminal infusion of a nonnutrient solution at high rates (≥12 ml/min) will also disrupt the MMC,[43] at least the former presumably mediated through neural pathways because complete abdominal vagotomy[21] and selective proximal gastric vagotomy[22] abolished distention-induced inhibition of the MMC. Nonnutrient gastric distention–induced inhibition of the upper gut MMC appears to be mediated via vagal pathways; this mechanism might explain the markedly decreased duration of inhibition of cyclic interdigestive patterns of motility in the neurally isolated upper gut by the smaller (50 g) liver meal. We have made similar observations with large and small meals after neural isolation of the jejunoileum alone,[24] again suggesting potential roles for hormonal and neural mechanisms. Our previous study with this same multivisceral in situ neural isolation did not show any obvious disruption of the MMC with a small liver meal; this discrepancy is probably related to variations in the short duration of postprandial disruption in relation to the MMC period of individual dogs.[11]

This study has several potential limitations that require acknowledgement. First, our canine preparation is a model of complete neural isolation. Although unlikely, some extrinsic nerves may innervate the gut by passing within the media of the celiac and superior mesenteric arteries and vena caval wall. We specifically stripped these vessels of all adventia under microscopic magnification but did not transect and re-

anastomose them because such a true autotransplantation would obligate an ischemic injury and a subsequent reperfusion injury, which would introduce confounding effects independent of the neural isolation. We have used this operative approach of in situ neural isolation to study the neurally isolated stomach,[10] the neurally isolated jejunoileum,[8] and the neurally isolated proximal colon[44] as well as the upper gut in the current study and have confirmed extrinsic denervation in the latter three preparations by demonstrating very low or unmeasureable catecholamine tissue concentrations postoperatively.

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Is Intra-Abdominal Drainage Necessary After Pancreaticoduodenectomy?

Martin J. Heslin, M.D., Lawrence E. Harrison, M.D., Ari D. Brooks, M.D., Steven N. Hochwald, M.D., Daniel G. Coit, M.D., Murray F. Brennan, M.D.

Closed suction drains after pancreaticoduodenectomy are theoretically used to drain potential collections and anastomotic leaks. It is unknown whether such drains are effective, harmful, or affect the outcome after this operation. Eighty-nine consecutive patients underwent pancreaticoduodenectomy for presumed periampullary malignancy and were retrospectively reviewed. Thirty-eight had no intra-abdominal drains and 51 had drains placed at the conclusion of the operation. We analyzed patient, nutritional, laboratory, and operating room factors with end points being complications and length of hospital stay. Intra-abdominal complications were defined as intra-abdominal abscess and pancreatic or biliary fistula. Postoperative interventions were defined as CT-guided drainage and reoperation. Analysis was by Student's *t* test and chi-square test. Two of eight surgeons contributed 92% of the patients without drains. The groups were equivalent with respect to demographic, nutritional, and operative factors. Time under anesthesia was significantly shorter in the group without drains ($P = 0.0001$). There was no statistical difference in the rate of fistula, abscess, CT drainage, or length of hospital stay. Intra-abdominal drainage did not significantly alter the risk of fistula, abscess, or reoperation or the necessity for CT-guided intervention after pancreaticoduodenectomy. Routine use of drains after pancreaticoduodenectomy may not be necessary and should be subjected to a randomized trial. (J GASTROINTEST SURG 1998;2:373-378.)

Use of closed suction drains after intra-abdominal surgical procedures is common. The intended purpose is to drain collections of fluid and control potential anastomotic leaks. It is largely unknown whether or not these drains serve their purpose or, conversely, increase postoperative morbidity. Randomized trials examining the role of postoperative drains after elective cholecystectomy, colectomy, or hepatectomy have not shown a benefit to the presence of a drain.[1-4] Retrospective, selected patients have been reported to have acceptable outcomes after pancreaticoduodenectomy without abdominal drainage; however, there was no comparison group in this report.[5] Others have reported cases suggesting closed suction drains have caused bowel perforations[6-8] and may increase the risk of postoperative infectious complications.[2] The purpose of this study was to determine whether the presence of a drain affected outcome after elective pancreaticoduodenectomy at a single institution. Specific outcome end points were postoperative morbidity and length of hospital stay.

PATIENTS AND METHODS

Between March 1, 1994, and August 30, 1996, eighty-nine patients underwent pancreaticoduodenectomy at Memorial Sloan-Kettering Cancer Center within the confines of a nutritional trial.[9] Criteria for entry into this analysis were no evidence of infection (white blood count <12,000 cells/mm^3, no fever, and no evidence of bacteremia), no immunosuppressive medication, no history of abdominal or pelvic radiation, and having undergone a pancreaticoduodenectomy for presumed or histologically proved periampullary malignancy. All patients had normal coagulation profiles prior to operation. A de-

From the Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, N.Y.
Dr. Heslin is the recipient of a Kristen Ann Carr Fellowship, 1995-1996, Memorial Sloan-Kettering Cancer Center.
Presented at the Thirty-Eighth Annual Meeting of The Society for Surgery of the Alimentary Tract, Washington, D.C., May 11-14, 1997.
Present address and correspondence: Martin J. Heslin, M.D., UAB Department of Surgery, 1922 Seventh Ave. South, KB 321, Birmingham, AL 35294. E-mail: marty.heslin@ccc.uab.edu.
Reprint requests: Murray F. Brennan, M.D., 1275 York Ave., New York, NY 10021.

tailed history was obtained from all patients after they were admitted to the hospital. All patients also underwent physical examination, laboratory analyses, and nutritional assessment, and informed consent was obtained prior to operation.

Thirty-eight patients had no intra-abdominal drains placed and 51 had closed suction drains placed at the conclusion of the procedure. Careful analysis of factors associated with perioperative morbidity was undertaken. Patient factors analyzed were age, sex, and comorbidity. Comorbidity was defined as the presence of diabetes mellitus (insulin-dependent or non–insulin-dependent), coronary artery disease, pulmonary disease, or hypertension.

Nutritional factors analyzed included percentage weight loss, preoperative serum albumin, and nutrition risk index (NRI). The NRI is defined as (15.9 × albumin [g/L] +0.417 × % usual body weight [%UBW]).[10] Specific laboratory data analyzed included total bilirubin (mg/dl), blood urea nitrogen, serum creatinine, and white blood cell count (mm^3). When utilized, early enteral feeding was begun on postoperative day 1, advanced as tolerated to a target of 25 ml/kg per 24 hours, and continued until the patient was eating 1000 kcal or consuming at least 1000 ml of a liquid diet.

The majority of the operations were "standard" pancreaticoduodenectomies involving distal gastrectomy, with pancreaticojejunostomy, choledochojejunostomy, and gastrojejunostomy. There were subtle variations including Roux-en-Y reconstruction of the gastrojejunostomy and pylorus-preserving procedures with pancreaticogastrostomy reconstruction depending on surgeon preference. Operating room factors analyzed were time under anesthesia (which included preincision laparoscopy), time in the operating room, blood loss, and need for blood transfusion. Pathologic factors analyzed were the presence of malignancy, the presence of nodal metastasis, and the presence of a positive margin.

Complications were evaluated and recorded separately from the surgical team. This involved daily rounds by a surgeon not associated with the team to evaluate the patient, laboratory, and radiologic data where appropriate. A biliary or pancreatic fistula was defined as persistent bile or pancreatic fluid drainage that continued at a rate of 30 ml or more per day and did not resolve by postoperative day 7. Intra-abdominal abscesses were defined as an intra-abdominal collection associated with fever that required either percutaneous or operative drainage yielding positive cultures. Gastrointestinal anastomotic leaks were defined as a leak from either the gastrojejunostomy or jejunojejunostomy. Other complications included in the minor or major category were cellulitis, superficial wound infection, deep wound infection, fascial dehiscence, pneumonia (defined as radiologic and culture evidence of infection), reexploration for any reason (specifically bleeding, hepatic artery thrombosis, bowel necrosis, and wound debridement) and death (hepatic artery thrombosis), prolonged ileus, gastric atony, atelectasis, pleural effusion, urinary tract infection, abdominal wall infection at the J-tube site, leak at the J-tube site, evisceration, intestinal obstruction, pulmonary embolus, myocardial infarction, cerebral vascular accident, deep venous thrombosis, chylous leak, renal failure, congestive heart failure, gastrointestinal hemorrhage, bowel necrosis, pneumothorax, necrotizing fasciitis, infectious colitis, line sepsis, acute cardiac arrhythmias, tracheobronchitis, or abdominal wall hematoma. Length of stay was measured from the date of operative until discharge home in days, regardless of social service constraints.

Statistical analyses were performed using the SPSS for Windows version 7.0 statistical package (SPSS, Chicago, Ill.). Continuous variables were compared by Student's t test, categorical variables were compared by chi-square or Fisher's exact test, and multivariate analysis utilized logistic regression. Length of hospital stay was compared using the log-rank test and plotted by the Kaplan-Meier method. The mean length of stay was compared by Student's t test. Significance was defined as $P < 0.05$.

RESULTS

Demographics

Eighty-nine patients underwent pancreaticoduodenectomy during a 2½-year period within the confines of a prospective trial to evaluate the benefit of early enteral feeding. Demographic data are presented in Table I. There were no significant differences between the groups.

Nutritional and Biochemical Parameters

Table II lists the preoperative nutritional and biochemical parameters measured. There were no statistically significant differences between the groups. It is important to note that although there was approximately a 6% to 7% average preoperative weight loss, the nutrition risk index was within the normal range, suggesting that this group was nutritionally intact. Equal percentages in each group received early feeding. There were no other significant differences in biochemical abnormalities between the groups.

Operative and Pathologic Factors

Two of eight surgeons contributed 92% of the patients without drains. Operating room and final pathology factors are depicted in Table III. Time un-

Table I. Demographics/comorbidity

	No drain (n = 38)	Drain (n = 51)	*P* value
Age (yr)*	65 ± 2	65 ± 2	0.91
Female (%)	53	37	0.20
Comorbidity			
Diabetes mellitus (%)	21	24	0.99
Hypertension (%)	29	33	0.81
Coronary artery disease (%)	8	18	0.22
Pulmonary disease (%)	3	8	0.39
Preoperative stent (%)	53	59	0.60

*Mean ± standard error of the mean.

Table II. Nutritional and biochemical parameters

	No drain (n = 38)	Drain (n = 51)	*P* value
Nutritional			
Weight loss (%)	6 ± 1	7 ± 1	0.39
NRI	104 ± 1	101 ± 1	0.10
Early enteral feeding (%)	61	57	0.83
Biochemical			
WBC (mm^3)	7.4 ± 0.3	7.4 ± 0.3	0.99
BUN (mg/dl)	15 ± 1	15 ± 1	0.85
Creatinine (mg/dl)	1 ± 0.04	1 ± 0.05	0.97
Preoperative bilirubin (mg/dl)	3.7 ± 0.8	5.1 ± 0.9	0.24
Albumin (g/dl)	4.1 ± 0.07	3.9 ± 0.06	0.17

NRI = nutritional risk index; WBC = white blood cell count; BUN = blood urea nitrogen.
Values are mean ± standard error of the mean unless otherwise indicated.

Table III. Operative and pathologic evaluation

	No drain (n = 38)	Drain (n = 51)	*P* value
Operating room			
Anesthesia time (min)	292 ± 13	386 ± 20	0.0001
Blood loss (L)	1.1 ± 0.01	1.1 ± 0.01	0.92
Blood transfusion (%)	37	35	0.99
Pathology			
Malignant (%)	82	92	0.20
Node positive (%)	53	74	0.15
Margin positive (%)	29	31	0.82

Mean ± standard error of the mean unless otherwise indicated.

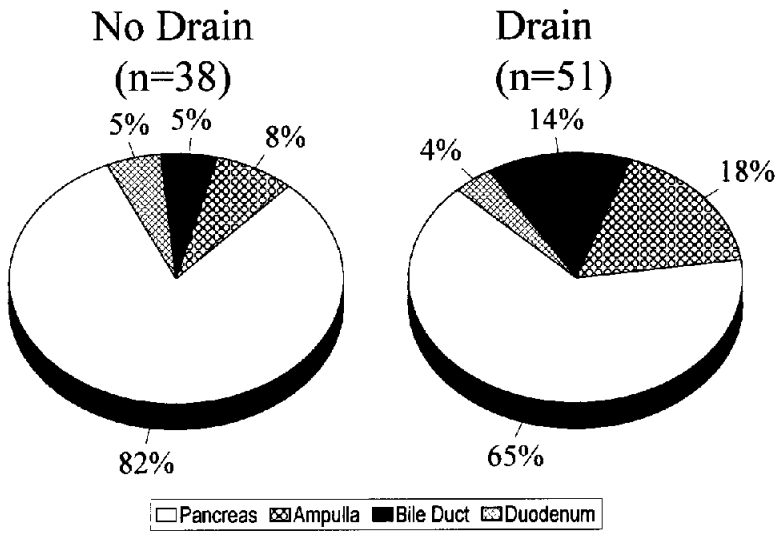

Fig. 1. Anatomic location of the lesion.

Table IV. General complications and length of hospital stay

	No drain (n = 38)	Drain (n = 51)	*P* value
Minor complications	9	13	0.85
Major complications	8	14	0.49
Any complication	15	23	0.60
Hospital stay (days)	12 ± 1	12 ± 1	0.71

Table V. Complications and interventions potentially affected by drains

	No drain (n = 38)	Drain (n = 51)	*P* value
Fistula	1	3	0.30
Abdominal abscess	0	3	0.13
Reoperation	3	1	0.45
Reoperation for bleeding	0	1	0.31
CT-guided drainage	1	2	0.28

der anesthesia (which included preincision laparoscopy in most cases) was significantly shorter in the group without intra-abdominal drains. There were no statistical differences in the percentage of masses that were malignant, node positive, or margin positive. Fig. 1 depicts the anatomic location of the lesions in each group.

Complications, Interventions, and Length of Hospital Stay

Table IV depicts the complications and length of hospital stay. There were no significant differences between the groups. Table V depicts the complications and interventions that potentially could be affected by the presence of an intra-abdominal drain. Although there were fewer total complications potentially affected by a drain in the "No drain" group, there were no statistical differences present.

DISCUSSION

The proposed purpose of intra-abdominal drainage is to drain collections of fluid and control potential anastomotic leaks. We have demonstrated that eliminating intra-abdominal drainage after pancreaticoduodenectomy did not increase the rate of fistula, intra-abdominal abscess, CT-guided percutaneous drainage, or reoperation for bleeding. Although the time under anesthesia was significantly shorter among the group without drains, which may suggest a bias in favor of the group without drains, this factor did not prove to be significantly correlated to the rate of postoperative complications on multivariate analysis. Thus operative intra-abdominal drainage in this series does not appear to help and therefore may not be necessary after pancreaticoduodenectomy.

The benefit of operative intra-abdominal drainage has been examined previously after a number of intra-abdominal procedures. Randomized studies af-

ter open cholecystectomy have either not shown a benefit[3] to drainage or have suggested an increase in wound infections and collections in the area where drainage was attempted.[2] Similarly, randomized studies after abdominal colectomy have demonstrated no decrease in the incidence of postoperative complications and reported that when anastomoses leaked, "neither pus nor feces came out of the drain."[1] Last, a recent prospective randomized trial from our institution evaluated the effect of intra-abdominal drainage after 120 elective hepatic resections.[4] This study demonstrated that operative drainage produced no significant difference in outcome including complication rate, mortality rate, or length of hospital stay.

In recent years the postoperative mortality rate after pancreaticoduodenectomy has declined to the low single digits, especially at centers where this operation is frequently performed.[11,12] Postoperative morbidity after pancreaticoduodenectomy is approximately 40% and the major complications reported include intra-abdominal abscess, postoperative bleeding, and pancreatic or biliary fistula.[11] Operative drainage of the peritoneal cavity after pancreaticoduodenectomy is thought by many to be necessary to allow early detection and possibly nonoperative treatment of these complications. Unfortunately adhesions or the omentum often exclude the peritoneal drain from anastomotic sites and therefore the drain does not function appropriately. Additionally, the development of interventional radiologic techniques has allowed nonoperative drainage of collections identified on CT or ultrasound scans, therefore reducing the need for reexploration in many cases. The prevalence of a pancreatic fistula after pancreaticoduodenectomy ranges from 5% to 18%.[11,13,14] Part of the differences in the range may depend on the definition of a leak. Cullen et al.[14] included in the definition of a pancreatic leak the presence of amylase in the drainage fluid. As they defined a pancreatic leak, most of these patients (73%) had "clinically insignificant leaks" and were managed by prolonged drainage. We would argue that these patients may not have needed drainage at all and a clinically insignificant leak may have remained insignificant regardless of drainage. In the same series 27% of the patients who presumably had drains in place had clinical symptoms associated with a leak requiring some form of postoperative intervention and therefore the drain did not function to prevent these complications.[14]

If an intervention does not necessarily help to decrease morbidity or mortality, then one must ask whether it is detrimental. The majority of randomized trials in other procedures that have examined this question have not shown closed suction drains to be of particular detriment,[1,3,4] although one trial found increases in morbidity after cholecystectomy.[2] The present study demonstrated no specific complication that could be attributed to the presence of a drain, although there were more intra-abdominal abscesses and fistulas in the group that had drains placed. There have been a number of case reports that have documented bowel perforation caused by closed suction drains.[6-8] Clearly the suction pressure generated by such closed systems can be of a level to cause injury to visceral tissues.[15] This was not reported in the present study. Last, it would be conjecture to suggest that the presence of a drain caused fistulas to remain open, as this is not a randomized study.

In summary, the present study has documented that intra-abdominal drainage after pancreaticoduodenectomy may not be necessary. There was no increased risk of intra-abdominal abscess or pancreatic or biliary fistula and no increased risk of postoperative interventions such as percutaneous drainage or reoperation without intra-abdominal drainage. The risks and benefits of intra-abdominal drainage after pancreaticoduodenectomy would be best answered within the context of a randomized trial.

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Endothelin-1 Mediates the Alcohol-Induced Reduction of Pancreatic Capillary Blood Flow

Thomas Foitzik, M.D., Hubert G. Hotz, M.D., Birgit Hotz, Michael Kirchengast, Ph.D., Heinz J. Buhr, M.D., F.A.C.S.

Increased plasma endothelin-1 (ET-1) levels in rats after alcohol administration and increased endothelin receptor expression in the pancreas in chronic alcoholic pancreatitis have led to the hypothesis that ET-1 may play a critical role in the pathogenesis of ethanol-induced pancreatic injury through impairment of perfusion. To further test the hypothesis that ET-1 mediates an alcohol-induced reduction of pancreatic perfusion, the present study compares the effect of intravenous alcohol and ET-1 on pancreatic capillary blood flow (PCBF) and investigates whether endothelin receptor blockade prevents the alcohol-induced reduction in PCBF. Anesthetized rats were randomly assigned to receive one of the following: a 1-hour infusion of 2 g/kg alcohol or the volume equivalent of saline solution plus ET-1 (1.25 μg/kg), a specific endothelin-A receptor antagonist (50 mg/kg), or saline solution (volume equivalent). The pancreas was exposed for intravital microscopy; PCBF was determined at the same location before the test solutions were given, after the infusion, and 1 hour thereafter. Alcohol and ET-1 significantly decreased PCBF from 2.0 nl/min/cap to 1.7 nl/min/cap. The reduction in PCBF was even more pronounced when alcohol and ET-1 were combined (1.5 nl/min/cap), whereas the ET receptor antagonist increased PCBF in saline-treated rats to 2.2 nl/min cap and maintained stable PCBF in alcohol-treated animals. The observation that PCBF is reduced by both alcohol and ET-1 and that the alcohol-induced reduction of PCBF can be aggravated by ET-1 and prevented by a specific endothelin-1 antagonist supports the hypothesis that ET-1 is the mediator of the alcohol-associated reduction of pancreatic perfusion. (J GASTROINTEST SURG 1998;2:379-384.)

Experimental evidence suggests that the alcohol-induced reduction of pancreatic capillary blood flow (PCBF) and oxygenation is an important factor in the pathogenesis of alcohol-related pancreatic injury.[1-4] The mechanism by which alcohol reduces pancreatic perfusion and oxygen supply, however, is still unknown. One hypothesis accounting for an alcohol-related reduction in regional blood flow is that high concentrations of blood ethanol cause vasoconstriction and thereby reduce capillary blood flow in the splanchnic circulation.[5,6] Among the vasoactive substances that may mediate these vascular disturbances, endothelin has recently been shown to be released from endothelial cells in response to alcohol and to disturb regional perfusion, for example, in the stomach and liver.[7-9] Lewis et al.[10] reported that alcohol also stimulates the release of endothelin in the pancreas. This observation together with the finding that the pancreas is especially susceptible to (exogenous) endothelin[11,12] has led to the hypothesis that endothelin may mediate alcohol-induced changes in pancreatic perfusion. The present study further tests this hypothesis by comparing the effects of alcohol and exogenous endothelin on pancreatic capillary blood flow and by evaluating whether the alcohol-induced reduction in PCBF can be prevented by a specific endothelin-A receptor blocker.

MATERIAL AND METHODS

All experiments were conducted in accordance with the national guidelines for the use and care of laboratory animals and approved by the local ethics committee. After overnight fasting, female Wistar rats

From the Department of Surgery, Benjamin Franklin Medical Center, Freie Universität Berlin, and Knoll AG (M.K.), Berlin and Ludwigshafen, Germany.
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Reprint requests: Dr. Thomas Foitzik, Abteilung für Allgemein, Gefäß-und Thoraxchirurgie, Klinikum Benjamin Franklin, Freie Universität Berlin, Hindenburgdamm 30, D-12 200 Berlin.

(225 to 275 g) were anesthetized with intraperitoneal pentobarbital (20 mg/kg) and ketamine (40 mg/kg). Polyethylene catheters (inside diameter 0.5 mm) were inserted into the right jugular vein (two lines) and the left carotid artery (one line) for infusions, blood sampling, and hemodynamic monitoring. To assess PCBF, the pancreas was exposed by minilaparotomy, placed in an immersion chamber with lactated Ringer's solution maintained at 37° C, and positioned under a fluorescence microscope (Leitz, Wetzlar, Germany) with a heat protection and excitation filter (450 to 490 nm). The animals received an intravenous injection of 0.5 ml/kg erythrocytes labeled with fluorescein isothiocyanate (FITC; Sigma, Deisenhofen, Germany). After a stabilization period of 10 to 20 minutes, a randomly chosen area in the head of the pancreas (400 to 325 μm) was recorded for off-line analysis. In this area, blood flow was calculated in all capillaries based on the concentration of fluorescent erythrocytes per unit of arterial blood at the time of the recording, the capillary hematocrit value, and the number of FITC-labeled erythrocytes passing through the respective vessel.[13,14] Cardiorespiratory monitoring included repeated measurements of mean arterial pressure, heart rate, and arterial blood gases.

After baseline measurements of PCBF and the systemic cardiorespiratory parameters, animals were randomly divided into two groups for intravenous infusion of either 2 g/kg ethanol or the equivalent volume of 0.9% sodium chloride (via intravenous line 1). Both groups were further divided into three subgroups for additional treatment with 1.25 μg/kg endothelin-1, 50 mg/kg of the specific endothelin-A receptor blocker *LU-135252* (both provided by Knoll AG, Ludwigshafen, Germany), or the volume equivalent of normal saline solution (via intravenous line 2). All infusions were given over 60 minutes using Harvard pumps (Harvard Apparatus, Inc., S. Natick, Mass.).

We chose intravenous administration of the test solutions to shorten intravital microscopy of the exposed pancreas and to separate direct effects of ethanol on pancreatic microcirculation from indirect effects on the splanchnic circulation that might result from contact with the gastric mucosa and small bowel.[15] The alcohol dose and infusion rate had previously been shown to produce ethanol blood levels between 1500 and 2000 mg/L without significantly altering systemic hemodynamic and respiratory parameters.[4,16] Reevaluation of blood ethanol levels in the present experiment performed in 6 of the 18 animals that received alcohol revealed concentrations of 1795 ± 83 mg/L at the time of PCBF measurements. The observation time was limited to 2 hours because healthy control animals maintain stable cardiorespiratory parameters and blood flow in the exposed pancreas throughout this time.[13,14] The microscope was not moved during this time to allow repeated assessment of blood flow in the same capillaries. PCBF and systemic cardiorespiratory parameters were recorded before the start of the test solutions (t_0), after the infusion (t_1), and 1 hour thereafter (t_2). Computer-assisted off-line analysis of the recordings was performed by a blinded member of the team (B.H.) at the conclusion of the experiments. Since systemic circulatory derangement may influence regional perfusion, only animals with stable cardiorespiratory function (mean arterial pressure >90 mm Hg, pO_2 >80 mm Hg, pCO_2 <50 mm Hg, pH >7.25 and <7.55) were included in the final analysis. At the end of the experiment, animals were killed with an overdose of intravenous pentobarbital.

All results are expressed as mean ± standard error of the mean. Changes in continuous variables over time within the same group of animals were analyzed using the paired Student's *t* test, which enabled multiple comparison. Differences among groups were compared by analysis of variance. A P value <0.05 was considered significant.

RESULTS

Systemic cardiorespiratory parameters during the 2-hour observation period remained stable in all but one rat treated with alcohol and endothelin; this animal was subsequently excluded from further analysis. There were no significant differences in these parameters between the experimental groups at any time point (Table I). PCBF before the administration of the test solutions was within the normal range of 1.95 to 2.05 nl/min/cap in all experimental groups. After infusion of the test substances, PCBF was unchanged in saline-treated control animals (2.0 ± 0.05 nl/min/cap) and significantly reduced in animals given alcohol or ET-1 (1.7 ± 0.04 nl/min/cap). The combination of alcohol and ET-1 further reduced PCBF (1.5 ± 0.06 nl/min/cap), whereas the endothelin antagonist increased PCBF in the saline-treated animals (2.2 ± 0.05 nl/min/cap) and prevented the reduction of PCBF in animals given alcohol (2.0 ± 0.05 nl/min/cap). Analogous differences between the experimental groups were found 1 hour after infusion (Fig. 1).

DISCUSSION

Reduced regional perfusion and microcirculatory disturbances are believed to play an important role in the pathogenesis of alcohol-associated damage in

Table I. Systemic cardiorespiratory parameters before (t_0) and 1 hour after (t_1) administration of test solutions

Group	(n)	Map (mm Hg)		pO_2 (mm Hg)		pCO_2 (mm Hg)		pH	
		t_0	t_1	t_0	t_1	t_0	t_1	t_0	t_1
Sal + Sal	(6)	121 ± 6	118 ± 5	96 ± 6	90 ± 4	40 ± 2	41 ± 2	7.41 ± 0.01	7.39 ± 0.01
Sal + ET-1	(6)	119 ± 7	122 ± 4	98 ± 5	88 ± 8	39 ± 2	42 ± 3	7.40 ± 0.01	7.36 ± 0.01
Sal + ET-Ag	(6)	120 ± 5	111 ± 5	101 ± 7	93 ± 5	41 ± 2	44 ± 3	7.38 ± 0.02	7.31 ± 0.03
ETOH + Sal	(6)	121 ± 5	116 ± 9	95 ± 7	86 ± 4	40 ± 2	42 ± 5	7.40 ± 0.01	7.35 ± 0.02
ETOH + ET-1	(6)	120 ± 6	117 ± 5	97 ± 6	89 ± 6	38 ± 2	43 ± 2	7.39 ± 0.02	7.34 ± 0.01
ETOH + ET-Ag	(6)	122 ± 7	117 ± 6	99 ± 5	92 ± 4	41 ± 2	44 ± 3	7.39 ± 0.02	7.31 ± 0.02

Sal = saline solution; ET-1 = endothelin-1; ET-Ag = endothelin-A receptor antagonist; ETOH = ethanol.
There were no significant differences between the experimental groups at either time point.

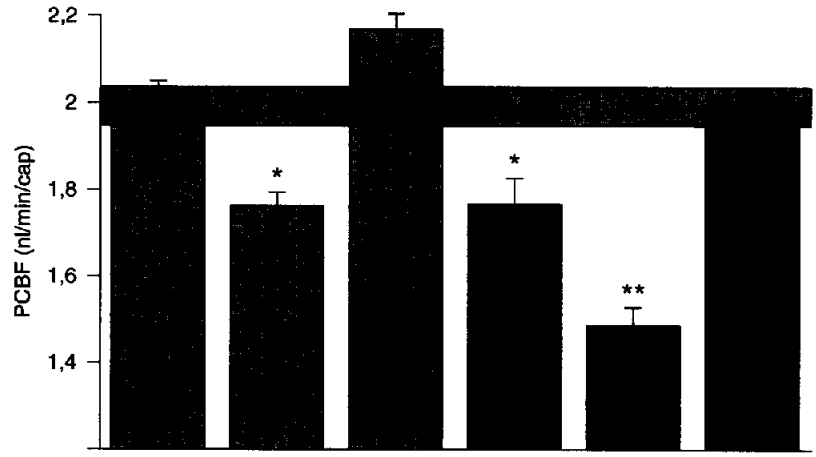

Fig. 1. Pancreatic capillary blood flow *(PCBF)* 1 hour after infusion of test solutions. *=P <0.05 compared to baseline (before administration of test solutions; all baseline values were in the range of 1.95 to 2.05 nl/min/cap) and control animals *(Sal + Sal)*. **=P <0.05 compared to Sal + ET-1 and ETOH + Sal.

many organs including the heart, brain, stomach, and liver.[5,6,17-21] Infusion of ethanol into the portal vein, for example, has been shown to cause vasoconstriction and microvascular disturbances in the liver with subsequent tissue hypoxia and hepatocellular injury[22,23]; in the gastric mucosa intragastric as well as intravenous ethanol administration decreases hemoglobin oxygen saturation and causes mucosal injury.[21,24] The mechanism of the vasoactive ethanol effect, however, is still unknown. Recently it has been demonstrated that alcohol-induced vasoconstriction and microcirculatory changes in the liver and stomach are associated with an increase in the vasoactive peptide endothelin.[9,25,26] This, together with the observation that ethanol-induced vasoconstriction can be reduced by endothelin antibodies,[24] has suggested that the effect of alcohol on the vasculature is mediated by endothelin.

The hypothesis that alcohol-associated changes in pancreatic perfusion may also be mediated by endothelin is based on the following three observations: (1) alcohol causes the release of ET-1 from the feline pancreas,[10] (2) endothelin receptor expression is increased in endothelial and ductal pancreatic cells in patients with chronic alcoholic pancreatitis,[27] and (3) small doses of (exogenous) endothelin reduce pancreatic and pancreatic capillary blood flow without altering systemic hemodynamic parameters, indicating high pancreatic susceptibility to endothelin.[12,28] These observations, together with the present findings of (1) a comparable PCBF decrease by alcohol and (exogenous) endothelin and (2) prevention of the alcohol-induced reduction in PCBF by endothelin receptor blockade, strongly support the hypothesis that endothelin mediates the alcohol-induced reduction in PCBF and may therefore play a critical role in the

pathogenesis of ethanol-induced pancreatic injury through impairment of perfusion and subsequent ischemia.

The 2 g/kg alcohol dose used in the present study was chosen based on data from previous investigations showing that this dose of alcohol produces blood ethanol levels between 1500 and 2000 mg/L and reduces PCBF and oxygenation without significantly altering systemic cardiorespiratory parameters in young female Wistar rats.[4,16,29] Despite the difference from the clinical situation with comparable blood ethanol concentrations often causing respiratory acidosis or episodes of hypo- or hypertension, we required stable cardiorespiratory function since derangement of these parameters may compromise the interpretation of local microcirculatory changes. The doses of endothelin-1 and the specific endothelin A receptor blocker *LU-135252* were also chosen on the basis of previous experiments since they likewise did not significantly alter systemic hemodynamic parameters in rats.[30,31] Increased PCBF in animals treated with the endothelin antagonist (as compared to saline-treated control animals) could be explained by the blockade of endogenous endothelin, which may be increased after laparotomy and exposure of the pancreas.

The finding that the combination of alcohol and (exogenous) endothelin further reduced PCBF (compared to the already decreased values found in animals treated with alcohol or ET-1 alone) indicates that endogenous endothelin released by alcohol and exogenous endothelin has a synergistic effect and agrees with the observation that the effect of endothelin on the vascular system is dose dependent.[8] Despite this additive effect on pancreatic perfusion, the systemic hemodynamic parameters remained stable (in all but one animal treated with alcohol plus ET-1) indicating that there is no correlation between the local and the systemic action of endothelin. This agrees with endothelin measurements in different organs, which appeared to be too low to affect more than the regional vascular beds.[18,21,26] In addition, endothelin susceptibility of different vascular beds varies, probably in relation to endothelin receptor expression.[32,33]

In the present study we did not measure the release of endothelin or compare regional and systemic plasma levels since other groups previously demonstrated increased endothelin in cultured endothelial cells, perfused vessels, and regional perfusion including pancreatic venous blood a few minutes after alcohol administration.[8-10,34] The temporal course of changes in regional ethanol and ET concentrations, however, which have not yet been investigated in detail either, would be of great interest because it is not known at present why low blood alcohol concentrations cause an increase in splanchnic blood flow.[35] Furthermore, it has not yet been determined whicl member of the endothelin family and which receptor: are mainly involved. Our findings suggest that ET-1 i: the main mediator of alcohol-induced changes, a: least in pancreatic microcirculation, since the en· dothelin antagonist effective in the present study i: specific to the endothelin A receptor, which selec· tively binds ET-1.[36]

The mechanism by which endothelin may cause the reduction in regional blood flow is not clear. Firs described as a vasoconstrictor,[37] endothelin is pres· ently considered to be a multifunctional cytokine.[32,3: Masuda et al.[21] observed gastric mucosal congestior in association with alcohol and endothelin and sug· gested that endogenous endothelin (released by alco· hol) may concentrate in the venules and cause venula: constriction. In the present experiment we did not de· tect any vasomotion at the capillary level or notable vasoconstriction or vasodilatation when examining vessels upstream of the capillaries. Therefore we tenc to support the concept that endothelin leads tc changes within the endothelial cells. This hypothesi: is in agreement with the observation of Kvietys e al.[38,39] who demonstrated (endothelin-mediated? leukocyte endothelial interaction and neutrophil· associated endothelial cell injury after alcohol admin· istration in the gastric mucosa. These changes coulc easily cause a reduction of capillary blood flow and a: increase in microvascular permeability, which ha: been observed in other experiments in rats exposed tc ET-1.[40,41]

CONCLUSION

Although we cannot draw any firm conclusions re· garding mechanisms, our observation that PCBF i comparably decreased by alcohol and small doses o exogenous ET-1 and that the alcohol-induced reduc tion in PCBF can be aggravated by ET-1 and pre vented by a specific endothelin-1 antagonist provid· strong evidence that ET-1 is the mediator of alcohol associated pancreatic perfusion disturbances. Thes· findings suggest that ET-1 produced in response tc alcohol plays a critical role in the pathogenesis o ethanol-induced pancreatic injury through pancreati· microcirculation impairment, thereby increasing th· susceptibility of the pancreas to injury.

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Discussion

Dr. S. Strasberg (St. Louis, Mo.). I noted that the dose of alcohol you used was 2 g/kg/hr. For a 70 kg man, that works out to be the equivalent of approximately half a bottle of whiskey in an hour. Since you only experienced a moderate 25% reduction in blood flow, have you performed dose-response studies for alcohol?

Dr. Foitzik. We have done this in previous studies. If higher doses of alcohol are used, there will be changes in the systemic cardiorespiratory parameters. We do not perform experiments under these conditions because then the effect of alcohol on the local microcirculation cannot be interpreted. A dose of 2 g/kg alcohol given over 1 hour is the maximal dose that we found can be used in these animals without causing significant alterations in the systemic cardiorespiratory parameters. You are indeed correct—the dose is relatively high.

Dr. Strasberg. What effect do you think a 25% reduction in pancreatic blood flow has on the induction of inflammation in the organ. You can reduce blood flow to the liver by 25% and the liver does not seem to be adversely affected.

Dr. Foitzik. The pancreas is very susceptible to isc emia. Nevertheless, a 25% reduction in pancreatic blc flow does not injure the pancreas, per se. However, if al hol is superimposed on other noxious agents, for examp exocrine hyperstimulation, pancreatic cell injury will occ such is not the case when animals are exposed to exocr hyperstimulation alone. To my knowledge, the liver is 1 adversely affected by this amount of alcohol, but the g tric mucosa is.

Dr. M. Sunamura (Sendai, Japan). I think this red tion in pancreatic microcirculation is very significant. I you detect any change in pancreatic parenchyma after t reduction in pancreatic microcirculation.

Dr. Foitzik. I completely agree with you. We are 1 speaking of microcirculation. I only presented the data PCBF. Microcirculation comprises several other factors a we are evaluating these parameters right now. We : studying leukocyte endothelial interaction, vascular perm ability, and functional capillary density, which I think are important as capillary blood flow.

Evolving Management of Mild-to-Moderate Gallstone Pancreatitis

Sadeesh K. Srinathan, M.D., Jeffrey S. Barkun, M.D., Shailesh N. Mehta, M.D., Jonathan L. Meakins, M.D., Alan N. Barkun, M.D.

The objective of this study was to describe recent trends in the management of mild-to-moderate gallstone pancreatitis and assess patient outcomes. Acute gallstone pancreatitis has traditionally been managed with open cholecystectomy and intraoperative cholangiography during the initial hospitalization. The popularization of endoscopic retrograde cholangiopancreatography (ERCP) and laparoscopic cholecystectomy has made a reassessment necessary. Two consecutive time periods were retrospectively analyzed: prior to laparoscopic cholecystectomy (prelaparoscopic era [PLE]) and after the diffusion of laparoscopic cholecystectomy (laparoscopic cholectomy era [LCE]). There were 35 patients in the PLE group and 58 in the LCE group. LCE patients waited 37.1 ± 63 days from admission until cholecystectomy, compared to 9.8 ± 14.8 days in the PLE group ($P = 0.04$). Biliary-pancreatic complications occurred in 24% of LCE patients and only 6% of PLE patients ($P = 0.05$), nearly always while they were awaiting cholecystectomy ($P = 0.009$). Patients in either time period who underwent cholecystectomy with intraoperative cholangiography developed less pancreatic-biliary complications than those who underwent ERCP prior to cholecystectomy, with or without sphincterotomy. Delaying the interval from pancreatitis to laparoscopic cholecystectomy beyond historical values is associated with a greater risk of recurrent biliary-pancreatic complications, which are not prevented by the use of ERCP. Early cholecystectomy with intraoperative ductal evaluation is still the approach of choice. (J GASTROINTEST SURG 1998;2:385-390.)

Although the incidence of acute pancreatitis depends on the referral population, the prevalence ranges from 54 to 238 cases per million.[1,2] Approximately one half of all cases of pancreatitis are attributable to biliary tract stone disease and serious complications may occur in 20% to 30% of cases with an overall case-fatality rate of approximately 10%.[2-4]

The traditional management of mild-to-moderate gallstone pancreatitis is based on the principle of early supportive care followed by an open cholecystectomy during the initial admission once the acute manifestations have resolved.[5-7] Since the early 1990s, however, laparoscopic cholecystectomy has rapidly emerged as the procedure of choice for the treatment of patients with symptomatic cholelithiasis.[8-10] The introduction of this modality, along with the popularization of other minimally invasive diagnostic and therapeutic techniques, has made it necessary to reevaluate the treatment of many biliary tract diseases. A retrospective study was thus undertaken to describe the impact of these modalities in the management of gallstone pancreatitis and their effect on patient outcome.

PATIENTS AND METHODS

Data were collected from the prospective McGill University endoscopic retrograde cholangiopancreatography (ERCP) and laparoscopic cholecystectomy (LC) databases. Supplemental information was obtained from retrospective hospital chart review and telephone interviews. All patients with a diagnosis of acute gallstone pancreatitis treated at one of three

From the Department of Surgery (S.K.S., J.S.B., and J.L.M.) and the Division of Gastroenterology (S.N.M. and A.N.B.), The McGill University Health Center, McGill University, Montréal, Québec, Canada.

Drs. J.S. Barkun and A.N. Barkun are Chercheurs Cliniciens Boursier of the Fonds de la Recherche en Santé du Québec.

Dr. S.M. Mehta is the recipient of an American Digestive Health Foundation/American Society of Gastrointestinal Endoscopy Training Award.

Reprint requests: Dr. Jeffrey S. Barkun, Department of Surgery (Room S10.30), The Royal Victoria Hospital, 687 Pine West, Montréal, Québec, Canada H3A 1A1.

McGill University teaching hospitals between April 1991 and July 1992 were candidates for the study if they had survived the initial episode of pancreatitis.

The diagnosis of acute gallstone pancreatitis required all of the following criteria: epigastric pain, hyperamylasemia (serum amylase level greater than twice normal), and a documented gallstone or common bile duct stone on ultrasound, or at cholangiography. Patients were excluded from the study if a history of alcohol abuse was reported or if hypertriglyceridemia or hypercalcemia was noted on laboratory testing. Attempts were made to contact all patients for follow-up by phone. Patients were classified as belonging to the prelaparoscopic cholecystectomy era (PLE) if they were admitted before September 1, 1991, and to the laparoscopic cholecystectomy era (LCE) if they were admitted after September 1, 1991. This particular date was chosen as the cutoff because it allowed us to compare the *approach* to gallstone pancreatitis across both time periods rather than simply the effects of the method of cholecystectomy. It represents the time by which the laparoscopic technique had become the modality of choice for the management of cholelithiasis at the McGill University hospitals and by which all surgeons involved in this study had concluded their LC "learning curve."

Demographic information included age and sex. Baseline variables included serum bilirubin, amylase, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) values and the duration of symptoms prior to admission. The premorbid condition of patients was assessed by determining the American Society of Anesthesiologists (ASA) physical status score.[11] The clinical severity of the attack of pancreatitis was graded according to the number of Ranson's criteria that were present in the first 24 hours.[12] The severity of disease on CT scans was graded from A to E, as previously reported in the literature.[13] Imaging results from ultrasound, CT scans, and ERCP examinations as well as operative findings were reviewed and recorded. Procedural variables across both time periods included the number of cholecystectomies performed (laparoscopic or open), intraoperative cholangiograms, ERCPs, and the time interval between the index case of pancreatitis and cholecystectomy.

Patient outcome variables included total duration of hospital stay, duration of postoperative stay, and total complications. Total complications included biliary-pancreatic and ERCP-related complications. A biliary-pancreatic complication was defined as any complication related to the presence, persistence, or recurrence of cholelithiasis or choledocholithiasis (severe biliary colic, cholecystitis, cholangitis, pancreatitis, or pseudocyst). ERCP-related complications were those temporally related to the performance of the procedure including bleeding, pancreatitis, cholangitis, perforation, and basket impaction.[14] To avoid the confounding related to the increased use of ERCP in the LCE group, we did not count ERCP-related pancreatitis as a "biliary-pancreatic complication" when comparing the PLE and LCE groups.

Statistical Analysis

Normally distributed continuous variables are expressed as mean ± standard deviation. Continuous variables with a skewed distribution are described with medians and ranges, or mode. Wherever possible, 95% confidence intervals (CI) around point estimates of proportions were given using the normal approximation of the binomial distribution.

Between-group comparisons of continuous variables were carried out using Student's t test or nonparametric testing where applicable. Between-group comparisons of categorical variables were performed using contingency table analysis: Chi-square or Fisher's exact tests, where appropriate. A P value of 0.05 or less was considered significant.

RESULTS

Of the 476 charts reviewed, 93 satisfied the criteria for inclusion in the study. The remaining 383 patients were excluded because of an erroneous diagnosis, inability to satisfy the study definition of gallstone pancreatitis, the presence of chronic pancreatitis, or missing data. There were 35 patients in the PLE group and 58 patients in the LCE group. The mean follow-up time was 20.9 months (range 8.5 to 38.1 months) with a follow-up rate of 76.3%, as achieved by means of telephone interviews. There were no differences between the two groups in terms of age, sex distribution, or premorbid condition (ASA score) (Table I). Nearly all patients presented with mild pancreatitis, as reflected by the 24-hour Ranson score and CT grade. There was no difference in biochemical or ultrasound findings across time periods (data not illustrated).

The proportion of patients undergoing cholecystectomy was similar in the PLE and LCE groups (71% vs. 74%, respectively). In the LCE group the majority of patients underwent LC, although 28% still had an open cholecystectomy either as a primary procedure or as a conversion (Table II).

Patients in the LCE group underwent intraoperative cholangiography less frequently than did those in the PLE group: 26% (95% CI = 10% to 31%) vs. 68% (95% CI = 31% to 66%) (P <0.05), but they underwent ERCP more often: 57% (95% CI = 43%

Table I. Baseline variables

	PLE (n = 35)	LCE (n = 58)	*P* value
Age (yr)*	60.5 ± 19.9	59.5 ± 19.0	NS
Females	21 (60%)	40 (69%)	NS
Ranson's score at 24 hours†	1	1	
CT grade†	A	A	
ASA score†	1	1	
Telephone follow-up	23 (66%)	42 (72%)	NS
Time to follow-up (mo)*	29.2 ± 4.6	15.1 ± 5.0	

NS = not significant.
*Mean ± standard deviation.
†Mode.

Table II. Procedural variables

	PLE (n = 35)	LCE (n = 58)	*P* value
Cholecystectomy	25 (71%)	43 (74%)	NS
Open	25 (100%)	12 (28%)	
Laparoscopic	0	31 (72%)	
Intraoperative cholangiogram	17 (68%)	11 (26%)	<0.05
ERCP	7 (20%)	33 (57%)	<0.05
No sphincterotomy	2 (28%)	14 (42%)	<0.05
Sphincterotomy	5 (72%)	19 (58%)	NS

Table III. Measures of outcome

	PLE	LCE	*P* value
Mean total hospital stay (days)	16.3 ± 16.4	14.5 ± 11.5	NS
Hospital stay due to pancreatitis (days)	7.0 ± 3.4	9.1 ± 4.6	NS
Median postoperative hospital stay (days)	9	4	NS
Interval from admission to cholecystectomy (days)	9.8 ± 14.8	37.1 ± 63	0.04

Values are mean ± standard deviation except where indicated.

Table IV. Complications

	PLE	LCE	*P* value
Total complications	11 (31%)	27 (47%)	NS
Biliary-pancreatic complications	2 (6%)	14 (24%)	0.05
Biliary-pancreatic complications during interval to cholecystectomy	0	12 (87%)	0.009

to 70%) vs. 20% (95% CI = 8% to 37%) (P <0.05). The use of ERCP in LCE patients did not depend on the type of cholecystectomy performed (P = 0.85). Of the LCE patients undergoing ERCP, 58% (95% CI = 29% to 96%) had a sphincterotomy compared to 72% (95% CI = 39% to 75%) in the PLE group (P >0.05) (see Table II). An endoscopic sphincterotomy was performed in all cases where a common bile duct stone was suspected at ERCP.

There were no differences between groups in the durations of total hospital stay or hospital stay required for the episode of pancreatitis (Table III). Patients in the LCE group waited a mean of 37.1 ± 63 days from admission until cholecystectomy as compared to 9.8 ± 14.8 days for the PLE group (P = 0.04). The total complication rates (Table IV) were similar in both groups: 27 (47%) in the LCE group and 11 (31%) in the PLE group. ERCP was responsible for one complication in a PLE patient (14%; 95% CI = 0.4% to 58%) and two complications in the LCE group (6.1%; 95% CI = 0.7% to 20%); these were all episodes of mild pancreatitis.

Table V. Relationship between ERCP and occurrence of a biliary-pancreatic complication during the interval to cholecystectomy*

	Biliary-pancreatic complications	No biliary-pancreatic complications
ERCP	7	33
No ERCP	3	50

*$P = 0.09$.

Table VI. Relationship between ERCP with sphincterotomy and occurrence of a biliary-pancreatic complication during the interval to cholecystectomy (all patients except two with post-ERCP pancreatitis)*

	Biliary-pancreatic complications	No biliary-pancreatic complications
Sphincterotomy	4	13
No sphincterotomy	6	70

*$P = 0.08$.

As defined in the Methods section, biliary-pancreatic complications were compared without including ERCP-related pancreatitis. Fourteen patients (24%; 95% CI = 17% to 41%) in the LCE group had a biliary-pancreatic complication vs. two (6%; 95% CI = 2% to 23%) in the PLE group ($P = 0.05$). The biliary-pancreatic complications in the PLE group consisted of recurrent pancreatitis in one patient and pseudocyst formation in another. Complications in the LCE group included recurrent pancreatitis in six, cholecystitis in four, and cholangitis, biliary colic, and pseudocyst in one case each. One patient in the LCE group died of multiple organ failure following recurrent pancreatitis.

In assessing the timing of the biliary-pancreatic complications, 87% of those in the LCE group occurred while the patients were awaiting cholecystectomy. In the PLE group both biliary-pancreatic complications occurred postoperatively and none occurred during the interval to cholecystectomy ($P = 0.009$).

The relationship between ERCP and the occurrence of a biliary-pancreatic complication during the wait for cholecystectomy was then analyzed in all patients irrespective of the time period to which they belonged. The performance of an ERCP did not protect patients against the subsequent occurrence of a biliary-pancreatic complication (Table V). In fact, performing an ERCP with or without a sphincterotomy (Table VI) was associated with a three times greater likelihood of developing a biliary-pancreatic complication (not significant). Furthermore, this is true even though all ERCP-related episodes of pancreatitis h been excluded from this part of the analysis, thus u derestimating the overall incidence of pancreatitis these patients.

DISCUSSION

For many years now, early cholecystectomy duri the index admission has supplanted prolonged cc servative management with delayed operative int vention in the treatment of mild-to-moderate ga stone pancreatitis.[15-18] This approach was shown have reduced both the likelihood of recurrent pa creatitis and the total duration of hospital stay.[5-7] reassessment of this treatment algorithm has be brought about by the recent emergence of LC and t suggested role of ERCP in early acute severe bili pancreatitis.[19-21] The purpose of the present study v to assess the impact of these on the management mild-to-moderate acute gallstone pancreatitis wh emphasizing changes specifically occurring with t introduction of LC.

Data from the McGill University LC and ER registries confirmed that the baseline characterist of the patient populations and the episodes of panc atitis were similar before and after the introduction LC. However, a clear change in the treatment alg rithm was observed across time periods. In the P period, patients underwent cholecystectomy with traoperative cholangiography during the initial a mission; in the LCE period, however, most patie underwent preoperative ERCP and were then sch uled for elective LC, often during a subsequent a mission. This change in utilization of ERCP v characteristic of all patients in the LCE group, ir spective of whether they had undergone a lapa scopic or open cholecystectomy, thus reflectin change in overall *approach* rather than an isolated fect of the use of LC. This phenomenon was also su ported by the finding that the increased referral of tients for ERCP was not related to any specific b chemical, radiologic, or clinical factor. This change philosophy expectedly resulted in more liberal use ERCP as a diagnostic modality for patients in LCE group, which is reflected by a drop in the int operative cholangiography rate when compared to PLE group. Some of these observations have alre been made by others.[22-24]

The total duration of hospital stay in both time riods was principally related to the index episode pancreatitis. Although the duration of postoperat hospitalization was similar in both groups, a shor stay in the LCE group (median of 9 vs. 4 days, spectively) may have been obscured by the small sa ple size (see Table III).

The most notable difference between the PLE and LCE groups was the mean duration of time from the initial admission for acute gallstone pancreatitis to subsequent cholecystectomy (9.8 ± 14.8 days vs. 37.1 ± 63 days, respectively; $P = 0.04$). Because of the retrospective nature of this study, reasons for the delay in performing LC can only be hypothesized, although they represent an important characteristic of the philosophy guiding the approach in the LCE group. They may relate to any of the following: operating room or ERCP scheduling conflicts, institutional pressure for early patient discharge, or the erroneous perception that the acute pancreatic inflammatory process may somehow lead to an increase in the rate of conversion to open surgery (indeed this has been disproved).[25] A further possibility, which we favor, may have been the belief that performing an ERCP soon after resolution of the pancreatitis would protect against recurrent episodes of pancreatitis, thus allowing the surgeon to delay the date of the cholecystectomy.[26,27] This last point will specifically be addressed later on in this article.

The overall complication rates were similar for both time periods (see Table IV). However, a significant increase in pancreatitis and other biliary-pancreatic complications was identified in LCE patients as compared to PLE patients (24% vs. 6%, respectively; $P = 0.05$). This difference was primarily related to complications occurring while patients were waiting to undergo cholecystectomy. Because episodes of pancreatitis related to ERCP were excluded from these calculations, the occurrence of biliary-pancreatic complications was most likely related to the duration of time between the episode of pancreatitis and cholecystectomy, as suggested in the prelaparoscopic literature[5-7,15-18] and recently confirmed by others.[22,28,29] There were 12 such complications in the LCE group and none in the PLE group ($P = 0.009$).

The results summarized in Tables V and VI address the hypothesis that performing an ERCP (with or without sphincterotomy) might keep a patient from developing biliary-pancreatic complications in the interval prior to cholecystectomy. ERCP did not reduce the incidence of such complications, even though ERCP-related pancreatitis had been excluded from the analysis. In fact, there was a trend suggesting that patients referred for ERCP in the interval to cholecystectomy were at increased risk for the development of biliary-pancreatic complications, even though the reasons for referral for ERCP were similar to those among patients requiring intraoperative cholangiography. Although this finding may not be surprising for diagnostic ERCP, performing a sphincterotomy also did not appear to decrease the likelihood of developing a biliary-pancreatic complication among patients awaiting cholecystectomy (see Table VI). Possible reasons for this may relate to factors such as the inadequacy of the size of the sphincterotomy,[30] or other variables that were not readily available for comparison in our study population.

These findings, in conjunction with other reports, cast doubt over the role of preoperative ERCP in the routine management of mild-to-moderate acute gallstone pancreatitis for several reasons. In this context, ERCP has been reported to exhibit a low diagnostic yield in detecting common bile duct stones,[31-33] even though this rate was high in the present series of patients. Furthermore, ERCP carries significant morbidity; indeed it was associated with a 7.5% (3/40; 95% CI = 1.6% to 20.4%) overall rate of post-ERCP pancreatitis in the present study. Last, we have shown in our patients that neither diagnostic nor therapeutic ERCP appeared to protect against recurrent biliary complications.

A more judicious approach is thus to perform intraoperative cholangiography at the time of LC; if a common bile duct stone is found, the patient may then undergo either operative clearance or postoperative ERCP. These conclusions have been confirmed by preliminary results from decision analysis modeling.[34]

CONCLUSION

Although LC is safe in the treatment of patients with mild-to-moderate gallstone pancreatitis, a delay in performing LC is associated with a significantly greater and unacceptable risk of recurrent biliary-pancreatic complications. Furthermore, preoperative ERCP in this context does not appear to reduce the incidence of these complications in the interval to cholecystectomy. Early LC with intraoperative ductal evaluation should therefore be the preferred approach. If these observations are confirmed by others, ERCP in the context of acute mild or moderate gallstone pancreatitis can be reserved for patients with proven concomitant choledocholithiasis or for postoperative patients with a suspected common bile duct stone.

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Effect of Endotoxin on Canine Colonic Motility and Transit

Stephen T. Spates, M.D., Joseph J. Cullen, M.D., Kimberly S. Ephgrave, M.D., Marilyn M. Hinkhouse, B.S.

Diarrhea is a common problem in patients who have episodes of sepsis and are being fed enterally. Endotoxemia results in gastrointestinal motor dysfunction characterized by slowed gastric emptying and rapid intestinal transit; however, the effect of endotoxin on colonic motility is unknown. The aim of our study was to determine the effects of a single sublethal dose of endotoxin on colonic motility and transit. Seven dogs underwent construction of a 50 cm colonic Thiry-Vella fistula. Five manometry catheters were sewn into the colonic lumen at 8 cm intervals along the fistula. Following recovery, the fistula was perfused with an isotonic solution at 2.9 ml/min, and fasting and postprandial colonic motility was determined. Liquid transit was assessed by bolus of a nonabsorbable marker instilled into the proximal end of the Thiry-Vella fistula. Recordings of gastrointestinal contractile activity were made digitally to determine contractile frequencies and motility indexes. Following completion of the baseline studies, each dog was given a single dose of *E. coli* lipopolysaccharide, 200 μg/kg intravenously, and studies were repeated daily for the next 3 days. Endotoxin doubled the fasting colonic contractile frequency on postendotoxin day 1 and also increased motility indexes on that same day. Fasting motility indexes and contractile activity were decreased on postendotoxin days 2 and 3. The postprandial frequency of contractions and motility indexes were decreased on postendotoxin day 3. Fasting colonic liquid transit was rapid on postendotoxin day 1, whereas postprandial liquid transit was rapid on both postendotoxin days 1 and 2. Endotoxin temporarily speeds liquid transit and increases both the frequency and strength of colonic contractions. These effects may contribute to the diarrhea that occurs during episodes of sepsis. (J GASTROINTEST SURG 1998;2:391-398.)

Diarrhea is a common problem in patients who have episodes of sepsis and are being fed enterally and has been estimated to occur in 10% to 20% of critically ill patients.[1] Common causes of diarrhea in critically ill patients include hypoalbuminemia, hyperosmolar formulas, antibiotics, and infections.[2] Unfortunately, more than 50% of patients cannot be adequately treated by changing dietary factors to correct osmotic diarrhea.[3] Thus other pathophysiologic causes of the diarrhea, including secretory or exudative factors or diarrhea secondary to altered intestinal motility, must be considered.

The mechanisms of altered motility in diarrhea states are poorly understood, but it is likely that disordered colonic motility plays a role in many types of diarrhea. Colonic transit is rapid during diarrheal states, even though overall colonic motor function may be decreased. Colonic contractile activity includes individual phasic contractions, which are organized into groups of contractions separated by quiescent periods. These contractile states, sometimes referred to as colonic motor complexes,[4] propagate variable distances, mostly in the aborad direction but sometimes in the orad direction. In diarrheal states the absence of phasic contractions, which slow transit and represent the majority of colonic activity, results in rapid colonic transit. Colonic contractile activity also includes large-amplitude contractions, sometimes referred to as giant migrating contractions. These contractions are of long duration and large amplitude, and propagate over a greater length of colon. Giant migrating contractions in the distal colon are thought

From the Department of Surgery, University of Iowa College of Medicine and Veterans Affairs Medical Center, Iowa City, Iowa.
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Reprint requests: Joseph J. Cullen, M.D., 4622 JCP, University of Iowa Hospitals and Clinics, Iowa City, IA 52242.

to provide the force to rapidly move feces during defecation. These large-amplitude contractions, especially in distal colonic segments, can also influence transit resulting in frequent defecation. Thus loss of phasic contractions (resulting in rapid colonic transit) or an increase in the number of large-amplitude migrating contractions (propelling the fecal stream aborad) may both contribute to the motility disorder resulting in diarrhea.

Endotoxemia results in gastrointestinal motor dysfunction characterized by slowed gastric emptying and rapid intestinal transit. However, little is known about the effect of endotoxin on colonic motility and transit. Inflammatory disorders of the colon are frequently accompanied by diarrhea, which is related to changes in motor activity of the sigmoid colon.[5] Previous studies from our laboratory have indicated that endotoxemia has profound effects on colonic transit.[6] Colonic transit of solids in dogs was slowed 24 hours after a single sublethal dose of endotoxin. Thus the aim of our study was to determine the effects of a single sublethal dose of endotoxin on colonic motility and transit.

MATERIAL AND METHODS

Preparation of Animals

All procedures, care of animals, and conduct of experiments were carried out according to the protocol approved by the Veterans Administration and University of Iowa Animal Care and Use Committees. Surgical procedures and experiments were performed in accordance with the "Guide for the Care and Use of Laboratory Animals," published by the United States Public Health Service. Seven conditioned mongrel dogs weighing 12 to 18 kg were anesthetized with thiopental sodium (25 mg/kg) and halothane. Under aseptic operating conditions, dogs underwent construction of a colonic Thiry-Vella fistula. Briefly, a 50 cm segment of colon starting at the cecum and ending at the sigmoid colon was isolated, and the anatomic continuity of the remaining bowel was reestablished by an ileorectostomy end-to-end anastomosis. Five perfused, noncompliant intraluminal polyethylene manometry catheters (outer diameter = 2.25 mm; internal diameter = 1.25 mm) were sewn into the colonic lumen of the TVF at 8 cm intervals beginning 5 cm from the proximal end. All manometry catheters were embedded in a stainless steel cannula positioned in and anchored to the anterior abdominal wall. A metal perfusion cannula was inserted through the appendiceal orifice into the proximal end of the colonic loop, and the proximal end was oversewn. The distal end of the colon was connected to a modified Thomas cannula, which was then brought through the abdominal wall. The animals were allowed to recover for 2 weeks before baseline studies were begun. During the recovery period, the cannulas were flushed periodically with 0.9% NaCl to prevent mucous plugging.

Conduct of Experiments

After an 18-hour fast, the dogs were placed in a Pavlov sling, and fasting and fed studies of absorption and transit were begun. The distal cannula was irrigated with 30 ml of 0.9% NaCl to clear it of debris. The Thiry-Vella fistula was perfused via the proximal cannula at 2.9 ml/min using a pump (Masterflex pump, Cole-Parmer Instrument Co., Niles, Ill.) with warmed (37° C) solution containing 120 mmol/L NaCl, 20 mmol/L $NaHCO_3$, 5 mmol/L KCl, 5.6 mmol/L glucose, and 5 g/L polyethylene glycol (PEG). The concentration of PEG in the sample was determined by a turbidimetric method[14] using a spectrophotometer (Coleman Junior II, Coleman Instruments, Maywood, Ill.) to calculate the recovery of PEG. At the beginning of each study, the manometric catheters (perfused at a rate of 0.3 ml/hr with a low-compliance capillary infusion system using deionized, degassed water) were connected to pressure transducers (Viggo-Spectramed model DT-XX, Spectramed Inc., Oxnard, Calif.). Manometry signals were converted from an analogue to a digital signal and relayed directly into a real-time digital acquisition system controlled by a personal computer (Hewlett-Packard 75000 series B mainframe, Mountain View, Calif.), which allowed display of the data as they were acquired at a rate of 3 Hz and later storage of the data on hard disk. The stored data were analyzed using the DADiSP software package (DADiSP/windows version 3.01C; DSP Development Corp., Cambridge, Mass.). The infusion was continued for 1 hour before any measurements of motility or transit were done. Following measurement of colonic motility for 2½ hours, liquid transit was determined by instilling a bolus of a nonabsorbable marker, phenolsulfonphthalein (PSP, 0.125 mg in 0.5 ml volume) into the proximal perfusion catheter. Sampling was continued from the distal cannula, and content was collected every 2 minutes for the next 25 minutes. Aliquots were assayed for determination of PEG and PSP concentrations. Recovery of PSP was corrected for fractional intraluminal content recovery determined using PEG recovery. The time from bolus to recovery of one half of the PSP was chosen as the transit time for the 50 cm colonic segment. Following the fasting studies, the dogs were gavage fed a 240 kcal liquid meal consisting

of 240 ml of Sustacal (Mead Johnson & Company, Evansville, Ind.), and measurements of colonic motility and transit were repeated as in the fasting periods.

Following completion of two baseline studies on each dog, the endotoxin studies were begun. After an overnight fast, each dog was given a single bolus infusion of *Escerichia coli* lipopolysaccharide, serotype 055:B5 (Sigma Chemical Co., St. Louis, Mo.), 200 μg/kg intravenously. Colonic infusions and the fasting and fed studies were then begun (postendotoxin day 1) and repeated each day for 2 more days. All dogs were fasted with free access to water throughout the postendotoxin period. After completion of the endotoxin studies, all dogs were killed and postmortem examinations were performed. No dog had any evidence of mechanical small bowel obstruction, perforations, or other intestinal abnormalities.

Analysis of Data

Determination of Colonic Motility. Motility recordings were analyzed with a personal computer using the DADiSP software program to quantitate motility events. Movement artifacts in the manometric data were eliminated by computerized deletion of pressure waves that occurred simultaneously across all manometric recording sites. At each channel, pressure waves greater than 10 mm Hg were recognized and their duration, amplitude, and frequency were recorded. The amplitude of each peak was defined as the distance from the baseline to the apex of the unfiltered peak. The motility index (MI) was defined as $MI = \log_e$ (sum of amplitudes × number of pressure waves + 1) and was calculated separately for the fasting and fed colonic motility and transit studies.

A computer program was created within the DADiSP environment to determine the frequency of giant migrating contractions as previously described by Karaus and Sarna.[4] Briefly, peaks greater than 50 mm Hg from baseline, which occurred in three to five consecutive channels, within a 5-second time window, were considered giant migrating contractions.

Determination of Transit Time. The transit of the liquid isotonic solution was expressed as the time from bolus to recovery of one half of the PSP from the distal cannula and expressed as the half-time (T½) for the 50 cm colonic transit. Two baseline transit studies were performed in each dog and a mean value was obtained.

Statistical Analysis. All results are expressed as mean ± standard error of the mean. One-way analysis of variance with Tukey's test and Wilcoxon rank-sum test were performed to compare the baseline studies with the postendotoxin studies for the motility and transit parameters. Chi-square analysis using a Mann-Whitney U test was used to determine the difference in giant migrating contractions during the baseline and postendotoxin periods. All statistical tests were performed with the Systat statistical software program (Systat Inc., Evanston, Ill.).

RESULTS

Colonic Motility

During the baseline studies, both short- and long-duration contractions were seen throughout the length of the colon (Fig. 1). Immediately following endotoxin bolus, the frequency and amplitude of colonic contractions increased (Fig. 2). Additionally, giant migrating contractions were seen that were also associated with defecation in the dog. No giant migrating contractions were seen during these baseline studies; however, giant migrating contractions were present in four of the seven dogs during the fasting period on postendotoxin day 1 ($P < 0.01$). Giant migrating contractions were absent for the rest of the postendotoxin study days. After 24 hours, during postendotoxin day 2, there was a decrease in both the frequency and strength of colonic contractions and no giant migrating contractions were seen.

Endotoxin doubled the fasting contractile frequency on postendotoxin day 1 when compared to baseline values (Table I). Contractile frequency during fasting decreased on postendotoxin day 2 and remained decreased on postendotoxin day 3 when compared to baseline values. During the fed periods, the same trends were seen with contractile frequency after endotoxin, but not to the same extent as during the fasting period.

Endotoxin also increased the amplitude of colonic contractions, which was reflected in increases in the colonic motility index. The fasting motility index was increased on postendotoxin day 1 as seen with the frequency of contractions. Likewise, the motility index was decreased on postendotoxin day 2 and remained so on postendotoxin day 3. Once again during the fed periods, the same trends were seen with the motility index after endotoxin, but not to the same extent as during the fasting period.

Endotoxin seemed to have a similar effect on both the proximal and distal colon. Compared to the baseline studies, the fasting motility index of the proximal colon was increased on postendotoxin day 1, whereas the contractile frequencies were decreased on both postendotoxin days 2 and 3 (Fig. 3). Compared to the baseline studies, both the fasting contractile frequencies and motility indexes were increased during postendotoxin day 1 in the manometric ports situated in

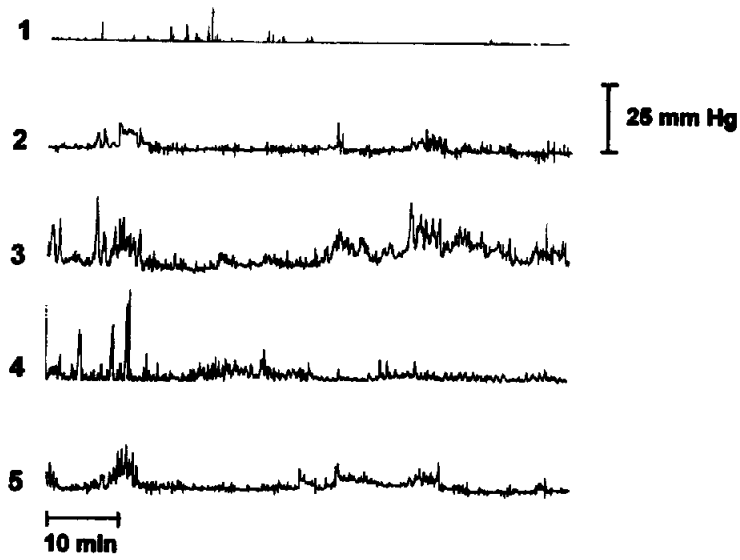

Fig. 1. Baseline fasting colonic motility demonstrating the pattern of colonic motor complexes found in the healthy state.

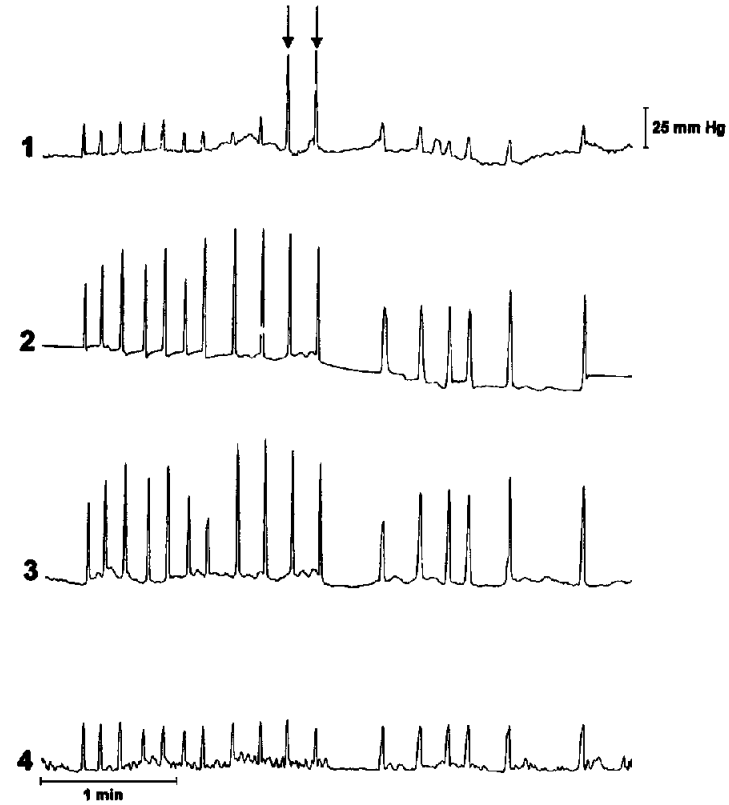

Fig. 2. Effect of endotoxin on canine fasting colonic motor activity (postendotoxin day 1). *E. coli* lipopolysaccharide, 200 μg/kg intravenously, results in an increase in both the frequency and strength of colonic contractions. Additionally, two giant migrating contractions (arrows) occur beginning at the first manometry site and propagating distally to the third manometry site.

Table I. Effect of *E. coli* lipopolysaccaharide on fasting and fed colonic motility and transit

	Baseline	Day 1	Postendotoxin Day 2	Postendotoxin Day 3
Fasting				
Contractions/10 min	12.6 ± 1.3	25.3 ± 6.5	6.8 ± 1.3*	5.8 ± 1.0*
MI/10 min	225 ± 17	730 ± 254*	133 ± 39*	110 ± 23*
T½ (min)	13.3 ± 2.4	7.5 ± 1.4*	12.5 ± 3.2	13.0 ± 2.8
Fed				
Contractions/10 min	9.3 ± 1.5	14.5 ± 5.6	5.85 ± 1.3	5.2 ± 1.0*
MI/10 min	152 ± 25	508 ± 290	104 ± 25	80 ± 17*
T½ (min)	13.1 ± 1.7	9.1 ± 1.7*	11.6 ± 1.6*	16.7 ± 3.6

MI = motility index; T½ = half-time.
Values are means ± standard error of the mean; n = 7.
*$P < 0.05$.

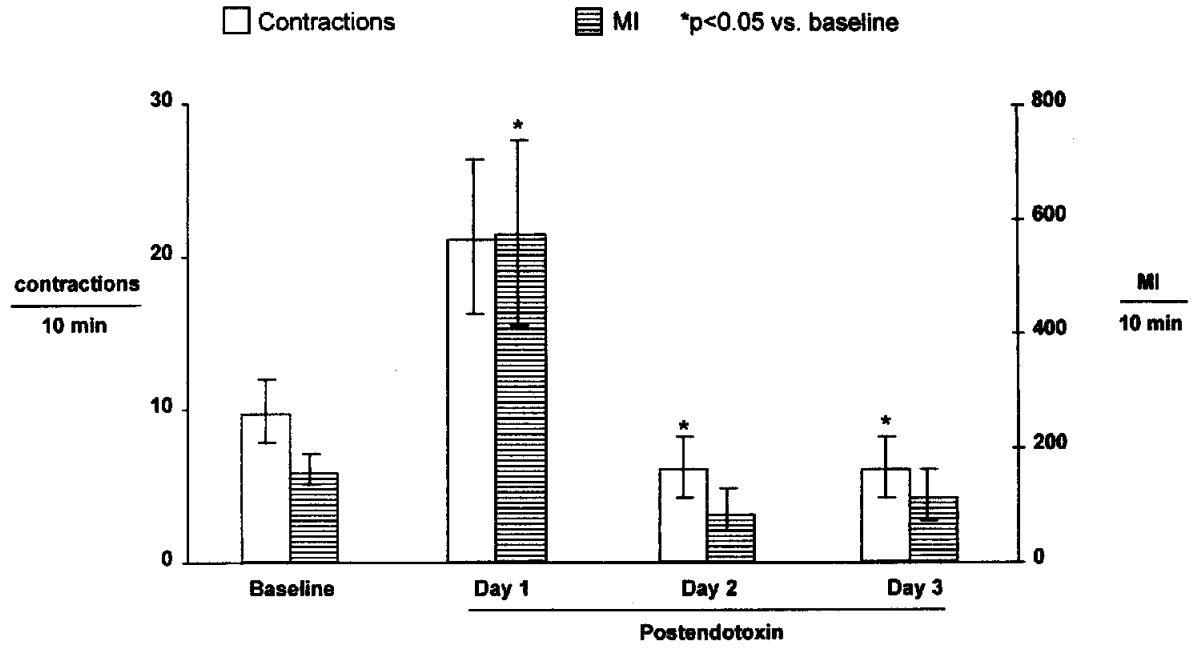

Fig. 3. Effect of *E. coli* lipopolysaccharide on fasting proximal colonic motility patterns (means ± SEM; n = 7). MI = motility index.

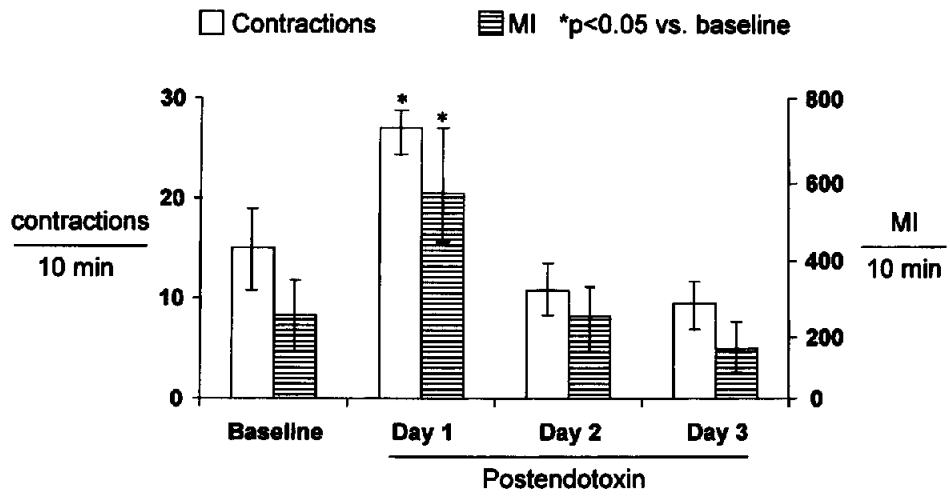

Fig. 4. Effect of *E. coli* lipopolysaccharide on fasting distal colonic motility patterns (means ± SEM; n = 7). MI = motility index.

the distal colon (Fig. 4). In the distal colon there were no differences in the frequency of strength of the contractions during postendotoxin day 2 or 3 when compared to the baseline studies.

Colonic Transit

Transit of the liquid marker was rapid during the fasting period on postendotoxin day 1 when compared to the baseline studies (see Table I). During the digestive period, colonic liquid transit was rapid on both postendotoxin days 1 and 2 when compared to the baseline studies.

DISCUSSION

Diarrhea is not an uncommon occurrence in critically ill patients. Large studies investigating the complications of tube feedings in critically ill patients reveal that up to 68% of patients may have diarrhea.[7] No single variable has been consistently identified as causing tube-feeding diarrhea; however, multiple organ failure and sepsis can be factors associated with diarrhea in this setting.[8,9]

Our study demonstrates that endotoxemia has profound effects on colonic motility. Endotoxin increased the fasting motility index on postendotoxin day 1. Fasting motility indexes and contractile activity were decreased on postendotoxin days 2 and 3. The postprandial frequency of contractions and motility indexes were decreased on postendotoxin day 3. Endotoxin seemed to have similar effects on the proximal and distal portions of the colonic Thiry-Vella loop. Fasting colonic liquid transit was rapid on postendotoxin day 1, whereas postprandial liquid transit was rapid on both postendotoxin days 1 and 2.

Our study correlates with a number of other previous observations regarding colonic motility and transit during diarrheal states. Giant migrating contractions have been seen in dogs in response to pharmacologic agents such as neostigmine and guanethidine.[4] Similarly, in humans, oral administration of diarrhea-producing agents induces multiple mass movement and giant migrating contractions.[10,11] Additionally, clinical diarrhea due to an excessive number of giant migrating contractions has been reported in ulcerative and experimental colitis.[12-14]

Postprandial colonic transit continued to be rapid during postendotoxin day 2, even though the frequency of contractions and the motility index were decreased from baseline studies. Although contractions of the gut propel the ingested food in the caudad direction, an increase in frequency, amplitude, or duration of colonic contractions does not necessarily speed transit. Many reports suggest that motor activity during diarrhea is characterized by a decreased amplitude and a decrease in the percentage duration of contractile activity and yet a more rapid transit.[15-17] Thus the phasic contractions in the normal colon actually delay transit, so that when they decrease in quantity, the transit becomes faster. The rapid transit seen on postendotoxin day 1 may be attributed to the appearance of giant migrating contractions seen on that day, whereas the postprandial transit changes seen on postendotoxin day 2 may be due to the decrease in phasic activity observed on that day.

A recent report from our laboratory suggests that endotoxin temporarily impairs colonic absorption of

water and electrolytes, which may further contribute to the diarrhea seen on postendotoxin day 1.[18] A previous study from our laboratory also demonstrated that a single sublethal dose of endotoxin caused diarrhea on postendotoxin day 1 but a delay in colonic transit of *solids* on postendotoxin day 2.[6] Once again we demonstrated that the dose of lipopolysaccharide used is diarrheogenic in this canine preparation with colonic transit of *liquids* being rapid on postendotoxin day 1. There are, however, major differences in the experimental preparation used in the two studies. Our present study used a colonic Thiry-Vella loop to study both motility and transit, whereas the previous study was performed using the intact unprepared colon of the dog.

CONCLUSION

Endotoxin temporarily speeds liquid transit and increases both the frequency and amplitude of colonic contractions following a sublethal dose of endotoxin. Endotoxin seemed to have a similar effect on the distal colon when compared to the proximal colon. The frequency and strength of colonic contractions decreased on postendotoxin day 2. The changes in colonic motility and the resulting rapid liquid colonic transit may contribute to the diarrhea that occurs during episodes of sepsis.

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Discussion

Dr. B. Schirmer (Charlottesville, N.C.). Your data on transit of liquids would suggest that these contractions probably are coordinated. Have you done the study looking at solid material and have you controlled for the fact that you can often appreciate significant contractions in the colon, but the actual motility is not propagated in a forward pattern?

Dr. J.J. Cullen. We have not studied propagation in the colon. We previously conducted a study, the results of which were presented last year, in which we looked at propagation in the small intestine, and in that study the normal propagation from proximal to distal was disrupted in the small intestine. I do not know of a motility program that looks at propagation of colonic contractions, so we did not study that. We studied transit of solids a number of years ago and found that on postendotoxin day 2 the transit of these spheres is markedly delayed, but that was in a model where the colon was intact and there was no Thiry-Vella loop.

Dr. J.A. Bastidas (Palo Alto, Calif.). What happens to these dogs when they are given the endotoxin? Do these dogs get diarrhea without their colons?

Dr. Cullen. They do get diarrhea. With regard to physiologic parameters, their heart rate increases somewhat, they become somewhat tachypneic, and this is only for the first couple of hours after they receive the endotoxin. Most notably, though, for the first 2 hours they do have wretching and some vomiting, so they have to be given the liquid meal later. We previously showed that gastric emptying of liquids is delayed for 2 days. We have not studied gastric emptying of solids, but I suspect the pattern would be the same.

Dr. J. Pemberton (Rochester, Minn.). It seemed that the first day after endotoxin administration the motility index and the contractions were increased but your $T_{1/2}$s were shorter; if the hypothesis is that short- and long-term contractions in the colon delay transit, then how can you report that result? If indeed this is a mechanism for diarrhea in the critically ill patient, besides giving antibiotics, what type of therapeutic intervention might have an impact on that mechanism?

Dr. Cullen. We showed a marked increase in giant migrating contractions, which were never even seen in the baseline studies, and I think that's what is driving the rapid transit. Then on day 2 we could only look at rough estimates of the motility index and frequency of contractions, and those were both decreased, and I think that resulted in the somewhat rapid transit on that day, so I think there are two mechanisms involved. As far as employing anything other than antibiotics, I am not sure. On day 1 smooth muscle relaxation would be beneficial. Other than that I am not quite sure. We have looked at a number of mediators in smooth muscle neural transmission, including nitric oxide and vasoactive intestinal peptide, and we have not shown an increase of these mediators in the colon as we have shown in the jejunum of dogs.

Dr. L. Anestidou (Houston, Tex.). How long did you record?

Dr. Cullen. The motility tracings go for about 6 hours.

Dr. D. Dempsey (Philadelphia, Pa.). Is there a species difference?

Dr. Cullen. In the small bowel of dogs, endotoxin causes rapid jejunal transit and a definite change in the propagation of jejunal contractions. In the rat, rapid transit is also seen in the jejunum, which is associated with the production of nitric oxide. We have not studied colonic motility in the rat, but I suspect it would be similar to that in the dog. A number of groups studying diarrhea and changes in motility, as mentioned previously, have shown similar contractile frequency and activity in dogs and in humans, as far as experimental colitis and ulcerative colitis are concerned.