Should Pancreaticoduodenectomy Be Performed in Octogenarians?

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As the population in the United States ages, an increasing number of elderly patients may be considered for pancreaticoduodenal resection. This high-volume, single-institution experience examines the morbidity, mortality, and long-term survival of 727 patients undergoing pancreaticoduodenectomy between December 1986 and June 1996. Outcomes of patients 80 years of age and older (n = 46) were compared to those of patients younger than 80 years. In these older patients, pancreaticoduodenectomy was performed for pancreatic adenocarcinoma (n = 25; 54%), ampullary adenocarcinoma (n = 9; 20%), distal bile duct adenocarcinoma (n = 5; 11%), duodenal adenocarcinoma (n = 2; 4%), cystadenocarcinoma (n = 2; 4%), cystadenoma (n = 1; 2%), and chronic pancreatitis (n = 2; 4%). When compared to the 681 concurrent patients younger than 80 years who were undergoing pancreaticoduodenectomy, the two groups were statistically similar with respect to sex, race, intraoperative blood loss, and type of pancreaticoduodenectomy performed. Patients 80 years of age or older had a shorter median operative time (6.4 hours vs. 7.0 hours; $P = 0.02$) but a longer postoperative length of stay (median = 15 days vs. 13 days; $P = 0.01$) and a higher complication rate (57% vs. 41%; $P = 0.05$) when compared to their younger counterparts. Pancreaticoduodenectomy in the older group resulted in a 4.3% perioperative mortality rate compared to 1.6% in the younger group (P = NS). In the subset of patients undergoing pancreaticoduodenectomy for periampullary adenocarcinoma (n = 495), patients 80 years of age or older (n = 41) had a median survival of 32 months and a 5-year survival rate of 19%, compared to 20 months and 27%, respectively, in patients younger than 80 years (n = 454; $P = 0.77$). These data demonstrate that pancreaticoduodenectomy can be performed safely in selected patients 80 years of age or older, with morbidity and mortality rates approaching those observed in younger patients. Based on these data, age alone should not be a contraindication to pancreaticoduodenectomy. (J GASTROINTEST SURG 1998;2:207-216.)

Advancing age is one of the risk factors for the development of pancreatic cancer. Approximately three quarters of the patients with pancreatic adenocarcinoma are 60 years of age or older. The annual incidence of pancreatic cancer increases from two per 100,000 in the 40- to 44-year age group to 100 per 100,000 in the 80- to 84-year age group.[1] In a recent report from The Johns Hopkins Hospital, the median age of patients undergoing pancreaticoduodenal resection for periampullary adenocarcinoma in the 1990s was 67 years, with 39% of the patients being 70 years or older.[2]

Persons aged 65 years and older account for the fastest growing subset of the United States population. As the United States population ages, increasing numbers of elderly patients are being referred for pancreaticoduodenal resection. A number of recent series have reported that pancreaticoduodenectomy can be performed safely, with mortality rates of less than 5%.[2-7] Several recent studies have evaluated pancreaticoduodenal resection in the elderly population. In these studies the authors specifically compared patients age 70 years or older to those younger than 70 years. The results indicate that resection can be per-

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formed safely and long-term survival is possible in this age group.[8-14]

Given that almost 40% of the patients with periampullary malignancies referred to our institution fall into this 70 years of age or older category, and that prior studies have yielded acceptable results, the decision to consider patients in the 70- to 80-year age range for resection is no longer controversial. In an attempt to determine whether pancreaticoduodenal resection is justified in patients 80 years or older, we retrospectively reviewed our pancreaticoduodenectomy database to ascertain whether these carefully selected octogenarians have morbidity, mortality, and outcomes comparable to their younger counterparts.

METHODS

The records of 727 consecutive patients undergoing pancreaticoduodenectomy at The Johns Hopkins Hospital between December 1986 and June 1996 were retrospectively reviewed. Outcomes of patients 80 years or older and patients younger than 80 were compared.

The surgical techniques have been described previously.[7,15,16] Briefly, the biases at our institution have been as follows: (1) to perform a pylorus-preserving resection, reserving distal gastric resection for lesions involving the distal stomach or first portion of the duodenum; (2) to perform standard pancreaticoduodenectomy, without extended retroperitoneal lymph node dissection; and (3) to perform partial pancreatectomy and restore pancreatic-enteric continuity via pancreaticojejunostomy or pancreaticogatrostomy. Vagotomy, tube gastrostomy, tube jejunostomy, prophylactic octreotide, and total parenteral nutrition are not routinely used.

All pathology specimens were reviewed by a single pathologist (R.H.H.) to determine the primary pathologic diagnosis and the extent of disease. As previously described for periampullary adenocarcinoma, the site of origin of the tumor was determined by looking for an in situ component in the specimen, as well as where the tumor was centered.[7]

Perioperative mortality was defined as in-hospital death or death within 30 days of surgery. The specific complications examined included delayed gastric emptying, pancreatic fistula formation, intra-abdominal abscess formation, cholangitis, pneumonia, wound infection, peptic ulceration, and the need for reoperation in the immediate postoperative period. Delayed gastric emptying was defined by means of previously stated criteria.[17] Pancreatic fistula formation and bile leak required 50 ml or more of amylase- or bilirubin-rich fluid from the drains in the region of the pancreatic-enteric anastomosis or the hepaticojejunostomy on or after postoperative day 10.[18] Pneumonia, cholangitis, and wound infection required positive cultures from the specified site, fever, and radiographic evidence of an infiltrate in the case of pneumonia.

The demographics, intraoperative factors, postoperative diagnoses, tumor characteristics, and outcomes of the 46 patients 80 years of age or older were compared with those of the 681 patients younger than 80 years who were undergoing pancreaticoduodenectomy during the same time period. Chi-square analysis, Student's t test, or Fisher's exact test was used to compare the groups, when appropriate.

Among patients undergoing pancreaticoduodenectomy for periampullary adenocarcinoma, long-term survival in those 80 years of age or older was compared with survival in those younger than 80 years. Survival curves for the two groups were generated using the Kaplan-Meier method.[19] Differences in long-term survival between subgroups were compared using the log-rank test. Results are reported as mean ± standard deviation. Significance was accepted at the 5% level.

RESULTS

During the 9½ years of this study, 727 patients underwent pancreaticoduodenectomy for a variety of benign and malignant diseases of the periampullary region. The annual distribution of these resections is depicted in Fig. 1. Forty-six patients were 80 years of age or older (mean age 82.9 ± 2.7 years; median 82.0, range 80.0 to 90.0). Nineteen of these 46 patients were male (41%) and 27 were female (59%), with 44 whites (96%), one black (2%), and one Asian (2%). There were no statistical differences in the sex and race distributions between the older (≥80 years) and younger (<80 years) subgroups (Table I), although the older age group had a larger percentage of women, which is consistent with the trends seen in the general population.

Data depicting postoperative pathologic diagnoses are summarized in Table II. Of the 46 pancreaticoduodenal resections performed in the older age group, 43 (93%) were for malignant disease. Forty-one (89%) of the 43 malignancies were periampullary adenocarcinomas, with 25 of pancreatic origin (54%), nine of ampullary origin (20%), five of distal bile duct origin (11%), and two of duodenal origin (4%). The remaining two malignant tumors were pancreatic cystadenocarcinomas. Of the three older patients oper-

ated on for benign disease, two had chronic pancreatitis (4%) and one had a pancreatic cystadenoma (2%). The distribution of postoperative diagnoses in the older age group is significantly different when compared to that in patients younger than 80 years because pancreaticoduodenectomy was performed for benign disease in a much higher percentage of the younger patients (20%; $P = 0.01$).

Table III depicts the intraoperative factors analyzed in these patients. In the older patients, pancreaticoduodenectomy was performed within a median time of 6 hours and 25 minutes. This was significantly shorter than the time in the younger patients, who had a median operative time of 7 hours ($P = 0.02$). Older patients had an average estimated intraoperative blood loss of 642 ml and an average number of

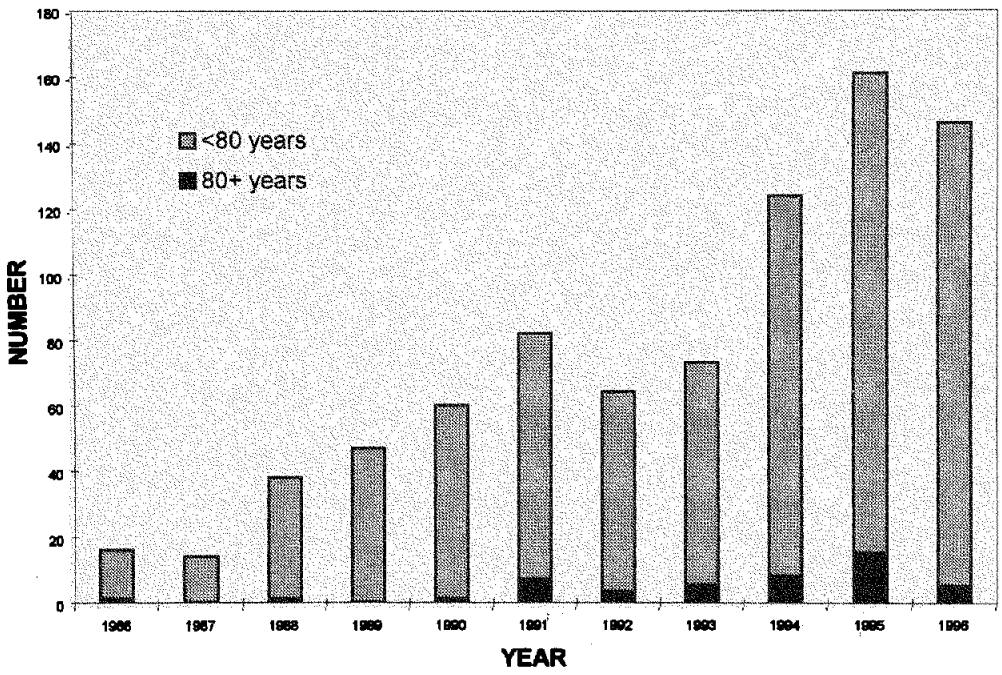

Fig. 1. Annual distribution of patients undergoing pancreaticoduodenectomy at The Johns Hopkins Hospital. Shown are the total number of patients per year, as well as the number of patients 80 years of age or older, as compared to those younger than 80 years.

Table I. Pancreaticoduodenectomy: Comparison of demographic features for entire cohort

Demographics	≥80 years (n = 46; 6%)	<80 years (n = 681; 94%)	*P* value
Age (yr)			
Average	82.9 ± 2.7	60.9 ± 12.4	<0.0001
Median	82.0	64.0	
Range	80.0 - 90.0	18.0 - 79.0	
Sex			
Male	19 (41%)	377 (55%)	0.06
Female	27 (59%)	304 (45%)	
Race			
White	44 (96%)	607 (89%)	0.23
Black	1 (2%)	55 (8%)	
Other	1 (2%)	19 (3%)	

Table II. Pancreaticoduodenectomy: Postoperative diagnoses

Diagnosis	≥80 years		<80 years		*P* value
	No. of patients	%	No. of patients	%	
Benign	3	7	138	20	0.01
Malignant	43	93	543	80	
Specific diagnosis					
Pancreatic cancer	25	54	282	41	0.003
Ampullary cancer	9	20	74	11	
Distal bile duct cancer	5	11	69	10	
Duodenal cancer	2	4	29	4	
Chronic pancreatitis	2	4	80	12	
Cystadenocarcinoma	2	4	16	2	
Cystadenoma	1	2	26	4	
Periampullary adenoma	0	0	22	3	
Malignant neuroendocrine tumor	0	0	24	3	
Benign neuroendocrine tumor	0	0	10	1	
Gastrointestinal stromal tumor	0	0	11	2	
Other	0	0	38	6	

Table III. Pancreaticoduodenectomy: Intraoperative factors

Intraoperative factors	≥80 years	<80 years	*P* value
Operative time (hr:min)			
Average	6:34 ± 1:31	7:11 ± 1:44	0.02
Median	6:25	7:00	
Intraoperative blood loss (ml)			
Average	642 ± 490	961 ± 1179	0.07
Median	500.0	650.0	
Transfusions (units of PRBCs)			
Average	0.8 ± 1.1	1.1 ± 2.4	NS
Median	0.0	0.0	
Type of resection			
Pylorus-preserving	41 (89%)	559 (82%)	NS
Classic	5 (11%)	122 (18%)	
Extent of pancreatectomy			
Partial	45 (98%)	652 (96%)	NS
Total/Completion	1 (2%)	29 (4%)	
Anastomosis			
Pancreaticojejunostomy	32 (71%)	476 (74%)	NS
Pancreaticogastrostomy	13 (29%)	167 (26%)	
Vein resection			
Yes	2 (4%)	25 (4%)	NS
No	44 (96%)	656 (96%)	

PRBCs = packed red blood cells; NS = not significant.

red blood cells transfused of 0.8 units. These values are smaller but not statistically different from the values in the younger group. Eighty-nine percent of patients 80 years or older underwent a pylorus-preserving resection and 98% had a partial pancreatic resection. Among the 45 older patients undergoing partial pancreatic resection and requiring pancreatic-enteric anastomosis, reconstruction was via pancreaticojejunostomy in 32 (71%) and via pancreaticogastrostomy in 13 (29%). In the older group, only 4% of patients (n = 2) underwent vein resection to achieve adequate surgical margins. There are no differences between the two groups with regard to type of resection, extent of pancreatectomy, type of pancreatic-enteric anastomosis, or incidence of vein resection.

Data on perioperative mortality and morbidity are summarized in Table IV. The perioperative mortality rates were 4.3% for the older patients and 1.6% for the younger patients ($P = 0.21$; not significant [NS]). Although these mortality rates are not statistically different, the older subgroup experienced a significantly higher incidence of postoperative complications (57% vs. 41%; $P = 0.05$). The most common complications among the older patients were delayed gastric emptying (33%), pancreatic fistula (15%), wound infection (11%), and cholangitis (11%). In the younger cohort, delayed gastric emptying (18%), pancreatic fistula (14%), and wound infection (11%) were the most common complications. The incidence of delayed gastric emptying was significantly higher in the older age group ($P = 0.03$), and cholangitis was also more common, although this difference did not achieve significance ($P = 0.08$). The elderly patients had a significantly longer postoperative length of stay with a median of 15 days, compared to 13 days in the younger cohort ($P = 0.02$).

In 41 of the 46 older patients, pancreaticoduodenectomy was performed for periampullary adenocarcinoma. Table V presents the data comparing the demographic information and intraoperative factors in the two cohorts, considering only those patients with periampullary adenocarcinoma. There were significantly more female patients in the older cohort (61% vs. 43%; $P = 0.03$), and the operative time was significantly shorter in the older cohort (median 6 hours and 20 minutes vs. 7 hours; $P = 0.01$). All other comparisons including racial distribution and other intraoperative factors were comparable in the two groups. Considering only those patients with periampullary adenocarcinoma, the two groups were similar with respect to site of origin of the tumor, tumor diameter, resection margin status, lymph node status, and tumor differentiation (Table VI).

For patients 80 years and older, the overall 1-, 2-, and 5-year survival rates were 73%, 65%, and 20%, respectively (median = 38 months), compared to

Table IV. Pancreaticoduodenectomy: Postoperative course

Postoperative course	≥80 years	<80 years	*P* value
Mortality			
Yes	2 (4.3%)	10 (1.6%)	0.21
No	44 (96%)	671 (98%)	
Overall complications			
Yes	26 (57%)	270 (41%)	0.05
No	20 (44%)	383 (59%)	
Specific complications			
Delayed gastric emptying	15 (33%)	120 (18%)	0.03
Pancreatic fistula	7 (15%)	89 (14%)	0.77
Wound infection	5 (11%)	69 (11%)	0.92
Intra-abdominal abscess	1 (2%)	35 (5%)	0.30
Cholangitis	5 (11%)	29 (5%)	0.08
Endoscopy required	2 (4%)	26 (4%)	0.88
Pneumonia	3 (7%)	21 (3%)	0.27
Bile leak	1 (2%)	19 (3%)	0.77
Pancreatitis	0 (0%)	14 (2%)	0.17
Ulcer	1 (2%)	5 (3%)	0.38
Reoperation	3 (7%)	22 (3%)	0.34
Postoperative length of stay (days)			
Average	21.1 ± 15.9	16.9 ± 10.5	0.02
Median	15.0	13.0	

Table V. Pancreaticoduodenectomy for periampullary adenocarcinoma: Comparison of demographics and intraoperative factors for entire cohort

	≥80 years (n = 41; 8%)	<80 years (n = 454; 92%)	*P* value
Demographics			
Age (yr)			
Average	83.0 ± 2.7	63.4 ± 10.2	<0.00005
Median	82.0	65.0	
Range	80.0 - 90.0	33.0 - 79.0	
Sex			
Male	16 (39%)	258 (57%)	0.03
Female	25 (61%)	196 (43%)	
Race			
White	39 (95%)	410 (90%)	NS
Black	1 (2%)	30 (7%)	
Other	1 (2%)	14 (3%)	
Intraoperative factors			
Operative time (hr:min)			
Average	6:25 ± 1:24	7:05 ± 1:36	0.01
Median	6:20	7:00	
Intraoperative blood loss (ml)			
Average	616 ± 411	930 ± 1140	NS
Median	500.0	687.5	
Transfusions (units of PRBCs)			
Average	0.8 ± 1.0	1.1 ± 2.3	NS
Median	0.0	0.0	
Type			
Pylorus-preserving	36 (88%)	376 (83%)	NS
Classic	5 (12%)	78 (17%)	
Extent of pancreatectomy			
Partial	40 (97%)	437 (96%)	NS
Total/completion	1 (3%)	17 (4%)	
Anastomosis			
Pancreaticojejunostomy	29 (73%)	318 (73%)	NS
Pancreaticogastrostomy	11 (27%)	119 (27%)	
Vein resection			
Yes	2 (5%)	20 (4%)	NS
No	39 (95%)	434 (96%)	

Abbreviations as in Table III.

81%, 62%, and 45%, respectively, for patients younger than 80 years (median = 48 months, $P = 0.12$; Fig. 2). Such a comparison of overall survival is clearly influenced by the significant differences in age between the two groups, as well as the significantly higher percentage of patients in the younger group undergoing resection for benign disease (20%), as compared to only 7% of patients undergoing resection for benign disease in the older group ($P = 0.01$). In patients undergoing pancreaticoduodenectomy for periampullary adenocarcinoma, long-term survival was compared (Fig. 3) between patients younger than 80 years (n = 454) and those 80 years or older (n = 41). The older cohort had a median survival of 32 months with 1-, 2-, and 5-year survival rates of 71%, 63%, and 19%, respectively, compared to a median survival of 20 months and 1-, 2-, and 5-year survival rates of 73%, 47%, and 27% in the younger cohort ($P = 0.77$). Survival curves were also generated for pancreatic adenocarcinoma alone, the most common pathologic diagnosis (Fig. 4). The older group had a median survival of 17 months as compared to 18 months in the younger group ($P = 0.57$). The numbers of octogenarians with ampullary,

Table VI. Pancreaticoduodenectomy for periampullary adenocarcinoma: Comparison of pathologic findings and postoperative diagnosis for entire cohort

	≥80 years (n = 41; 8%)	<80 years (n = 454; 92%)	*P* value
Pathologic diagnosis			
Pancreatic cancer	25 (61%)	282 (62%)	NS
Ampullary cancer	9 (22%)	74 (16%)	
Distal bile duct cancer	5 (12%)	69 (15%)	
Duodenal cancer	2 (5%)	29 (6%)	
Tumor characteristics			
Diameter (cm)			
Average	2.7 ± 1.8	3.0 ± 1.8	NS
Median	2.0	2.5	
Range	0.8 - 10.0	0.3 - 15.0	
Margins			
Positive	5 (12%)	96 (21%)	NS
Negative	36 (88%)	358 (79%)	
Nodes			
Positive	23 (56%)	286 (63%)	NS
Negative	18 (44%)	168 (37%)	
Differentiation			
Well	2 (5%)	27 (6%)	NS
Moderate	26 (70%)	276 (65%)	
Poor	9 (25%)	122 (29%)	

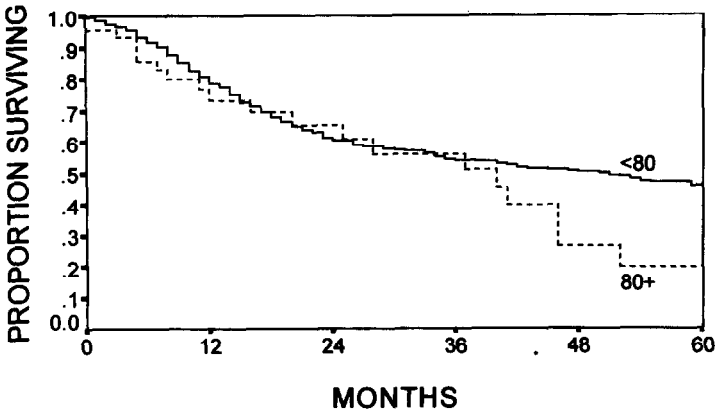

Fig. 2. Actuarial survival curves comparing all patients 80 years of age or older (n = 46; median survival = 38 months; 5-year survival = 20%) undergoing pancreaticoduodenal resection to those patients younger than 80 years (n = 681; median survival = 48 months; 5-year survival = 45%; $P = 0.12$). Of note, the older patient group had a 93% incidence of resections for malignancy, which is significantly higher than the rate in the younger group (80%; $P = 0.01$).

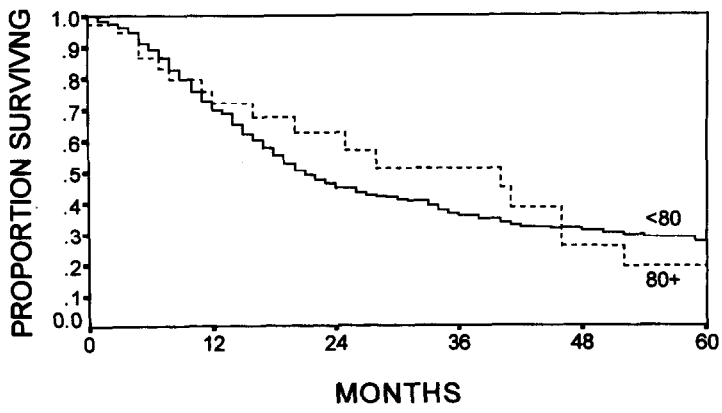

Fig. 3. Actuarial survival curves comparing patients 80 years of age or older undergoing pancreaticoduodenectomy for periampullary adenocarcinoma (n = 41; median survival = 32 months; 5-year survival = 19%) to those younger than 80 years (n = 454; median survival = 20 months; 5-year survival = 27%; $P = 0.77$).

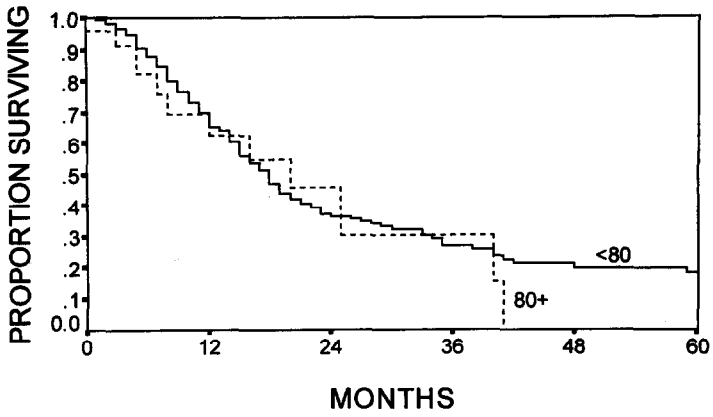

Fig. 4. Actuarial curves comparing patients 80 years of age or older undergoing pancreaticoduodenectomy for pancreatic adenocarcinoma (n = 25; median survival = 18 months; 2-year survival = 46%) to those younger than 80 years (n = 282; median survival = 17 months; 2-year survival = 36%; $P = 0.57$).

distal bile duct, and duodenal adenocarcinoma were too small to generate meaningful Kaplan-Meier survival curves. Of the nine older patients operated on for ampullary adenocarcinoma, four are alive at 94, 61, 15, and 8 months, respectively. The remaining five died at 52, 47, 46, 28, and 11 months, respectively, following resection. Four of the five elderly patients with distal bile duct tumors remain alive at 47, 8, 5, and 4 months, respectively, whereas the final individual died at 5 months postoperatively. Both of the patients age 80 years or older who had primary lesions of the duodenum are alive at 36 months and 13 months, respectively, after resection.

DISCUSSION

As the United States population ages, an increasing number of elderly patients will develop periampullary malignancies, making them eligible for pancreaticoduodenal resection. This trend can be demonstrated by the age distribution of patients undergoing pancreaticoduodenal resection at our institution over the past two decades. During the period of this study, there has been a general increase in the number of pancreaticoduodenal resections performed per year (see Fig. 1), as well as an increase in the number of octogenarians being treated by pancreaticoduodenectomy. In the 1980s, only 2% of the patients undergoing pancreaticoduodenal resection for periampullary cancer were 80 years of age or older. In contrast, of the 443 patients undergoing resection for periampullary adenocarcinoma between January 1990 and July 1996, 35% were between 60 and 69 years of age, 30% were between 70 and 79 years, and 9% were 80 years or older.

Many recent series have demonstrated that pancreaticoduodenectomy can be performed safely, with mortality rates of less than 5%.[2-7] In recent years several groups have specifically analyzed the results of pancreaticoduodenal resection in older populations.[5] Morbidity and mortality of elderly patients undergoing major pancreatic resection were recently examined by Fong et al.[8] In this large series, pancreatic resection in 138 patients age 70 years or older resulted in a mortality rate of 6%, a median hospital stay of 20 days, and a morbidity rate of 45%. The actuarial 5-year survival rate in these older patients was 21%, which is significantly lower than the 29% 5-year survival seen in the younger patients. An earlier report by Delcore et al.[11] noted that the operative mortality rate for pancreaticoduodenectomy in patients older than 70 years was 5%, with a 32-month median survival. A study by Katoh et al.[14] in patients older than 70 years demonstrated a 50% operative morbidity rate after resection for pancreatic cancer, with a 5-year survival rate of 15%; in patients who had primary lesions in the bile duct the morbidity rate was 40%, with a 40% 5-year survival rate. Several other authors have also described series of patients older than 70 years of age who have undergone major pancreatic resection,[9,10,12,13] with acceptable outcomes.

From the preponderance of data available, it appears that pancreaticoduodenal resection can be performed safely in patients 70 years of age or older, and it can be associated with long-term survival. However, the role of pancreaticoduodenectomy in octogenarians, a group with a more limited life expectancy, is unclear. The aim of the current study was to ascertain whether pancreaticoduodenectomy can be performed safely in patients 80 years of age and older and to de-

termine whether these patients benefit from a similar prolongation in survival, especially in the case of periampullary adenocarcinoma.

This study demonstrates that pancreaticoduodenal resection can be safely performed in selected patients 80 years of age or older. When compared to their younger counterparts, patients 80 years or older have statistically shorter operative times, with comparable intraoperative blood loss and transfusion requirements. Pancreaticoduodenectomy in these older patients is, however, associated with a significantly higher postoperative morbidity rate, leading to statistically longer postoperative hospital stays. Yet half of these older patients are discharged from the hospital by postoperative day 15. Of note, the mortality rate in this series was only 4.3% in the older patients, which is not significantly different from the 1.6% mortality rate in the concurrent younger patients, again emphasizing the safety of the procedure when it is properly applied. There should be no arbitrary age cutoff for patients who require pancreaticoduodenectomy. For example, we recently performed a successful pancreaticoduodenal resection in a 105-year-old woman with an obstructing duodenal cancer. The patient tolerated the procedure well and was discharged from the hospital on postoperative day 10, without postoperative morbidity.

Candidates for pancreaticoduodenctomy at The Johns Hopkins Hospital are carefully evaluated preoperatively for cardiopulmonary and anesthetic considerations, as well as other risk factors. No additional or specialized preoperative testing was performed in these older patients compared to younger patients. In reality, the decision to offer the opportunity for resection was made by the attending surgeon, based on routine testing, independence of lifestyle, and general performance status. Postoperatively, appropriate attention is paid to expediting the return to preoperative performance status, often assisted by physical therapy. Undoubtedly the care of these patients demands additional attention and can take full advantage of the multidisciplinary team assembled to guide these patients through their postoperative recovery.

Finally, of great importance, the octogenarians undergoing pancreaticoduodenectomy for periampullary adenocarcinoma had an actuarial 5-year survival rate of 19%, which is not significantly different from the survival rate seen in the younger group. Since pancreaticoduodenectomy offers the only hope for long-term survival for patients with periampullary cancers, healthy patients 80 years of age and older clearly should be considered for potentially curative resection. Furthermore, it has been our bias to recommend postoperative adjuvant chemoradiation therapy to our patients following pancreaticoduodenectomy, regardless of age. This recommendation is based on data from the Gastrointestinal Tumor Study Group[20,21] and our institution,[22] which have indicated that 5-fluorouracil–based chemoradiation therapy improves postoperative survival.

Long-term survival after pancreaticoduodenectomy is possible in carefully selected octogenarians. The operation can be performed safely, with a mortality rate comparable to that observed in younger patients. Based on these data, an aggressive approach toward resection, especially for periampullary malignancies, is justified in this population. Age alone is not a contraindication to pancreaticoduodenectomy.

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Utility of a Linear Array Ultrasound Endoscope in the Evaluation of Suspected Pancreatic Disease

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Endoscopic ultrasonography (EUS) is currently being used to evaluate and stage pancreaticobiliary malignancies and neuroendocrine tumors, and to perform aspiration for cytologic diagnosis. There are currently two different commercially available EUS systems for clinical use. One system uses a mechanical radial sector scanner oriented in a plane perpendicular to the long axis of the endoscope, and the other uses an electronic convex scanner that is oriented in the long axis of the endoscope. The vast majority of the current literature reports experience using the radial scanning device in the evaluation of pancreaticobiliary abnormalities. We prospectively evaluated the linear probe as the sole instrument for EUS in 26 patients with suspected pancreatic disease. The results of the endoscopic ultrasound examination were compared with the results of surgery or long-term clinical follow-up. The sensitivity and specificity of linear array EUS for benign pancreatic disease were 93.8% and 88.2%, respectively. The sensitivity and specificity for malignant disease of the pancreas were 80.0% and 88.9%, respectively. The linear array echoendoscope, employed as the only instrument for evaluation of the pancreas, is accurate in the evaluation of pancreatic disease. The addition of EUS-guided pancreatic biopsy would be anticipated to improve the sensitivity of the linear array instrument for detecting malignancy. (J GASTROINTEST SURG 1998;2:217-222.)

Currently two different endoscopic ultrasound (EUS) systems are commercially available for clinical use. One system employs a mechanical radial scanner that is oriented in a plane perpendicular to the long axis of the endoscope, whereas the other uses an electronic convex scanner that is oriented in the long axis of the endoscope. The vast majority of the current literature reports experience with the use of the radial scanning device, and no large series have been reported in the literature relaying the utility of the linear scanning device in the evaluation of pancreatic disease. Because of the limited (100-degree) view provided by the sector scanner, the radial scanning instrument, with its 360-degree view, is believed by some endosonographers to allow more rapid recognition of anatomic structures. We prospectively evaluated the linear probe in 26 patients with suspected pancreatic disease and found that the linear probe was efficacious in the hands of experienced endosonographers.

PATIENTS AND METHODS

EUS examinations were requested by the patient's attending physician to evaluate suspected disease of the pancreas. Over a 6-month period, 26 consecutive patients presenting with either clinical histories (n = 6) or abnormal prior imaging studies (n = 20) consistent with pancreatic disease were prospectively evaluated by means of endoscopic ultrasonography (Table I). We evaluated 19 men and seven women whose mean age was 60.4 years (range 10 to 87 years). Informed consent was obtained from all patients. No complications were encountered during this study.

Standard practices of conscious sedation for endoscopy at our institution were followed, with no

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Table I. Patient data

Patient	Age/Sex	Indication	EUS findings	Surgery/Additional studies	Diagnosis
1	76/F	Pancreatic mass on CT	Well-circumscribed, benign-appearing mass	None	Benign inflammatory mass; clinical f/u >12 mo
2	70/M	CBD dilated on US and PTC	No mass, dilated CBD with normal IHD	ERCP Choledochoduodenal fistula	Benign obstruction secondary to passed stone
3	49/M	Pancreatic mass on CT	T3N1Mx	T3N1M0 Pancreatic carcinoma	Pancreatic carcinoma
4	63/M	PD cut off on ERCP	Normal	None	Pancreas divisum
5	73/M	Chylous ascites, r/o pancreatic mass	Diffusely abnormal pancreatic parenchyma, no focal mass	Schirrous pancreatic carcinoma	Pancreatic carcinoma
6	52/M	Dilated PD on CT	Chronic pancreatitis, dilated MPD and PD stones	None	Chronic pancreatitis
7	59/F	Acute pancreatitis	Chronic pancreatitis	CT-guided biopsy and ERCP, chronic pancreatitis, cytology (−)	Chronic pancreatitis
8	68/M	Pancreatic mass on CT	T3NxMx—unable to enter duodenum	T3NxM1 peritoneal metastasis	Pancreatic carcinoma
9	67/M	Double duct sign on ERCP	T2N0Mx, SMV encased but not invaded	T3N0M0 Pancreatic carcinoma	Pancreatic carcinoma
10	60/F	PD cut off on ERCP	Chronic pancreatitis	Pancreaticojejunostomy	Chronic pancreatitis
11	49/M	Dilated CBD and PD on CT	Chronic pancreatitis with MPD stricture	Choledochojejunostomy	Chronic pancreatitis
12	87/M	Jaundice	T1bN0 cholangiocarcinoma	None; ERCP, cytology (+)	Cholangiocarcinoma
13	74/M	Enlarged HOP on CT	Normal pancreas	None	Normal pancreas; >12 mo f/u

14	38/M	Jaundice	T2N0Mx Pancreatic carcinoma	T2N0M1 Pancreatic carcinoma	Pancreatic carcinoma
15	60/M	Ampullary mass on ERCP	T2N0Mx Ampullary carcinoma	T2N0M0 Ampullary carcinoma	Ampullary carcinoma
16	79/F	CBD dilated on CT	No mass, dilated CBD and MPD	None	Ampullary stenosis; >12 mo f/u
17	68/M	Evaluate jaundice	T3N0Mx Pancreatic carcinoma	T3N0M0 Pancreatic carcinoma	Pancreatic carcinoma
18	67/F	Pancreatic mass on CT	Chronic pancreatitis with pancreatic mass, T1b *if malignant*	Chronic pancreatitis with benign mass	Chronic pancreatitis with focal benign mass
19	61/M	Ampullary mass on ERCP	T4N1Mx Ampullary carcinoma	T4N1M0 Ampullary carcinoma	Ampullary carcinoma
20	45/M	Dilated PD on CT	Pancreatic pseudocyst, chronic pancreatitis	Acute and chronic pancreatitis	Acute and chronic pancreatitis
21	53/M	Abnormal pancreas on CT	Chronic pancreatitis and pseudocyst	Chronic pancreatitis, pancreaticojejunostomy	Chronic pancreatitis and pseudocyst
22	10/F	Recurrent pancreatitis	Pancreatic pseudocyst with pseudoaneurysm	MRI; pancreatitis with hematoma	Pancreatic pseudocyst with hematoma
23	45/M	PD cut off on ERCP	Chronic pancreatitis	None	Chronic pancreatitis; >12 mo f/u
24	64/M	Abdominal pain, weight loss	Normal	None	Normal; >12 mo f/u
25	63/F	HOP mass on CT, increased serum amylase	Normal	None	Normal; >12 mo f/u Salivary amylasemia
26	43/M	CBD dilated on CT	Chronic pancreatitis	Unresectable pancreatic adenocarcinoma	Pancreatic carcinoma

CBD = common bile duct; US = ultrasonography; PTC = percutaneous transhepatic cholangiography; IHD = intrahepatic duct; ERCP = endoscopic retrograde cholangiopancreatography; SMV = superior mesenteric vein; PD = pancreatic duct; HOP = head of pancreas; f/u = follow-up; r/o = rule out.

modification for endosonography. All patients underwent topical pharyngeal anesthesia, followed by conscious sedation using diazepam and meperidine. In some instances droperidol was also used for sedation.

The Pentax FG32-UA echoendoscope (Pentax Precision Instruments, Orangeburg, N.Y.) was the sole EUS instrument used to image all patients. It is an electronic linear array sector scanning instrument with a 100-degree viewing angle oriented parallel to the axis of the endoscope. The instrument was used at both the 5 and 7.5 MHz scanning frequencies. The Hitachi EUB-515C console (Hitachi Corp., America, Philadelphia, Pa.) was used to generate and capture the images. Images were primed to thermal prints, radiographic film, and S-VHS tape and saved for review.

A practiced endosonographer, experienced with the use of both types of EUS systems, was present for all examinations. In addition, an experienced extracorporeal ultrasonographer was present in most instances. Imaging was accomplished using the balloon technique and luminal instillation of deaerated water as needed. Examinations were begun distal to the ampulla when technically feasible, and sequential images of the pancreas and surrounding structures were examined on instrument withdrawal. Typically the ampulla and the head of the pancreas were imaged from the duodenum, and the body and tail were imaged through the stomach wall. The Doppler capacity of the instrument was employed to confirm the identity of vascular and avascular peripancreatic structures such as the components of the portal triad (portal vein, bile duct, and hepatic artery).

Surgical evaluation of the imaged lesion or long-term clinical observation (16 to 24 months) combined with additional imaging studies was used as the "gold standard" for diagnosis.

RESULTS

Transduodenal and transgastric imaging of the entire pancreas was successfully accomplished in 25 cases. In one patient a pancreatic mass compressed the duodenum (see Table I, patient 8), which precluded passage of the echoendoscope, and images were obtained only from the transgastric position. TNM staging was not adversely affected in this patient.

The final clinical diagnoses (see Table I) for the patients in our series included pancreatic carcinoma (n = 7; Fig. 1), chronic pancreatitis (n = 8), ampullary carcinoma (n = 2), benign papillary stenosis (n = 2), cholangiocarcinoma (n = 1), pancreatic pseudocyst (n = 1), pancreas divisum (n = 1), acute pancreatitis (n = 1), and normal pancreas (n = 3).

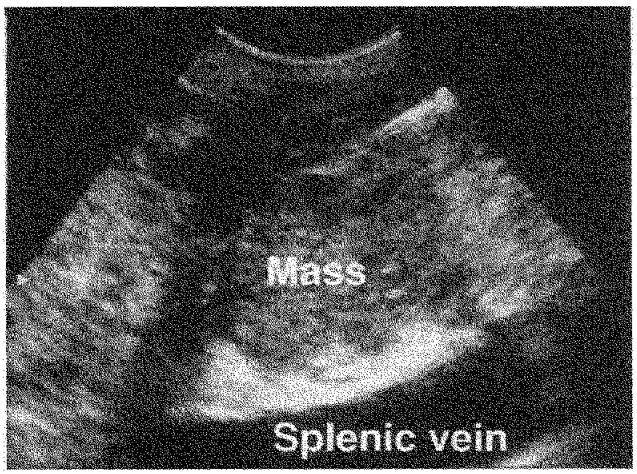

Fig. 1. EUS view of the head of the pancreas demonstrating a 2.5 cm homogenous mass that proved to be pancreatic carcinoma.

Table II

	EUS impression		
Diagnosis	**Benign**	**Malignant**	**Sensitivity**
Benign	15	1	93.8%
Malignant	2	8	80.0%
Specificity	88.2%	88.9%	

The sensitivity and specificity of linear array EUS for benign pancreatic disease were 93.8% and 88.2%, respectively. The sensitivity and specificity for malignant diseases were 80.0% and 88.9%, respectively (Table II). Nine of the patients with benign pancreatic disease had tissue confirmation obtained at operation or by means of biopsy and/or cytologic studies, along with clinical follow-up examination. In the patients with malignancies whose lesions were staged at operation, accuracy of the endoscopic ultrasound TNM staging was 83% (5 of 6) for tumor (T) stage and 100% (6 of 6) for node stage.

DISCUSSION

Vilmann et al.[1] first described an initial experience with the linear array echoendoscope and found that the resolution of the images and the sector scanning orientation may make image interpretation difficult. In our prospective evaluation, the linear array echoendoscope was accurate for the evaluation of pancreatic disease. We did not encounter any subjective difficulties with the resolution of the transducer at either 5 or 7.5 MHz. The linear array ultrasound instrument af-

fords an excellent view of the head of the pancreas because of the natural orientation of the scan plane when the instrument is placed in the proximal duodenum. The ability to visually place the probe near the ampulla aids in this orientation. The body and tail of the pancreas can be adequately imaged with this instrument, and no errors were attributable to poor visualization of a lesion in the body or tail of the pancreas.

Our group has employed the linear array echoendoscope in the preoperative assessment of patients with pancreatic neuroendocrine tumors and found it to be accurate and equivalent to results achieved with the radial array instrument.[2,3] Only one other series has been reported in the English language literature detailing the utility of the sector-scanning echoendoscope in the evaluation of both benign and malignant pancreatic disease. Using the linear array instrument, Giovannini and Seitz[4] demonstrated an overall diagnostic accuracy of 88% in their series of patients with surgical confirmation, which is similar to the findings in our study.

We did not use the radial scanning echoendoscope in any of the patients in the series, and we believe that any potential imaging or case selection bias was therefore eliminated. Other reports detailing a head-to-head comparison of the two types of systems have revealed no significant differences in terms of diagnostic accuracy. Rösch et al.[5] recently reported results of a study comparing the two types of echoendoscopes in the preoperative evaluation of ampullopancreatic carcinoma and found similar results for T staging (75%). In the evaluation of pancreatic neoplasms, most of the other published literature on the radial scanning instrument indicates similar, comparable accuracy to the results achieved with the sector scanner.[6,7]

Using the radial array instrument in the evaluation of chronic pancreatitis and pseudocysts, Wiersema et al.[8] demonstrated a high degree of sensitivity, but with some loss of specificity, when this device was compared with a clinical gold standard. Our results with the linear instrument demonstrate a similar degree of sensitivity, although the relatively small size of the study sample will require a larger study for validation for the utility of the sector-scanning instrument in the evaluation of benign pancreatic disease.

A benefit of the linear array instrument is the ability to perform directed biopsies under direct real-time ultrasound guidance.[9-12] Under EUS guidance, a fine-needle aspiration catheter is passed through the working channel of the echoendoscope and introduced into the lesion of interest. The linear array echoendoscope, because of its ease in evaluation of the pancreatic head, is ideally suited for directed biopsy of mass lesions. Further studies evaluating this technique and the Doppler capability of the probe are in progress. The ability to biopsy lesions allows for improved histologic diagnoses, but the published literature contains an accuracy range of only 85% to 90% for detecting malignancy. This is a particular problem in the pancreas, where even intraoperative biopsies may be negative in the setting of malignancy. Our study indicates that an experienced endosonographer can be accurate in the prediction of the malignant or benign nature of a lesion without a tissue biopsy.

Recent developments in the design of sector-scanning echoendoscopes may allow for entry of additional manufacturers into the field. Mechanical array scanning in the longitudinal plane has recently been demonstrated.[13] A switchable dual-plane scanner was also recently demonstrated, which would have the benefit of allowing the radial scanning plane to orient the user and to search for lymph nodes, in addition to performing EUS-guided biopsy.

CONCLUSION

Our series demonstrated a diagnostic accuracy of 88% in patients with surgical confirmation of the nature of the pancreatic lesion. The linear array ultrasound endoscope affords excellent viewing capability of the pancreas and allows accurate diagnostic interpretation, comparable to reports with the radial scanning instrument. EUS examination of the pancreas with the linear array echoendoscope was sensitive and specific. No significant differences were noted in comparison with the published literature on the use of the radial sector-scanning instruments. Additional studies are needed to confirm these findings and to assess the utility of this echoendoscope in the performance of therapeutic endosonography.

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Outcome of Lateral Pancreaticojejunostomy in the Management of Chronic Pancreatitis With Nondilated Pancreatic Ducts

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Lateral pancreaticojejunostomy has demonstrated variable success in the management of chronic pancreatitis associated with ductal dilation, but its role in patients with nondilated ducts is poorly defined. The aim of this study was to assess the outcome of lateral pancreaticojejunostomy in chronic pancreatitis with nondilated pancreatic ducts. The records of all patients who underwent lateral pancreaticojejunostomy with a pancreatic duct measuring less than 7 mm in diameter were reviewed. Seventeen patients underwent lateral pancreaticojejunostomy for chronic pancreatitis and intractable pain between 1995 and 1996. Endoscopic retrograde cholangiopancreatography demonstrated features of chronic pancreatitis that were mild in seven patients, moderate in five, and severe in four. Postoperative complications occurred in two patients (11.7%). There were no deaths. Mean length of follow-up was 10.3 months (range 3 to 16 months). Rehospitalization for recurrent pancreatitis or pain was necessary in 59% of patients. Emergency room visits were reported by 76%. Narcotic use continued in 88%, with 76% of the patients reporting their pain as the same or worse than before the operation, and 65% continuing to view their health status as poor. In chronic pancreatitis patients with a nondilated pancreatic duct, lateral pancreaticojejunostomy appears to be of little benefit with respect to pain relief, subsequent hospitalization, continued narcotic use, or overall health status. (J GASTROINTEST SURG 1998;2:223-229.)

The surgical management of chronic pancreatitis has included various modifications of drainage procedures patterned after that of Peustow and Gillesby.[1] In contrast to resective operations, drainage procedures offer the advantage of conservation of pancreatic tissue, thus preserving endocrine and exocrine function. The side-to-side lateral pancreaticojejunostomy (LPJ) has become the operation of choice for patients whose pancreatic ducts are markedly dilated and who do not have a mass in the head of the pancreas. The operation has a low morbidity and mortality rate and achieves significant pain relief as demonstrated in several reports.[2-4] The definition of a dilated pancreatic duct varies widely. In a large retrospective study, Leger et al.[5] reported excellent results with pancreaticojejunostomy when the pancreatic duct was greater than 10 mm, but a worse prognosis if the duct measured less than 5 mm.

Delcore et al.[6] reported their experience with the use of LPJ in 25 patients whose pancreatic ducts measured less than 8 mm. All of them experienced complete pain relief in the immediate postoperative period with 86% remaining free of pain after a mean follow-up of 3.5 years. The mean pancreatic duct diameter in that group of patients was 6 mm (range 4 to 8 mm), which was considered by some critics to be a dilated rather than a narrow duct.

Frey and Amikura[7] performed local resection of the head of the pancreas and LPJ in patients with chronic pancreatitis including those with nondilated pancreatic ducts measuring 4.5 to 5 mm in diameter. The average diameter of the main pancreatic duct was 6.3 mm at the neck of the pancreas. Although good results were reported in terms of pain relief for the entire group, no specific reference was made to the outcome of those patients with nondilated ducts.

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Another technique introduced by Beger and Buchler[8] is duodenum-preserving resection of the head of the pancreas. This procedure has been used in patients with nondilated ducts in whom the disease appears to be confined to the head region. It involves removal of most of the head and uncinate process of the pancreas while leaving the duodenum intact. Significant pain relief is achieved in most of these patients. Total pancreatectomy with preservation of the duodenum and pylorus has shown good results in relieving pain associated with chronic pancreatitis; however, the associated high morbidity[9] has tempered the authors' enthusiasm for this procedure in the management of chronic pancreatitis.

The group of patients with chronic pancreatitis and a nondilated duct comprises a large subset for whom effective surgical therapy has not yet been defined. The aim of this study was to assess the outcome of LPJ in patients with chronic pancreatitis associated with a nondilated pancreatic duct.

PATIENTS AND METHODS

The records of all patients who underwent LPJ for chronic pancreatitis and intractable pain with a pancreatic duct measuring less than 7 mm in diameter between 1995 and 1996 at our institution were reviewed. The diagnosis of chronic pancreatitis was made on the basis of clinical examination and endoscopic retrograde cholangiopancreatography (ERCP) findings. All patients had a history of recurrent epigastric abdominal pain radiating into the posterior upper back requiring hospitalization. ERCP and endoscopic ultrasound evaluation of the pancreas were conducted prior to any endoscopic therapeutic interventions with sphincterotomy or pancreatic stent placement. Patients with prior pancreatic ductal or resectional surgery were excluded.

Demographics, perioperative complications, and clinical outcome data were collected. A previously validated and employed quality-of-life questionnaire was administered to all patients and included questions on frequency of attacks, self-rated pain score (0 to 10), number of medical encounters, number of pain pills taken per week, and a five-point Likert scale to assess changes in health status.[10] All questions were intended to reflect the condition of the patient before and after LPJ.

Preoperative Assessment and Interventions

Pancreatic enzyme supplementation and diabetes status were assessed prior to the operation. Serum amylase and lipase levels measured during each hospital admission. Serum cholecystokinin (CCK) and trysinogen levels were obtained in most of the patients.

ERCP demonstrated features of chronic pancreatitis that were mild in seven patients, moderate in five, and severe in four, as defined by the Cambridge classification.[11] Two patients had a history of pseudocysts drained percutaneously prior to LPJ. All patients, with the exception of one, had undergone endoscopic treatment prior to surgical management. This included stenting of the main pancreatic duct (76%), biliary sphincterotomy (58%), and pancreatic sphincterotomy (64%). The three patients with pancreas divisum had undergone trials of minor orifice stenting (3 of 3), minor papilla dilation, and minor papilla sphincterotomy (2 of 3).

Operative Procedure

The Partington-Rochelle modification of LPJ was used.[12] The pancreatic duct was located and incised transversely in the body of the gland in proximity to its junction with the head, then opened longitudinally, proximally, and distally. An incisional biopsy was obtained at this transverse incision site. Incision of the tail was carried the full length of the duct and into the head to within 2 cm of the duodenum. A 60 cm Roux-en-Y jejunal limb was used to construct the LPJ anastomosis with a single layer of interrupted 3-0 silk sutures. The inner layer was placed through the full thickness of the jejunal wall and well into the substance of the opened gland but not routinely into the pancreatic duct. Closed suction drainage was used to drain the area.

RESULTS

Patient Demographics and Characteristics

Seventeen consecutive patients with intractable pain due to chronic pancreatitis associated with a pancreatic duct measuring less than 7 mm in diameter underwent LPJ between 1995 and 1996 at our institution. The mean age of the group was 36.9 years with a range of 24 to 48 years. Eleven of the patients were women and six were men. The mean follow-up time was 10.3 months with a range of 3 to 16 months. The etiology of the disease was defined based on clinical and ERCP data; five patients (29%) had a history of alcohol addiction. Three patients (18%) had pancreas divisum, eight patients (47%) with no other etiology identified had sphincter of Oddi dysfunction as determined by sphincter manometry studies, and one patient was considered to have familial pancreatitis.

The main complaint by the patients was abdominal pain; 76% also complained of nausea and vomiting and 35% had a history of weight loss. The mean du-

ration of symptoms was 6.5 years with a range of 1 to 25 years. All patients required some form of narcotic analgesia on a regular basis to control their pain.

Postoperative Outcome

Early postoperative complications occurred in two patients and included a drain tract infection and an external pancreatic fistula, both of which were treated nonoperatively and resolved within 2 weeks. There were no deaths in the group.

Elevated serum enzyme levels were documented in 15 of the 17 patients preoperatively, with mean lipase and amylase concentrations of 780 and 1940 IU/L, respectively. Baseline serum CCK and trypsin levels were obtained in 14 patients and found to average 1.5 pmol and 24.5 ng/ml, respectively. More than half of the patients required pancreatic enzyme supplements, and five patients were diabetic and required insulin preoperatively. There were no changes in the use of pancreatic enzyme supplements or insulin requirements after the operation. Two patients had common bile duct stenosis managed by choledochoduodenostomy at the time of LPJ.

Microscopic examination of the incisional biopsy of the pancreas showed chronic pancreatitis in all 19 specimens. Other descriptive abnormalities on pathologic analysis included fibrosis in eight of the specimens (47%), atrophy in five (29%), calcification in three (18%), and acute inflammation in one. Operative findings of gross pancreatic inflammation were described as severe in one patient, moderate in 10, and mild in six.

Postoperative ERCPs were performed in 12 of the 17 patients. A stricture at the pancreatojejunal anastomosis was seen in two patients, wheres strictures distally in the head of the pancreas were evident in four patients. The remainder had no change from preoperative studies. Six patients have undergone additional operations, four of which were pylorus-preserving Whipple procedures and two feeding jejunostomies.

On average, the number of hospitalizations, emergency room visits, frequency of attacks, or number of pain pills taken by the patients did not differ significantly before and after the operation (Table I). Overall, 59% of the patients have required hospitalization for recurrent pancreatitis or pain, 76% have visited the emergency room for the same reason, although they did not require hospital admission at the time. Narcotic use continued in 88% of the patients, even though 24% had characterized their pain as improved compared to before surgery. Seventy-six percent of the patients reported their pain as the same or worse than before LPJ, with 65% continuing to view their health status as poor.

Table I. Lateral pancreaticojejunostomy (LPJ): Clinical outcome

	Before LPJ	After LPJ
Hospitalizations*	3	2
Emergency room visits*	4	2
Frequency of attacks	Daily	Weekly
No. of pain pills (per week)	25	20

*Average number of hospitalizations or emergency room visits for the entire group during the 6 months before and after LPJ.

DISCUSSION

The etiology of pain in chronic pancreatitis remains uncertain. There are currently two main theories: hypertension of the ductal system or parenchyma and perineural inflammation. Hypertension of the main pancreatic ductal system has been observed in patients with chronic pancreatitis.[13,14] Relief of the elevated pressures by means of drainage has provided results supporting the theory of ductal hypertension. Pancreatic tissue pressures have been measured to be as high as 20 mm Hg in chronic pancreatitis, returning to normal (7 to 15 mm Hg) after surgical decompression.[15] This has also been demonstrated in several animal models.[16] However, some patients are not relieved of pain despite a documented reduction in ductal dilation, suggesting that other mechanisms such as perineural inflammation may be an important part of their pain syndrome.[17] Intraoperative measurement of pancreatic ductal pressures would have been valuable in this group of patients but was not attempted because of the number of patients with pancreatic stents in place.

Endoscopic Therapy

Placement of endoscopic stents to achieve decompression of the ductal system has been suggested to be safe and effective in relieving pain in some patients and a possible predictor of the success of surgical drainage. Problems with stent occlusion and migration remain a major concern. In addition, it has been observed in animal models that lesions in the ductal system are induced by the stent itself.[18,19] Some investigators have found that radiographic changes were reversible in most patients after stent retrieval.[20] The indications for endoscopic drainage of the pancreatic duct are obstruction of flow due to strictures, stones, or other conditions and recurrent pain or pancreatitis. When combined with other procedures such as stent placement, sphincterotomy results in alleviation of symptoms in some patients, but late stenosis of the pancreatic sphincterotomy site can occur in as many

as 14% of the cases.[20] Such was the case in at least three of our patients in whom repeated sphincterotomies were performed. Variable degrees of pain relief were obtained after endoscopic treatment in this group.

The typical patient in our group was a young white female with a history of sphincter of Oddi dysfunction. To date, studies have been inconclusive in establishing the exact nature of the relationship between chronic pancreatitis and sphincter of Oddi dysfunction. Papillary fibrosis and other pathologic changes have been evident in up to three fourths of patients with chronic pancreatitis.[21] A recent study from our institution supports a clear association between sphincter of Oddi dysfunction and early changes of chronic pancreatitis in patients with unexplained pancreatic pain.[22] An important question is that of the relationship between permanent pancreatic duct strictures and pancreatic stenting. An unfortunate scenario is that of a patient with pain of unknown etiology who has an ERCP and then develops post-ERCP pancreatitis. Subsequent stent placement leads to permanent duct stricture and chronic pancreatitis associated with narcotic abuse. The critical question becomes: Did this patient ever have pancreatic pain? The patients included in this study were judged to have pancreatic pain on the basis of clinical evaluation by experienced pancreaticobiliary endoscopists, as well as evidence of chronic pancreatitis on ERCP and endoscopic ultrasonography prior to any endoscopic therapeutic intervention.

Laboratory Evaluation

Traditionally, lipase and amylase serum levels have been used in the diagnosis of pancreatitis. These enzymes are markedly elevated in the acute setting but may be below normal in the chronic setting as a result of severe acinar atrophy. Most of our patients had at least one documented episode of highly elevated amylase and lipase levels during periods of pain. These elevations were short in duration and not consistent with symptoms at all times.

Some studies have shown an elevated serum CCK level in patients with chronic pancreatitis, whereas others have failed to demonstrate such an increase.[23] It is proposed that CCK may increase pancreatic enzyme secretion and thus indirectly contribute to pain. Gomez et al.[24] compared plasma CCK levels in three study groups (chronic pancreatitis with pain, chronic pancreatitis without pain, and normal controls) and found statistically significant differences between the groups with pancreatitis with and without pain. Patients with pain had a higher basal level of CCK. The CCK levels of the patients in our study were only mildly elevated. This may be suggestive of pronounced exocrine insufficiency.

Low serum trysin levels have been demonstrated in patients with chronic pancreatitis and pancreatic insufficiency.[25] A proposed mechanism of injury in pancreatitis is the release of active trypsin, resulting in activation of proenzymes and subsequently leading to autodigestion of the gland. Trypsin levels are particularly high in patients with alcoholic pancreatitis and have been touted as a sensitive and specific marker for alcoholic disease, even when amylase levels are normal.[26] Trysin levels were not markedly elevated in our group of patients, including those with a history of alcohol addiction. One patient had an extremely high trypsin level, but the etiology of her disease was considered to be sphincter of Oddi dysfunction.

Assessment of Pain

There is no standard method of assessing pain relief and quality of life in chronic pancreatitis. Several authors have emphasized the need to improve the reporting of outcomes of procedures aimed at relieving pain and decreasing the incidence of complications from chronic pancreatitis.[27] We used a quality-of-life questionnaire that was previously validated and used specifically in patients with chronic pancreatitis.[10] The questionnaire included questions intended to assess subjective well-being as well as objective questions such as the specific type of pain medicine taken, the number of pills per week, and the number of visits to a health care provider.

Although the number of hospitalizations, emergency room visits, and pain pills taken per week appeared to decrease somewhat after the operation in our group of patients, we believed these changes were minimal and constituted no meaningful alteration of their overall status. Some patients reported relief of pain in the immediate postoperative period but this was short-lived, and within weeks to months complaints of pain and requests for narcotics were practically unchanged from prior to the intervention. Three subjects have remained free of pain, and one of them was able to resume work activity. No other common characteristics were discernible among these three patients.

Narcotic use continued in 88% of the patients even though 24% characterized their pain as improved when compared to preoperative pain. In some patients, persistent narcotic use is a reflection of continued drug dependency. Assessment of pain is difficult in these patients because they use pain to justify the continued use of narcotics. The mean duration of

symptoms in this group was substantial (6.5 years) and ranged to as long as 25 years. This speaks for the chronicity of pain in this particular group.

Possible Reasons for Failure of the Operation

There are several possible reasons for the failure of LPJ in this series of patients. Inability to completely drain the head of the pancreas is a problem. Many of these patients had a very atrophic and fibrotic pancreas with severely diseased ducts that extended well into the proximity of the duodenum, making the drainage difficult. Likewise, narrowing of the duct in the body and tail and the presence of multiple obstructed side branches made it difficult to assess what in fact could be drained. In addition, LPJ fails to drain the duct of Santorini or ducts of the uncinate process. Morever, the dominant duct in the head of the pancreas, which courses in an anteroposterior direction, remains undrained with LPJ. Better results may be achieved with pancreatic head resection in patients whose underlying disorder is failure to drain the head and uncinate process.

Recurrence of stricture at the anastomotic site has been reported previously.[5,28] This occurred in two patients in our group in whom postoperative ERCP was performed. The strictures were successfully dilated endoscopically with a balloon through the scope, with no change in the clinical course marked by recurrent pain leading to hospitalization. Progression of the disease may have contributed to the recurrence of strictures in the head region apart from those found at the pancreatojejunal anastomosis. Some have reported success after reoperation to decompress residual dilated ducts and revision of LPJ.[29] Others have advocated pancreaticoduodenectomy if a long stricture of the main duct in the head of the pancreas is evident after operation.[30,31] Outcome has been favorable in the four patients who underwent subsequent pancreaticoduodenectomy; however, the number of patients and the length of follow-up prohibit drawing conclusions from these early results.

The etiology of pancreatic pain is complex explaining the variable results achieved with current treatment modalities. Although ductal hypertension was hypothesized to be the cause of pain in the patients presented herein, it is impossible to exclude other pathophysiologic factors that remain unrecognized. In addition, the contribution of perineural inflammation as the cause of pain remains difficult to qualify.

Last, this was a very difficult group of patients who were highly dependent on narcotics to control their pain and in whom true assessment of pain relief remains elusive. Pain associated with chronic pancreatitis is more complicated than improving ductal drainage of the pancreas.

The final question to which these data point is not why the operation failed but why it succeeded in some of the patients. None of the patients who had a good outcome had sphincter of Oddi dysfunction as the underlying cause of pancreatitis. Two patients had chronic fibrocalcific pancreatitis associated with alcohol abuse, and one had chronic pancreatitis associated with pancreas divisum. All three of these patients had ducts that did not measure more than 7 mm in diameter but were on the upper scale of duct dimension measuring more than 6.5 mm.

In summary, this group of patients is unique in their distribution in terms of the etiology of chronic pancreatitis. The majority of these patients did not have chronic pancreatitis associated with alcohol abuse. In patients with chronic pancreatitis with a nondilated pancreatic duct, LPJ appeared to be of little benefit with respect to pain relief, subsequent hospitalizations, continued narcotic use, or overall health status. LPJ was of no benefit in patients with nondilated pancreatic ducts and chronic pancreatitis associated with sphincter of Oddi dysfunction.

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Discussion

Dr. A.L. Warshaw (Boston, Mass.). I wonder whether one additional reason for failure that might have been on your list would be patient selection. You call this a study of chronic pancreatitis yet fewer than half of your patients showed fibrosis on their biopsies, which to my understanding is what defines chronic pancreatitis. More than half of your patients had elevated amylase and lipase levels, which is uncommon for chronic pancreatitis. More than half of them had definition of the pathogenesis etiology of sphincter of Oddi dysfunction or pancreas divisum, which is presumably lesser ampulla stenosis, and that again is not the usual cause of chronic pancreatitis. This is a group of patients that most of us struggle with and have not found a way to ameliorate. Can you tell us if the alcoholic chronic pancreatitis group did better than the others? I was not sure if you specified whether you performed a single-layer anastomosis to the pancreatic capsule vs. a duct-to-mucosa anastomosis. I wonder whether you obtained any information concerning relative patency of your anastomosis once you were finished.

Dr. G. Rios. Of the patients who were alcoholics, one did well; the others did not. We had 3 out of 17 patients who did get some pain relief from the operation. With this small a number, it is difficult to say why they did better than the others. All of the patients were classified as having chronic pancreatitis based on ERCP and some of them also had endoscopic ultrasound, which confirmed the diagnosis of chronic pancreatitis. The lipase and amylase levels were elevated on at least one occasion, although there were several times when patients were admitted for pain attacks with no elevation, but on at least one occasion we did document elevation of the enzymes. The anastomosis we used was a capsular single-layer anastomosis. Follow-up ERCPs in two patients showed complete obstruction. Immediately after the surgery, it was assumed that the anastomoses were patent.

Dr. L.W. Traverso (Seattle, Wash.). Your study suggests that in order to determine the results of an operation for chronic pancreatitis, it is very important to quantitate the amount of chronic pancreatitis present in the patient preoperatively. Dr. Cotton, at the Cambridge Conference in 1983, developed the anatomic classification of pancreatitis. None of these patients had what would be called severe chronic pancreatitis according to imaging studies. Can you

provide us with a composite picture of the pancreas derived not only from the ERCP findings but also from the CT findings? Do you have any more data on the specific ductal anatomy?

Dr. Rios. The measurements were made at the widest part of the pancreatic duct. The one patient classified as "severe" had features of stricturing in the duct. The others had changes in side branches and in the contour of the duct. I do not have any more details from the CT scans.

Dr. M. Sarr (Rochester, Minn.). We have all seen patients who despite having had a total pancreatectomy still have pain. We have reviewed our experience and found that many of these patients were addicted to narcotics preoperatively. How many of your patients were truly addicted?

Dr. Rios. It is difficult to say which patients were truly addicted. Most of them have enrolled in programs in one of our pain service clinics. Overall, some of the patients whom we treat with chronic pancreatitis are very difficult to manage and continue to call for prescriptions. We tried to establish some sort of a contract with them in an effort to reduce the number of narcotics they were prescribed. I cannot really say how many were truly addicted, but we do believe that most of them were.

Dr. S. Strasberg (St. Louis, Mo.). I think we may be seeing a new kind of patient within the past few years, and that is the patient who has fairly significant epigastric pain, undergoes a number of investigations, and finally has an ERCP. During the ERCP the patient undergoes a pancreatic sphincterotomy or perhaps a stent is placed. If you go back and review the history of these patients, the first time they ever have an elevated amylase level is after the first ERCP, and sometimes those patients end up with strictures in the pancreatic duct. These strictures are managed with surgery satisfactorily on an anatomic basis. They no longer come in with attacks of pancreatitis, but if they are evaluated later, they still have the same pain they had originally, which suggests that sometimes the source of these problems may be related to complications of the ERCP itself. Since some of your patients had elevated amylase levels, have you gone back and tried to determine if they had elevated serum amylase levels before they ever had their first ERCP.

Dr. Rios. No, we have not.

Dr. K. Lillemoe (Baltimore, Md.). Dr. Warshaw commented on the high incidence of sphincter dysfunction in your group, which is certainly atypical for most series and probably reflects your referral pattern with Dr. Cotton. Was anything done to the sphincter either preoperatively, endoscopically, with attempts at stenting? Did you consider adding a surgical sphincteroplasty in these patients, in addition to the pancreaticojejunostomy?

Dr. Rios. The typical patient in our group was a young white female with sphincter of Oddi dysfunction, which is not the typical etiology for pancreatitis. A number of these patients have had endoscopic interventions, sphincterotomies, sphincteroplasties, and stent placement in the biliary and pancreatic ducts, with variable degrees of pain relief. We did not consider performing surgical sphincteroplasty at that time.

Effect of Gut Transposition on the Expression of the Endocrine Gene Neurotensin

Xiao-Min Wang, M.D., Ph.D., Robert P. Thomas, M.D., B. Mark Evers, M.D.

Expression of the gene encoding neurotensin (NT/N) is developmentally regulated in the adult small bowel with maximal expression noted in the distal ileum; the mechanisms responsible for this strict spatial-specific expression pattern are not known. The purpose of this study was to determine whether NT/N expression is altered by ileojejunal transposition. Rats underwent either sham operation or ileojejunal transposition and were killed 2 months after operation. The transposed (either jejunum or ileum) and sham-operated segments of gut were removed, a portion was processed for histologic examination, and the remainder was extracted for total RNA and analyzed by ribonuclease protection using a rat NT/N probe. For comparison, expression of another gut endocrine gene, peptide YY, and an enterocyte-specific gene, sucrase-isomaltase (SI), was also determined. Expression of the gut endocrine genes, NT/N and peptide YY, were minimally affected by transposition of either the jejunum or ileum. In contrast, SI expression was markedly altered in both the transposed jejunum and ileum compared with corresponding sham gut segments. Expression of the NT/N gene is minimally altered after jejunoileal transposition despite marked adaptive and morphologic changes in the transposed segments. These findings provide further support that the strict pattern of NT/N expression is "imprinted" in the gut and maintained regardless of location along the cephalocaudal gut axis. (J GASTROINTEST SURG 1998;2:230-237.)

The mucosa of the small bowel is a dynamic tissue that is profoundly affected by luminal nutrients, dietary alterations, or disuse.[1-3] Despite their overall gross similarities, the jejunum and ileum possess a number of important functional, histologic, and molecular differences that occur in a well-defined pattern along both the vertical (crypt-to-villus) and longitudinal (duodenum-to-ileum) axis.[4-6] For example, there is a progressive decrease in villus height and mucosal mass from pylorus to ileocecal valve.[6] Transposition of the distal ileum to the proximal jejunum, however, results in hyperplastic villi that resemble the jejunum.[7-9] In contrast, transposition of a jejunal segment to the distal ileum results in a modest but significant hypoplasia of the villi. These findings suggest that villus growth is profoundly influenced by the luminal environment and position in relation to transit of intraluminal nutrients. In addition to the well-defined morphologic gradient that occurs in the small bowel, a number of gut-specific genes are localized in a strict expression pattern along the longitudinal gut axis.

Neurotensin (NT), an important regulatory hormone of the gut, is localized in the gastrointestinal tract to specialized enteroendocrine cells (N cells) of the adult small bowel.[10] NT, which is released by intraluminal fats,[11] facilitates translocation of fatty acids from the intestinal lumen[12] and affects numerous aspects of gastrointestinal function including pancreatic secretion,[13] gut motility,[14] and gut mucosal growth.[15,16] We have shown that the gene encoding NT and the structurally related hexapeptide neuromedin N (designated NT/N) is developmentally regulated in the gut of both rats and humans in a distinctive temporal- and spatial-specific pattern.[17] NT/N expression is initially low in the fetus but rapidly increases after birth to assume the distinctive adult topographic distribution of increasing NT/N

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expression along the longitudinal axis of its small bowel. We have evaluated the effect of different liquid diets and chow on NT/N expression and found that the relative distribution of NT/N messenger RNA along the jejunoileal gut axis was maintained independent of the diet administered,[18] thus suggesting that NT/N gene expression may be "imprinted" to a particular gut segment and not influenced by luminal nutrients. In contrast, the enterocyte-specific sucrase-isomaltase (SI) gene is altered dramatically depending on the type of diet. Unlike the well-characterized changes in villus morphology associated with transposition of gut segments, the effects of gut transposition on the expression of terminally differentiated genes has not been defined.

The purpose of our study was to determine the effect of transposing gut segments on expression of the NT/N gene. For comparison, expression levels of another gut endocrine gene, peptide YY (PYY), and the enterocyte-specific SI gene were determined in the transposed and sham gut segments.

MATERIAL AND METHODS

Experimental Design

Two-month-old male Fischer 344 rats (180 to 220 g; Harlan Sprague-Dawley, Inc., Indianapolis, Ind.) were housed for at least 1 week prior to operation at a constant temperature (22° C) with 12-hour light/dark cycles. During this period all rats were fed standard laboratory chow (Rat Chow; Ralston Purina, St. Louis, Mo.) ad libitum. After acclimation, the rats were fasted overnight with free access to water. The next morning, rats were weighed, randomized into four groups, and then anesthetized with Nembutal (40 mg/kg intraperitoneally). The peritoneal cavity was entered through a midline incision and either transposition of a jejunal segment to the distal ileum, transposition of an ileal segment to the proximal jejunum, or corresponding sham operations were performed as described below (n = 10 to 11 rats/group).

Jejunal Transposition (Fig. 1, *A*). A jejunal segment (5 to 8 cm in length) was divided approximately 3 to 4 cm distal to the ligament of Treitz and mobilized with its corresponding neurovascular supply to the distal ileum where the ileum was transected 2 to 3 cm from the ileocecal valve. The proximal jejunal segment was then anastomosed in an end-to-end fashion to the distal ileum with interrupted 6-0 silk sutures. Similarly, intestinal continuity of the proximal jejunum was restored by end-to-end enteroenterostomy with interrupted sutures. The midline abdominal wound was closed in one layer with 3-0 silk. The small bowel of the sham group of rats for this procedure was transected at three places (i.e., distal ileum and two places in the proximal jejunum) with immediate reanastomosis without jejunal transposition.

Ileal Transposition (Fig. 1, *B*). A 5 to 8 cm segment of distal ileum (2 to 3 cm from the ileocecal valve) was divided and mobilized as described above to the proximal jejunum (3 to 4 cm from the ligament of Treitz), where it was anastomosed end-to-end to the proximal jejunum. The distal ileum was reanastomosed, and the abdominal wound was closed. The small bowel of the sham group of rats for this procedure was transected at three places (i.e., proximal jejunum and two places

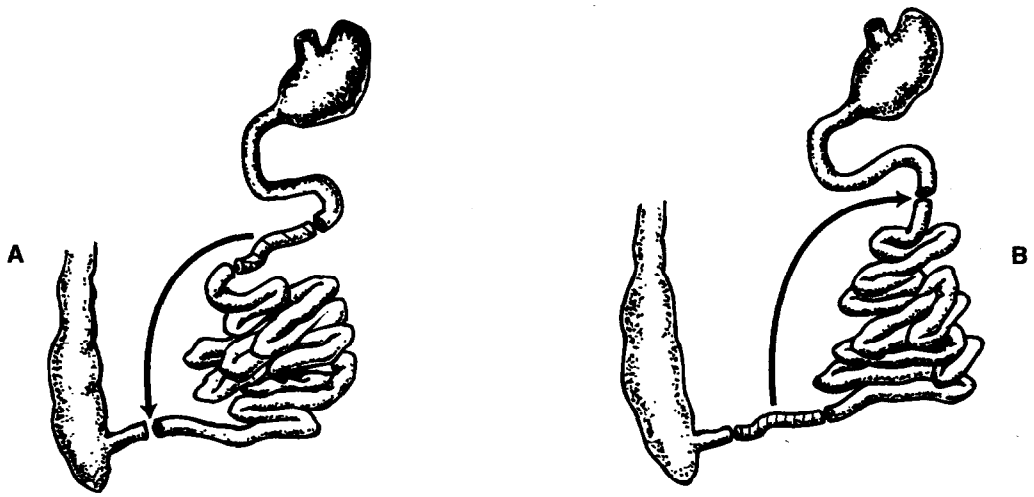

Fig. 1. Schematic diagram of jejunal transposition **(A)** and ileal transposition **(B)**. A 5 to 8 cm segment of gut (hatched lines) was transposed either to the distal ileum or proximal jejunum as described in Material and Methods.

in the distal ileum) with immediate reanastomosis without ileal transposition.

After the operation, all rats were allowed free access to water. On postoperative day 2, rats were allowed chow ad libitum for the remainder of the experiment. Two months after operation, the rats were weighed, sacrificed, and the transposed and corresponding sham gut segments were removed, immediately frozen in liquid nitrogen (N_2), and stored at −70° C. A small piece from each segment was fixed in 10% buffered formalin and embedded in paraffin. Sections (5 μm) were stained with hematoxylin and eosin, and villus height (from villus tip to the crypt-villus, junction) was measured with a calibrated eyepiece. Three random sites from each section (total of three gut sections per group) were observed and mean villus height was calculated. The time of 2 months after the procedure was selected so that the rats had fully recovered from the transcription procedure as determined by the fact that they were tolerating their regular chow diet and gaining weight consistent with the respective control groups.

RNA Extraction, Probe Preparation, and Ribonuclease Protection Assays

Total RNA was extracted from all full-thickness gut samples by the method of Chomcyznski and Sacchi,[19] using Ultraspec II (Biotecx Laboratories, Inc., Houston, Tex.) according to the manufacturer's specifications. To analyze NT/N gene expression in the gut, a rat genomic probe (pGEM E1I-NT) was used.[20] ^{32}P-labeled antisense RNA was transcribed by using T7 RNA polymerase and [α-^{32}P]cytidine triphosphate (CTP) after linearization with *Eco*RI. For PYY RNA analysis, a [α-^{32}P]CTP–labeled antisense RNA probe was synthesized from a *Stu*I linearized rat PYY probe.[21] For analysis of SI expression, a [α-^{32}P] CTP–labeled cRNA probe was synthesized from a *Xho*I linearized rat SI probe (pRSI-1).[22] To ensure the integrity of the cRNA samples, as well as equal loading, a linearized rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe from Ambion (Austin, Tex.) was used.

Ribonuclease (RNase) protection experiments were carried out by using the RPA-II kit (Ambion) according to the instructions of the supplier and by following our previously described methods.[23] Briefly, total RNA (50 μg) representing pooled samples from three rats was hybridized with the ^{32}P-labeled antisense RNA probes (2×10^5 cpm) overnight at 45° C, followed by digestion with a 1:250 dilution of RNase A/RNase T1 mixture for 30 minutes at 37° C. RNA was pelleted and resuspended in 8 μl of loading buffer. RNase-resistant fragments were separated on a 5% polyacrylamide-8 *M* urea gel and visualized by autoradiography. The signals were analyzed quantitatively using a Lynx 5000 digital image analysis system.

Statistical Analysis

Values for body weight and villus height are expressed as mean ± standard error of the mean and analyzed using Student's *t* test at the 0.05 level of significance.

RESULTS

Body Weight and Effect of Gut Transposition on Villus Height

There were no differences in initial or final body weights between the rats undergoing transposition and their respective sham controls (data not shown). As shown in Fig. 2, jejunum transposed to the ileum produced villi that were shorter and thicker and appeared similar to native ileum. Similarly, transposition of the ileum to the proximal jejunum produced villi that were longer and thinner than native ileum and morphologically resembled native jejunum. Actual measurements of the villus height demonstrated that the villi of the transposed jejunum were significantly shorter than those of the nontransposed sham jejunum (0.09 ± 0.13 mm vs. 0.69 ± 0.03 mm). Furthermore, the villus height of transposed ileum was significantly longer than the corresponding segment of nontransposed sham ileum (0.82 ± 0.03 mm vs. 0.56 ± 0.02 mm). These findings confirm the morphologic and adaptive changes noted by other investigators after gut transposition.[7-9]

Effect of Gut Transposition on NT/N and PYY Gene Expression

RNase protection analysis was used to assess the expression pattern of the gut-specific NT/N gene after transposition. The expected 169b protection product was noted in both the jejunum and ileum (Fig. 3, *A*). As previously described, NT/N expression is greater in the ileum compared to the jejunum.[24] Transposition of the ileum to the jejunal location produced an approximate 27% decrease in NT/N gene expression compared to sham ileum; however, the expression levels were still approximately sevenfold greater than in the jejunum (sham or transposed) (Fig. 3, *A* and *B*). Therefore even though an approximate 27% decrease was noted compared to the sham ileum, expression levels remained high compared with the jejunum and never reached the low level of NT/N expression normally found in this portion of gut. Transposition of the jejunum to the distal ileum produced

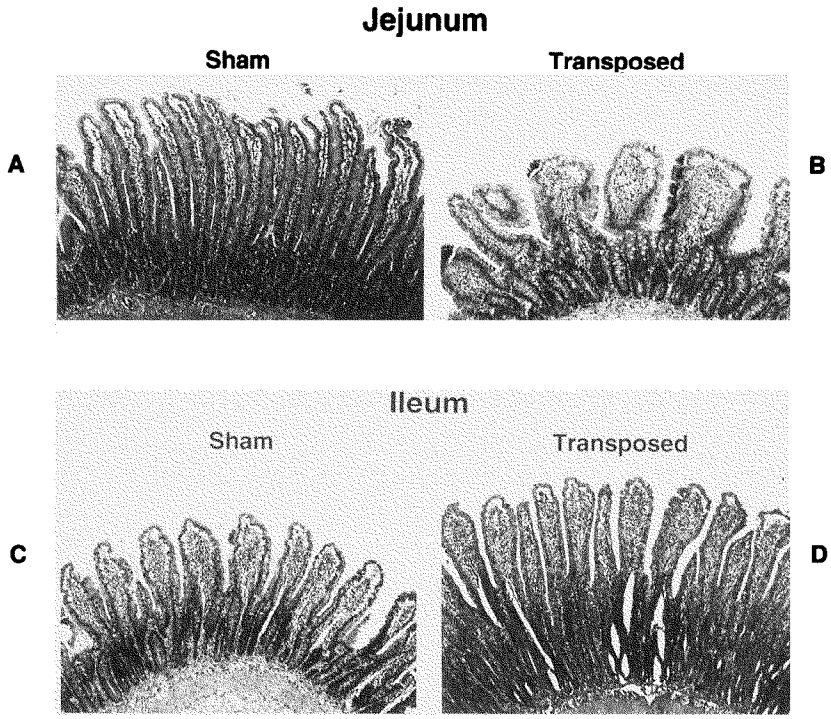

Fig. 2. Photomicrographs of cross sections of small intestine from sham **(A)** and transposed **(B)** jejunum and sham **(C)** and transposed **(D)** ileum demonstrated morphologic changes in villus architecture by transposition of gut segments to different locations. (Original magnification for all sections, ×64.)

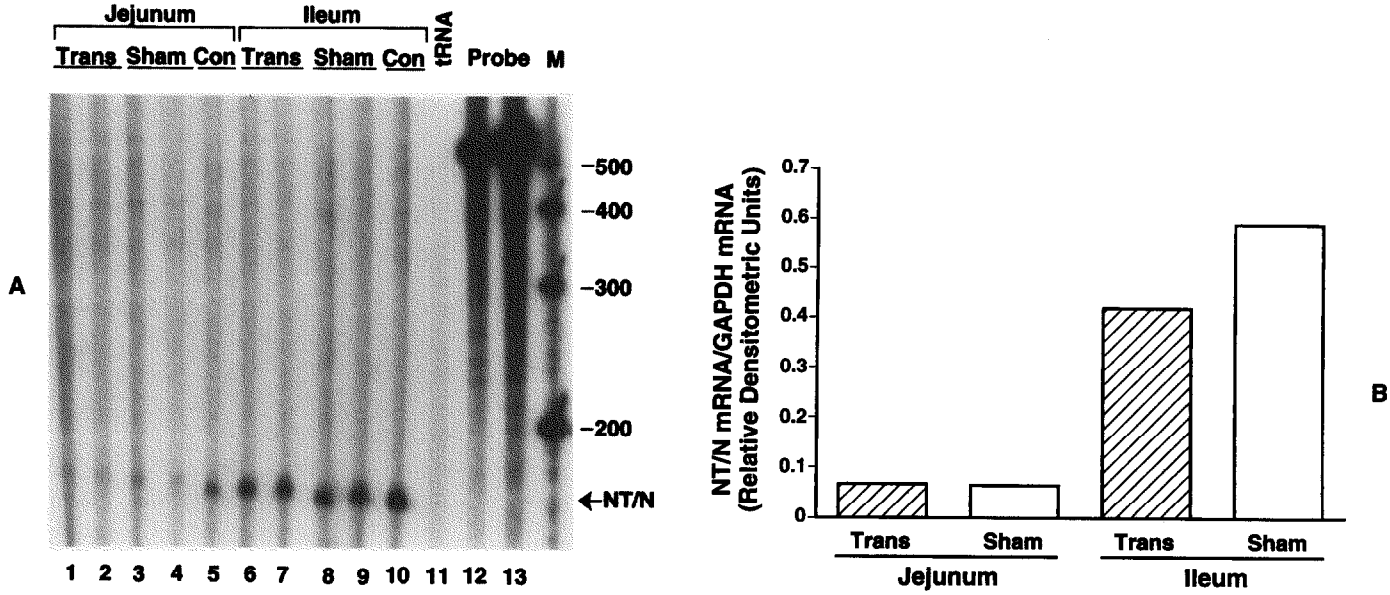

Fig. 3. RNase protection analysis of NT/N expression. **A,** RNase protection analysis of 50 μg total RNA from transposed *(Trans)*, sham, or control *(Con;* nonoperated) jejunum and ileum (each lane represents RNA pooled from three rats). RNA was hybridized with the ^{32}P-labeled antisense rat NT/N probe, and protected products (~169b) were separated on a gel. M = RNA molecular size marker (Ambion); *Probe* = probe alone without RNase; tRNA = transfer RNA (2 μg) + probe + RNase. **B,** Scanning densitometry of NT/N corrected for GAPDH mRNA (run on separate gel) and expressed as the mean in relative densitometric units. Open bar represents sham gut; single-hatched bar represents transposed gut.

no change in NT/N expression. The reason for the apparent increase in NT/N expression in the control (nonoperated) jejunum is not known since NT/N expression levels in the sham and transposed segments are more consistent with the expression of NT/N noted in other studies.[18] In addition, we compared NT/N expression with that of another gut endocrine gene, PYY, which is expressed in the distal ileum but not in the jejunum.[25] As expected, PYY was not expressed in the sham jejunal segments (Fig. 4, *A*). Furthermore, PYY was not expressed in the jejunum transposed to the ileal location; the expression of PYY in the ileum was not altered by transposition (Fig. 4, *A* and *B*).

To ensure the integrity of the RNA samples, separate RNase protection analyses were performed using

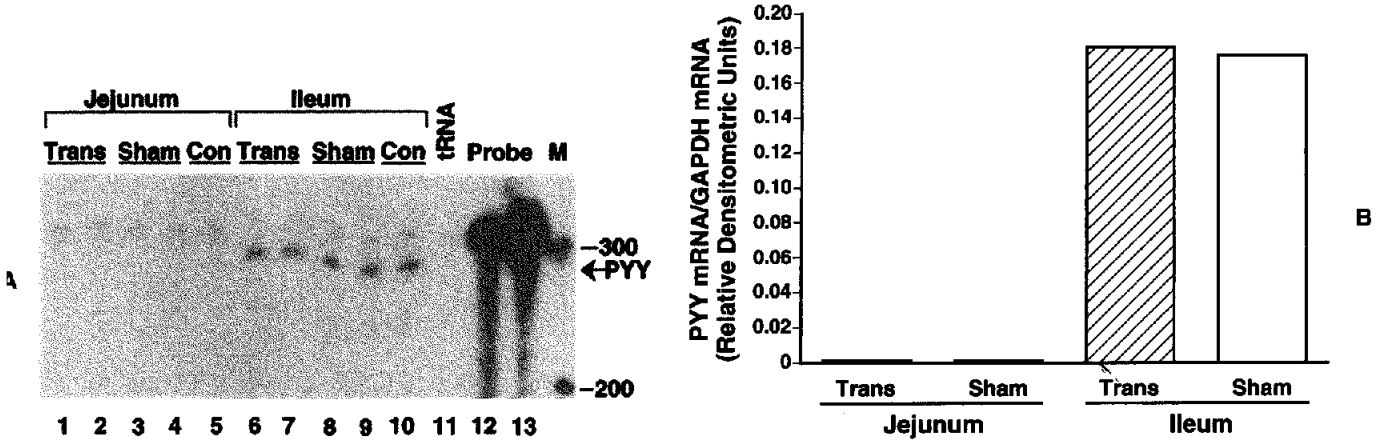

Fig. 4. RNase protection analysis of peptide YY *(PYY)* expression. **A,** RNase protection analysis of 50 μg total RNA from transposed *(Trans)*, sham, or control (*Con;* nonoperated) jejunum and ileum (each lane represents RNA pooled from three rats). RNA was hybridized with ^{32}P-labeled antisense rat PYY probe, and protected products (~290b) were separated on a gel. M = RNA molecular size marker (Ambion); Probe = probe alone without RNase; tRNA = transfer RNA (2 μg) + probe + RNase. **B,** Scanning densitometry of PYY corrected for GAPDH mRNA (run on separate gel) and expressed as the mean in relative densitometric units. Open bar represents sham gut; single-hatched bar represents transposed gut.

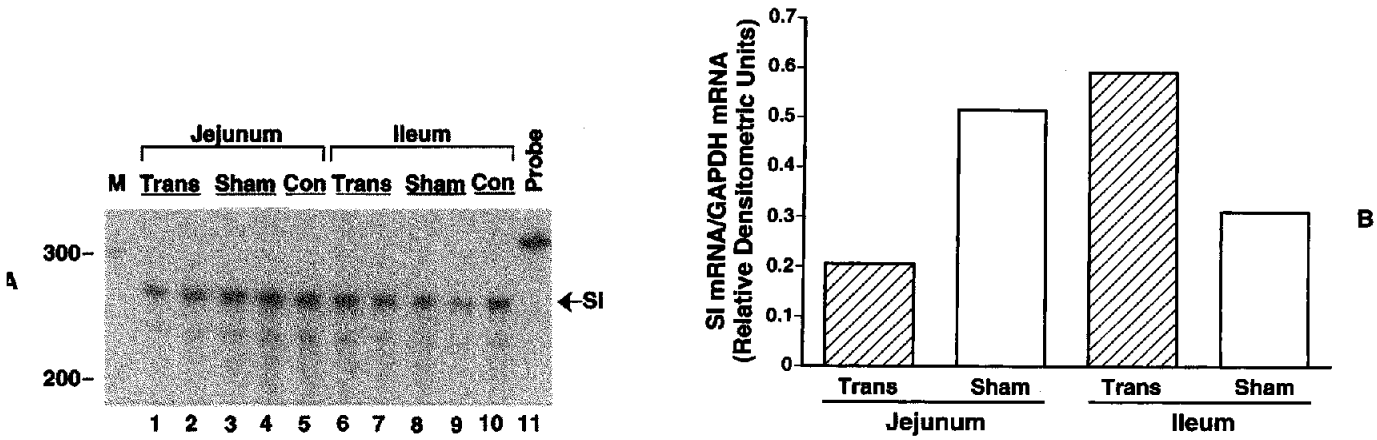

Fig. 5. RNase protection analysis of sucrase-isomaltase *(SI)* expression. **A,** RNase protection analysis of 50 μg total RNA from transposed *(Trans)*, sham, or control (*Con;* nonoperated) jejunum and ileum (each lane represents RNA pooled from three rats). RNA was hybridized with the ^{32}P-labeled antisense rat SI probe, and protected products (~269b) were separated on a gel. M = RNA molecular size marker (Ambion); Probe = probe alone without RNase. **B,** Scanning densitometry of SI corrected for GAPDH mRNA (run on separate gel) and expressed as the mean in relative densitometric units. Open bar represents sham gut; single-hatched bar represents transposed gut).

a rat GAPDH probe. All samples were intact, and the results for NT/N and PYY were normalized to those of GAPDH. Collectively these findings demonstrate that, despite the marked morphologic changes associated with transposing gut segments to different locations along the longitudinal axis, expression of the endocrine genes NT/N and PYY are minimally affected.

Effect of Transposition of SI Gene Expression

Expression of SI, an enterocyte-specific gene, is also maintained in a strict pattern in the gut with expression levels highest in the jejunum and decreased in the distal ileum.[26] In contrast to the endocrine genes, NT/N and PYY, SI mRNA levels were dramatically altered by gut transposition (Fig. 5, *A*). SI mRNA levels were greater in the transposed ileum and lower in the transposed jejunum compared to their respective sham gut segments, which were not transposed. In fact, SI expression levels of ileum transposed to the jejunal location and jejunum transposed to the ileal location approximated values found in the native (sham) jejunum and ileum, respectively (Fig. 5, *B*). Taken together, these findings further demonstrate that the expression of the enterocyte-specific SI gene is markedly affected by location along the longitudinal axis as opposed to endocrine genes in which expression is maintained despite changes in gut position and luminal composition.

DISCUSSION

In our present study we have confirmed the marked morphologic and adaptive changes that occur in gut mucosa of small bowel segments transposed to different locations along the luminal stream.[7-9] Characteristically the villi of the small bowel progressively decrease in size along the cephalocaudal gut axis; however, transposition of a segment of ileum to the proximal jejunum results in a dramatic hyperplasia of the villi and, in contrast, transposition of jejunum to the distal ileum results in a relative villus hypoplasia. Despite these marked adaptive changes, which occur with transposition, the terminally differentiated genes expressed in these gut segments have not been extensively analyzed. Determining whether changes occur in the strict expression patterns of these genes will better delineate the mechanisms regulating the well-defined gene expression patterns in the gut, as well as provide a better understanding of the complex process involved in gut differentiation and development. The strict expression patterns noted for the terminally differentiated genes of the gut may be "imprinted" and therefore cannot be altered despite transposition of the gut to different locations. Alternatively, the luminal environment and position along the gut axis may greatly affect the expression of these genes.

Despite the marked adaptive changes in the gut mucosa following transposition, expression of the NT/N gene was minimally affected compared to sham segments of gut, which had not been transposed. Previously we have shown that the NT/N gene is not altered by feeding rats different elemental diets or by ectopic placement of gut segments into the flanks of athymic nude mice.[18] Collectively these results support the notion that expression of the NT/N gene in the gut is a preprogrammed event and appears "imprinted" to the particular segment of gut with only minor influences of luminal factors and position along the gut axis on the final pattern of NT/N gene expression. Moreover, these findings lend further support for the molecular analysis of the terminally differentiated NT/N gene as a possible model for better elucidation of the factors regulating gut development and differentiation since similar factors controlling expression of NT/N may also affect these cellular processes.

For comparison we analyzed the expression of another gut endocrine gene, PYY, a 36-amino acid peptide released into the circulation and lumen of the small intestine from mucosal endocrine cells localized to the terminal ileum and colon.[25,27] Consistent with these reports, we did not detect PYY gene expression in the sham (nontransposed) jejunum. Furthermore, PYY was not expressed in jejunum transposed to the ileal position, and the expression of PYY in the distal ileum was not altered by transposition to the jejunal location. Therefore our finding that endocrine gene expression is only minimally affected by transposition suggests that expression of these differentiated genes is not altered by changing the location of the gut segment in relation to the luminal stream. Consistent with our results, Rubin et al.[28,29] have noted that the complex program of endocrine cell differentiation could be maintained in the absence of luminal contents using transgenes of rat liver fatty acid binding protein *(fabp)* linked to the human growth hormone reporter gene.

In marked contrast to the NT/N and PYY genes, expression of SI was markedly altered by transposition of either jejunum or ileum. SI gene expression, which is normally elevated in the jejunum, was decreased when jejunum was transposed to the ileal location. Conversely, transposition of the ileal segment, which normally has a low level of SI expression, produced an increase in SI gene expression when transposed to the proximal jejunum. Once again, these findings are consistent with our previous report,[17] as well as the reports of others,[30,31] demonstrating that

SI gene expression is greatly influenced by both dietary components and the location of the gut segment along the cephalocaudal axis, thus confirming the fact that SI gene expression is dramatically affected by environmental influences.

CONCLUSION

Jejunoileal transposition produced marked adaptive and morphologic changes in the mucosal architecture of the small bowel. Despite these changes in morphology, expression of the gut endocrine genes, NT/N and PYY, remain relatively unaltered, suggesting that the specific pattern of expression noted for these terminally differentiated endocrine genes appears to be imprinted and maintained in the gut regardless of location along the longitudinal gut axis. In contrast, the enterocyte-specific gene SI was altered depending on its location, thus further demonstrating the dependence of environmental factors on the pattern of SI gene expression in the gut. Collectively these results provide a better understanding of mechanisms regulating the intricate and complex gene expression patterns in the small bowel. Further studies will better elucidate the molecular factors responsible for this apparent "preprogrammed" expression pattern of these endocrine genes. The fact that the expression of the small bowel–specific NT/N gene changes in an orderly fashion during normal gut development, but is only minimally affected by environmental influences, suggests that NT/N will continue to provide a useful molecular model to achieve a better understanding of the factors responsible for gut differentiation.

We thank Drs. Paul Dobner (University of Massachusetts, Worchester, Mass.), Peter Traber (University of Pennsylvania, Philadelphia, Pa.), and Andrew Leiter (New England Medical Center, Boston, Mass.) for providing the rat NT/N, sucrase-isomaltase, and peptide YY probes, respectively. In addition, we thank Eileen Figueroa and Karen Martin for manuscript preparation.

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Discussion

Dr. B. Schirmer (Charlottesville, N.C.). It would seem as though your data negate the theory that PYY acts as an ileal break based on luminal content.

Dr. B.M. Evers. I do not think our data negate that theory because neurotensin, like PYY, is released by fats. We expected to find that dietary influences would affect neurotensin gene expression, but it did not. I think we are addressing two different issues. The gene expression appears not to be affected by these various environmental influences, whereas the protein that can be released is dependent on the intraluminal contents.

Dr. R.A. Hoden (Boston, Mass.). I am wondering about the effect of cell number. Do you have any data on the number of endocrine cells depending on the surgical manipulation?

Dr. Evers. I do not have those data. We certainly need to perform some immunohistochemical studies.

Dr. D. Soybell (Boston, Mass.). Suppose that you take the distal ileum out of continuity entirely and make a Thiry-Vella loop and infuse in that loop just proximal succus vs. distal succus. Would that be a better model?

Dr. Evers. I do not know. We have taken segments out, we have placed them into nude mice, and we see the same sort of temporal- and spatial-specific expression pattern that we see in situ. We have placed rats on various elemental diets for several months and the pattern does not change.

Dr. Soybell. One thing that I think would be very important is to take the distal ileum out of continuity and let it atrophy and prove that the neurotensin gene will not be expressed. It would at least tell you that luminal nutrients play some permissive role.

Dr. Evers. That is a great suggestion.

Dr. G. Telford (Milwaukee, Wis.). What happens to the expression of this gene if you resect the distal bowel and try to drive it to express itself in the proximal bowel.

Dr. Evers. We have tried that. Expression is minimally altered. The protein goes way up, but the gene expression is not changed so we have speculated that there is a translational or at least a post-transcriptional effect of resection, but it does not appear to affect the actual expression of the gene.

Selective Increase in Gastric Mucosal mRNA Encoding Basolateral Na-K-2Cl Cotransporter Following Ileostomy in the Rat

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Results of previous studies suggest that major surgical resections or reconstructions of the distal small intestine can alter morphologic and functional properties of the stomach. Little is known about the effect of lesser surgical alterations, such as construction of an ileostomy, on the morphology and transport properties of the gastric mucosa. To evaluate the effects of ileostomy, Sprague-Dawley rats underwent sham laparotomy (n = 10) or loop ileostomy construction (n = 10). After body weights had stabilized (~21 days), the animals were killed. Gastric mucosal scrapings were prepared for Northern blot analysis of messenger RNA levels for (1) H/K ATPase, found in parietal cells; (2) Na-K-2Cl cotransporter, found in both parietal and surface cells; and (3) Na/K ATPase, found in all gastric mucosal cells. Gastric mucosa from ileostomy animals was visibly hypertrophied compared to sham-operated animals. There was a 145% increase in the mRNA levels of the Na-K-2Cl cotransporter in gastric mucosa of the ileostomy group but no significant changes in H/K ATPase or Na/K ATPase mRNA levels. Construction of an ileostomy selectively enhances expression of the Na-K-Cl cotransporter in the gastric mucosa. Further studies are required to understand the neurohumoral stimuli underlying this selective response. (J GASTROINTEST SURG 1998;2:238-243.)

It is well recognized that extensive surgical resections and reconstructions of the distal small intestine can alter the morphologic and functional characteristics of the stomach and proximal regions of the small intestine.[1-4] In humans and experimental animals, massive small bowel resection induces significant compensatory hypertrophy and hyperplasia in the mucosa of the remaining small intestine and stomach.[1,3-5] In addition, massive small bowel resection has been shown to cause hypergastrinemia, gastric mucosal hyperplasia, and increased basal acid secretion.[6,7] Surgical procedures involving bypass of extensive regions of small bowel do not elicit significant alterations in morphology of the mucosa in the intestinal segments proximal to the bypass. Such bypass procedures are associated with G (gastrin)-cell hyperplasia and increased levels of basal acid secretion by the stomach.[5,8] These observations indicate that extensive resection or bypass of different regions of the small intestine leads to the changes in the neurohumoral milieu, which in turn cause morphologic and functional changes in the mucosa of the most proximal intestine and stomach.

There is little information on adaptive responses of the stomach or more proximal intestinal regions after nonresective surgical alterations of the distal small intestine. In this study we used a rat model to explore the response of the gastric mucosa to construction of a loop ileostomy. To evaluate the functional consequences of ileostomy on the gastric mucosa, we measured mRNA levels encoding for ion transporters expressed by both the acid-secreting parietal cells and

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the mucus/HCO_3^-–secreting surface epithelial cells. These transporters include (1) the H/K ATPase expressed exclusively in the apical membranes of parietal cells and responsible for secretion of H^+ ions into the gastric lumen, (2) the Na-K-2Cl cotransporter expressed in the basolateral membranes of parietal cells and surface epithelial cells and responsible for Cl^- uptake from the serosal or nutrient compartment during periods of active secretion,[9-11] and (3) the Na/K ATPase expressed by all epithelial cells and not as directly responsible for secretory activity by the parietal or surface epithelial cells. Our findings indicate that construction of an enterostomy in the distal ileum results in selective increases in mRNA levels of the Na-K-2Cl cotransporter, but not of the H/K ATPase or Na/K ATPase, that are expressed in the gastric mucosa.

MATERIAL AND METHODS

Animal Model

The study protocol was approved by the Harvard Medical Area Standing Committee on Animals at Harvard Medical School, Boston, Massachusetts. Twenty adult, 4- to 6-month-old, male Sprague-Dawley rats had operative procedures. Ten animals randomly underwent a sham laparotomy. The remaining 10 animals underwent construction of a loop ileostomy modified from the procedure described by Bleyl et al.[12] All animals underwent similar preoperative and postoperative care. This entailed food deprivation for 24 hours before and 24 hours after operation. All animals had access to water before surgery, and to both water and 0.25 normal saline solution *ad libitum* after surgery. Animals were anesthetized using a combination of ketamine (40 mg/kg) and xylazine (5 mg/kg) administered intramuscularly. After induction of anesthesia, a 22-gauge, 1-inch intravenous catheter was placed in the tail vein, and a syringe was attached to provide intravenous fluid during the operative and postoperative recovery period. All animals received a total of 3.0 ml in 0.25 ml boluses of normal saline solution prior to removal of the intravenous cannula. Body temperature was monitored with a rectal thermistor. During all operations, each animal's body temperature was maintained above 35° C by use of a warming blanket. Postoperative pain control was achieved by subcutaneous injection of Buprenex (buprenorphine hydrochloride, 0.1 mg/kg) administered 4 hours after the animals were fully recovered from the anesthetic and then again 12 hours from that injection. Additional doses were given only if animal's behavior indicated discomfort. Postoperatively animals' weights were recorded daily. Ileostomy animals were given daily baths on postoperative days 2 to 9 to prevent skin injury from the caustic ileostomy effluent.

All operations were performed under sterile conditions. After the induction of anesthesia, each animal's abdomen was shaved and cleansed with 95% ethanol. In both sham and ileostomy animals, the abdomen was entered through a 4 cm midline incision. In the sham laparotory animals (N = 10), once the abdomen was entered, the distal small intestine and cecum were identified. The distal small intestine and cecum were then brought out of the midline incision and draped with warm saline-soaked gauze. Ten minutes after exteriorization, the distal small intestine and cecum were returned to the abdominal cavity. The midline incision was then closed with a running 4-0 Vicryl suture. The skin edges were approximated and closed with animal skin staples. In ileostomy animals (N = 10), after the abdomen was entered, a 4 to 6 mm circular stoma was created in the abdominal musculature and skin approximately 1.0 cm to the right of the midline incision. A loop of distal ileum, 5.0 cm proximal to the ileocecal valve, was delivered through the stoma. The loop of ileum was opened along the antimesenteric border for a distance of 1.0 cm. The free edges of this loop were matured to the abdominal musculature and skin with interrupted 4.0 silk sutures. Prior to closure of the abdomen, both afferent and efferent limbs of the loop ileostomy were examined for patency by infusion of warm saline solution through a 22-gauge intravenous catheter. The abdominal cavity was closed as previously described.

Tissue Preparation for mRNA

Animals were killed by a pentobarbitol overdose (100 mg/kg). Their stomachs were harvested, discarding the forestomach, and the mucosa was separated rapidly from the underlying muscularis by sharp dissection. This mucosal tissue was frozen and stored in liquid nitrogen until processed for mRNA. Messenger RNA from the samples was isolated by RNA-zol B (Tel-test, Inc., Friendswood, Tex.). This isolation kit was slightly modified by the addition of the following steps. After the RNA pellet was resuspended in diethyl pyrocarbonate (DEPC), water, chloroform, and isoamyl alcohol were added in a 2:1:1 ratio, vortexed for 30 seconds, and then centrifuged at 14,000 rpm. The supernate was washed with a 1:1 ratio of chloroform to isoamyl alcohol. The supernate was then added to a mixture of sodium-acetate, pH 5.2 and 2 volumes of 100%, and allowed to precipitate overnight at −80° C. The solution was centrifuged and the pellet was washed in 70% ethanol followed by a resuspension in DEPC-treated water. The RNA samples were then stored at −80° C until use.

Northern Blot Analysis

Total RNA (10 μg per lane) was run on a 1% agarose gel containing 0.63% formaldehyde. The RNA was then transferred to nylon membranes and underwent ultraviolet cross-linking. The membranes were prewashed at 50° C in a NaCl, sodium dodecyl sulfate (SDS) solution for 30 minutes, then prehybridized for 2 hours at 42° C in a NaCl, formamide, and dextran sulfate solution. The membranes were hybridized overnight with 10^6 counts × min^{-1} × ml^{-1} ^{32}P-labeled cDNA probe for each of the following three ion transporters: (1) bumetanide-sensitive Na-K-2Cl cotransporter (1200 base pairs, transmembrane domain, provided by Eric Delpire, Ph.D., Children's Hospital, Boston, Mass.), (2) omeprazole-sensitive H/K ATPase (200 base pairs, alpha subunit, provided by Bruce Kone, Ph.D., Gainesville, Fla.), and (3) ouabain-sensitive Na/K ATPase (3500 base pairs, alpha subunit, provided by Bruce Kone). The membranes were washed twice at low stringency (room temperature, 300 mmol/L NaCl) and once at higher stringency (65° C, 30 mmol/L NaCl). The membranes were then exposed to x-ray film (Reflexion NEF; Du Pont Company, Boston, Mass.) at −80° C for 1 to 3 days. Quantification of relative mRNA abundance was performed via scanning densitometry (Microtek Image Scanner, Microtek International, Inc., Hsinchu, Taiwan; Image 1.49 software from National Institutes of Health, Bethesda, Md.). Membranes were allowed several weeks between probing so as to not require stripping. All membranes were probed with ^{32}P-labeled cDNA encoding the housekeeping enzyme, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), to provide additional confirmation of the equivalence of gel loading.[11]

Statistical Analysis

Data were recorded and analyzed with a standard software statistical package (Excel, Microsoft Corp., Redmond, Wash.). Comparisons of measurements between experimental groups were carried out by means of analysis of variance (ANOVA) for multiple measurements.

RESULTS

Animal Response to Ileostomy

Animal weight was monitored daily throughout the study. As shown in Fig. 1, *A*, there was no significant difference between the two operative groups prior to the operations. The sham group had a preoperative weight of 431 ± 13 gm and ileostomy animals weighed 484 ± 26 gm. Postoperatively the sham animals had an initial weight loss but regained weight by the end of the first postoperative week. By the time of death, the sham laparotomy animals were approximately 7% above their preoperative weight (Fig. 1, *B*). Ileostomy animals had severe postoperative diarrhea from postoperative day 2 to approximately day 9. During this time period they had significant weight loss. By the time their weights had stabilized (range day 18 to 24) they had lost nearly 30% of their body weight but maintained normal, healthy behavior (see Fig. 1, *B*). Weight stabilization was defined by 5 days of a stable daily weight with variation no greater than 5 gm or by observation of a gain in weight. Ileostomy animals' weights usually stabilized between 2 to 3 weeks after the operation. A randomly selected sham animal was killed whenever an ileostomy animal was killed.

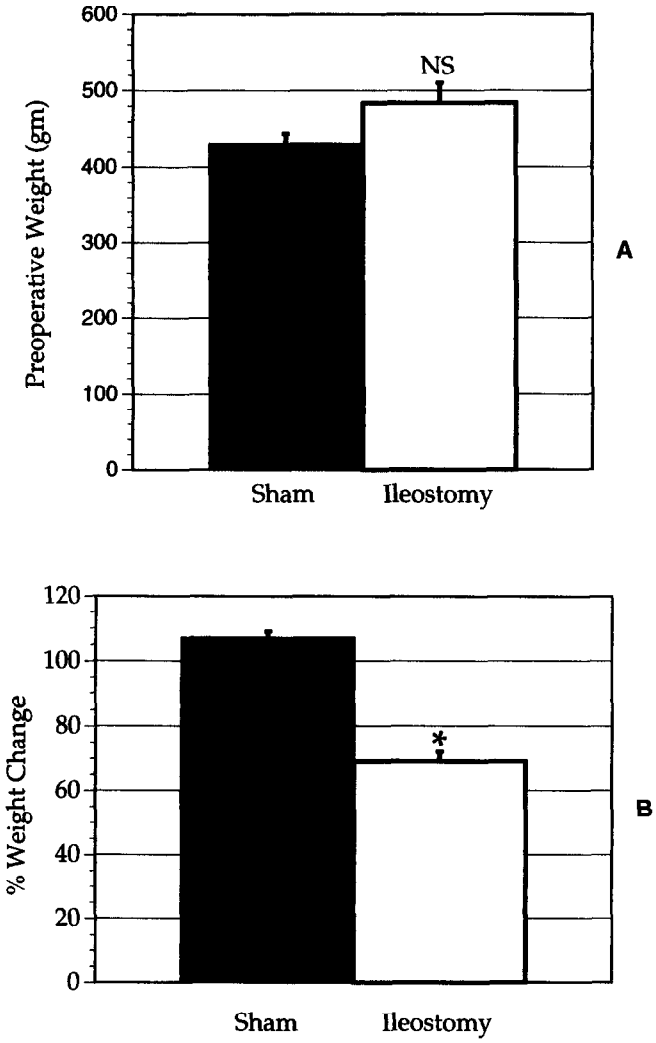

Fig. 1. A, Results are expressed in grams as means ± standard error (N = 10). Statistical analysis by ANOVA (NS = nonsignificant). **B,** Results are expressed as percentage of weight change from preoperative weight as means ± standard error (N = 10). Statistical analysis by ANOVA (* = $P < 0.05$).

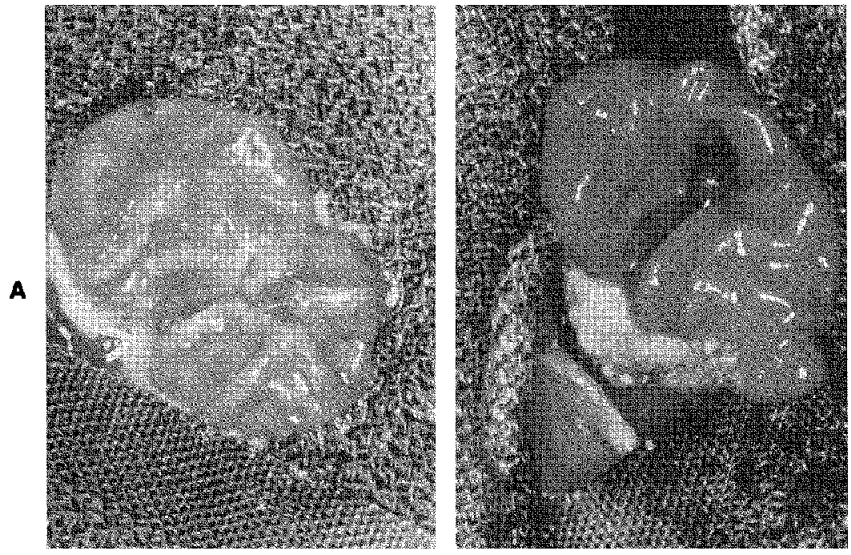

Fig. 2. Comparison of the gastric mucosa between a sham animal **(A)** and an ileostomy animal **(B)**.

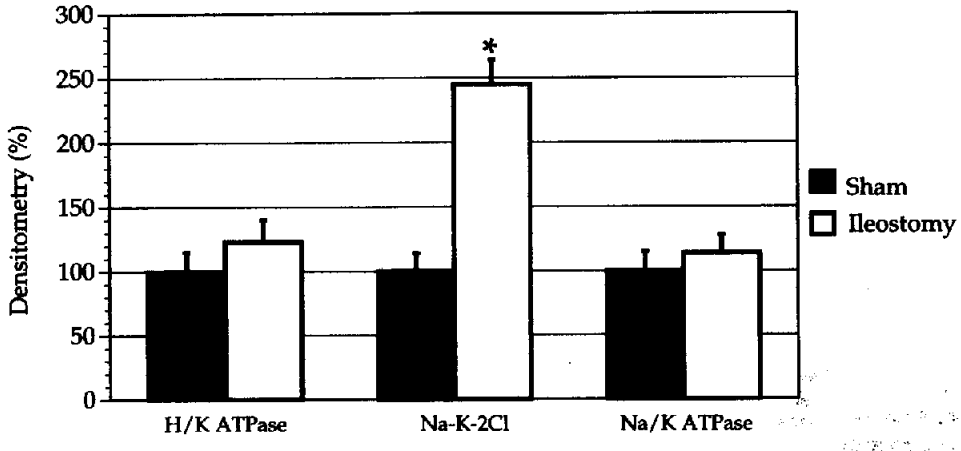

Fig. 3. Comparison of the total mRNA levels of the selected ion transporters in sham vs. ileostomy animals. Results are expressed as means ± standard error (N = 5). Statistical analysis by ANOVA (* = $P < 0.05$).

Mucosal Appearance and Transporter mRNA Levels

At the time of death, the stomach and intestines of each animal were inspected. In sham animals the stomach typically appeared full of solid food. Variable amounts of succus entericus were visible in the small intestine. The cecum and colon were invariably full of semisolid fecal matter. In the ileostomy group the stomach was typically enlarged, with a mildly dilated lumen. The small intestine proximal to the ileostomy was mildly dilated. The ileum distal to the stoma, the cecum, and colon were devoid of luminal contents. In the ileostomy group, following opening of the stomach and intestines, just prior to harvest of mucosal scrapings, inspection revealed a mild but noticeable thickening of the mucosal surface all along the regions proximal to the stoma. The stomach mucosa itself appeared to have an increased number of rugae. Distal to the ileostomy, the mucosa was noticeably atrophied and thinned, compared to sham animals (Fig. 2).

Densitometric analysis of Northern blots revealed a mean 145% increase in the mRNA expression of the basolateral Na-K-2Cl cotransporter in the ileostomy group above levels measured in the sham-operated group ($P < 0.001$). Messenger RNA levels of the H/K ATPase and Na/K ATPase were not significantly different in the ileostomy animals, compared to levels measured in the sham-operated group (Fig. 3).

DISCUSSION

The principal finding in this study was that construction of a distal loop ileostomy in the rat results in a mild but visible alteration in the size and appearance of the mucosa of the stomach. In addition, we observed an increase in the levels of mRNA encoding for the basolateral Na-K-2Cl cotransporter in gastric mucosa scrapings. We did not detect associated alterations in levels of mRNA encoding for H/K ATPase or Na/K ATPase.

Previous experimental studies have focused on the impact of extensive resections or complex reconstructions on the physiology and morphology of the intestines and stomach.[2,8,13] With regard to the effect of massive small bowel resection, the most consistent finding has been a period of prolonged hypergastrinemia resulting in gastric mucosal hyperplasia and increased acid secretion.[6,7] In contrast, distal small intestinal bypass has not been associated with consistent morphologic changes in the gastric mucosa, in clinical or experimental studies. Nevertheless, in one experimental study, increased acid secretion was observed after distal intestinal bypass.[8] These observations have been taken to suggest that there is a feedback mechanism that communicates the state of the distal small intestine to the more proximal regions of the gastrointestinal tract. Our preliminary findings indicate that a nonresectional surgical alteration of the distal intestine, such as ileostomy, may influence the function in the most proximal regions of the gastrointestinal tract.

In this study we observed a selective increase in mRNA encoding for the basolateral Na-K-2Cl cotransporter. The fact that mRNA levels of the Na/K ATPase did not change suggests that the response to ileostomy does not represent a nonspecific increase in transporter expression throughout the gastric mucosa. Our previous work suggested that when acid secretion is stimulated by feeding or elevation of circulating gastrin levels, mRNA levels of both the H/K ATPase and the Na-K-2Cl cotransporter increase together.[10,11] The increases in expression of these key transporters involved in acid secretion can be elicited by hypergastrinemia, even if acid secretion is inhibited by administration of omeprazole.[14] Thus, if ileostomy was associated with increases in circulating gastrin or sustained increases in basal levels of acid secretion, we would have expected that mRNA levels for the apical H/K ATPase would have been increased along with those of the basolateral Na-K-2Cl cotransporter. Our findings would suggest that it is not the parietal cells but rather the surface cells that are reponsive to the changes caused by ileostomy. The cellular source of the increase in mRNA encoding for the Na-K-2Cl cotransporter will require further exploration, preferably with the use of in situ hybridization techniques and immunocytochemistry using specific antibodies to the cotransporter.

Although we have demonstrated a selective increase in the mRNA expression of the gastric mucosal Na-K-2Cl cotransporter in response to distal loop ileostomy, the physiologic significance of such an increase is not immediately obvious. It is known that in other mucus-secreting epithelium that apical Cl^- secretion is essential for proper hydration of the secreted mucus.[15] It is thus tempting to speculate that the selective increase in the Na-K-2Cl cotransporter may be occurring in the mucus-secreting surface cells in response to a neurohumoral stimulus to mucus secretion. In this regard we would note that the general dilatation and mucosal thickening of the stomach and intestines proximal to the ileostomy was a consistent finding. Confirmation of this qualitative observation by rigorous morphometric techniques would support the hypothesis that adaptation of the stomach and proximal intestine after ileostomy leads to selective hypertrophy of mucosal elements, possibly the surface epithelium that provides mucosal resistance to injury by prolonged contact with noxious substances in the lumen.

In summary, we have shown that construction of a distal small intestine loop ileostomy can alter physiologic processes in the most proximal region of the alimentary tract. The loop ileostomy in the rat may serve as a useful model for studying the neurohumoral feedback mechanisms between the distal and proximal regions of the gastrointestinal tract. This model may thus provide insight into physiologic adaptations that occur in disease processes affecting the ileum or after ileostomy in humans.

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Prognostic Significance of Intraperitoneal Free Cancer Cells Obtained by Laparoscopic Peritoneal Lavage in Patients With Gastric Cancer

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Laparoscopy is a safe and useful method for examining the local extent and regional spread of disease in patients with gastric cancer. Peritoneal dissemination remains a frequent type of recurrence after surgical treatment. The aim of this study was to determine the prognostic value of intraperitoneal free cancer cells (IFCCs) detected by laparoscopic peritoneal lavage. Forty-nine patients with advanced gastric cancer underwent laparoscopy with cytologic examination for staging. Peritoneal lavage was performed when ascites was not present. Aspirated fluid from the peritoneal cavity was centrifuged and subjected to cytologic examination using Giemsa and Papanicolaou staining methods. Patients were surgically treated and followed for a minimum of 5 years. IFCCs were detected in 41% of the patients. In eight cases (16.3%) laparoscopy revealed carcinomatosis and/or multiple liver metastases, so laparotomy was not performed. Patterns of recurrence after curative resection included the following: peritoneal (n = 3), local (n = 4), liver (n = 1), and other (n = 1). All patients who tested positive for IFCCs had peritoneal recurrence. The absence of IFCCs was associated with improved overall survival (21 months for a 95% confidence interval of 7.4 to 34.6 vs 4 months for a 95% confidence interval of 2.4 to 5.6). Overall survival adjusted for type of resection also demonstrated a favorable outcome for patients who were negative for IFCCs. The following conclusions were drawn: (1) laparoscopic peritoneal lavage cytology may be useful in identifying patients at high risk for peritoneal relapses and may alter treatment, and (2) IFCCs provide additional prognostic information in patients with gastric cancer. (J GASTROINTEST SURG 1998;2:244-249.)

Gastric cancer carries an overall poor prognosis[1] and even after potentially curative resection, approximately 50% of the patients die of recurrent disease during the first 2 years of follow-up.[2,3] Peritoneal dissemination is the most common type of recurrence in gastric cancer after curative surgery.[3-8] Such recurrences may originate from intraperitoneal dissemination of malignant cells or they may be the result of surgical manipulation.[3,5,7,9,10]

Peritoneal lavage cytology has not been routinely incorporated into the management of gastrointestinal carcinomas, although pancreas, colon, and gastric cancers are quite often associated with ascites and/or the presence of free tumor cells at the time of the initial diagnosis.[9] Therefore the search for intraperitoneal free cancer cells (IFCCs) may be pertinent in the staging and management of gastric carcinomas. Laparoscopy can be utilized in this regard. It is a safe and useful method for examining the local extent and regional spread of this disease and peritoneal lavage can be performed.[11-17] Laparoscopy allows easy access to intra-abdominal structures for biopsy and determining local resectability, thereby avoiding unnecessary high-risk laparotomy procedures.[11-17] Furthermore, it can aid in the diagnosis of occult intra-abdominal M1 disease that might otherwise go undetected by standard radiologic methods with implications for treatment and quality-of-life decisions.

We have previously shown that IFCCs are commonly present when invasion of the gastric serosa is greater than 3 cm^2 or when adjacent organs and structures are invaded. IFCCs are also more frequent in Borrmann class IV tumors, in diffuse type carcinomas, and in patients with advanced TNM stage disease.

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There appears to be no association between lymph nodes and liver metastases and the presence of IFCCs.[17]

Patients who test positive for IFCCs by means of laparoscopic peritoneal lavage cytology may be at high risk for peritoneal recurrence, which may correlate with poor prognosis. Thus the purpose of this study was twofold: (1) to correlate results of cytologic examinations with patterns of recurrence following resection and (2) to determine the prognostic value of IFCCs.

PATIENTS AND METHODS

Forty-nine patients (34 men and 15 women; mean age of 62.4 years, range 29 to 82.4 years) with advanced gastric carcinoma underwent laparoscopy for staging between June 1991 and June 1992 and then underwent prospective follow-up. Laparoscopic assessment of each patient included the presence of ascites, gastric serosal invasion, liver, peritoneal, and lymph nodes metastases, tumor fixation, and cytologic examination of the peritoneal fluid.

Peritoneal lavage cytology was performed as previously described.[4-8,17] Briefly, the peritoneal cavity was washed with 100 ml of physiologic saline solution, kept at 37° C, instilled into the upper abdomen, and allowed to collect. After dispersion in the peritoneal cavity, 10 to 20 ml of fluid was aspirated under direct vision from the subhepatic and/or subdiaphragmatic space. The fluid was immediately centrifuged at 2000 rpm for 5 minutes. The nucleated cell layer was smeared onto a glass slide and stained using Giemsa and Papanicolaou methods.

The slides were interpreted by experienced cytologists and the results were classified as positive, negative, or suspicious for IFCCs, considering either one or both stains. The following cellular characteristics were used to determine the presence of malignant cells: number, size, shape, type of cytoplasm, cytoplasmic vacuoli, nuclear abnormalities, nuclear chromatin, nuclear-cytoplasmic ratio, mitotic figures, and nucleolar prominence (Fig. 1). Decisions regarding resectability were made on the basis of laparoscopic findings and without regard to cytologic status.

Curative surgical treatment was characterized by resection of all gross tumor at operation (including patients with removed clinically enlarged nodes) and no evidence of metastatic disease. Patients underwent a D2 radical dissection with en bloc resection. No other forms of oncologic treatment, including immunotherapy, chemotherapy, or radiotherapy, were offered to these patients.

All patients were followed for a minimum of 5 years, to the present time or until death, using clinical, endoscopic, CT scan, and/or ultrasound examinations.

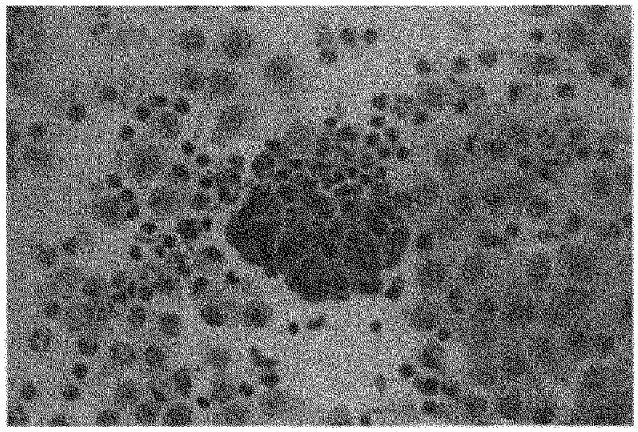

Fig. 1. Papanicolaou staining of intraperitoneal free cancer cells. (×40.)

Chi-square or Fisher's exact tests were used to analyze the data; a two-tailed P value <0.05 was considered statistically significant. Survival was calculated by means of the Kaplan-Meier method and survival curves were compared using the log-rank test. All statistical analyses were performed with the SPSS 5.0 program (SPSS Inc., Chicago, Ill.).

RESULTS

The overall prevalence of positive IFCCs was 20 (40.8%) of 49—11 (64.7%) of 17 patients with ascites and 9 (28%) of 32 patients without ascites who underwent peritoneal lavage. Eight patients (16.3%) were spared having to undergo a laparotomy because M1 disease was diagnosed laparoscopically (carcinomatosis and/or multiple hepatic metastases). The detection rate of IFCCs and its association with tumor stage (TNM), subsequent extent of surgery, and pattern of recurrence following curative resection (n = 17) are presented in Table I. Positive cytologic findings were identified in 87.5% of patients who were not operated on because of laparoscopic contraindications, 40% who underwent palliative surgery without resection, 42.8% who underwent a palliative resection, and 17.6% who underwent curative gastrectomy ($P = 0.004$). The operative and pathologic findings that denoted a palliative resection (n = 14) included lymph node involvement in four, liver involvement in four, and local tumor invasion in six patients.

All patients undergoing curative resection who had positive cytologic findings had peritoneal recurrences (3 of 17 17.6%), whereas no peritoneal recurrences

were observed in the patients with negative cytologic findings as the first manifestation of the disease.

Forty-one patients were dead and eight were alive at a minimum follow-up of 60 months and maximum of 67 months. All patients with positive cytology died (n = 20) and only 3 of 20 survived more than 1 year after diagnosis.

The median overall survival for the whole group of patients was as follows: negative cytology, 21 months (95% confidence interval [CI] = 7.4 to 34.6); positive cytology, 4 months (95% CI = 2.4 to 5.6); and suspicious cytology, 5.1 months (95% CI = 1.8 to 6.6); $P = 0.0001$. Considering only those patients under-

Table I. Peritoneal lavage cytology results in 49 patients with gastric carcinoma

Variable	Positive (%)	Negative (%)	Suspicious (%)
Cytology status	20 (40.8)	26 (53.0)	3 (6.1)
Tumor stage (UICC, 1987)			
Ib		2	
II		2	
IIIa	3	5	
IIIb	3	2	
IV	14	15	3
Extent of surgery			
Curative	3 (17.6)	14 (82.4)	
Palliative			
Resection	6 (42.8)	8 (57.2)	
No resection	4 (40)	3 (30)	3 (30)
No surgery	7 (87.5)	1 (11.1)	
Pattern of recurrence after curative surgery			
No recurrence		8	
Peritoneal	3	0	
Local	0	4	
Liver	0	1	
Other sites	0	1	

UICC = International union against cancer staging system.

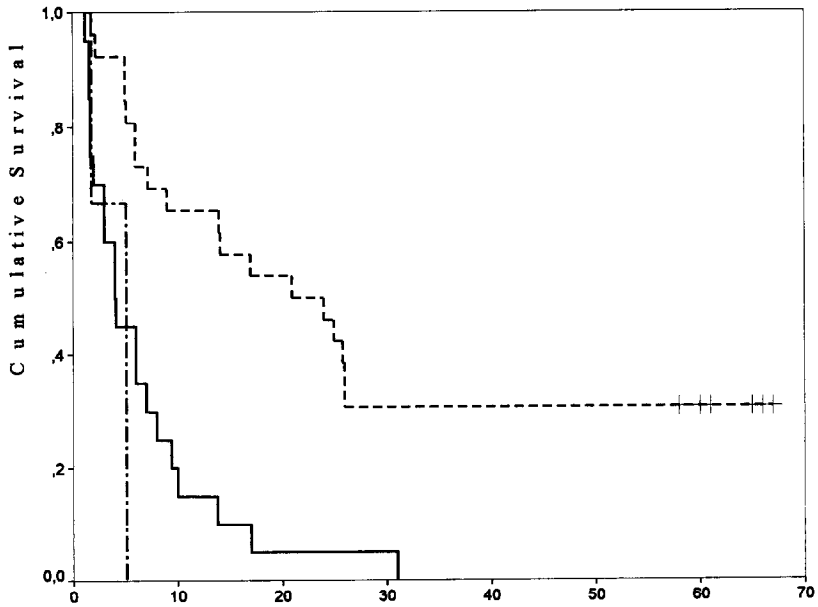

Fig. 2. Cumulative curves of overall survival for positive (solid line), negative (broken line), and suspicious IFCC patients (n = 49).

going curative resection (n = 17), the median overall survival for those with negative cytology has not been reached, whereas the patients with positive cytology had median survival of 9.4 months (95% CI = 0.8 to 18.0); $P = 0.0002$. The median overall survival for stage III patients (n = 13) was as follows: negative cytology, 26 months (95% CI = 17 to 45) and positive cytology, 9.4 months (95% CI = 0.8 to 18); $P = 0.0006$.

The survival curves of patients with either positive, negative, or suspicious cytologic findings are shown in Figs. 2, 3, and 4.

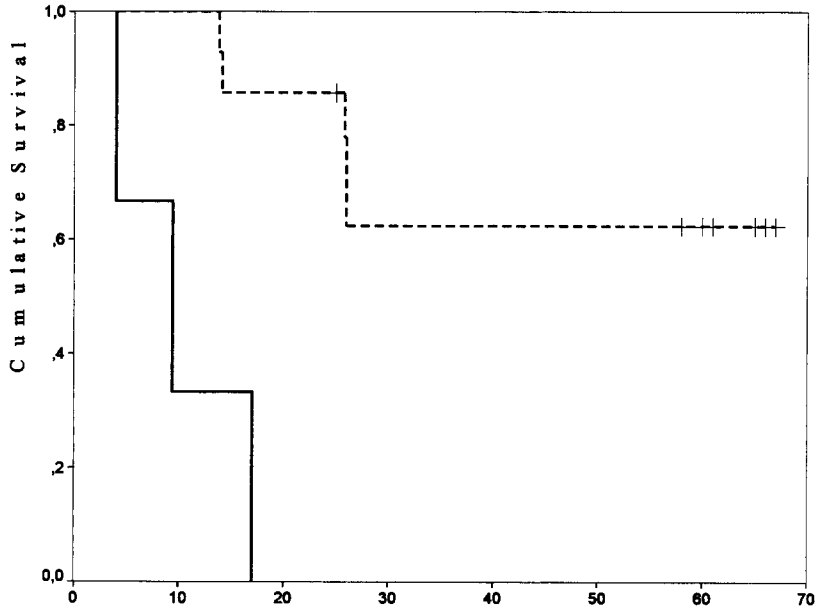

Fig. 3. Cumulative curves of overall survival for positive (solid line) and negative (broken line) IFCC patients who underwent curative resections (n = 17).

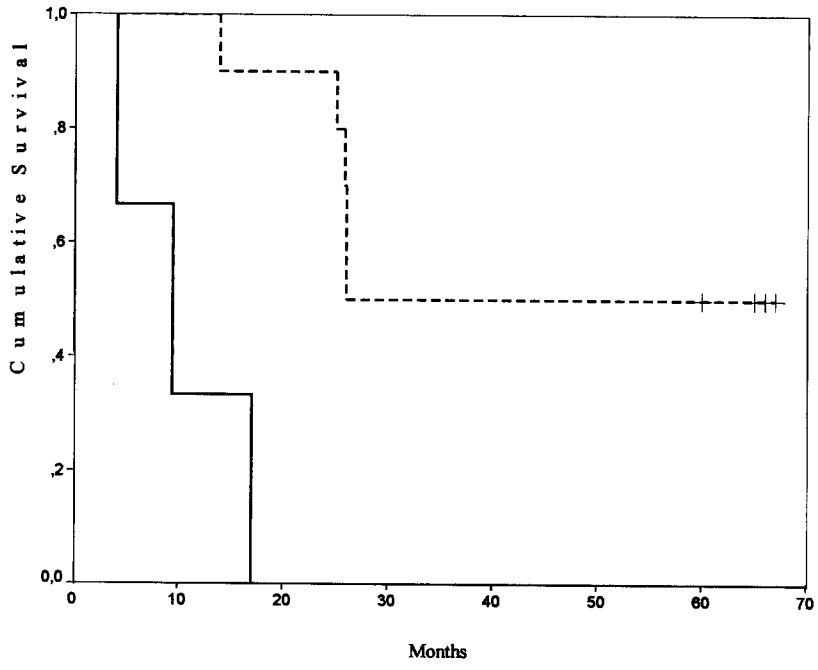

Fig. 4. Cumulative curves of overall survival for positive (solid line) and negative (broken line) IFCC stage III patients (n = 13).

DISCUSSION

The pretherapeutic diagnostic process with the aim of accurate staging is essential for planning therapy and minimizing both unnecessary discomfort and needless laparotomy in patients with gastric cancer, especially in the context of multimodality management of cancer.[11-18] Detailed staging not only enables individualized and tumor stage–oriented resection but also opens the door to neoadjuvant therapy.[18] When patients are offered the opportunity to choose among surgical resection, investigational neoadjuvant chemotherapy, palliative chemotherapy, or symptomatic relief alone, the need for precise preoperative staging becomes apparent.[15]

Laparoscopy has been shown to be a remarkably accurate staging method.[11-16] Our data show that laparotomy could have been avoided in 16.3% of patients in whom M1 disease was diagnosed. IFCCs might play an important role in the development of peritoneal metastases, which is a frequent event in patients with gastric carcinoma. The peritoneal cavity can be a route for dissemination of malignant cells by direct continuity with the lesion or can act as a receptacle for lymphatic spread.[10,17,19] Our data show that patients with IFCCs do not escape postoperative peritoneal recurrence.

IFCCs were detected in 41% of patients during staging laparoscopy. The prevalence of a positive cytologic outcome in patients with laparoscopically obtained lavage is similar to the results reported in the literature using the open technique.[17] The rate of detection of IFCCs in the literature ranges from 7.1% to 47%, depending on the methodology used, the extent of the resection, the type of tumor, the tumor stage, and whether there is gastric serosal invasion.[3-10,19]

Laparoscopy with peritoneal cytologic examination might be used to predict the extent of surgical treatment in patients with gastric cancer. Patients with unresectable tumors had a higher positivity for IFCCs than those who underwent palliative or curative gastrectomy. Warshaw[20] studied 40 patients with potentially resectable pancreatic ductal adenocarcinoma and found similar results. Only 1 of 10 patients with cancer of the pancreatic head associated with positive peritoneal cytology was able to undergo resection, whereas 13 of 25 patients with negative cytologic findings successfully underwent resection. Thus laparoscopic peritoneal lavage cytology may improve the selection of patients suitable for curative or palliative resection.

It is noteworthy that nonclinical malignant disease manifested by IFCCs was observed in 3 (17.6%) of 17 patients who were treated with curative surgery. Similar results have been obtained by other investigators.[4,6,10] These subclinical micrometastases may have the potential to develop into recurrent disease and influence both treatment and survival. In general, survival after surgery for gastric cancer is not significantly improved by adjuvant chemotherapy[21]; however, it might be beneficial in this subgroup of patients, since micrometastases are more susceptible to chemotherapy than macroscopic disease.[4] Abnormal cytologic findings may also serve as a guide to chemotherapy or investigational therapies.[3,20] These tumors might be treated by local administration of chemo- or immunotherapeutic agents injected directly into the peritoneal cavity, which might lead to a higher concentration in the peritoneum than is possible with systemic chemotherapy, thus improving therapeutic results.[22] Hagiwara et al.[23] showed a significant improvement in survival among cytology-positive patients treated with mitomycin adsorbed on carbon particles delivered intraperitoneally.

Previous reports have stated that the prognosis in surgically treated patients with gastric carcinoma is significantly affected by the presence of IFCCs at the time of gastrectomy.[4,6-10] Our data demonstrate that regardless of the type of resection, a positive cytologic test result is a significant prognostic factor for survival. This is in accordance with the Japanese experience and suggests that this procedure should be routine in the investigation of patients with gastric cancer.[10] In the Western world, the Dutch Gastric Cancer Group has reported their cytologic results in 457 patients undergoing curative resections who had randomized D1 or D2 dissections. Positive cytologic findings were found to be indicative of a poor prognosis, with a median survival of 13 months.[10]

Patients who tested positive for IFCCs had essentially stage IV outcome, even in the absence of distant metastases or macroscopic peritoneal disease. As a result of peritoneal lavage cytology, 3 (23.1%) of 13 patients were reclassified from stage III to stage IV, depending on the IFCC results. Survival curves showed a significant difference between the two groups, with a decreased median overall survival for patients who tested positive for IFCCs.

CONCLUSION

Laparoscopy with peritoneal lavage cytology should be included as an integral part of the staging, evaluation, and classification of patients with gastric cancer. This method can be of great value in detecting microscopic intra-abdominal spread of gastric cancer and may be useful in identifying patients at high risk for peritoneal recurrence. Furthermore, positive laparoscopic peritoneal lavage cytology is a good predictor of poor outcome in patients with advanced gastric cancer and may be used in planning treatment.

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Discussion

Dr. A. Warshaw (Boston, Mass.). These findings are identical to what we have been seeing in patients with pancreatic cancer who are subjected to laparoscopic peritoneal lavage. Patients who have positive cytologic findings have the identical poor survival that patients with gross metastatic disease have. As I look at your data, I see there is no survival advantage to the subsequent resection in patients with positive cytologic findings. Would you interpret your data as indicating that in patients who do not need an operation to relieve an obstruction or stop bleeding, the resection should be omitted?

Dr. U. Ribeiro. My feeling is that in patients with positive cytologic findings, surgery should be avoided unless there is some complication of the tumor that would necessitate an operation.

Dr. Riddell (Hamilton, Ontario). You mentioned that the finding of premalignant cells in the peritoneal cavity seems to correlate with the extent of serosal involvement or involvement of adjacent organs. Is this really an independent factor for prognosis or could you have actually made the same determination by inspecting the serosal surface and noting whether there was involvement of adjacent organs? In short, is a cytologic examination really necessary?

Dr. Ribeiro. I believe that IFCCs represent an independent prognostic factor.

Dr. Quan-Yang Duh (San Francisco, Calif.). Did you observe any incisional or trochar site recurrence?

Dr. Ribeiro. No, we did not.

Proctocolectomy With Jejunal Pouch–Distal Rectal Anastomosis: An Alternative to Ileal Pouch Reconstruction

Michinaga Takahashi, M.D., James W. Williams, M.D., Keith A. Kelly, M.D.

The aim of this study was to determine whether a jejunal pouch would have a lower resting pressure, be more distensible, and have more interdigestive migrating myoelectric complexes and less fecal bacterial overgrowth than would an ileal pouch after proctocolectomy and pouch–distal rectal anastomosis. In six conscious dogs with a jejunal pouch–distal rectal anastomosis and six with an ileal pouch–distal rectal anastomosis (controls), pouch distensibility and motility were measured using a barostat and perfused pressure–sensitive catheters passed per anum, pouch electrical activity was recorded using chronically implanted electrodes, and the number of bacteria per gram of stool was assessed by culture. Dogs with a jejunal pouch had lower resting pouch pressures, more distensible pouches, faster frequencies of pacesetter potentials in the pouch, more phase 3 intervals of the interdigesive migrating myoelectric complex reaching the pouch, but similar numbers and types of bacteria in their stools compared to the dogs with an ileal pouch. We concluded that jejunal pouches have a lower resting pressure, are more distensible, have more cleansing contractions, but a similar fecal flora compared to ileal pouches. A jejunal pouch has features that make it an attractive alternative to an ileal pouch for pouch–distal rectal or pouch–anal canal anastomosis after proctocolectomy. (J GASTROINTEST SURG 1998;2:250-259.)

Proctocolectomy and ileal pouch–anal canal anastomosis has become the operation of choice for many patients with chronic ulcerative colitis and familial adenomatous polyposis.[1] Various shapes of pouches, J-shaped, S-shaped, and W-shaped, all constructed from ileum, have been used to reduce the frequency of bowel movements and achieve reasonable fecal continence after this operation.[2] Nonetheless, diarrhea and incontinence occur in approximately 15% of patients postoperatively.[2,3] Incontinent patients characteristically have high resting pressures in their pouches, low anal canal pressures, and no increase in anal canal pressures when the pouches are distended compared to continent patients.[4] The ileal pouch also exhibits few, if any, phase 3 intervals of the interdigestive migrating myoelectric complex (MMC).[5-7] Phase 3 intervals are electrical events that trigger periodic propulsion, emptying, and cleansing of the gastrointestinal tract during fasting. In addition to these problems with diarrhea and incontinence, approximately one half of patients with ileal pouches develop inflammation of their pouches ("pouchitis"), a condition that can produce diarrhea, hematochezia, and systemic symptoms.[3]

We wondered whether a jejunal pouch might provide a better reservoir for fecal contents than an ileal pouch in patients undergoing proctocolectomy. The jejunum is larger and has fewer propulsive waves than does the ileum.[8,9] It should provide a better reservoir, a lower resting pressure in the pouch, more distensibility, and therefore better continence than an ileal pouch.

A jejunal pouch may also be less susceptible to pouchitis. For example, the jejunum is seldom inflamed or ulcerated in ulcerative colitis and Crohn's disease, whereas the ileum is more commonly so involved; approximately 10% of patients with chronic ulcerative colitis have backwash ileitis, and more than 50% of patients with Crohn's disease have Crohn's ileitis. The luminal pH of the jejunum is slightly more

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acidic than that of the ileum, and the acidic pH may discourage the growth of bacteria. A more alkaline pH in pouch effluent has been associated with pouchitis.[10,11] The resistance of the jejunum to inflammation and ulceration may also be due, in part, to the type of mucus it produces, but this is unknown. Of interest is that the jejunum is also more resistant to peptic ulceration than the ileum. Peptic ulceration has been shown recently to involve a complex interaction between a luminal bacteria, *Helicobacter pylori*, the corrosive powers of the luminal content, the protective mucous layer, and the resistance of the mucosa to ulceration. A similar interaction may exist in ulcerative colitis.

In addition, prolonged stasis that encourages bacterial overgrowth may not occur in jejunal pouches, because they likely would have MMCs that would periodically cleanse them during fasting. The ileal pouch has few MMCs.[5] Fewer MMCs mean more stasis and more bacterial overgrowth. Overgrowth of both anaerobic and aerobic organisms does occur in ileal pouches, and both classes of organisms have been implicated in the villous atrophy, crypt elongation, and inflammation that occurs in ileal pouches.[12,13]

A straight jejunal-anal anastomosis (no pouch) has been used successfully in a 9-year-old girl who required proctocolectomy for ulcerative colitis.[14] Moreover, a suggestion had been made earlier that a jejunal pouch–anal canal or a jejunal pouch–distal rectal anastomosis may have clinical applicability.[15] However, we have found no published experimental investigation of a jejunal pouch used as a rectal substitute after proctocolectomy.

Our hypothesis is that a jejunal pouch would have a lower resting pressure, be more distensible, have more interdigestive MMCs and have less susceptibility to pouchitis than an ileal pouch. This hypothesis was tested by comparing motility, electrical activity, bacterial flora, and histologic changes in the two types of pouches.

METHODS

Experimental Preparation

Twelve female mongrel dogs weighing between 16 and 23 kg were fasted overnight and given 100 mg of cephalothin intravenously. They underwent general anesthesia with methohexital sodium (12.5 mg/kg), 1.5% isoflurane, and atropine sulfate (0.04 mg/kg). Using a sterile operative technique, a midline celiotomy was made, and a colectomy and proximal proctectomy were accomplished. The proctectomy extended distally to a point 3 cm proximal to the pelvic floor.

In six dogs, a J-shaped pouch was constructed from the distal jejunum. The small bowel was divided at a site one-half the distance between the ligament of Treitz and the ileocolic valve. The distal 30 cm of jejunum immediately proximal to the transection was used to form the pouch. Two layers of sutures were used: an inner continuous layer of 3-0 polyglycolic acid and an outer interrupted layer of 3-0 silk. The jejunum was partially transected 10 cm proximal to the pouch. A 3 cm neuromuscular bridge on the mesenteric border was left intact to maintain neuromuscular continuity from the duodenal pacemaker through the proximal jejunum to the newly formed pouch.[16] The proximal ileum was sutured closed and anastomosed side to end to the midjejunum proximal to the bridge. The lumen of the jejunum distal to the bridge was also sutured closed. The distal ileum was anastomosed end to side to the midjejunum downstream from the closure and 5 cm proximal to the pouch. The newly formed jejunal pouch was then anastomosed to the distal rectum, end to end. This operation established a jejunal J pouch and yet maintained electrical, motor, and luminal continuity between the proximal bowel and the pouch, just as is the case with a conventional ileal J pouch (Fig. 1). In the other six dogs (controls), 30 cm of terminal ileum was used to form a J-shaped ileal pouch, and the pouch was anastomosed to the distal rectum as is done in a conventional ileal pouch–anal canal anastomosis (Fig. 1).

Both types of pouch were anastomosed to the distal rectum instead of to the anal canal at the dentate line because of the greater ease of a distal rectal anastomosis and to preserve completely the anal canal sphincter and its innervation. This study was designed to explore pouch function and not the fate of retained distal rectal and proximal anal canal mucosa.

In both groups of dogs, monopolar Ag-AgCl recording electrodes were applied to the pouch at 9 cm intervals and to the small bowel at 4 cm intervals, as in Fig. 1. Ten electrodes were used in dogs with an ileal pouch and 11 electrodes in dogs with a jejunal pouch. The electrodes were connected by insulated copper wires to a multioutlet connector embedded in a stainless steel cannula and positioned in and anchored to the left anterior abdominal wall. The dogs were allowed to recover for 5 weeks before tesing was begun.

Conduct of Experiments

Small intestinal motility and electrical activity were measured three times on different days in each conscious animal at 5 to 7 weeks and at 10 to 12 weeks after operation, respectively, except as noted. Bacteriologic cultures of feces from the pouch were also obtained at 5 to 7 weeks and 10 to 12 weeks after op-

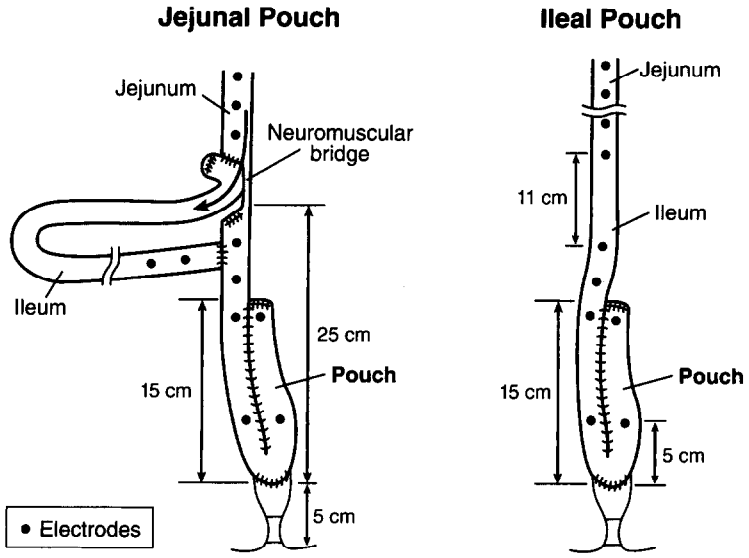

Fig. 1. Canine experimental preparations.

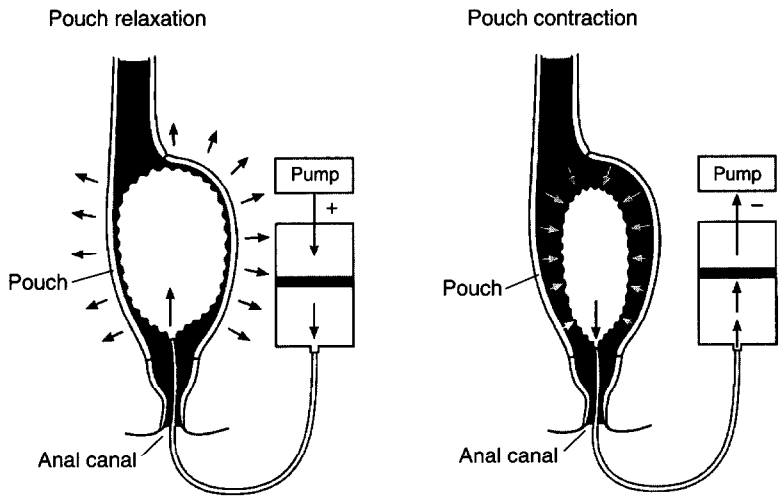

Fig. 2. Electric barostat in place in canine enteric pouches.

eration, whereas gross and histologic examinations of the small bowel and pouch were performed at autopsy.

Resting Pressure Motor Patterns. These patterns were assessed in each dog using an electronic barostat.[17,18] A compliant latex bag was attached to the distal end of a 16 F double-lumen plastic tube, one end of which was connected to rigid bellows containing air, whereas the other end was connected to the pressure sensor. The bag, catheter, and bellows formed a closed system (Fig. 2). An electronically driven motor continuously adjusted the bellows to maintain the air pressure within the system at a value specified by the operator.

After an 18-hour overnight fast, the dogs were placed fully conscious in a Pavlov stand. The bag and tube were inserted transanally for approximately 10 cm, so as to locate the bag at the center of the pouch. Air pressure within the system was set at 3 mm Hg to expand the bag to fill the pouch to the extent that the muscular tone of the pouch allowed. The pressures recorded by the barostat were measured over 90 minutes.

Pressure-Volume Response. Pressure-volume responses in the pouch were evaluated after the barostat study. The bag and tube were disconnected from the barostat, and both were filled with water. The tube was connected to a low-compliance perfusion

system and the bag was distended with water at a rate of 15 ml/min in 20 ml increments. The pressure was measured for 1 minute at each increment. The bag was distended from 0 ml to the maximum volume, the volume at which the dog showed signs of discomfort or evacuated the bag. The water in the bag was then withdrawn and the bag was removed. Thereafter the bag was placed on the Pavlov sling at the same height at which it was located in vivo in the dog. The bag was again distended with water in 20 ml increments, and the pressure was recorded for 20 seconds at each increment until the volume in the bag reached the maximum volume. The pressure data were digitized and stored on disk for later computer analysis.

Pouch Pressure Waves. On other days, pouch pressure waves were measured manometrically by three pressure-sensitive, open-type, perfused polyethylene catheters. After an 18-hour overnight fast, the catheters were passed transanally so that the open tips were located 5, 10, and 15 cm proximal to the anal verge. The catheters were connected to a low-compliance perfusion system, pressure transducers, and a pressure-recording monitor for each channel,[19] and pressures were recorded for 4 hours. The pressure data were digitized and stored on disk for later computer analysis. On still other days, again after an 18-hour overnight fast, the dogs were given 312 g of canned pet food (394 calories; protein 8.5%, fat 5.25%) per os. The motility of the pouch was recorded as above for 2 hours. The fed studies were performed only twice in each animal.

Electrical Activity. Electrical activity was recorded during fasting and with feeding concurrently with the pressure wave studies. The electrodes were connected by leads to an electrical amplifier/computer acquisition system. Using the stainless steel cannula as a ground, myoelectrical signals were amplified utilizing Mayo custom-built, analogue amplifiers. The amplified analogue signals were then converted to digital signals sampled at 100 Hz. The signals were displayed in real time on a VGA monitor, while simultaneously stored on magnetic media to be analyzed later.

Bacteriology. Quantitative aerobic and anaerobic measurement of bacterial flora of the pouch was performed. A 1 g sample of stool was harvested via an anoscope or from just-passed feces. The sample was immediately serially diluted by 10-fold increments with sterile Ringer's lactate to a dilution of 10^{-8}. Inoculation of appropriate culture plates was performed using a sterile pipette to place 0.1 ml of each dilution on the culture plate. For aerobic incubation, each dilution was inoculated onto sheep blood agar, and the 10^{-4}, 10^{-6}, and 10^{-8} dilutions were also inoculated onto eosin-methylene blue agar and colistin-nalidixic acid (Columbia base) agar. Similarly for anaerobic incubation, each dilution was placed onto rabbit blood agar (brucella base) and the 10^{-4}, 10^{-6}, and 10^{-8} dilutions were also inoculated onto gentamycin-vancomycin agar and phenylethyl alcohol agar. The anaerobic plates were incubated in Gaspak jars (Becton Dickinson Microbiology Systems, Cockeysville, Md.). The aerobic cultures were incubated overnight. The anaerobic cultures were first assessed at 48 hours, then again at 5 to 7 days. A Gram stain was done on each colony type, and the colonies were divided into four categories: gram-positive coccus, gram-negative coccus, gram-positive bacillus, and gram-negative bacillus. The total number of colonies in each classification was recorded. For detection of true anaerobes, a representative of each colony type was subcultured to sheep blood and chocolate agar plates and incubated aerobically and anaerobically in CO_2. Growth in CO_2 and/or O_2 disqualified a colony as anaerobic.

Postmortem Examination. After completion of the experiments (12 weeks after operation), all animals were killed with an overdose of pentobarbital sodium, and the areas of operation were inspected. In particular, gross evidence of inflammation and ulceration of the pouch was noted. The integrity of the luminal closure at the site of the neuromuscular bridge was also examined by distending the lumen of the bowel on one side of the closure with a 1% solution of methylene blue to 200 mm Hg pressure, while the lumen on the other side was inspected for the appearance of the methylene blue. The pouches were fixed in formalin for histologic assessment.

Analysis of Data

Motility Data. The barostat volumes were averaged electrically over each successive 15-minute period. The computer calculated the average volume of the balloon at every quartile (minimum, 25%, 50%, 75%, and maximum). The *pressure-volume curves* were obtained by subtracting the values when the bag was distended ex vivo from the values in vivo at each point. The *pressure waves* were analyzed using programs developed in our laboratory.[19,20] Briefly, the programs allow computer identification of pressure waves. The frequency, amplitude, and area under the curve were determined. A motility index (MI) was calculated ($MI = \log_{10}$ [amplitude $\times$ frequency/2]). Means and standard error of the mean (SEM) during the experimental periods were calculated.

Electrical Data. Digitized myoelectric signals were analyzed using a VAX/VMS platform by a previously described method developed in our laboratory.[21] The three basic steps in the analysis were: (1) identification of the precise time (within 1/100th of a second) of each pacesetter potential (PP) in all recording channels during each experiment; (2) use of the PP

event times in a single recording channel to calculate the instantaneous and the mean PP frequency in each channel during the experiment; and (3) use of the PP event times in adjacent recording channels (spatially separated by 4 cm on the bowel wall) to determine the propagation direction of each PP detected in each channel. PP frequency and direction of propagation were examined during phase I, phase II, phase III, and phase IV of the interdigestive MMC, as identified by inspection of the tracings, and using the criteria of Code and Marlett,[22] and during feeding. Analysis was focused on the recordings from the pouch and from the bowel just proximal to the pouch. Means and SEM of data from each experimental period and condition were calculated.

Histologic Assessment. Inflammation in the pouches was scored as absent, mild, moderate, and severe based on histologic assessment. Furthermore, crypt elongation, villous atrophy, and the abundance of mucous cells were also noted.

Statistical Analysis. Data from the two groups of dogs were compared using Student's *t* test for unpaired data and the Bonferroni correction where indicated. Data within a group were compared using paired Student's *t* test. When data were compared between groups, analysis of variance (ANOVA) was employed. For bacterial flora, data from the two groups were compared by means of the Mann-Whitney U test.

RESULTS

The motor, electrical, and bacteriologic data at 5 weeks after operation were, in general, similar to those at 10 weeks after operation. Thus only the 10-week data are presented in this report. The 5-week data are available at the authors' laboratory.

Condition of the Dogs

Three of the six dogs with a jejunal pouch remained in good health during the experiments. They ate well and had soft, foamy stools. On a few occasions one of these dogs had bloody stools; otherwise blood was not noted in the stools. The dogs maintained their weight well and lost a mean ± SEM of only 0.4 kg by the end of the experiment, from 21.5 ± 1.9 kg at operation to 21.1 ± 0.6 kg at autopsy.

The other three jejunal pouch dogs had only fair health after operation. They ate well but had profuse, watery, often bloody stools and had perianal excoriation because of them. They lost a mean of 2.5 kg of body weight during the experiment, from 20.5 ± 1.2 kg at operation to 18.0 ± 1.1 kg at autopsy.

The dogs with an ileal pouch remained in good health during the experiment. They ate well, but they did have watery diarrhea. Their stools were more watery than the foamy stools of the healthy dogs with a jejunal pouch. These dogs gained a mean of 0.6 kg of weight during the experiment, from 17.8 ± 0.5 kg at operation to 18.4 ± 0.7 kg at autopsy.

Pouch Motility, Electrical Activity, and Bacteriology

The motility, electrical activity, and bacteriology of the jejunal pouch dogs with good health after operation were similar to those of jejunal pouch dogs with poor health after operation. Thus the data from all six dogs were compared as a group to the data from the six dogs with ileal pouches.

Pouch Motility

Barostat Studies. The barostatic pressures and volumes varied slightly and rhythmically in both jejunal pouches and ileal pouches, but the amplitude of the variations was greater in the jejunal pouches (Fig. 3). The overall mean volume present in the jejunal pouches with the barostat set at 3 mm Hg pressure was greater (42 ± 3.2 ml) than in the ileal pouches (29 ± 2.9 ml; $P < 0.05$). Moreover, the maximal volume present in the pouches during the 90-minute study was also greater in the jejunal pouches (84 ± 3.3 ml) than in the ileal pouches (63 ± 5.5 ml; $P < 0.05$). Thus the jejunal pouches periodically relaxed more fully than the ileal pouches during mild distention (Fig. 4). The minimum volume, however, was similar in the two types of pouches (jejunal 4.8 ± 1.5 ml, ileal 6.3 ± 1.5 ml; $P > 0.05$).

Pressure-Volume Curves. Dogs with a jejunal pouch had a lower resting basal pressure in the pouch (3.1 ± 0.2 mm Hg) than dogs with an ileal pouch (7.7 ± 1.5 mm Hg). As the pouches were distended, the pressures at each volume of distention were less in jejunal pouches than in ileal pouches ($P < 0.05$; Fig. 5). The maximal volume to which the pouch could be comfortably distended, however, was similar in the jejunal pouches (115 ± 3.4 ml) and the ileal pouches (114 ± 8.7 ml; $P > 0.05$). At maximal volume, the dogs sometimes evacuated the intrapouch bag.

Pressure waves were recorded by the pressure-sensitive catheters from both jejunal pouches and ileal pouches. The MI in jejunal pouches was greater in the proximal pouch than in the distal pouch (Table I). The MI was also greater in the fed state than in the fasting state. In contrast, the MI in the proximal pouch was similar to that in the distal pouch in dogs with ileal pouches, and the MI changed little with feeding.

Electrical Activity. The frequency of the PPs in the jejunum just proximal to the neuromuscular bridge (15.3 ± 0.5 cpm) was similar to that just distal to the bridge (14.5 ± 0.5 cpm; $P > 0.05$), showing that the

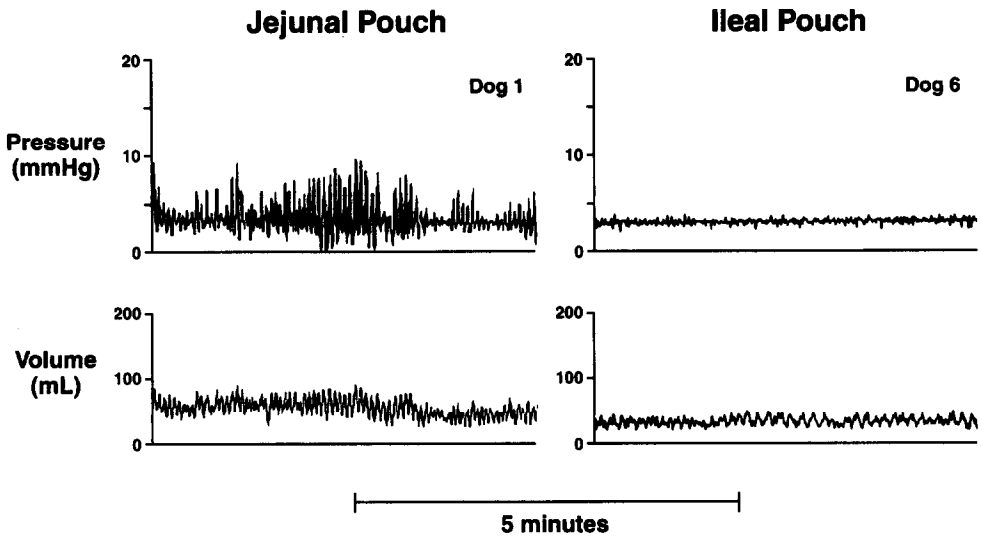

Fig. 3. Barostat recordings of canine enteric pouches at 3 mm Hg pressure.

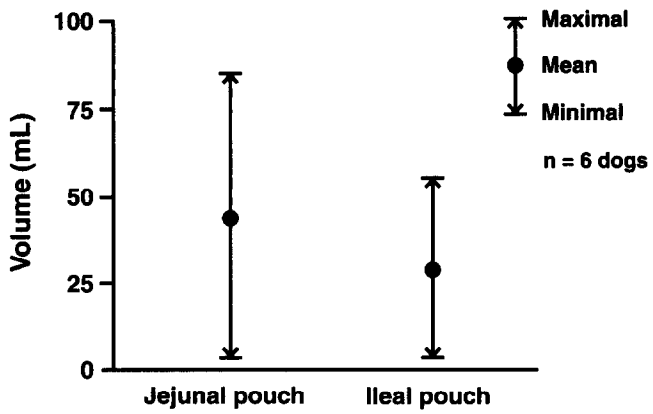

Fig. 4. Grand maximal, mean, and minimal volumes noted in canine enteric pouches during 90 minutes of barostat recording at 3 mm Hg pressure.

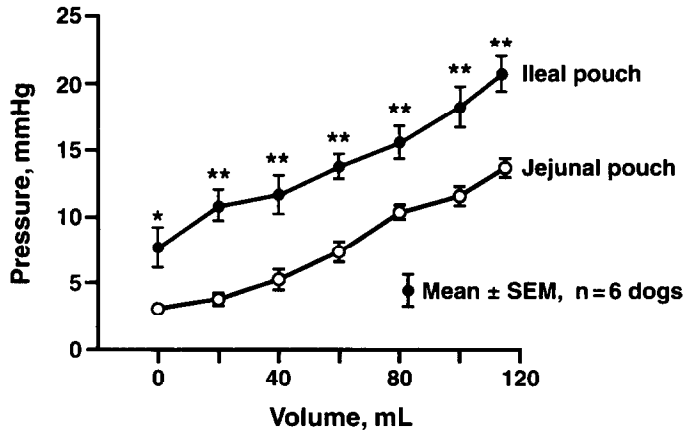

Fig. 5. Pressure-volume curves in canine enteric pouches. * = $P < 0.05$; ** = $P < 0.01$.

Table I. Mean ± SEM motility index in the pouch after proctocolectomy with jejunal pouch (n = 6 dogs) or ileal pouch (n = 6 dogs) reconstruction

	Motility index			
	Distance from the anus			
State, type of pouch	15 cm	10 cm	5 cm	Overall mean
Fasting				
Ileum	6.4 ± 0.8	4.2 ± 0.9	3.8 ± 0.9	4.9 ± 0.8
Jejunum	7.5 ± 0.5*	6.4 ± 0.5†	4.9 ± 0.5	6.3 ± 0.4
Fed				
Ileum	6.5 ± 0.8	5.4 ± 1.1	4.1 ± 1.4	5.4 ± 0.7
Jejunum	8.4 ± 0.6*	7.1 ± 0.5‡	5.6 ± 0.7	7.1 ± 0.6‡

*P <0.01 vs. 5 cm.
†P <0.05 vs. 5 cm.
‡P <0.05 vs. fasting.

Table II. Mean ± SEM enteric electrical parameters after protocolectomy with ileal pouch (n = 6 dogs) or jejunal pouch (n = 6 dogs) reconstruction

	Proximal to pouch		Pouch
State, type of pouch	Frequency of PPs (cpm)	Aborad PP propagation (%)	Frequency of PPs (cpm)
Fasting			
Ileum	12.4 ± 0.3*	62.7 ± 3.3	12.0 ± 0.7*
Jejunum	14.5 ± 0.5	63.0 ± 2.2	14.6 ± 0.4
Fasting, MMC phase 3			
Ileum	14.3 ± 0.2*	59.5 ± 2.5	13.5 ± 0.5*
Jejunum	15.5 ± 0.3	58.3 ± 3.8	15.5 ± 0.3
Fed			
Ileum	12.0 ± 0.4*	66.7 ± 2.1	11.3 ± 1.1*
Jejunum	14.5 ± 0.5	64.3 ± 2.0	14.4 ± 0.7

PP = pacesetter potential.
*Differs from value just below; $P < 0.05$.

bridge maintained neuromuscular continuity between the proximal bowel and the bowel used to form the pouch. The dogs with jejunal pouches had faster frequencies of PPs during the fasting state and after feeding, both in the pouch and in the afferent limb just proximal to the pouch, than did dogs with ileal pouches (Fig. 6 and Table II). The percentages of PPs propagating aborally did not differ between the two types of pouches during fasting or feeding. Interdigestive MMCs were found in the bowel proximal to both types of pouches and in the pouches themselves. However, phase 1 of the MMCs, the quiescent phase with few or no action potentials, was often not present or of short (<5 min) duration in both types of pouches. Nonetheless, the period of the MMCs in the small bowel did not differ between dogs with ileal pouches (114 ± 7.5 min) and dogs with jejunal pouches (101 ± 4.8 min; $P > 0.05$). In contrast, 35 (88%) of 40 MMCs propagated from the bowel just proximal to the pouch into the jejunal pouches, whereas only 6 (19%) of 32 MMCs propagated into ileal pouches ($P < 0.05$). Phase 3 intervals of the MMCs were nearly always abolished by feeding in both types of pouches. Only four phase 3 intervals were found in the pouch after feeding in three of the dogs with jejunal pouches, whereas none were found in the other three dogs with jejunal pouches and in the six dogs with ileal pouches.

Pouch Bacteriology. No clear-cut differences in the numbers or types of luminal bacteria present in the pouches were noted between the jejunal pouches and the ileal pouches. Jejunal pouches had 8.0 ± 0.3 $\log_{10}$ colony-forming units (cfu) of aerobic bacteria per gram of stool and 7.1 ± 1.4 $\log_{10}$ cfu of anaerobic bac-

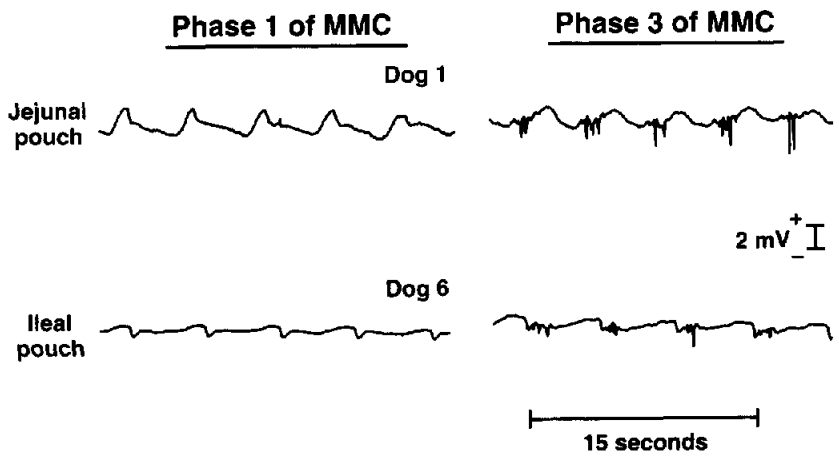

Fig. 6. Electrical recordings from canine enteric pouches during fasting. MMC = interdigestive migrating myoelectric complex.

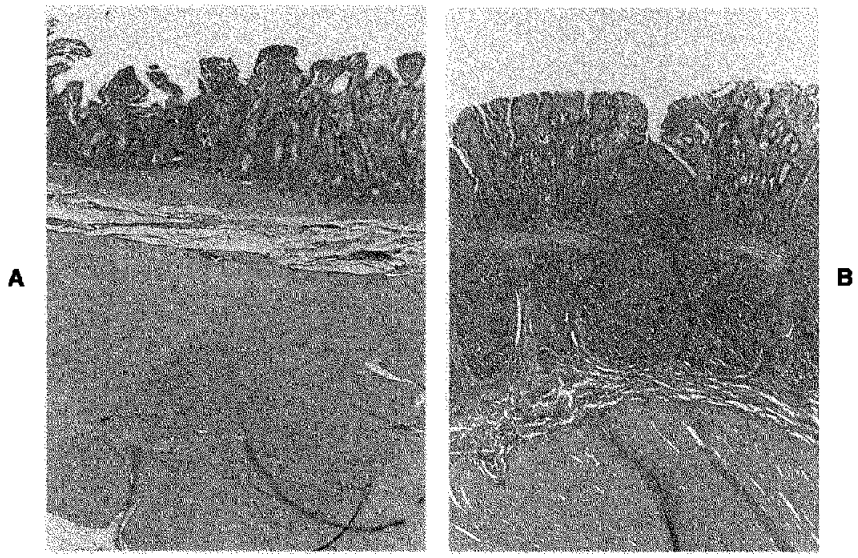

Fig. 7. Photomicrograph of canine enteric pouches. **A,** Jejunal pouch. **B,** Ileal pouch. (Hematoxylin and eosin stain; ×20.)

teria per gram of stool, whereas dogs with ileal pouches had 8.3 ± 0.3 $\log_{10}$ cfu of aerobic bacteria per gram of stool and 7.8 ± 0.5 $\log_{10}$ cfu of anaerobic bacteria per gram of stool ($P > 0.05$).

Postmortem Findings

A fistula between the proximal jejunum and the distal jejunum at the site of the neuromuscular bridge was found in five of the six dogs with a jejunal pouch. Three of these fistulas were large (>5 mm in diameter). The fourth dog had a small fistula, 3 mm in diameter, whereas the fifth dog had a fistula only pinpoint in size. The sixth dog with a jejunal pouch and the dogs with ileal pouches had no fistulas. The three dogs with large fistulas were those with only fair health after operation. The three with small or no fistulas and the dogs with ileal pouches were those with excellent health after operation.

On gross examination the mucosa of the pouch appeared hemorrhagic in the three dogs with jejunal pouches and large fistulas. The mucosa of the other jejunal pouches and that of the ileal pouches appeared normal. On microscopic examination, more mucus-secreting mucosal cells and more mucosal and submucosal lymphoid follicles were present in the ileal pouches than in the jejunal pouches (Fig. 7). Both types of pouches, however, had short, blunted villi and elongated mucosal crypts. The jejunal pouches with fistulas had chronic inflammation and fibrosis sur-

rounding the fistulas, but neither type of pouch showed microscopic evidence of acute or chronic pouchitis.

DISCUSSION

This experiment shows that dogs with jejunal pouch–distal rectal anastomosis after proctocolectomy had lower basal intrapouch pressures, more distensible pouches, faster frequencies of PPs in the pouch, more phase 3 intervals of the interdigestive MMC reaching the pouch, and similar numbers of bacteria in the pouch than did dogs with ileal pouch–distal rectal anastomosis. These features suggest that jejunal pouches might have advantages over ileal pouches for patients who undergo pouch–distal rectal or pouch–anal canal anastomoses after proctocolectomy.

The main advantage of the jejunum over the ileum as a pouch lies in the jejunum's larger volume, lower basal pressure, and greater distensibility. These factors should mean that subjects with a jejunal pouch would have less incontinence than would those with an ileal pouch. Others have shown that a low basal pouch pressure in concert with a capacious, distensible pouch and an adequate anal sphincter means less incontinence.[4,5,23]

The jejunal pouch also had more rapid PPs than the ileal pouch. This means that more contractions are possible in the jejunal pouch each minute. Moreover, a gradient of motor activity was present in jejunal pouches, with the proximal part of the pouch showing a greater MI than the distal part. Motility was also enhanced in the jejunal pouches with feeding. These motor patterns in jejunal pouches might, at first glance, be thought to result in an adverse effect of more propulsion and more bowel movements. However, jejunal contractions typically mix and churn, rather than propel, especially postprandially. Thus the enhanced motility in a jejunal pouch after feeding might not necessarily lead to more frequent bowel movements.

The presence of more phase 3 intervals of the MMC in the jejunal pouch, however, likely would initiate more bowel movements. Indeed, when phase 3 intervals did appear in either type of pouch, the dogs did sometimes immediately empty their pouches. Emptying of the pouch during fasting, especially complete emptying, however, likely could have a beneficial effect rather than a detrimental effect. With more complete emptying during fasting, less prolonged stasis of bowel contents would be present in the pouch, and less bacterial overgrowth would be expected. Nonetheless, this factor, that is, more MMCs, likely was not a major factor influencing bacterial growth, because the number of bacteria per gram of stool was similar in the two types of pouches.

The cause of pouchitis in distal enteric pouches in humans is unknown. Bacterial overgrowth, altered immune responsiveness, and lack of short-chain fatty acids in the lumen are a few of the hypotheses regarding etiology that have been put forward.[10,13,24,25] Bacterial overgrowth was similar in the jejunal pouches and ileal pouches in these canine studies, and neither type of pouch showed pouchitis. Bacterial overgrowth and other factors that may be responsible for a predisposition to pouchitis in humans apparently were not present in sufficient degree to cause pouchitis in dogs. Pouch metaplasia, however, was seen in the dogs, as it is in humans.

Although the jejunal pouch appears to have several physiologic advantages over the ileal pouch, the appearance of fistulas between the proximal bowel and the pouch along the neuromuscular bridge was a major problem with the type of jejunal pouch we constructed. Modifications of the construction are clearly needed before clinical application takes place.

In summary, dogs with a jejunal pouch–distal rectal anastomosis had lower basal intrapouch pressures, a more distensible pouch, faster frequencies of PPs in the pouch, more interdigestive MMCs reaching the pouch, and similar bacteria flora in the pouch compared to dogs with an ileal pouch–distal rectal anastomosis. A jejunal pouch modified to prevent fistulas between the proximal bowel and the pouch could be an attractive alternative to an ileal pouch, when restoring enteric continuity after proctocolectomy.

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Discussion

Dr. B. Schirmer (Charlottesville, N.C.). In your slide you showed a higher incidence of MMCs in the jejunal pouch vs. the ileal pouch. Were those MMCs from the proximal bowel that were transmitted through to the distal pouch.

Dr. M. Takahashi. Yes.

Dr. Schirmer. Why do you hypothesize that 80% made it through to a jejunal pouch, whereas a much lower percentage made it to the ileal pouch?

Dr. Takahashi. Perhaps because, even in health, fewer MMCs reach the terminal ileum than reach the distal jejunum.

Dr. Schirmer. But these are phase 3 proximally that then migrated through the anastomosis to the pouch.

Dr. Takahashi. We made a neuromuscular bridge, and MMCs can pass through the neuromuscular bridge.

Dr. Schirmer. Did you observe any animals for more than 3 months after which time anastomotic breaks in the bowel tend not to impede MMC transmission?

Dr. Takahashi. We observed our animals for only 10 weeks.

Dr. M.G. Sarr (Rochester, Minn.). Do you think the differences that you have shown are related to the differences in motility in the jejunum vs. the ileum or differences in the mechanical characteristics of the wall of the jejunum vs. the ileum?

Dr. Takahashi. I do not know exactly, but probably the diameter of the ileum is smaller than that of the jejunum. The jejunum is more distensible. I think then that the motility of the jejunum is greater than that of the ileum.

Dr. Sarr. Our group has been interested in maintaining the direction of the PPs by the neuromuscular bridge. It is very easy to do in dogs but very difficult in humans. Do you have any thoughts on how you might be able to use a jejunal patch in a human as opposed to dogs?

Dr. Takahashi. About 12 years ago, a 7-year-old girl was reported by others. The girl had reconstruction after proctectomy with a straight jejunal segment instead of an ileal pouch. No neuromuscular bridge was used and no jejunal pouch was made. She recovered well and had good function after the operation.

Dr. J.E. Fischer (Cincinnati, Ohio). Underlying the experiment is the hypothesis that pouches empty because of muscular activity. Do you believe that pouches really empty not because of pressure or passive action but because of motility?

Dr. Takahashi. I think phase 3 of the MMC can cause emptying of the pouch, but usually pouches are emptied by the Valsalva maneuver.

Duodenoesophageal Reflux Induces Esophageal Adenocarcinoma Without Exogenous Carcinogen

Martin Fein, Ph.D., M.D., Jeffrey H. Peters, M.D., Para Chandrasoma, M.D., Adrian P. Ireland, M.D., Stefan Öberg, M.D., Manfred P. Ritter, M.D., Cedric G. Bremner, M.D., Jeffrey A. Hagen, M.D., Tom R. DeMeester, M.D.

In the rat model, esophageal adenocarcinoma reproducibly develops following surgically induced duodenal reflux into the esophagus and administration of nitrosamine. In addition, decreasing gastric acid via partial or total gastrectomy increases the prevalence of adenocarcinoma in this model. We questioned whether carcinogen was necessary for cancer development in the gastrectomized model and whether esophageal acidification could reverse the effect of gastrectomy. Three groups of 26 rats each were randomized to a surgical procedure to produce one of the following reflux models: gastroduodenal reflux by esophagojejunostomy, duodenal reflux by total gastrectomy and esophagojejunostomy, or no reflux by Roux-en-Y reconstruction. In a second experiment, 42 rats were operated on to induce duodenal reflux. One week following surgery, they were randomized to receive acidified water (pH 1.8) or tap water. The animals were killed at 24 weeks of age, and the esophagus was evaluated histologically. All animals with reflux had severe esophagitis and 87% developed columnar lining of the distal esophagus. Nearly half (48%) developed adenocarcinoma at the anastomotic site 16 weeks postoperatively and without carcinogen administration. Cancer prevalence did not differ between animals with gastroduodenal or duodenal reflux but tended to be lower in animals receiving acidified water. Duodenoesophageal reflux is carcinogenic in the rat model. Exogenous carcinogen is not necessary for cancer development in gastrectomized rats. (J GASTROINTEST SURG 1998;2:260-268.)

The incidence of esophageal adenocarcinoma has risen faster than that of any other cancer, and is now estimated to be 1 per 100,000.[1,2] The reasons for this change are unclear. It is known that the presence of metaplastic specialized intestinal, or Barrett's epithelium, is the most important etiologic factor in the development of esophageal adenocarcinoma.[3] Longitudinal studies have documented progression from metaplasia to dysplasia to carcinoma, thereby linking the common malady of gastroesophageal reflux disease with esophageal cancer.[4] Evidence from both clinical studies[5-12] and animal models[13-19] have shown esophageal exposure to duodenal juice to be a key factor in the genesis of specialized intestinal metaplasia and likely the development of adenocarcinoma.

The pathophysiology of esophageal carcinoma has been studied in the rat model. Previous studies have shown that the administration of N-nitroso compounds including methyl-N-amylnitrosamine (MNAN) and dimethyl-N-amylnitrosamine (DMNM) results in the induction of squamous cell carcinoma of the esophagus in a dose-dependent fashion.[20] Surgically induced reflux of gastroduodenal juice into the esophagus increases the tumor yield and alters the histology to adenocarcinoma in most tumors.[14] Animals with reflux of gastroduodenal juice who did not receive a carcinogen rarely developed esophageal adenocarcinoma.[14,17] Recently it has been shown that decreasing gastric acid via partial or total gastrectomy increases the prevalence of adenocarcinoma in this model.[18] We questioned whether carcinogen was necessary for cancer development in gastrectomized rats with duodenoesophageal reflux and whether esophageal acidification could reverse the effect of gastrectomy.

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METHODS

Reflux Models

One hundred nineteen 8-week old male Sprague-Dawley rats (Harlan, Sprague-Dawley, Inc., Indianapolis, Ind.) were studied in two different experiments.

Experiment 1. Three groups of rats were operated on in a randomized fashion to produce the following reflux models (Fig. 1, *A*):

1. *Gastroduodenal Reflux* (n = 26). Esophagojejunostomy (EJ) with gastric preservation to induce reflux of gastric and duodenal juice into the esophagus. The anastomosis was performed 4 cm distal to the ligament of Treitz. The posterior vagal trunk and the left gastric artery remained uncut.
2. *Duodenal Reflux* (n = 26). Esophagojejunostomy and total gastrectomy (EJTG) to induce esophageal reflux of duodenal juice alone.
3. *No Reflux* (n = 25). Total gastrectomy and a Roux-en-Y reconstruction (EJRY) to divert duodenal content away from the esophagus. After completion of the end-to-side esophagojejunostomy, the afferent jejunal loop was ligated with silk, divided, and anastomosed end to side to the jejunum at least 15 cm from the esophagojejunal anastomosis.[21] This group served as a control group.

Experiment 2. This experiment was designed to study the effect of exogenous acid added to the drinking water of rats with esophagojejunostomy and total gastrectomy (Fig. 1, *B*). Forty-two animals were randomized 1 week after surgery to drink either acid syrup (duodenal reflux–acid) or tap water (duodenal reflex–no acid). Acid syrup was prepared with hydrochloric acid at a pH of 1.8 (0.016 mol/L) and sucrose 68 mg/ml and given ad libitum.

Surgical Procedure

Operations were performed after an acclimatization period of 3 days. Rats were kept in metal cages on a 12-hour light/dark cycle at a temperature of 70° F and a humidity of 60%. Water and standard solid chow (Teklan rodent diet 8604, Harlan) were given ad libitum. Before surgery, the animals were starved overnight, and water was discontinued on the morning of surgery. Rats were anesthetized with an intramuscular injection of xylazine hydrochloride (12 mg/kg) and ketamine (75 mg/kg). In all animals, an

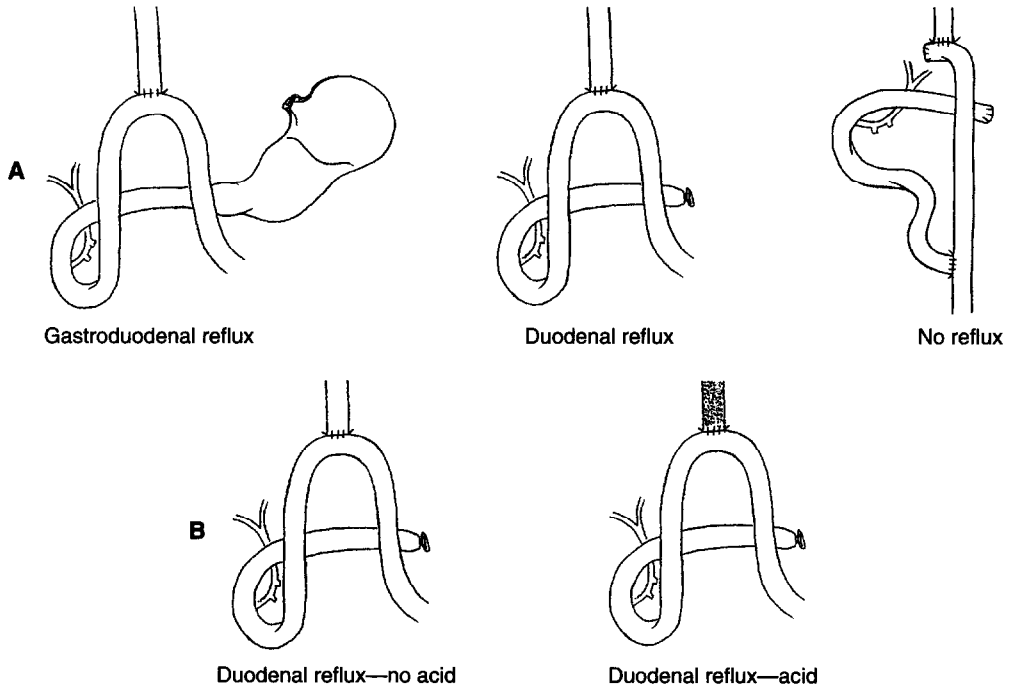

Fig. 1. A, Reflux models: Experiment 1. Esophagojejunostomy inducing gastroduodenal reflux, total gastrectomy and esophagojejunostomy inducing duodenal reflux, and total gastrectomy and Roux-en-Y reconstruction to divert reflux. **B,** Reflux models: Experiment 2. Total gastrectomy and esophagojejunostomy inducing duodenal reflux in both groups. Acidification of the esophagus by acid syrup given orally in one group.

end-to-side esophagojejunostomy was performed. The esophagus was divided above the gastroesophageal junction, and a 4 mm jejunostomy was made 4 cm distal to the ligament of Treitz. The esophagojejunal anastomosis was constructed in an antecolic manner with eight interrupted full-thickness 7-0 polypropylene sutures.[13,16,22] Before abdominal wall closure, 1 ml of 0.9% sodium chloride was instilled into the peritoneal cavity. Water was permitted when the rats awoke, and chow was provided the next day. Rats were weighed every 2 weeks during the course of the experiment. Any rats that became ill were killed. The experimental course was 16 weeks (24 weeks of age).

The total mortality rate was 8% (9 of 119). Two rats died from an accidental intravascular injection of the drugs for anesthesia. Three rats died within the first week after surgery for unknown reasons. Four animals died of reflux disease, two with severe pneumonia and two with upper gastrointestinal bleeding. Seventy-three rats were evaluated in experiment 1 (22 EJ, 26 EJTG, and 25 EJRY) and 37 rats in experiment 2 (18 EJTG-no acid and 19 EJTG-acid).

Gross and Histologic Evaluation

Sixteen weeks after surgery, the rats were killed with an overdose of phenobarbital. The esophagus was resected from below the larynx to the jejunum, and 2 mm of jejunal mucosa was left attached to the specimen. The esophagus was opened longitudinally and the length, the circumference, and the proportion of macroscopically inflamed to normal esophagus was measured and the specimen photographed. The esophagus was divided into two longitudinal strips that were rolled up similar to a jelly roll, fixed in 10% buffered formalin, and embedded in paraffin blocks. Macroscopically visible tumors were cut in half and embedded separately. Sections were stained with hematoxylin and eosin. Histologic sections were read by the pathologist (P.C.) in a blinded fashion.

Histologic changes in the squamous epithelium were classified as (1) hyperplastic, defined as an increased thickness of the squamous epithelium with normal maturation and hyperkeratosis, or (2) regenerative, where the squamous epithelium showed increased height of lamina propria papillae greater than 70% of mucosal thickness, basal cell hyperplasia greater than 20% of mucosal thickness, and absence of hyperkeratosis. Regenerative changes are identical to changes described for reflux esophagitis in humans. Hyperplastic changes were scored on a scale from 0 to 3 ranging from absent to severe.

Inflammation was classified as (1) intraepithelial, based on the presence of eosinophils and/or neutrophils in the squamous epithelium, or (2) subepithelial, based on the degree of inflammatory cell infiltration in the lamina propria below the epithelium. Both types of inflammation were scored on a scale from 0 to 3 (absent to severe). The presence or absence of epithelial ulceration was also noted.

Columnar metaplasia was defined as the presence of a histologically abnormal glandular mucosa proximal to the anastomotic line. This columnar lining was required to be different from the jejunal mucosa by virtue of having markedly shortened or absent villi and distorted crypt architecture. The presence of this metaplastic columnar epithelium admixed with squamous epithelium was recorded.

Neoplasms observed in this study were of a uniform histologic type with the characteristic features of a mucinous adenocarcinoma. These consisted of malignant infiltrative glands associated with lakes of extracellular mucin.

Statistics

The study population was designed based on a sample size analysis (power 0.8; $P = 0.05$). In experiment 1 a prevalence of 4% tumors in the control group and 40% tumors in animals with reflux was assumed. To detect a difference at these levels, 25 animals had to be analyzed. Data are reported as mean ± standard deviation unless otherwise stated. The chi-square test was used to compare proportions among three groups and the Fisher's exact test for two groups. The Kruskal-Wallis test was used to compare continuous data among three groups and the Mann-Whitney U test for two groups. A P value <0.05 was considered significant. The study protocol was approved by the Institutional and Animal Care and Usage Committee of the University of Southern California.

RESULTS

Experiment 1

Macroscopic Findings. Mean baseline and subsequent body weight for each group of rats is shown in Fig. 2. Animals with no reflux grew significantly faster than animals with either gastroduodenal or duodenal reflux. Esophageal specimens of rats with reflux were significantly shorter and more dilated than those of rats without reflux (Table I). Shortening of the esophagus was unlikely to be due to the differences in the growth of the animals, as the anastomotic site was pulled up to or above the hiatus in animals in the reflux groups and remained intra-abdominal in those without reflux. There was gross evidence of severe esophageal mucosal injury in all animals with surgically induced reflux. This included epithelial thickening and extensive hyperplasia of the lower two thirds

of the esophagus. Ulceration was frequently present in the area above the anastomosis. A nodular intraluminal tumor or thickening of the anastomotic site was seen in some animals (Fig. 3). The esophagus of animals without reflux was grossly normal, except for a short area of epithelial thickening adjacent to the anastomosis seen in some of the animals.

Histologic Findings. Histologic findings are shown in Table II. In both groups with reflux, histologic changes became progressively more severe from the pharynx to the anastomosis. The specimens showed marked hyperplastic changes with increased thickness of the squamous epithelium, hyperkeratosis, regenerative changes with papillomatosis, and basal cell hyperplasia (Fig. 4). Inflammation was severe and typically involved the complete esophageal wall. Epithe-

Table I. Experiment 1: Macroscopic findings

	Gastroduodenal reflux (n = 22)	Duodenal reflux (n = 26)	No reflux (n = 25)
Length (mm)	55 ± 8	53 ± 7	67 ± 9
Circumference (mm)	10.4 ± 2.3	11.0 ± 2.1	4.5 ± 1.6
Esophagitis (%)	69 ± 23	68 ± 20	2 ± 10

All parameters differ between the group with no reflux and the groups with reflux ($P < 0.001$).

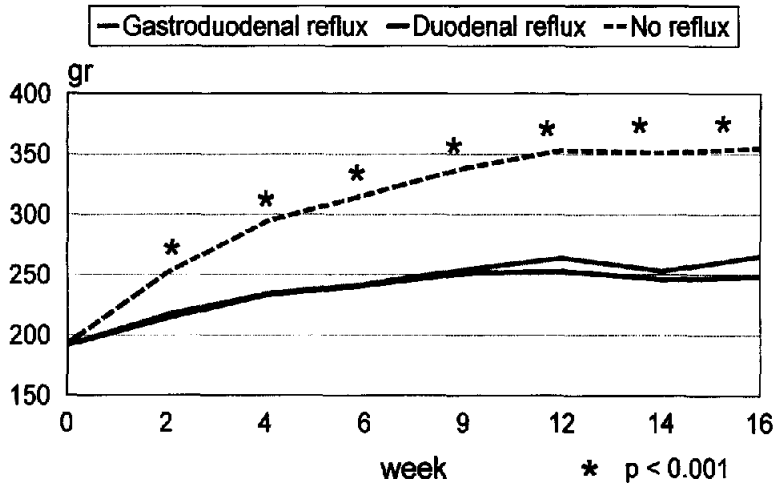

Fig. 2. Time course of weight changes. Significantly higher weights for the group with no reflux beginning in the second week.

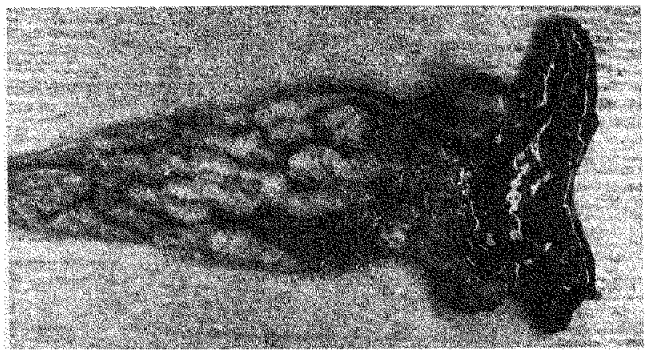

Fig. 3. Macroscopic appearance of a resected esophagus from a rat following gastrectomy with esophagojejunostomy. The tumor at the anastomotic site is cut in half. There is a large area of ulceration adjacent to the anastomosis and severe esophagitis with hyperplasia in the lower two thirds of the esophagus.

Table II. Experiment 1: Histologic findings

	Gastroduodenal reflux (n = 22)	Duodenal reflux (n = 26)	No reflux (n = 25)
Intraepithelial inflammation			
0/1	22 (100)	26 (100)	25 (100)
2	0	0	0
3	0	0	0
Subepithelial inflammation			
0	2 (9)	0	23 (92)
1	2 (9)	10 (39)	2 (8)
2	11 (50)	5 (19)	0
3	7 (32)	11 (42)	0
Hyperplasia			
0	0	1 (4)	6 (24)
1	1 (5)	1 (4)	17 (68)
2	9 (41)	15 (58)	2 (8)
3	12 (54)	9 (34)	0
Relation regenerative to hyperplastic changes	24 ± 17%	24 ± 16%	1 ± 4%
Ulceration	17 (77)	22 (85)	0
Columnar lining	20 (91)	21 (81)	6 (24)
Adenocarcinoma	12 (55)	11 (42)	4 (16)

Subepithelial inflammation and squamous hyperplasia were significantly more frequent in the groups with reflux compared to the group with no reflux, as was ulceration, columnar lining of the distal esophagus, and adenocarcinoma.

Fig. 4. Hyperplastic squamous epithelium on the right, defined as an increased thickness of the squamous epithelium with normal maturation and hyperkeratosis. Regenerative epithelium on the left with a height of lamina propria papillae greater than 70% of mucosal thickness, basal cell hyperplasia greater than 20% of mucosal thickness, and absence of hyperkeratosis.

lial ulceration and columnar lining were frequently present adjacent to the anastomosis (Fig. 5). Columnar lining was located above the anastomotic site and differed from the jejunal mucosa in that the villi were less dense and shorter and the crypts were irregular. Seventy percent had columnar lining mixed with squamous epithelium, suggesting that this was metaplastic (Barrett's) epithelium. Columnar lined metaplastic epithelium not adjacent to the anastomosis was seen in 10% of the animals in the reflux groups.

In the Roux-en-Y control group, there was a well-defined border between the jejunal epithelium and the squamous epithelium. The squamous epithelium adjacent to the anastomosis showed moderate hyperplasia in two, mild hyperplasia in 16, and no changes in six of these animals.

Relation Between Composition of Reflux and Tumor Yield. All tumors were adenocarcinomas. Squamous cell cancer was not seen. Adenocarcinoma occurred in 55% of the animals with gastroduodenal re-

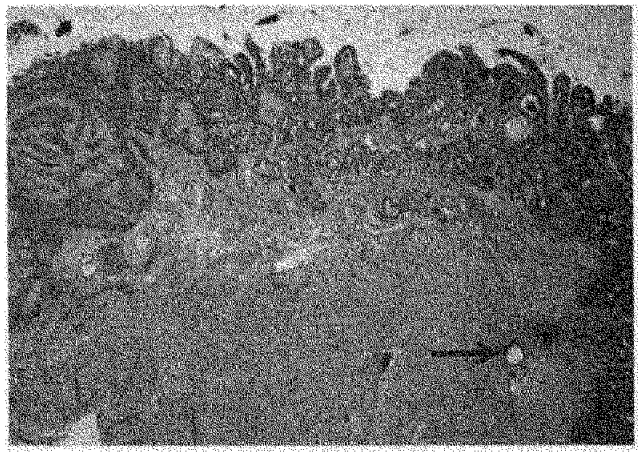

Fig. 5. Columnar lining of the distal esophagus. This differs from jejunal mucosa in that the villi are shortened or absent and crypt architecture is distorted. The columnar lining of the esophagus is mixed with squamous epithelium. Arrow indicates a stitch with the jejunum to the right and the esophagus to the left.

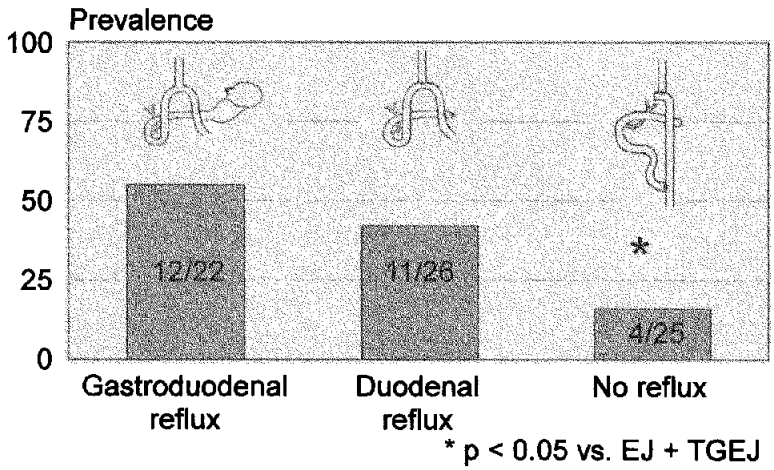

Fig. 6. Prevalence of adenocarcinoma in experiment 1. There are significantly fewer tumors in the group with no reflux compared to either the group with gastroduodenal or duodenal reflux. Cancer prevalence did not differ within the groups with gastroduodenal and duodenal reflux. EJ + TGEJ = esophagojejunostomy and total gastrectomy.

flux and 42% of those with duodenal reflux (Fig. 6). Histologically the tumors had a uniform appearance with the typical features of mucinous adenocarcinoma. Sixty percent developed within or adjacent to columnar lining of the distal esophagus (Fig. 7). Invasion of esophagus musculature and infiltration of neighboring organs, such as the liver, the pancreas, and the tissue surrounding the celiac artery, was frequently found. Although atypical cells in the regenerative squamous epithelium were found, there were no tumors identified above the anastomotic site.

The prevalence of adenocarcinomas (16%) was significantly lower in the Roux-en-Y group. All control animals with adenocarcinoma also had hyperplasia or columnar lining in the distal end of the esophagus, indicating that reflux was not completely abolished even with a Roux limb of 15 cm. Control animals with no hyperplasia (n = 6) did not develop adenocarcinomas. Furthermore, no cancer was found in animals with a Roux limb greater than 22 cm.

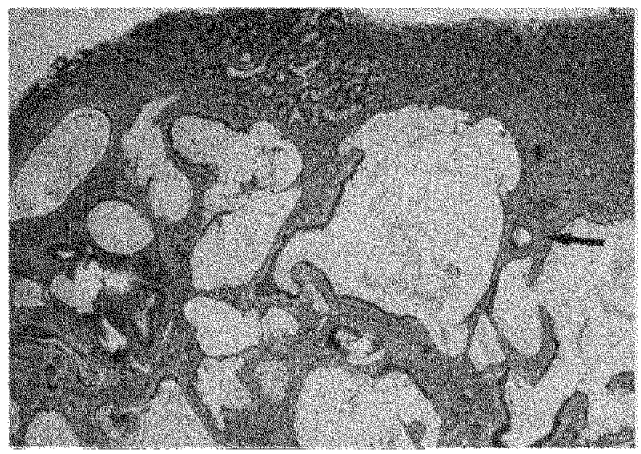

Fig. 7. Mucinous adenocarcinoma arising in columnar lining of the esophagus. This is characterized by malignant infiltrative glands associated with lakes of extracellular mucin. All adenocarcinomas were located at the anastomotic site. Arrow indicates a stitch with jejunum to the right and esophagus to the left.

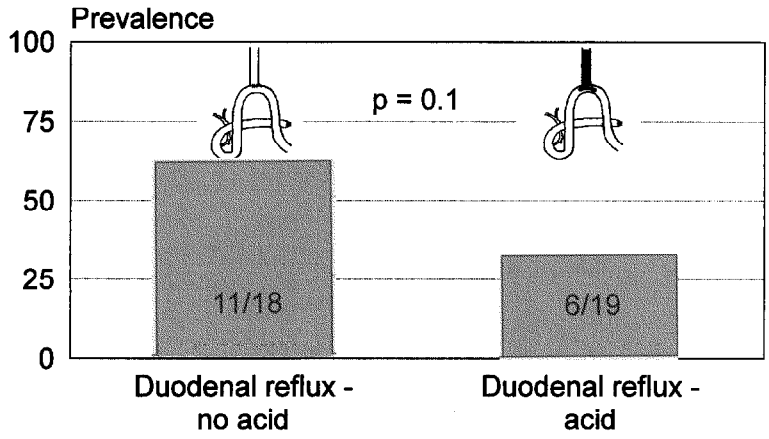

Fig. 8. Prevalence of adenocarcinoma in experiment 2. The cancer prevalence in the group with duodenal reflux was similar to that in experiment 1. Animals receiving acid syrup tended to have a lower prevalence of tumors.

Table III. Experiment 2: Histologic findings

	Duodenal reflux–no acid (n = 18)	Duodenal reflux–acid (n = 19)
Intraepithelial inflammation		
0/1	18 (100)	14 (73)
2	0	3 (16)
3	0	2 (11)
Subepithelial inflammation		
0	0	0
1	4 (22)	5 (26)
2	8 (45)	6 (32)
3	6 (33)	8 (42)
Hyperplasia		
0	0	0
1	2 (11)	3 (16)
2	15 (83)	12 (63)
3	1 (6)	4 (21)
Relation regenerative to hyperplastic changes	34 ± 23%	51 ± 27%
Ulceration	17 (94)	18 (95)
Columnar lining	15 (83)	18 (95)
Adenocarcinoma	11 (61)	6 (32)

Grade 2/3 intraepithelial inflammation, which is a common finding in reflux disease in humans, was only found in the group receiving acid. Otherwise there was no difference in esophageal injury between the two groups. Adenocarcinoma tended to be more frequent in the group with no acid (see Fig. 8).

Experiment 2

Histologic findings in the two groups of animals in experiment 2 are shown in Table III. The high tumor yield in animals with duodenal reflux was reproduced in this experiment. The addition of oral acid syrup tended to decrease the prevalence of tumors (Fig. 8).

DISCUSSION

The striking finding in this study is the presence and high prevalence of junctional adenocarcinomas, which averaged 48% after only 16 weeks' exposure to reflux of gastroduodenal or duodenal juice. The tumors were induced by reflux of duodenal juice into the esophagus and not by exogenous carcinogen, and confirmed in a second experiment. Miwa et al.[19] reported a 72% prevalence of mucinous adenocarcinomas in a similar study with exposure extending to 50 weeks. In this study there were also adenosquamous carcinomas and squamous cell carcinomas of the esophagus in 7 of 25 animals. In view of the severe changes observed in the squamous epithelium in our study, including basal cell hyperplasia, squamous atypia, and glandular metaplasia of the esophagus, development of carcinomas in the squamous-lined esophagus above the anastomotic site might be expected to occur with a more prolonged exposure time. The addition of exogenous carcinogen generally results in the development of adenosquamous carcinomas and squamous cell carcinomas.[13,14,17,18] At present there is no comparative data available on the number of mucinous adenocarcinomas developing with and without carcinogen administration.

The high incidence of adenocarcinomas after a relatively short time course raises the question of whether these tumors are truly malignant. The histologic findings of this study have been independently reviewed by three pathologists from three different centers, who had no knowledge of the experimental design. All three classified the tumors as mucinous adenocarcinomas. Cellular criteria of malignancy included pleomorphism, nuclear enlargement, hyperchromasia, abnormal chromatin pattern, and a high rate of mitosis. There was infiltration of the muscular layer of the esophagus and jejunum, and frequent in-

vasive growth into the liver, the pancreas, and the fat tissue around the celiac artery.

All tumors were located at the anastomotic site. The close relationship of the tumors to esophageal columnar lining suggests that they evolved from the columnar lining of the esophagus. The fact that jejunal mucosa is not altered by duodenal juice under physiologic circumstances suggests that the tumors are actually esophageal adenocarcinomas arising in areas of Barrett's metaplasia.

The cause-and-effect relationship of reflux to the development of adenocarcinoma was demonstrated by the significant reduction in the number of tumors in the control group. In contrast to the study of Miwa et al.,[19] adenocarcinoma did actually occur in the control group with a prevalence of 16%. All control animals with no inflammation had no tumors and all animals with adenocarcinoma had inflammation of the distal esophagus. This indicates that reflux was not completely abolished by the surgical technique used in the control group.

The role of the components of duodenal juice in carcinogenesis is not well understood. Trypsin, the major component of pancreatic juice, is an enzyme injurious to the esophagus and might therefore modify and promote carcinogenesis. However, it is unlikely that trypsin initiates cancer. In a mutagenesis assay, bile acids also fail to show a carcinogenic effect,[23] although a promoting effect of bile acids has clearly been demonstrated in colon cancer.[23,24] Esophageal carcinogenesis due to reflux of duodenal juice cannot be explained by a promotional effect alone.[25] Carcinogens, acting as initiators, must be present or develop in duodenal juice at some stage. This may occur when bile acids react with nitrite, producing carcinogenic N-nitrosamides.[26] N-nitroso–bile acids such as N-nitrosotaurocholic acid and N-nitrosoglycocholic acid have been shown to act as carcinogens.[27] Bacterial overgrowth is also important because it converts salivary and dietary nitrates to nitrites. Some bacteria can further combine nitrites with amides resulting in carcinogenic N-nitroso compounds.

The pH environment at the anastomotic site is also relevant because bile acids are modified by ionization or precipitation and the bacteria are altered. These factors might result in the observed reduction in tumor yield by gastric juice in our previous study with esophagoduodenostomy and carcinogen administration.[18] In contrast to this study, no modifying effect on carcinogenesis was found in experiment 1 after esophagojejunostomy with and without gastrectomy. A possible explanation for this difference is that the pH is neutral at the anastomotic site in both groups because gastric juice is completely neutralized before it reaches the anastomosis. In experiment 2, the trend to have fewer tumors in the acid syrup group is likely to show a similar effect as described in the previous study. However, the difference was not statistically significant. Further study is needed to draw conclusions about the role of acid in the model without exogenous carcinogen.

In conclusion, reflux of duodenal juice in rats induces carcinogenesis without exogenous carcinogen administration. In this model, carcinogens must be present or develop in duodenal juice.

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Discussion

Dr. F. Greene (Columbia, S.C.). You did not use the term dysplasia in your discussion. I was wondering if you were able to detect dysplastic lesions in some of these animals as a continuum to cancer.

Dr. M. Fein. We did not use the term dysplasia in this model because this was an animal model and pathologists define dysplasia in human terms, so the pathologist must first come up with a precise definition of what he or she thinks dysplasia is in this rat model. The definition would be based on the experimental model itself; therefore we did not talk about dysplasia.

Dr. H. Freiss (Bern, Switzerland). Did you also analyze the influence of gastrointestinal hormones on your interesting cancer model? It has been shown in previous studies that if surgical manipulation of the upper gastrointestinal tract takes place, there is upregulation, for example, of cholecystokinin (CCK) and gastrin, and these are hormones that influence tumor growth.

Dr. Fein. We did not look at the hormones in this study, but another previously mentioned study done 1 year earlier with carcinogens examined CCK and gastrin, but no significant influence was shown in that study. It was found that the levels of gastrin and CCK were altered, but although there were groups designed to have high gastrin and low gastrin levels, there was no difference in cancer prevalence or growth.

Dr. D. Fromm (Detroit, Mich.). Have you performed any measurements of other compounds, because I believe this is the first study that shows carcinoma appearing without carcinogen?

Dr. Fein. Indeed this is the first study showing this high prevalence of adenocarcinoma induced by duodenal juice without carcinogens in a time course of 16 weeks. Meanwhile, in a second study, published in the *International Journal of Cancer*, the same operation was performed and no carcinogens were administered. An observation period of 150 weeks was used and in this study the tumor prevalence, with the same type of tumor, was 72%. Thus that study confirmed our finding that there is actually carcinogenesis by reflux of duodenal juice without exogenous carcinogen.

Role of Cyclooxygenase-2 for Fluid Secretion by the Inflamed Gallbladder Mucosa

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Inflammatory fluid secretion by the gallbladder mucosa in experimental cholecystitis is induced by activation of cyclooxygenase, which leads to an increase in prostaglandin formation. Cyclooxygenase exists as a constitutive (cyclooxygenase-1) and an inducible (cyclooxygenase-2) isoform. The aim of this study was to demonstrate the role of cyclooxygenase-2 in inflammatory fluid secretion of the feline gallbladder. Experiments were performed 10 weeks after a surgical procedure in which chronic cholecystitis was induced in cats by ligation of the cystic duct and implantation of a gallstone in the gallbladder. Gallbladder fluid transport was continuously monitored via a perfusion system. In inflamed gallbladders the continuous fluid secretion was reversed to absorption by intravenous injection of the selective cyclooxygenase-2 blocker, NS 398 ($P < 0.001$). Increased levels of the inducible cyclooxygenase-2 were shown by immunoblotting in inflamed gallbladders. Selective pharmacologic blockade of cyclooxygenase-2 reduced the prostaglandin E_2 release to the inflamed gallbladder lumen ($P < 0.01$). These data suggest that cyclooxygenase-2 is involved in the inflammatory response during chronic cholecystitis. Selective cyclooxygenase-2 blockers may offer an alternative to traditional nonsteroidal anti-inflammatory drugs with fewer side effects in patients with cholecystitis who are awaiting operation. (J GASTROINTEST SURG 1998; 2:269-277.)

According to Jivegård et al.,[1] three different variables appear to be critical in controlling inflammatory secretion of fluid and electrolytes in a model of chronic cholecystitis: (1) nonadrenergic, noncholinergic (NANC) intrinsic secretomotor nerves, (2) prostaglandin E_2 (PGE_2), which results from the enzymatic breakdown of arachidonic acid by cyclooxygenase (COX),[2] and (3) nitric oxide (NO), which is derived from L-arginine via the inducible isoform of NO synthase.[3] Similar to nitric oxide synthase, COX appears to exist in different isoforms, of which COX-1 is expressed constitutively and COX-2 is induced by hormones, growth factors, and cytokines. Either pathway may be inhibited by aspirin-like drugs.[4] COX-1 is believed to be involved in the maintenance of normal cell integrity and function, for example, in platelets, vascular endothelium, stomach mucosa, and kidneys, resulting in the release of prostanoids.[4] Inflammatory stimuli, including endotoxin-lipopolysaccharides, elicit the release of cytokines (in particular interferon-γ, tumor necrosis factor-α, and interleukin-1β)[5] from a variety of cells such as macrophages, fibroblasts, and epithelial cells. The cytokines, in turn, induce the expression of COX-2, particularly in neutrophils and macrophages, resulting in the release of proinflammatory prostaglandins.[4,6,7]

The involvement of prostaglandins in the fluid secretion in chronic cholecystitis was partly inferred by the finding that the systemic injection of the unselective COX inhibitor, indomethacin, elicited a reversal of this variable into absorption.[8] This compound does not differentiate between the two isoforms of COX. The ability of commonly used nonsteroidal anti-inflammatory drugs (NSAIDs) to inhibit COX-2 may well explain their therapeutic utility as anti-inflammatory drugs, whereas inhibition of COX-1 may explain their unwanted side effects such as gastric and renal damage.[6]

The aim of the present study was to investigate whether the administration of the selective inhibitor

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of COX-2, NS-398,[9] mimics the antisecretory effect of indomethacin in the chronic cholecystitis model, thereby corroborating an involvement of COX-2 in the pathogenesis of inflammatory induced fluid secretion.

METHODS

The experiments were approved by the animal ethics committee of Göteborg University.

Induction of Cholecystitis

Experimental cholecystitis was induced by means of a validated technique.[10] To summarize, acute surgical procedures were performed in 17 cats of either sex under ketamine anesthesia. The gallbladder was opened under sterile conditions through a midline laparotomy. A portion of the gallbladder bile was evacuated and one human gallstone, 4 × 4 mm, was implanted. The opening in the gallbladder was closed with a pursestring suture, and the cystic duct was subserosally ligated after careful dissection, avoiding damage to vessels and nerves. The animals were treated prophylactically with intramuscular bensylpenicillin (0.5 g) and streptomycin (0.5 g) at the time the gallstones were implanted intramuscularly to guard against infection. No complications (wound infection, gallbladder perforation, or wound hernia) occurred postoperatively.

Investigation of Gallbladder Fluid Transport

The acute experimental procedures, performed 8 to 10 weeks after induction of cholecystitis, were identical to those described in previous reports.[10,11] The experiments were performed in 17 animals with chronic cholecystitis (see above) and, in addition, in nine (unoperated) control animals with normal gallbladders. The cats were fasted for 24 hours before the experiment but given free access to water. After induction with xylacine (Rompun, 1.5 mg/kg of body weight intramuscularly), anesthesia was maintained with chloralose (30 to 50 mg/kg intravenously, given as a single dose; a supplementary injection of 15 mg/kg was given at least once during the course of each experiment). Each animal was placed on an operating table with a thermostatically regulated heating pad to maintain body temperature at 38° C. After tracheotomy, artificial respiration was maintained with a Harvard ventilator (model 683, Harvard Apparatus, Inc., South Natick, Mass.). A femoral artery was cannulated and connected to a Statham P23DC transducer (Statham Instruments, Oxnard, Calif.) for recording of arterial blood pressure on a Grass polygraph (Grass Instrument Co., Quincy, Mass.). The abdomen was opened through a midline incision and the pylorus was ligated to prevent gastric juice from reaching the duodenum. The adrenal glands were ligated to prevent the release of catecholamines into the bloodstream. The common bile duct was cannulated in the direction of the liver with a plastic cannula (1.7 mm outside diameter) for drainage; the cystic duct was ligated in the control animals (this procedure already having been performed in the animals that had been subjected to gallstone implantation). The gallbladder was cannulated in situ with two cannulas (1.7 mm outside diameter), one in the fundus and the other close to the cystic duct, and secured with pursestring sutures. The gallbladder was continuously perfused using a roller pump at a rate of 10 ml/hr in an open perfusion system, with the intraluminal hydrostatic pressure adjusted to 7 cm H_2O by the height of the balance. The perfusate was an electrolyte solution (comprised of the following [in mmol/L]: 135 Na^+, 5.0 K^+, 105 Cl^-, and 35 HCO_3^-; osmolality 270 mOsm/L; pH 7.4) containing ^{14}C-labeled polyethylene glycol (PEG 4000) as a nonabsorbable marker.

Experimental Protocol

After the operation was completed, the abdomen was closed with clips and the animals were allowed to recover for 60 minutes, whereupon guanethidine (3 mg/kg intravenously) and atropine (1 mg/kg intravenously, followed by an intravenous infusion of 0.5 mg/kg/hr) were administered to eliminate any possible influence of noradrenergic and cholinergic nerves of the gallbladder on the variables under investigation. The animals were then left undisturbed for another hour to stabilize cardiovascular and hepatic function.

The various parameters investigated were thereafter monitored for at least 60 minutes to register steady-state conditions (basal period). Then either indomethacin (2 mg/kg intravenously) was administered to six operated and three control animals or the selective COX-2 blocker, N-[2-(cyclohexyloxy)-4-nitrophenyl] methanesulfonamide (NS-398) (1 mg/kg intravenously)[9,12] was injected into 11 operated and six control cats. In preliminary (unpublished) experiments we found that a dose of 0.1 mg/kg intravenously of the selective COX-2 blocker, NS-398, was ineffective, whereas a tenfold higher dose appeared to have undue effects on cardiovascular func-

tion. Therefore we chose to use 1 mg/kg intravenously. After 1 hour of "drug equilibration" (test period 1), the various parameters studied were then followed for another 60 minutes (test period 2). Samples of 3 ml of the buffer, taken from the influx to and the efflux from the gallbladder, respectively, were collected every 20 minutes during the three periods. The last sample in each period was used for estimation of PGE_2 and fluid transport.

Calculation of Net Fluid Transport

Gallbladder mucosal net fluid transport was calculated by two separate methods. First it was estimated from the change in weight on the balance (volume method), and second from the perfusion rate and the change in concentration of the marker in 200 μl samples of the perfusate (PEG method).[8] In a previous study it was shown that PEG 4000 does not escape from the perfusion system, even in inflamed gallbladders.[13] At the end of each experiment the pressure in the gallbladder was increased to test the patency of the perfusion system. The color of the perfusate after it had passed through the gallbladder was inspected to detect macroscopic bleeding from the mucosa into the gallbladder lumen.

Recording of Bile Flow

Changes in bile flow were monitored via an optical drop recorder on a Grass polygraph, and the average bile flow per 20 minutes was calculated in response to each drug administered after collecting the bile in preweighed vials.

Bile Salt Secretion Rate

The total bile salt concentration in bile was determined by an enzymatic method using 3α-hydroxysteroid dehydrogenase (Sterognost 3a, Nyegaard and Co., Oslo, Norway). Sodium chenodeoxycholic acid (Nyegaard and Co.) was used as a standard. The intra-assay coefficient of variation for the determination of sodium chenodeoxycholate was 2.8% and 1.9% at concentrations of 25 and 50 mmol/L, respectively.

Measurements of Radioactivity

The radioactivity of two samples (200 μl each) from the inflowing and outflowing buffer, respectively, was counted in a Tri-Carb 1500 scintillation counter (Packard Instrument Co., Meriden, Conn.) after the addition of 10 ml Opti-Fluor (Packard Instrument Co.). Correction for sample quenching was performed by the spectral index method.[14]

Prostaglandin E_2 (^{125}I) Assay

Sodium citrate was used as an anticoagulant and indomethacin (10 μg/ml) was used as an inhibitor of further breakdown of arachidonic acid. Specimens were kept on ice and centrifuged at 2500 × *g* for 10 minutes at 4° C. Following acidification, the addition of ethanol, and the centrifugation of plasma, PGE_2 was extracted on Amprep C18 minicolumns (Amersham RPN 1900, Amersham Corp., Arlington Heights, Ill.) according to the recommendations in the PGE_2 assay system (Amersham RPA 530). Tissues were homogenized in 0.1 mol/L Tris-HCl buffer, pH 7.4, containing indomethacin as an inhibitor. All tubes were kept on ice. Following centrifugation, supernates were treated as previously described and applied to C18 minicolumns for extraction of PGE_2. Following conversion of extracted PGE_2 by methyloximation according to the kit instructions, radioimmunoassay was performed within 6 days. Values were calculated from duplicate specimens.[15]

Immunoblotting

Soluble tissue extracts were prepared as previously described with minor modifications.[16] Briefly, the tissues were homogenized by a polytron (Kinematica, Littau/Lugern, Switzerland) in a PE buffer (10 mmol/L potassium phosphate buffer, pH 6.8, and 1 mmol/L EDTA) containing 10 mmol/L 3-(3-cholamidopropyl) dimethyl-ammonio) 1-propan sulfonate (CHAPS; Boehringer, Mannheim, Germany), aprotinin (1 μg/ml; Boehringer), Pefabloc (1 mg/ml; Boehringer), leupeptin (1 mg/ml; Boehringer), and pepstatin (1 mg/ml; Boehringer). The homogenate was sonicated (two times, 10 seconds each time) and centrifuged (12,000 ×g for 5 minutes); supernates were stored at −70° C until analysis. The samples were diluted in sodium dodecyl sulfate (SDS)–sample buffer before loading on one-dimensional SDS-polyacrylamide gel electrophoresis plates (4.5% stacking gel, 8% separating gel; Novex system, San Diego, Calif.). The amount of total protein was 35 μg/lane. The proteins were transferred to a polyvinyl difluoride membrane (Millipore Corp., Bedford, Mass.) by electroblotting (Novex system). The membranes were then incubated with blocking buffers containing rabbit polyclonal antibodies against COX.[17] These antibodies were affinity purified as described elsewhere.[18] This antibody has previously been demonstrated to preferentially recognize COX-2.[19] Several commer-

cial mono- and polyclonal antibodies against COX-1 and COX-2 did not recognize these enzymes in feline tissues (data not shown). Immunoreactive proteins were visualized by chemiluminescence using an alkaline-conjugated second antibody and CDP-star (Tropix, Inc., Bedford, Mass.) as substrate. The filters were exposed to enhanced chemiluminescence film (Amersham, Buckinghamshire, U.K.) at room temperature for 30 seconds to 2 minutes, and the films were subsequently developed.

Histopathologic Studies

The gallbladders were promptly fixed in 4% formalin and examined in paraffin-embedded, hematoxylin and eosin–stained sections (7 μm). The sections were studied under a light microscope (Leica DMRB/E, Wetglar Germany).

Bioactive Substances and Solutions

^{14}C-labeled polyethylene glycol (molecular weight approximately 4000) was obtained from Radiochemical Centre (Amersham). Other chemical used were atropine sulfate (Atropin, Kabi Pharmacia, Uppsala, Sweden), bensylpenicillin (Astra, Södertälje, Sweden), guanethidine sulfate (Ismeline, Ciba-Geigy AG, Basel, Switzerland), indomethacin (Confortid, Dumex Ltd., Copenhagen, Denmark), NS-398 (Cayman Chemical Co., Ann Arbor, Mich.), streptomycin (Heyl, Chempharma Fabrik GmbH & Co., Berlin, Germany), and xylacine (Rompun); Bayer AG, Leverkusen, Germany).

Statistical Analysis

Statistical analyses were performed by means of analysis of variance followed by Fisher's least significant difference test for repeated measurements. A P value <0.05 was considered significant. All quantitative data are presented as means ± standard error of the mean (SEM).

RESULTS

In the acute experiments blood pressure remained stable, mean arterial blood pressure being approximately 110 mm Hg in all animals despite guanethidine treatment. No animal had macroscopic bleeding from the mucosa into the gallbladder lumen.

Effects of Indomethacin on Inflammatory Fluid Secretion and Luminal Output of PGE_2

In *normal* gallbladders (i.e., from unoperated control animals; n = 3), the injection of indomethacin seemingly affected neither the basal fluid absorption (Fig. 1) nor the release of PGE_2 into the lumen (Fig. 2), in concert with earlier reports.[11]

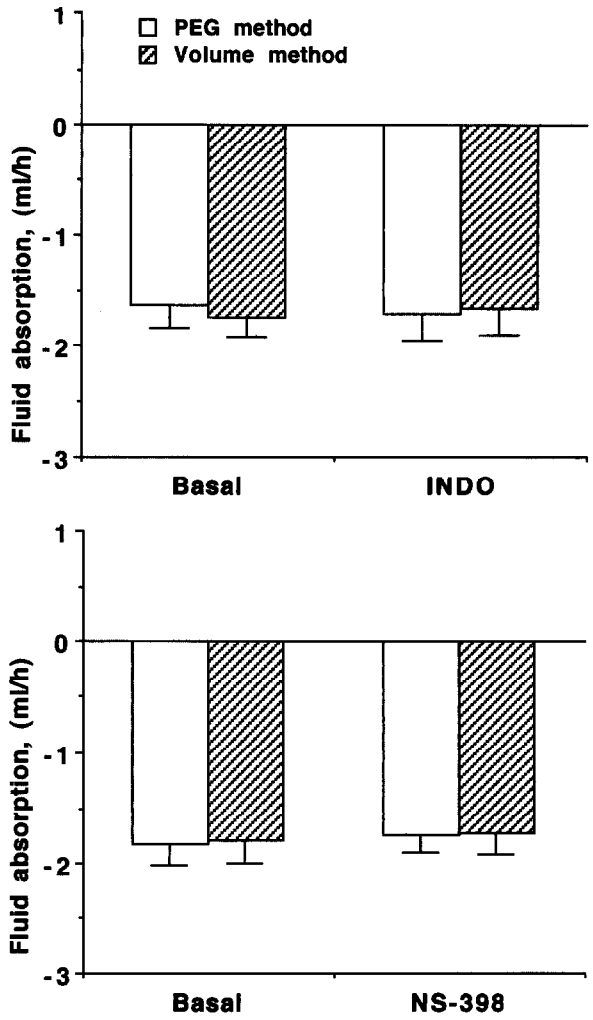

Fig. 1. Net fluid absorption across normal gallbladder mucosa after intravenous injection of indomethacin *(INDO)* (2 mg/kg) (n = 3) or NS-398 (1 mg/kg) (n = 6) as analyzed with the polyethylene glycol (PEG) and volume methods. Values are means ± SEM.

Conversely, in *inflamed* gallbladders (n = 6), indomethacin treatment reversed the prevailing fluid secretion to net fluid absorption (Fig. 3). The PGE_2 output into the gallbladder lumen was significantly reduced to approximately 30%, in comparison to untreated control animals with indomethacin treatment (see Fig. 2).

Effects of NS-398 on Inflammatory Fluid Secretion and Luminal Output of PGE_2

In normal (n = 6) (see Fig. 1) and inflamed (n = 11) (see Fig. 3) gallbladders, the selective COX-2 antagonist, NS-398, mimicked the effect of in-

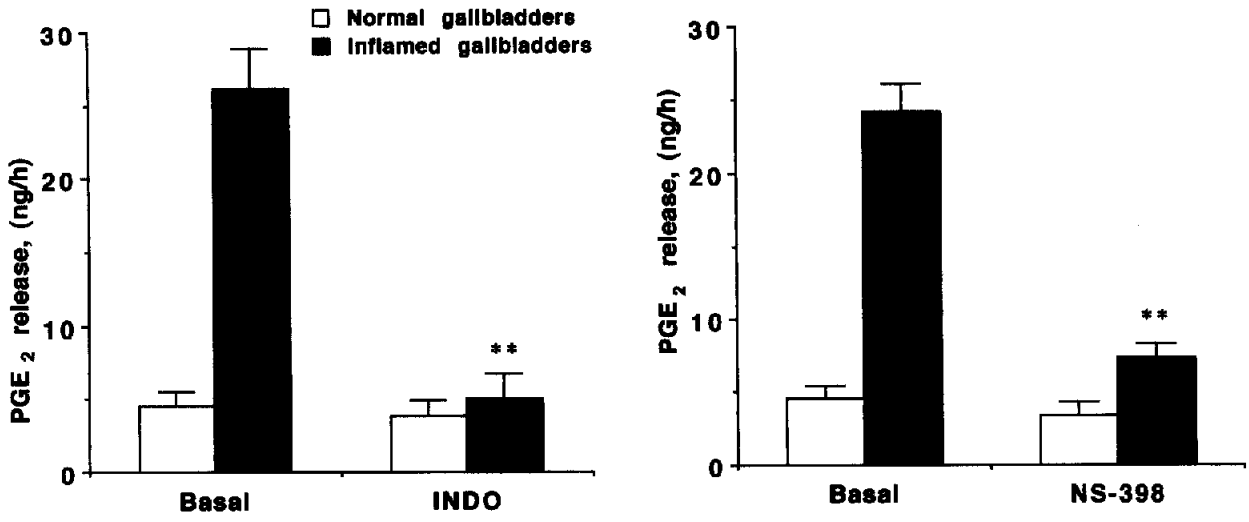

Fig. 2. PGE_2 release to the perfusing buffer from normal gallbladder mucosa after intravenous injection of indomethacin *(INDO)* (2 mg/kg) (n = 3) or NS-398 (1 mg/kg) (n = 6). PGE_2 release to the perfusing buffer from inflamed gallbladder mucosa after injection of indomethacin (n = 6) or NS-398 (n = 11). Values are means ± SEM. ** = $P < 0.01$ indomethacin period or NS-398 period compared to basal period.

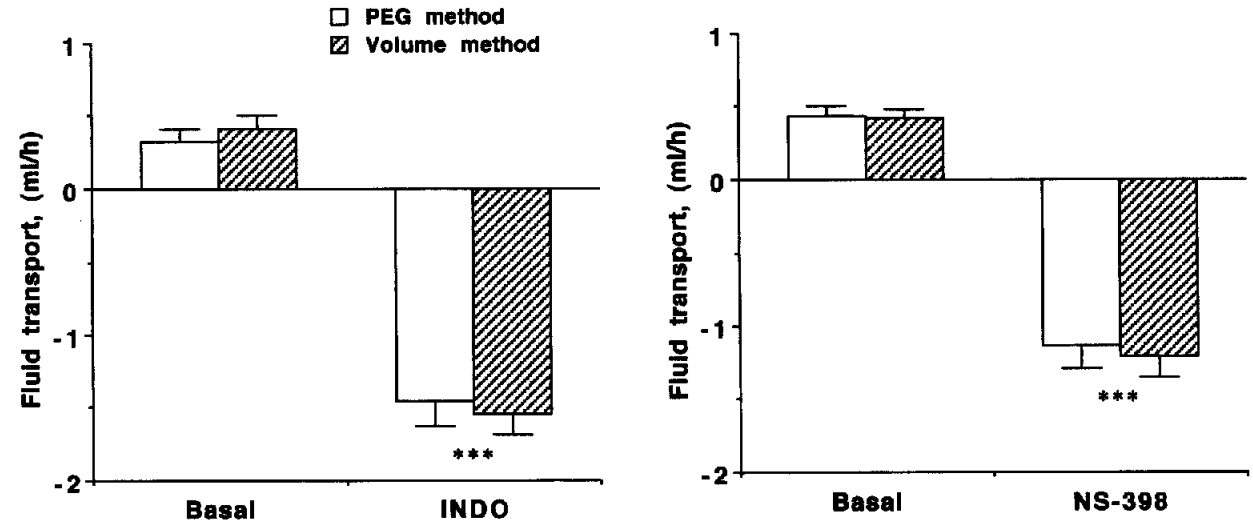

Fig. 3. Net fluid transport across inflamed gallbladder mucosa after intravenous injection of indomethacin *(INDO)* (2 mg/kg) (n = 6) or NS-398 (1 mg/kg) (n = 11). A positive sign denotes secretion and a negative sign denotes absorption. Fluid transport is analyzed with the polyethylene glycol *(PEG)* and volume methods. Values are means ± SEM. *** = $P < 0.001$, indomethacin period or NS-398 (COX-2 blocker) period compared to basal period.

domethacin on fluid transport (see Fig. 3) and PGE_2 release (see Fig. 2). No animal had macroscopic bleeding from the mucosa into the gallbladder lumen.

Bile Secretion in Normal and Inflamed Gallbladders

Neither in the experiments with normal nor inflamed gallbladders was the output from the liver of bile and bile acids changed by the administration of either indomethacin or NS-398 (Table I).

Immunoblotting

Immunoblot testing (Fig. 4) demonstrated that both isoforms of COX are present in the feline gallbladder, and an increased amount was detected in the inflamed mucosa. The remaining wall contained con-

Table I. Bile flow (ml/hr) and bile acid output (μmol/hr) in animals with normal and inflamed gallbladders*

Controls (n = 3) (unoperated)	Basal	INDO	Basal
Bile flow	1.47 ± 0.18	1.52 ± 0.21	1.48 ± 0.19
Bile acid output	15.5 ± 3.9	14.9 ± 4.1	15.9 ± 4.3
Controls (n = 6) (unoperated)	Basal	NS 398	Basal
Bile flow	1.45 ± 0.13	1.49 ± 0.16	1.51 ± 0.14
Bile acid output	15.3 ± 3.1	15.1 ± 2.9	14.9 ± 3.3
Test group (n = 6) (chronic cholecystitis)	Basal	INDO	Basal
Bile flow	1.62 ± 0.13	1.49 ± 0.16	1.58 ± 0.14
Bile acid output	16.5 ± 3.7	13.7 ± 4.1	15.9 ± 4.3
Test group (n = 11) (chronic cholecystitis)	Basal	NS 398	Basal
Bile flow	1.69 ± 0.09	1.58 ± 0.11	1.64 ± 0.13
Bile acid output	16.9 ± 3.2	14.6 ± 3.4	15.4 ± 3.7

INDO = indomethacin.
*All values are means ± standard error of the mean (SEM).
No statistical analysis was performed for the control group that received indomethacin. In the other groups there were no significant effects of either indomethacin or NS 398 (for statistical analysis, see Methods).

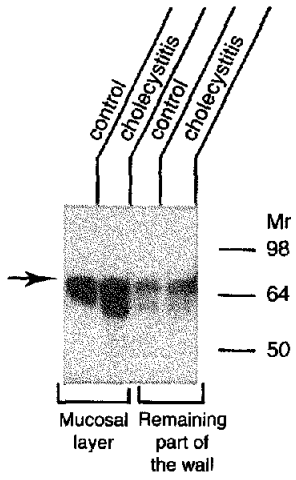

Fig. 4. Immunoblot demonstrating the presence of COX-1 and COX-2 in the mucosal layer and the remaining wall of the gallbladder of control animals and animals with chronic cholecystitis. Thirty-five micrograms of total protein was loaded into each lane.

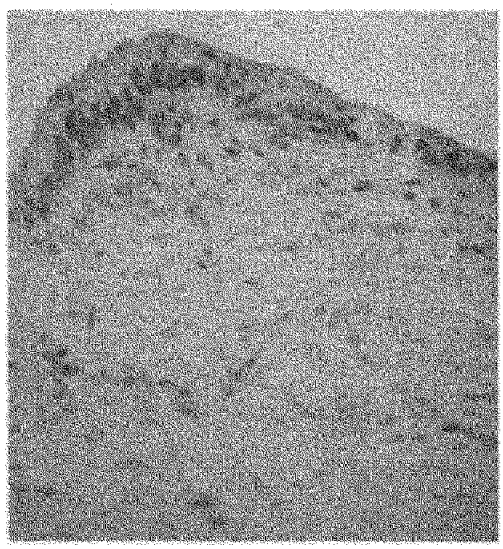

Fig. 5. Photomicrograph of a section of the wall of an inflamed gallbladder showing macrophages close to the epithelium, but no granulocyte infiltration. (Hematoxylin and eosin stain; original magnification × 400.)

siderably less compared to the mucosal layer, and there was no difference between the control animals and those with cholecystitis.

Histopathologic Examination

Sections of the wall of the normal gallbladders showed no macrophages or polymorphonuclear granulocytes. Sections of the wall of the inflamed gallbladders showed macrophages close to the epithelium but only sparse infiltration of granulocytes (Fig. 5).

DISCUSSION

The starting point for the present study was the well-established finding that inflammatory fluid secretion in experimental chronic cholecystitis may be reversed to absorption by the systemic administration

of the (unselective) COX antagonist, indomethacin.[8] Corroborating a role for prostaglandins in inflammatory induced fluid secretion, a luminal release of PGE_2 of approximately threefold the level in control animals was observed in animals with chronic cholecystitis.[2]

The aim of the present study was to investigate whether or not COX-2 is upregulated in a model of chronic cholecystitis in vivo. The effect of indomethacin on fluid secretion[8] and luminal PGE_2 release[2] was mimicked (qualitatively as well as quantitatively) by NS-398, which compound displays a high selectivity for COX-2.[9,12] We therefore suggest that most, if not all, of the PGE_2-induced inflammatory secretion is derived via the inducible pathway (i.e., COX-2).

In chronic inflammatory conditions, proinflammatory mediators such as cytokines and endotoxin induce the expression of COX-2, particularly in macrophages.[6,20] Both the present study and another recent report have demonstrated that in our model of chronic inflammation macrophages invade the border area between the mucosa and the muscle layer,[3] and it appears conceivable that such cells are the main source of the production of prostaglandins.

To our knowledge, the expression of different isoforms of COX in various tissues has not been described in the cat. We were able to detect the two isoforms with an affinity-purified polyclonal antibody, which was generated in the rabbit against the full-length protein. This antibody preferentially recognizes the COX-2 isoform, although COX-1 is also detected. The two isoforms of COX seem to migrate with apparent molecular weights of 70,000 and 72,000 daltons, respectively. The chronic cholecystitis resulted in a modest increase of COX. Interestingly the mucosal layer of the control gallbladders contained substantial amounts of both isoforms.

When previous data are taken together with the present results, the following *hypothetical* scheme for the inflammatory fluid secretion in our model of chronic cholecystitis may be outlined. Thus the secretory response is mostly neurogenic since it may be abolished by the neuron blocker, tetrodotoxin.[21] Tetrodotoxin seemingly is equipotent to any of the following compounds: the COX antagonists, indomethacin and NS-398 (present study); the antagonists of nitric oxide synthase (NOS), L-NNA and aminoguanidine, of which the latter displays selectivity for the inducible isoform of NOS (iNOS)[3]; and finally an antiserum to the putative neurotransmitter, vasoactive intestinal peptide (VIP).[22] This could imply that prostaglandins and NO act *in series* in one way or another, leading to the release of prosecretory VIP via a nervous link. The epithelium of the gallbladder is supplied mainly by nerves immunoreactive to either VIP or substance P,[23,24] and VIP receptors are demonstrated on the epithelial cells of the gallbladder in humans and in cats.[25] VIP induces a secretory response in the gallbladder,[26] and the finding that there are increased amounts of VIP[27] in the gallbladder lumen in experimental cholecystitis suggests that the nerves activated in chronic cholecystitis are "VIP-ergic."

It has recently been established that NO may induce the generation of prostaglandins via COX-2.[28] Therefore, in chronic cholecystitis macrophages, responding to endotoxin or cytokines such as interferon, tumor necrosis factor, and interleukin-1 could induce the expression of iNOS.[29] These stimuli could also induce the expression of COX-2, and an alternative explanation is that prostaglandin production is a consequence of the stimulation of COX-2 by NO itself. Thus, according to our hypothesis, the inflammatory stimuli lead to NO production (via iNOS), which in turn lead to PGE_2 generation (via COX-2); finally, PGE_2 acts as a strong stimulus for a continuously active secretomotor reflex utilizing VIP as a final mediator.

CONCLUSION

In the present study we have demonstrated that the selective COX-2 antagonist, NS-398, mimics the inhibitory effect of indomethacin on inflammatory fluid secretion and release of PGE_2. An hypothesis as to the role of NO, PGE_2, and efferent (VIP-ergic) nerves in this model of inflammation is presented. This study provides a rationale for symptomatic treatment of patients with cholecystitis who are awaiting operation with selective COX-2 inhibitors, with fewer side effects compared to unselective blockers.[30]

We gratefully acknowledge Christina Lönroth, M.D., Ph.D., for the prostaglandin E_2 (^{125}I) assay.

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Discussion

Dr. K. Lillemoe (Baltimore, Md.). There was a 10-week period between the surgical procedure and the analysis. There were no manipulations done on the gallbladders of the control animals. Is that correct?

Dr. Nilsson. That is correct. No manipulations at all were done on the control animals.

Dr. S. Strasberg (St. Louis, Mo.). Did you have a control group with stones implanted without cystic duct ligation or a control group that underwent simple cystic duct ligation without stone implantation? Also, you have a cholecystostomy-alone group, which would be necessary as a control for the surgical manipulation of stone implantation. I guess what I am trying to do is break down the components into those that contributed to cystic duct occlusion vs. the gallstones.

Dr. Nilsson. We have conducted bile duct experiments to determine the proper setting and we observed no difference between the animals operated on without ligation compared to our control group in which there were no operations at all. So we had one control group and we did not

operate until the acute experiment. In the group with inflamed gallbladders, we operated on them, ligated cystic ducts, and implanted two gallstones, but we did not interfere with the vessels to the gallbladder. These animals were left undisturbed for about 10 weeks and we then performed acute experiments in this group. In previous experiments we have used all of the control groups you are asking about, and they did not differ from the present control groups.

Dr. Strasberg. Does ligation of the cystic duct and implantation of stones result in acute cholecystitis, which then progresses to chronic inflammation at 10 weeks? Is this similar to a human model or is it different.

Dr. Nilsson. We tried to simulate what occurs in the human gallbladder during the phase following acute cholecystitis, and we also obtained sections from the gallbladders at 1, 2, 3, 4, and 5 weeks, and we noted that within the first 3 weeks, there was acute cholecystitis with infiltration of granulocytes but during the fifth week there were no granulocytes left, only mostly macrophages, so what we see is chronic cholecystitis after 10 weeks.

Laparoscopic Splenectomy: Reduction of Hospital Charges

Richard T. Schlinkert, M.D., Denise Mann, R.N., Amy Weaver, M.S.

Laparoscopic splenectomy has become our procedure of choice for the surgical management of immune thrombocytopenic purpura. Hospital charges for this procedure were analyzed for 24 consecutive patients undergoing laparoscopic splenectomy. Total charges have decreased over time and average a $233 decrease per patient treated. The decreased charges are related to decreased operating room charges. Furthermore, charges are shown to be related to the length of postoperative stay. Choice of instrumentation has kept intraoperative charges for disposables stable. (J GASTROINTEST SURG 1998;2:278-282.)

Assessing the value of a medical procedure requires analysis of quality and cost. Traverso[1] points out that quality must be assessed first and should be determined by physicians. Once the ideal procedure is identified, however, charges should be evaluated and efforts made to reduce costs.

Laparoscopic splenectomy was first performed in 1991.[2] Several authors have subsequently documented their experience with this procedure.[3-5] In 1995 we evaluated our early experience with laparoscopic splenectomy for patients with idiopathic thrombocytopenic purpura (ITP).[6] We concluded that laparoscopic splenectomy is safe and leads to a decreased use of parenteral narcotics, more rapid oral intake, and a shorter hospital stay when compared with historical controls undergoing open splenectomy. In 1996 we presented results of a follow-up study of 18 patients undergoing laparoscopic splenectomy for ITP.[7] That study demonstrated continued safety and also documented that 17 of 18 patients had appropriate responses of platelet counts following splenectomy. Furthermore, these 17 patients required no medications with a mean follow-up of 15 months. Based on these data we concluded that laparoscopic splenectomy should become the procedure of choice for ITP. Our current study evaluates the hospital charges for laparoscopic splenectomy and explores mechanisms for cost reduction.

TECHNIQUE

Our technique for laparoscopic splenectomy has evolved over time. The first two operations were performed with the patient supine and subsequent operations in the right lateral semidecubitus position. In our early experience, the spleen was retracted using disposable "fan" retractors. After division of the peritoneal attachments, the vessels of the gastrosplenic ligament were divided between clips. Individual vessels in the splenic hilum were isolated, clipped, and divided. The spleen was placed in a bag and removed through an incision that was enlarged slightly in some cases to facilitate morcellation. Currently we use 5 mm reusable retractors for splenic manipulation. The gastrosplenic ligament and short gastric vessels are divided using the Harmonic Scalpel (Ethicon Endo-Surgery, Inc., Cincinnati, Ohio). Endoscopic vascular staplers are used to transect the splenic hilum after an effort has been made to ligate the splenic artery. Incisions are not enlarged for splenic removal.

Our first two patients were hospitalized for 3 days; however, our current preference is to discharge patients on the day following surgery.

METHODS

All major surgery performed by surgeons at Mayo Clinic Scottsdale is performed at Scottsdale Memor-

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ial Hospital - North (SMHN). SMHN functions independently from Mayo Clinic Scottsdale. SMHN provided detailed data on charges for our patients undergoing laparoscopic splenectomy for ITP.

Between August 1992 and January 1997, a total of 24 patients underwent laparoscopic splenectomy for ITP. One patient who was converted to an open procedure to treat an unsuspected mucinous cystadenoma of the pancreas was excluded. Billing records were reviewed to obtain the following charges: *Preoperative*, which consisted of room charge, intravenous support, chemical analysis, hematologic evaluation, blood bank, and logistics; *intraoperative*, which consisted of operating room time charge, laparoscopic disposable instruments, and all other instruments used; and *postoperative*, which consisted of recovery room time, room charges, pharmacy, and laboratory and radiologic evaluations.

To make the charges comparable over time, all charges have been expressed in real 1996 dollars, which were calculated using the national Consumer Price Index-Urban (CPI-U) hospital and related services medical component.[8] Charges are reported as mean (± standard deviation). Simple linear regression analysis was used to assess whether the charges have been decreasing over time. A fitted regression line with a slope significantly different from zero indicated decreasing charges over time. Correlations between charges and length of stay were assessed using Spearman's rank correlation coefficient. All calculated P values were two sided, and P values <0.05 were considered statistically significant.

RESULTS

Mean overall charges were \$16,093 (± \$3,922). Preoperative, intraoperative, and postoperative charges as percentages of the total bill are shown in Fig. 1. There was no statistical difference in these proportions over time.

Over time there has been a significant trend toward a decrease in both total overall charges and intraoperative charges (Fig. 2; $P = 0.042$ [model $R^2 = 18\%$] and $P = 0.004$ [model $R^2 = 32\%$], respectively). For each additional patient over time, the average change in total charges was a decrease of \$233 and the average change in intraoperative charges was a decrease of \$152. Total charges for the first five patients in the series averaged \$17,522 (± \$1,679) compared to an average of \$12,843 (± \$429) for the five most recent patients in the series. Meanwhile the charges for preoperative and postoperative services have continued to fluctuate over time without a statistically significant decrease. The average length of stay in this series was 2 days. Seventy-five percent of the last 12 patients have had a length of stay of just 1 day. Total charges and postoperative charges were related to postoperative length of stay as shown in Fig. 3. Mean total charges for 1-, 2-, and 3-day stays were \$14,091, \$16,357, and \$17,818, respectively ($P < 0.001$). Mean postoperative charges for 1-, 2-, and 3-day stays were \$1,952, \$3,030, and \$3,765, respectively ($P < 0.001$). One patient with a length of stay of 8 days is not shown in Fig. 3. This patient had total overall charges of \$29,906 and total postoperative charges of \$13,442.

The percentage of intraoperative charges that were for laparoscopic disposables has not shown a statistically significant decrease over time ($P = 0.276$). The average percentage was 40.6% and ranged from 26.4% to 53.2%.

Operating room time has continued to decrease over time ($P < 0.001$), from 460 minutes for the second patient to 120 minutes for the two most recent

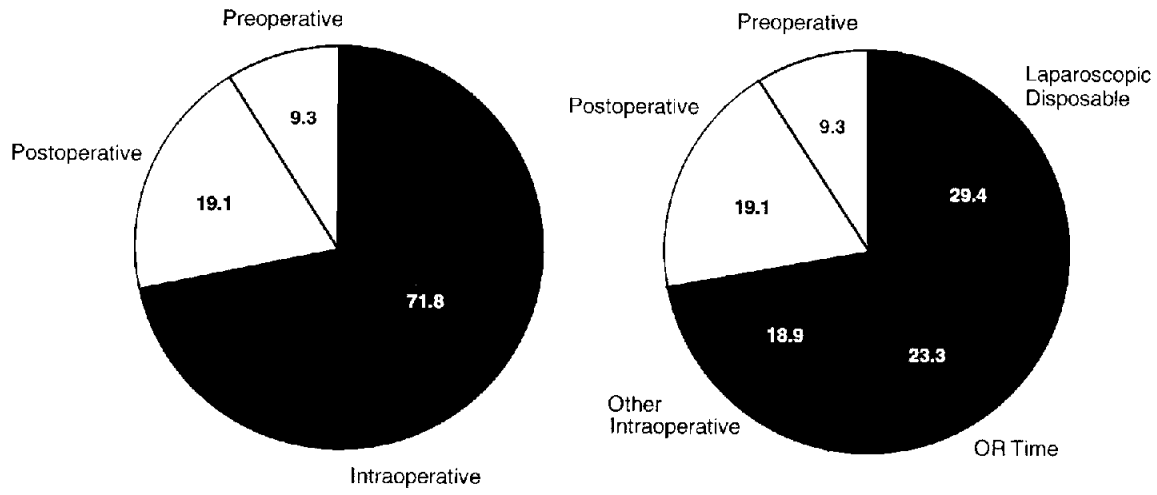

Fig. 1. Distribution of total charges for laparoscopic splenectomy.

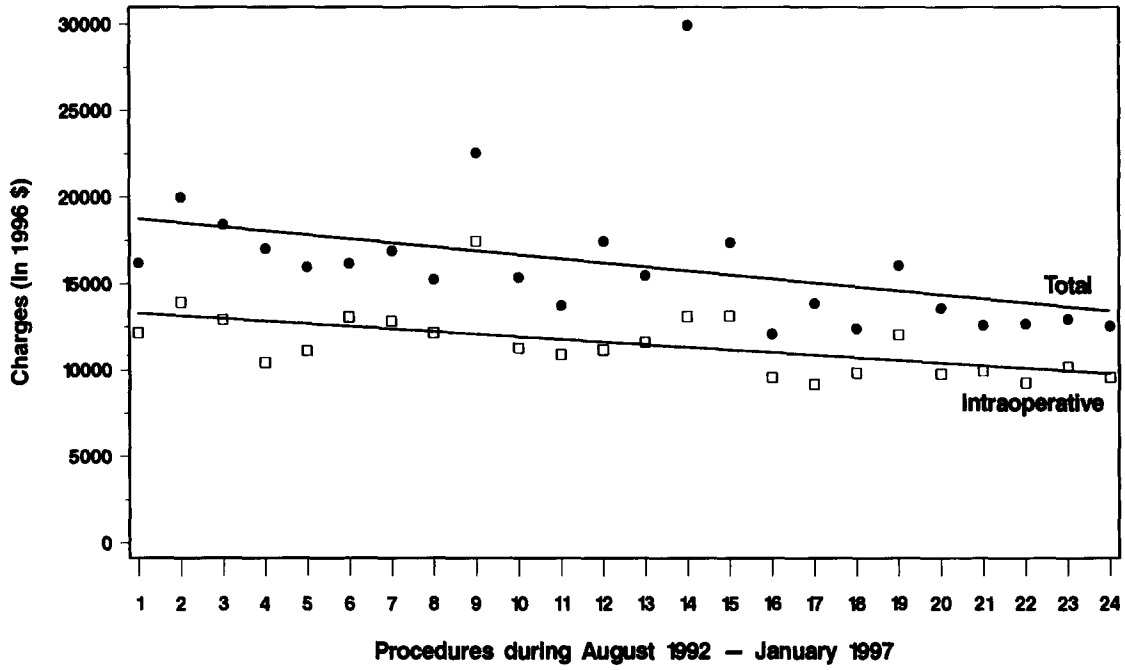

Fig. 2. Summary of total (○) and intraoperative (□) charges over time ($P = 0.042$, $P = 0.004$).

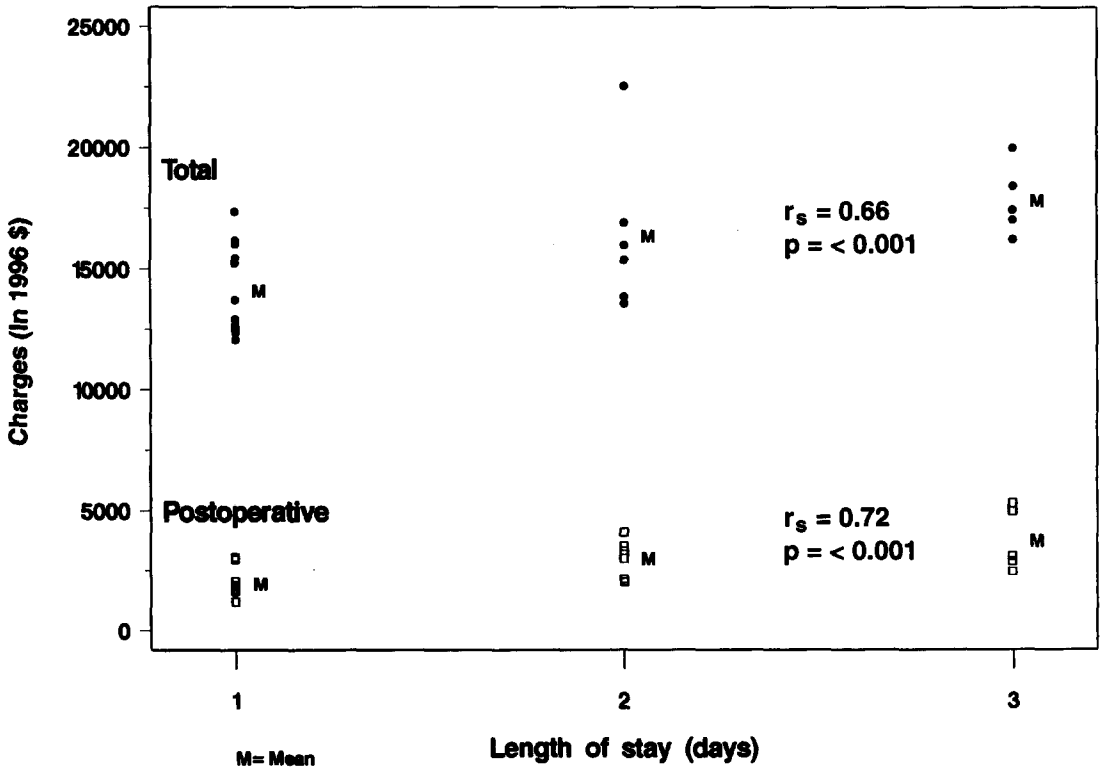

Fig. 3. Summary of total (○) and intraoperative (□) charges with respect to length of stay (total charges $r_s = 0.66$, $P = <0.001$; postoperative charges $r_s = 0.72$, $P = <0.001$).

patients (Fig. 4). The charges for operating room time have shown a trend toward decreased charges, but this did not reach statistical significance (Fig. 5; $P = 0.066$). The percentage of intraoperative charges that were for operating room time has remained fairly constant over the years.

DISCUSSION

Previous reports on the cost of laparoscopic procedures have focused primarily on a cost comparison of open and laparoscopic surgery. These studies have yielded differing results. In general, those studies that analyzed procedures with traditionally long postop-

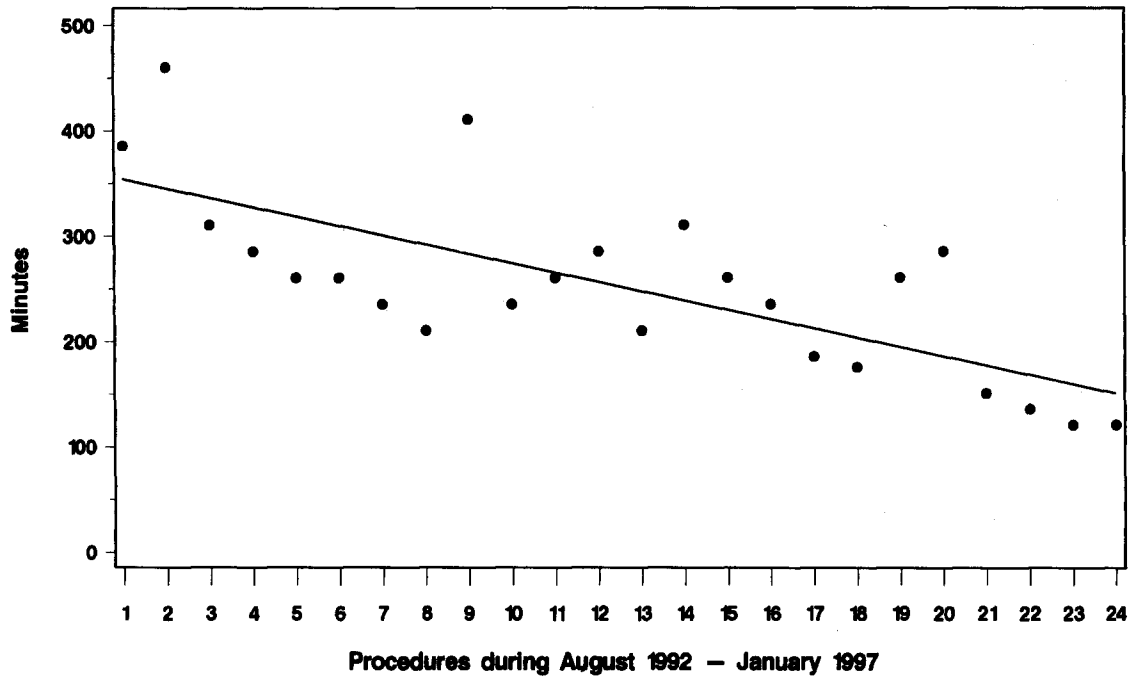

Fig. 4. Summary of operating room times per procedure ($P < 0.001$).

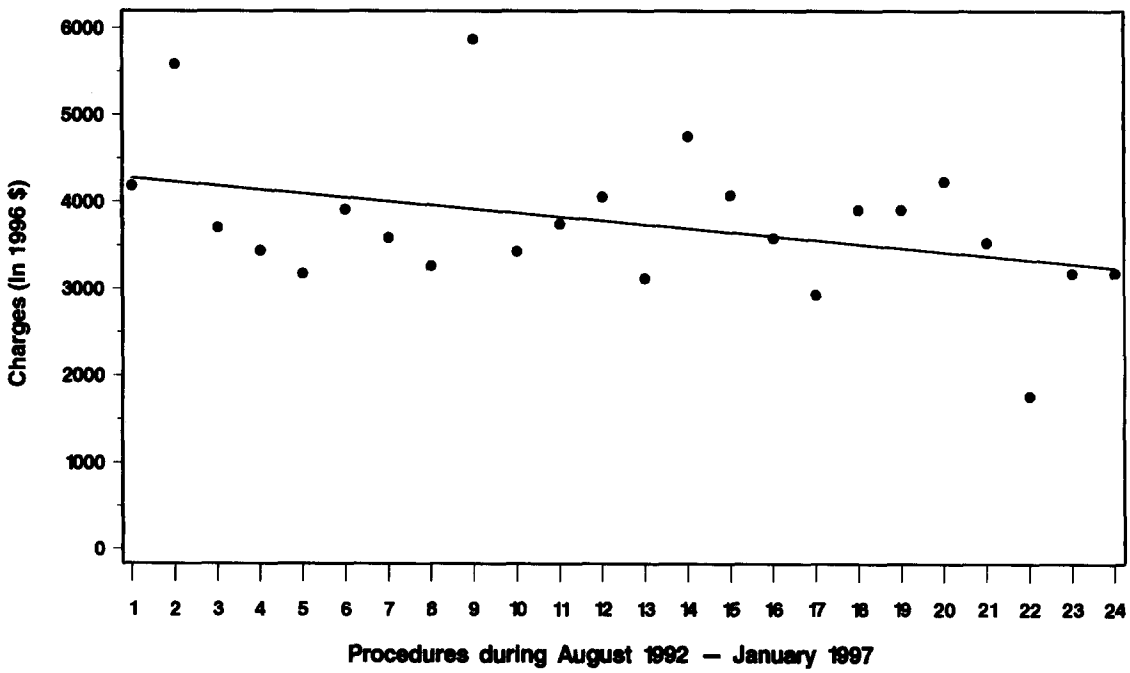

Fig. 5. Summary of charges for operating room time per procedure ($P = 0.066$).

erative recoveries (i.e., fundoplication,[9,10] adrenalectomy,[11] colon resection[12]) demonstrated either no difference in hospital charges or decreased cost for laparoscopic surgery. Procedures with shorter postoperative stays, such as open hernia repair, generally showed increased cost for the laparoscopic procedure.[13,14]

One report demonstrated that laparoscopic splenectomy had a decreased cost compared to the open approach.[15] We chose not to compare charges for open and laparoscopic splenectomy but rather to analyze charges for laparoscopic splenectomy over time. We found that total hospital charges per patient have decreased significantly over time, averaging a $233 decrease per patient treated. This was in part attributable to decreased operating room charges and a trend toward shortened postoperative stays. Postoperative and overall charges were related to length of stay and there was a trend toward decreasing length of stay over the study. Charges for operating room disposables did not change over time. By converting from disposable to reusable retractors, a significant cost savings was realized. In addition, the use of the Harmonic Scalpel has resulted in a decreased use of disposable clip appliers. Laycock et al.[16] demonstrated the effectiveness and potential cost savings of this device for the division of the short gastric vessels during laparoscopic fundoplication. The use of endosurgical vascular staplers on the splenic hilum further reduced the use of disposable clip appliers and saved an estimated 45 minutes to 1 hour of operating time per case over individual vessel transection of the splenic hilum. No patient to date has demonstrated clinical evidence of an arteriovenous fistula related to the use of staplers. The net effect of these choices in intraoperative instrumentation was decreased operating room times and charges with no increase in charges for operating room disposables.

Short postoperative stays have proved to be safe and are associated with reduced overall charges. Additional savings will likely be realized in the future. The high cost of disposables warrants an assessment of the need for them and a reevaluation of the cost of reusable products.

CONCLUSION

The hospital charges related to laparoscopic splenectomy can be decreased by improvement in technique and judicious use of instrumentation. Additional savings can be realized as reusable instrumentation continues to be improved and operating room times continue to decrease.

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Combined Cytosine Deaminase Expression, 5-Fluorocytosine Exposure, and Radiotherapy Increases Cytotoxicity to Cholangiocarcinoma Cells

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Cholangiocarcinoma is a malignancy that is resistant to current therapy. We applied the toxin gene therapy strategy of cytosine deaminase conversion of the nontoxic prodrug 5-fluorocytosine to 5-fluorouracil combined with radiotherapy to cholangiocarcinoma. The transduction efficiency of SK-ChA-1 cholangiocarcinoma cells was determined by fluorescence-activated cell-sorting analysis following infection with recombinant adenovirus AdCMVLacZ, which encodes the gene for β-galactosidase. To evaluate cytosine deaminase–mediated conversion of 5-fluorocytosine to 5-fluorouracil and subsequent cytotoxicity, SK-ChA-1 cells were infected with the recombinant adenovirus AdCMVCD, which encodes cytosine deaminase, and exposed to 5-fluorocytosine for 6 to 8 days. Additive cytotoxicity of radiation therapy was evaluated by cobalt-60 exposure following AdCMVCD infection and 5-fluorocytosine treatment. SK-ChA-1 cells were transduced (98.4%) by AdCMVLacZ at 100 plaque-forming units per cell. Following infection with AdCMVCD and exposure to 5 to 100 μg/ml of 5-fluorocytosine, 20% to 64% of SK-ChA-1 cells were killed. A combination of radiation and cytosine deaminase/5-fluorocytosine therapy resulted in enhanced cell killing (83.5% to 91.5%). Cholangiocarcinoma cells were transduced by recombinant adenoviral vectors and were killed by cytosine deaminase–mediated production of 5-fluorouracil. Enhanced cytotoxicity was seen with the addition of external beam radiation. These results provide a foundation for multimodality therapy for human cholangiocarcinoma that combines gene therapy technology with radiation therapy. (J GASTROINTEST SURG 1998;2:283-291.)

Since the description by Klatskin[1] of adenocarcinoma arising at the hepatic duct bifurcation, effective therapeutic options for cholangiocarcinoma remain to be discovered. Cholangiocarcinoma, a malignancy of the human biliary epithelium, continues to carry a poor long-term prognosis, inasmuch as curative therapeutic intervention is limited by the advanced disease stage of most patients at diagnosis.[2-5] In this regard, only 30% of patients are candidates for attempted curative surgical resection at presentation. Of these patients, another 70% are found to have occult metastatic or advanced local disease, precluding curative resection. Surgical cures do occur, yet most patients after undergoing attempted curative resection develop recurrent disease at the anastomotic site, within the liver, or as abdominal carcinomatosis.[6-8] Thus the overall 5-year survival rate following diagnosis of cholangiocarcinoma remains less than 10%.[6-8]

Adjuvant therapy with 5-fluorouracil (5-FU), a well-described radiosensitizing chemotherapeutic drug, has traditionally been ineffective as therapy for cholangiocarcinoma.[9] However, clinically effective antineoplastic treatment with 5-FU is generally limited by dose-related toxicity.[10] As an alternative, radiotherapy following attempted surgical resection for cholangiocarcinoma yields minimal prolongation in survival, on the order of 6 to 24 months, although there are conflicting reports regarding the efficacy of this treatment for cholangiocarcinoma.[2,4,5,10-14]

It is therefore clear that novel treatment strategies for cholangiocarcinoma are required. One such approach is gene therapy. A cancer gene therapy strat-

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egy, termed toxin gene/prodrug therapy, involves insertion and expression of a novel toxin gene in tumor cells and subsequent treatment with a nontoxic prodrug. The prodrug acts as substrate for the novel gene product and is converted to a cytotoxic substance by the toxin gene.[15,16] For this cancer gene therapy approach, most work has involved the herpes simplex virus thymidine kinase gene with ganciclovir (HSV-TK/GCV) and the *Escherichia coli* cytosine deaminase (CD) gene with 5-fluorocytosine (5-FC).[17-29]

CD is an enzyme from bacteria and fungi that normally catalyzes the formation of uracil by the deamination of cytosine. When 5-FC is the substrate, this enzyme will produce 5-FU, a potent radiosensitizing cancer chemotherapeutic drug.[15,16] Following introduction and expression of the CD gene in gastrointestinal carcinoma cell lines (colon and hepatic), in vivo and in vitro cytotoxicity induced by 5-FC conversion to 5-FU has been observed.[22-30] For practical applications of this approach, adenoviral vectors have been employed to deliver the CD gene in colon and hepatic cancer models.[31-33] In addition, recent studies involving combinations of radiation therapy with the HSV-TK/GCV toxin gene/prodrug system have shown efficacy in tumor cell control.[34-36] There have also been experimental combinations of CD gene transduction and radiation therapy for colon cancer and glioma models.[33,37]

Thus toxin gene/prodrug treatment with CD/5-FC combined with radiation therapy is a novel approach to the treatment of cholangiocarcinoma. An advantage to this approach is local production of the radiosensitizing and cytotoxic drug 5-FU, which may avoid systemic toxicities. In addition, tumor cell cytotoxicity induced by the locally produced 5-FU may be enhanced by the addition of radiation therapy. Therefore we sought to determine whether CD-mediated conversion of 5-FC to 5-FU was toxic to cholangiocarcinoma cells and whether this toxicity may be enhanced by radiation therapy.

MATERIAL AND METHODS

Cell Lines

The human cholangiocarcinoma cell line SK-ChA-1 was the gift of A. Knuth, Ludwig Institute for Cancer Research, London, United Kingdom. SK-ChA-1 cells were maintained in RPMI-1640 medium supplemented with L-glutamine (2 mmol/L), penicillin (10,000 IU/ml), streptomycin (10 mg/ml), and 10% heat-inactivated fetal bovine serum (FBS) (Summit Biotechnology, Ft. Collins, Colo.) at 37° C in a humidified 5% CO_2 atmosphere. HeLa cells (American Type Culture Collection, Rockville, Md.) were used as control cells and were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with L-glutamine (2 mmol/L), penicillin (10,000 IU/ml), streptomycin (10 mg/ml), and 10% heat-inactivated FBS at 37° C in a humidified 5% CO_2 atmosphere. The transformed human embryonal kidney cell line 293 is an E1A *trans*complementing cell line (Microbix, Toronto, Canada) used for viral propagation and titering and was maintained in DMEM-F12 supplemented with L-glutamine (2 mmol/L) and 10% heat-inactivated FBS at 37° C in a humidified 5% CO_2 atmosphere.

Recombinant Adenoviral Vectors

To analyze gene transfer efficiency, a recombinant adenoviral vector containing the LacZ reporter gene encoding β-galactosidase was employed. AdCMVLacZ is an E1A-deficient replication-incompetent adenoviral vector encoding the *E. coli* LacZ gene, under the control of the human cytomegalovirus (CMV) promoter/enhancer, which has been previously described.[38] The CD cDNA was provided by J. Harris (Imperial Cancer Research Fund, London, U.K.) and has been described.[17] A replication-deficient recombinant adenoviral vector encoding CD under the control of the CMV promoter/enhancer AdCMVCD was constructed using standard homologous recombination techniques.[39] Quantities of AdCMVCD suitable for in vitro studies were produced by infecting 293 cells with validated viral stock and purified by cesium chloride gradient ultracentrifugation and dialysis. The preparation of AdCMVCD was titered by plaque assay using 293 cells by standard techniques.[39]

Validation of AdCMVCD to Induce Functional CD Enzyme

To verify the effectiveness of the AdCMVCD vector to induce expression of CD within the SK-ChA-1 cells, the viral preparation was functionally validated. In a modification of the procedure used by Haberkorn et al.[40] to measure conversion of [6-^{3}H]-5-FC to [6-^{3}H]-5-FU as a result of CD expression in eukaryotic cells, SK-ChA-1 cells were infected with 100 plaque-forming units (pfu) per cell of AdCMVCD or AdCMVLacZ, then harvested after 48 hours' incubation at 37° C. The cells were lysed by four freeze-thaw cycles in 100 mmol/L Tris-HCl, 1 mmol/L EDTA/dithiothreitol (Sigma, St. Louis, Mo.), pH 7.8. Cellular debris was pelleted by centrifugation at 14,000 rpm for 2 minutes. The cytosolic fraction was separated, and 10 μl of each cell lysate was incubated with 0.5 μCi [6-^{3}H]-5-FC (Sigma) at 37° C

overnight. Each reaction mixture, as well as 5-FU/5-FC standards, was spotted on a silica gel thin-layer chromatography plate and developed in a butanol-water chamber. After separation of 5-FU and 5-FC, each region was visualized under ultraviolet light, and cut from the plate and placed in 5 ml of EcoLume scintillation fluid (ICN Pharmaceuticals, Inc., Costa Mesa, Calif.). Each region was counted for radioactivity in a Packard Tri-Carb 1900 TR liquid scintillation counter (Packard Instrument Co., Downers Grove, Ill). The [^{3}H] gate (0-18.6 keV) was used, with a counting efficiency of 60%. Percentage conversion of 5-FC to 5-FU was calculated as activity in the 5-FU region compared to the total counts in the 5-FC and 5-FU regions. Ability to induce conversion of [6-^{3}H]-5-FC to [6-^{3}H]-5-FU was verified prior to use of the viral preparation, and the same AdCMVCD viral preparation was used for all subsequent experiments. This reagent validation procedure revealed 61% vs. 1.7% conversion of [6-^{3}H]-5-FC to [6-^{3}H]-5-FU in SK-ChA-1 cells infected with 100 pfu/cell AdCMVCD and AdCMVLacZ, respectively.

Cellular Infections With Recombinant Adenovirus

Procedures regarding infection of tumor cells in vitro with recombinant adenovirus have been described previously.[38] Briefly, 1×10^6 cells were plated in six-well culture dishes (Costar Corp., Cambridge, Mass.) and infected with recombinant adenovirus (AdCMVLacZ or AdCMVCD) 24 hours later, when cells were at a confluency of 90% to 95%. Cellular infections were carried out in a minimal volume of serum-free Optimem (Gibco Laboratories, Grand Island, N.Y.) at 37° C. Adenoviral infection was terminated after 1.5 to 2 hours by the addition of RPMI-1640 with 20% FBS. Cells were infected with 10 or 100 pfu/cell of the indicated adenoviral vector.

Detection of Reporter Gene Expression

Forty-eight hours after infection with AdCMVLacZ, SK-ChA-1 and HeLa cells were analyzed by fluorescence-activated cell-sorting (FACS) analysis for β-galactosidase expression. HeLa cells were used for control purposes. Cells infected with AdCMVLacZ and uninfected control cells were harvested 48 hours following infection and resuspended at 1×10^7 cells/ml in FACS staining media, phosphate-buffered saline (PBS) solution (0.2 g/L KCl, 0.2 g/L KH_2PO_4, 8 g/L NaCl, 1.15 g/L Na_2HPO_4, pH 7.2) (Gibco) containing 10 mmol/L N-2-hydroxyethylpiperazine-N′-2-ethane sulfonic acid (HEPES) and 4% FBS. Aliquots were incubated at 37° C for 10 minutes, after which 100 μl of 2 mmol/L N-2-hydroxyethylpiperazine-N′-2-ethane sulfonic acid (FDG) (Sigma) was added to the tubes and incubated for an additional minute. FDG loading was terminated by adding 1 ml of ice-cold staining media. Cells expressing β-galactosidase cleave FDG to a colored product, allowing detection by FACS analysis. Cells were kept dark and on ice until FACS analysis was performed.

AdCMVCD-Induced Sensitivity to Cellular Production of 5-FU

SK-ChA-1 cells were infected with 10 pfu/cell of AdCMVCD and appropriate control cells (no viral infection and AdCMVLacZ as irrelevant virus control), as previously described. Twenty-four hours later, cells were trypsinized, counted, and plated (5000 cells/well) in 96-well microtiter plates (Costar) in 12 replicates. Media were supplemented with 0, 5, 10, 50, and 100 μg/ml of 5-FC (Sigma), respectively. Cell proliferation was determined by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium colorimetric assay (CellTiter 96 aqueous nonradioactive cell proliferation assay kit, Promega Corp., Madison, Wisc.) per manufacturer's recommendations, after 6 and 8 days' incubation in media containing 5-FC. The absorbance at 490 nm was then measured in a 96-well plate reader (Molecular Devices Corp., Menlo Park, Calif.). Data collected by the plate reader were analyzed by the SOFTmax software package (Emax, Molecular Devices).

Evaluation for Enhanced Cytotoxicity of Combined Toxin Gene Expression With Prodrug Exposure and Single-Fraction External Beam Radiation

To analyze increased cell killing of CD-expressing cells following a single fraction of external beam radiation, SK-ChA-1 cells were infected with AdCMVCD at 10 pfu/cell and appropriate control cells (no viral infection with and without 5-FC treatment, AdCMVLacZ infection with and without 5-FC treatment, and AdCMVCD infection without 5-FC treatment). Twenty-four hours later, media were aspirated and replaced with fresh RPMI-1640 media supplemented with 10 or 20 μg/ml of 5-FC. After 72 hours of exposure to 5-FC, cells were irradiated on ice at a dose of 8 gray (Gy) with a Picker C-9 80 cm isocenter cobalt-60 clinical irradiator (Picker International, Cleveland, Ohio). Following radiation treatment, infected and control cells were plated in 96-well microtiter plates (5000 cells/well) in media free of 5-FC.

MTS proliferation assays were performed 6 and 8 days following radiation treatment.

Statistical Analysis

The reporter gene expression in SK-ChA-1 cells represents the FACS cell counts of 10,000 cells per group. Percentages reported represent the fraction of cells expressing β-galactosidase.

To determine an interaction between viral infection (AdCMVCD, AdCMVLacZ, or no virus), dose of 5-FC, and duration of 5-FC exposure, a three-factor analysis of variance was employed. In addition, pairwise comparisons among all groups were performed to verify differences at a $P < 0.05$ level of significance. These experiments were repeated, and the data presented are a representative result.

A four-factor analysis of viral infection (AdCMVCD, AdCMVLacZ, or no virus), dose of 5-FC, radiation exposure, and time following radiation exposure was used to analyze the effects of a single radiation exposure on toxicity to SK-ChA-1 cells. Pairwise comparisons were also performed to verify differences at a $P < 0.05$ level of significance. These experiments were repeated, and the data presented are a representative result.

RESULTS

Cancer gene therapy strategies depend on efficient transfer of therapeutic genetic material and its effective expression within the target cell. Susceptibility of tumor cell types to adenoviral vector infection is variable, thereby necessitating verification on an individual cell line basis. To this end, Fig. 1 demonstrates the FACS analysis of the SK-ChA-1 cells and HeLa cells

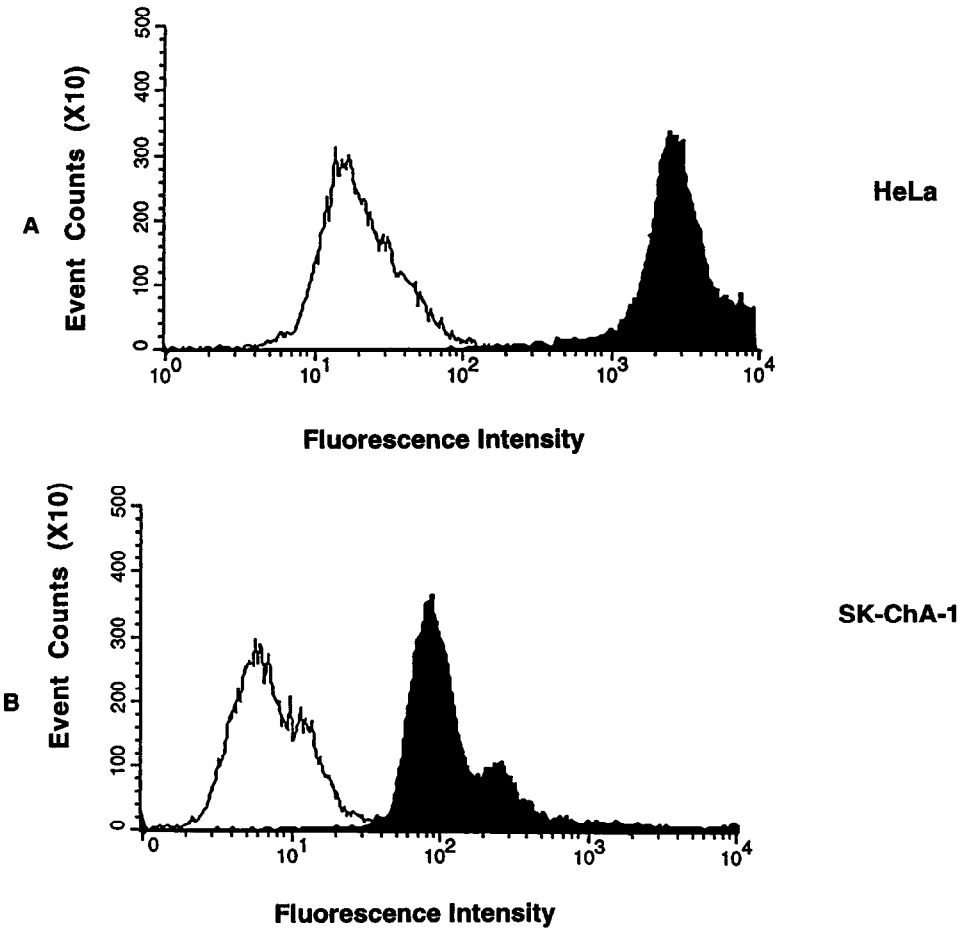

Fig. 1. Adenoviral infection efficiency for transduction of the SK-ChA-1 human cholangiocarcinoma cell line compared to HeLa cells. Cells were infected with AdCMVLacZ at 100 pfu/cell, and 2 days later harvested and stained with FDG for FACS analysis. Cells expressing β-galactosidase cleave FDG to form a colored product detectable by FACS. The open curve (□) represents uninfected stained cells, and the darkened curve (■) represents cells infected with AdCMVLacZ and exposed to FDG with subsequent development of colored product. **A**, HeLa cells—98.6% of cells were transduced. **B**, SK-ChA-1 cells—98.4% of cells were transduced.

infected with AdCMVLacZ. SK-ChA-1 cells were efficiently transduced to a level of 98.4% by the recombinant adenovirus AdCMVLacZ at 100 pfu/cell. Infectivity of this cholangiocarcinoma cell line was compared to HeLa cells, which were transduced at 98.6% efficiency by 100 pfu/cell of AdCMVLacZ. HeLa cells are a human cervical carcinoma cell line known to be highly susceptible to adenoviral infection. These results demonstrate that this cholangiocarcinoma cell line was transduced to a similar degree as HeLa cells with the reporter virus AdCMVLacZ.

After transduction and reporter gene expression was confirmed, sensitivity of this cholangiocarcinoma cell line to CD-mediated conversion of 5-FC to 5-FU was determined. In this regard, Fig. 2 shows the proliferation of SK-ChA-1 cells infected with no virus,

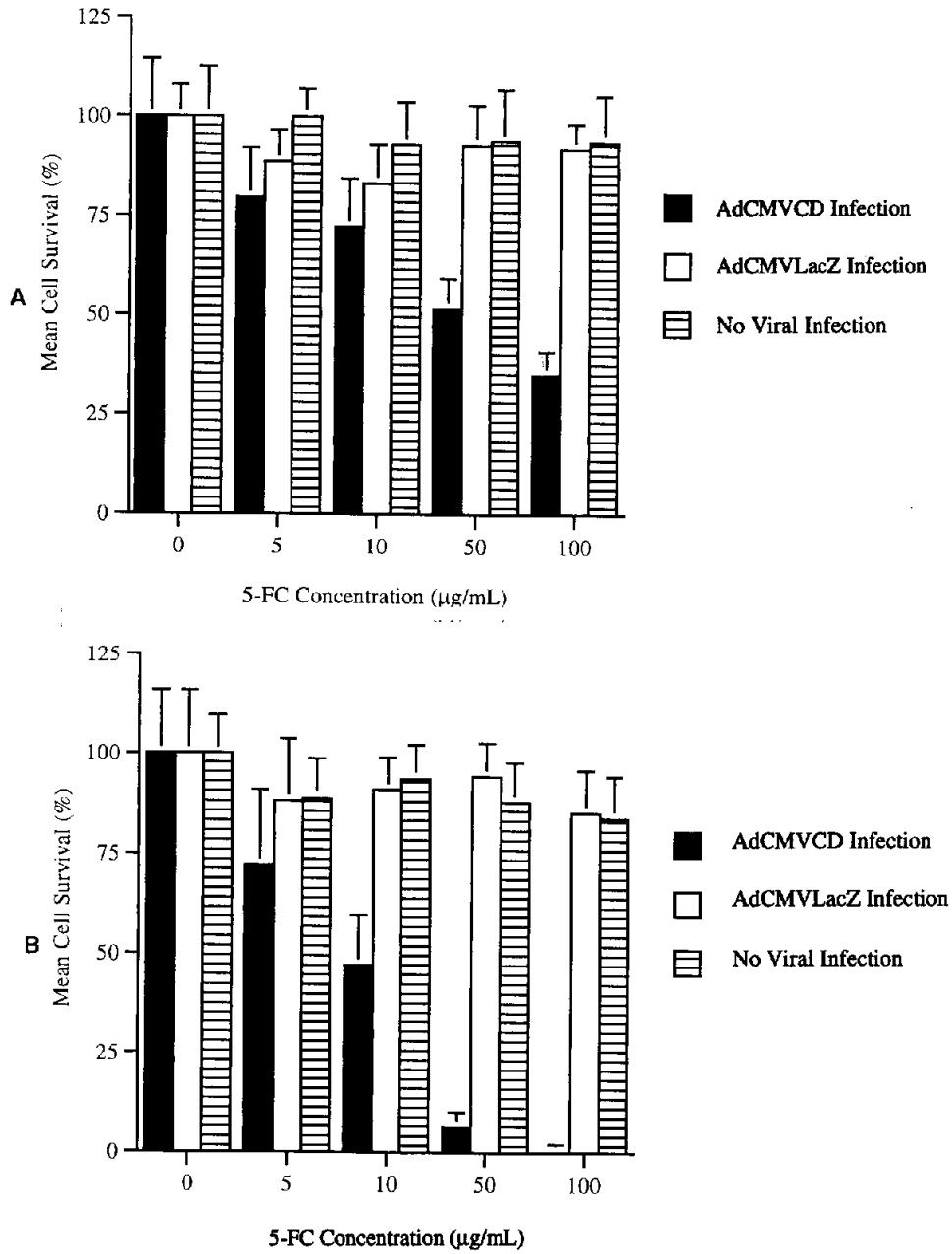

Fig. 2. Induction of 5-FU cytotoxicity mediated by CD conversion of 5-FC to 5-FU following adenoviral directed gene transfer. SK-ChA-1 cells were infected with no virus, AdCMVLacZ, or AdCMVCD, then exposed to various concentrations of 5-FC. MTS cell proliferation assays were performed. Results are normalized with respect to each viral infection control/0 μg/ml 5-FC. The data are derived from mean cell counts of six replicates. Bars at the top of each column represent standard deviation for each group. **A,** Six-day exposure to 5-FC. **B,** Eight-day exposure to 5-FC.

AdCMVCD, or AdCMVLacZ and exposed to various concentrations of 5-FC for 6 and 8 days' duration. With 6 days of prodrug exposure, AdCMVCD-infected cells exposed to 5, 10, 50, and 100 μg/ml of 5-FC were significantly reduced in cell number and represented 20.3%, 27.7%, 48.4%, and 64.7% cell killing relative to AdCMVCD infected/0 μg/ml 5-FC, respectively ($P < 0.05$). With 8 days of 5-FC exposure, AdCMVCD-infected groups exposed to 5, 10, 50, and 100 μg/ml of 5-FC had 28.1%, 52.8%, 93.7%, and 99.8% cytotoxicity compared to AdCMVCD infected/0 μg/ml 5-FC, respectively ($P < 0.05$). There

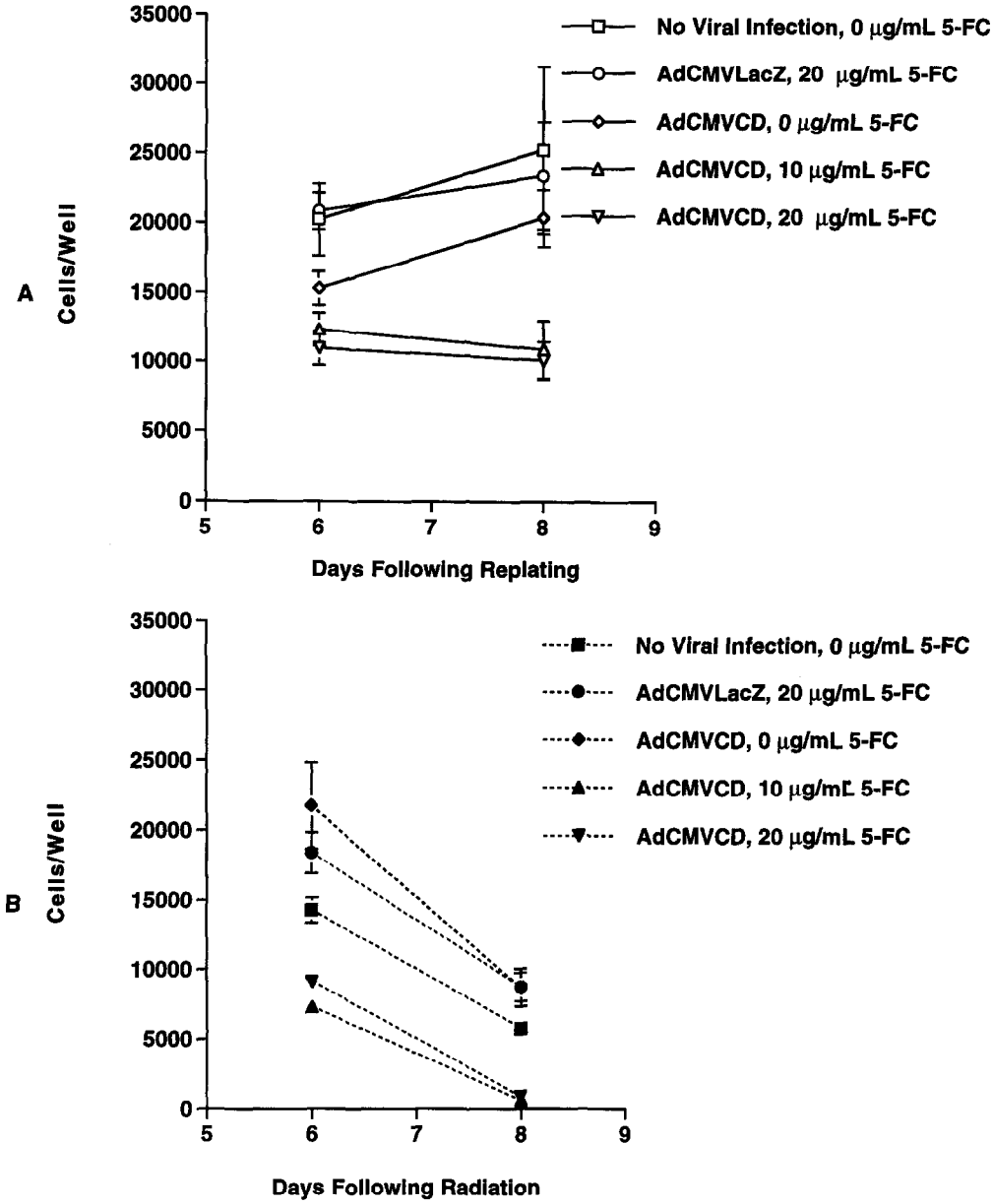

Fig. 3. Evaluation of cytotoxicity induced by the combination of AdCMVCD infection, 5-FC treatment, and external beam radiation. **A,** Nonirradiated SK-ChA-1 cells, infected 24 hours after plating with no virus, AdCMVLacZ, or AdCMVCD, treated with 5-FC on days 3 to 5 after plating, replated 24 hours later, and cell number determined 6 and 8 days later. **B,** SK-ChA-1 cells, infected 24 hours after plating with no virus, AdCMVLacZ, or AdCMVCD, treated with 5-FC on days 3 to 5 after plating, 24 hours later exposed to 8 Gy of cobalt-60 and replated, and cell number determined 6 and 8 days later. The data points are the mean cell counts of six replicates. Bars represent standard deviation.

was a plateau of cell proliferation in control groups by the 8-day time point (data not shown); however, only groups infected with AdCMVCD and exposed to 5-FC decreased significantly in cell number relative to the respective 0 μg/ml control. Cytotoxicity of 5-FU production with AdCMVCD-infected cholangiocarcinoma cells appeared to be progressive with time. In addition, cytotoxicity was more pronounced at higher 5-FC concentrations. The prodrug alone was nontoxic to uninfected cells at concentrations ranging from 5 to 100 μg/ml, and was also nontoxic in the presence of nonspecific viral (AdCMVLacZ) infection over the same 5-FC dose range.

An approach to improve tumor control and limit systemic toxicity of cancer treatment involves a combination of modalities. Inasmuch as systemic chemotherapy and radiotherapy have been minimally effective for cholangiocarcinoma, toxin gene therapy via CD-mediated production of 5-FU combined with radiation therapy represents a novel approach for treatment of this malignancy. In this regard, Fig. 3 shows the in vitro cytotoxic effects of AdCMVCD infection and 5-FC exposure alone and in combination with radiation treatment. As shown in Fig. 3, *A*, there was significant SK-ChA-1 cell cytotoxicity produced by AdCMVCD infection and exposure to 5-FC without radiation compared to nonirradiated control groups (no viral infection/0 μg/ml 5-FC exposure, AdCMVLacZ infection/20 μg/ml 5-FC exposure, and AdCMVCD infection/0 μg/ml 5-FC exposure) at 6 and 8 days following replating ($P < 0.05$). At 6 days following 8 Gy radiation, there was 39% and 45.9% greater cytotoxicity (Fig. 3, *B*) of SK-ChA-1 cells infected with AdCMVCD and treated with 10 or 20 μg/ml of 5-FC, respectively, compared to no radiation (see Fig. 3, *A*) ($P < 0.05$). At 8 days following radiation, this increased to 58.9% and 60.1% greater cytotoxity compared to no radiation treatment (see Fig. 3, *A*) ($P < 0.05$). There was significant killing of uninfected SK-ChA-1 cells by radiation exposure (see Fig. 3, *B*) representing 29.6% at 6 days and 77.2% at 8 days compared to nonirradiated uninfected SK-ChA-1 cells ($P < 0.05$). The results for other control groups (no viral infection/10 and 20 μg/ml 5-FC and AdCMVLacZ infection/0 and 10 μg/ml 5-FC) with or without radiation were similar to those of the control groups shown in Fig. 3. An increased cytotoxic effect was noted at 6 and 8 days in AdCMVCD-infected cells exposed to 10 or 20 μg/ml of 5-FC combined with radiation representing 42.5% and 91.5% cell killing, respectively, compared to AdCMVCD infection/0 μg/ml 5-FC treated and irradiated cells (see Fig. 3, *B*) ($P < 0.05$). Thus these results demonstrate increased cytotoxic effects of AdCMVCD-mediated intracellular conversion of 5-FC to 5-FU combined radiotherapy on cholangiocarcinoma cells.

DISCUSSION

Molecular chemotherapy via the CD/5-FC toxin gene/prodrug system represents a new and potentially efficacious treatment strategy for a multidisciplinary approach to hilar cholangiocarcinoma, as the prognosis remains poor for patients treated with traditional modalities. A limitation of current gene therapy technology involves difficulty in restricting novel gene expression only to the tumor cells.[25,26] In this regard, cholangiocarcinoma represents an attractive target for cancer gene therapy because of the inherent compartmentalization of the biliary system. The anatomically privileged nature of the biliary tree may allow minimal systemic dispersion of an adenoviral vector in a clinical setting. In addition to limiting dispersal of the vector, in situ cellular transduction is mandatory for solid tumor treatment. To this end, Vickers et al.[41] have demonstrated AdCMVLacZ gene transfer to intact biliary epithelium in a human liver. Cholangiocarcinoma presenting with numerous biliary obstructions may represent a difficult surface for vector delivery, although biliary malignancy occurring in this setting is usually associated with primary sclerosing cholangitis and comprises only 8% to 10% of cases.[42,43] However, the extent of most perihilar tumors at presentation is from the common hepatic duct bifurcation to the left or right hepatic ducts, often including the secondary biliary radicals.[4,6,8] We believe these latter tumors are amenable to delivery of an adenoviral vector via percutaneous or endoscopic cholangiography and placement of a stent, if necessary, for biliary drainage.

The reporter gene expression in the SK-ChA-1 cells exposed to recombinant adenovirus confirms delivery to, and expression of, novel genes in this cholangiocarcinoma cell line. This observation confirms our group's previous report of adenoviral directed gene transfer to gastrointestinal carcinoma cells, although this work involved the HSV-TK/GCV toxin gene prodrug system.[44] Tumor cell susceptibility to recombinant adenoviral infection is necessary for utilization of these vectors for human cancer gene therapy.

As a drug delivery mechanism, molecular chemotherapy has the advantage of intracellular production of chemotherapeutic drug as an alternative to systemic administration. The local production of 5-FU may aid in limiting systemic toxicities.[15,16] We observed that CD-mediated conversion of 5-FC to 5-FU was cytotoxic to SK-ChA-1 cells in relatively

low doses of the prodrug 5-FC. The cytotoxic effects were increased with longer duration of prodrug exposure, and with higher 5-FC concentrations. There was a lack of cytotoxic effects in the control cells (uninfected and AdCMVLacZ infected and exposed to 5-FC), demonstrating that cytotoxicity was limited to cells expressing CD and exposed to 5-FC.

Our results demonstrate that the cytotoxicity to SK-ChA-1 cells mediated by CD conversion of 5-FC to 5-FU was enhanced by external beam radiation. There was not an observable difference in cytotoxicity to cells infected with AdCMVCD and exposed to 10 or 20 μg/ml of 5-FC, either with or without radiation. The inability to distinguish treatment effects in these groups may have been the result of the limited sensitivity of the MTS assay system and the low cell numbers following treatment. Clonogenic survival analysis represents a more sensitive method to measure cell survival following highly cytotoxic treatments. This method was used in another study, and the results confirmed an additive cytotoxic interaction between AdCMVCD infection, 5-FC treatment, and radiation therapy.[45]

Although current clinical applications of chemotherapy and radiation therapy for cholangiocarcinoma yield minimal effects on long-term patient survival,[4,5] the drug delivery mechanism imparted by CD-mediated intracellular 5-FU production may provide a basis for improved disease treatment. We have begun to explore this model system for CD-mediated intracellular production of 5-FU and radiosensitization in an in vivo model of human cholangiocarcinoma.[45] The rationale for further characterization of this combined-modality approach is based on the promising results observed in these studies, the dose-limiting toxicity of currently used chemotherapy drugs, and their ineffectiveness in the treatment of cholangiocarcinoma.

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Hepatic Steatosis as a Potential Risk Factor for Major Hepatic Resection

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Hepatic steatosis is a recognized risk factor for primary nonfunction of hepatic allografts, but the effect of steatosis on postoperative recovery after major liver resection is unknown. Our aim was to determine if hepatic steatosis is associated with increased perioperative morbidity and mortality in patients undergoing major resection. A retrospective review of medical records of 135 patients who had undergone major hepatic resection from 1990 to 1993 was performed. Histopathology of the hepatic parenchyma at the resection margin was reviewed for the presence of macro- or microvesicular steatosis. The extent of steatosis was graded as none (group 1), mild with less than 30% hepatocytes involved (group 2), or moderate-to-severe with 30% or more hepatocytes involved (group 3). Outcome of patients was correlated with extent of steatosis. Patients with moderate-to-severe steatosis were obese (body mass index = 25.8 ± 0.5 vs. 26.5 ± 1.0 vs. 33.4 ± 2.9; $P \leq 0.05$ groups 1, 2, and 3, respectively) and had an increased serum bilirubin concentration preoperatively. Hepatectomy required a longer operative time for group 3 (290 ± 9 minutes vs. 287 ± 13 minutes vs. 355 ± 24 minutes; $P < 0.05$ groups 1, 2, and 3, respectively). Likelihood of blood transfusion was 51% in group 1, 52% in group 2, and 71% in group 3. Mortality was 14% in group 3 vs. 3% in group 1, and 7% in group 2; and liver failure occurred in 14% of patients in group 3 compared to 4% and 9% in groups 1 and 2, respectively. Patients in group 3 also had increased postoperative bilirubin levels compared to preoperative values. Moderate-to-severe hepatic steatosis may be associated with increased perioperative morbidity and mortality, and preoperative identification of steatosis warrants caution prior to major resection. (J GASTROINTEST SURG 1998;2:292-298.)

Hepatocyte steatosis is a common response to liver injury and is characterized by macro- and microvesicular fatty infiltration of hepatocytes, especially in the perivenular region. These fatty changes may be accompanied by hepatocyte ballooning, an inflammatory infiltrate, Mallory bodies, fibrosis, and even cirrhosis.[1,2] Such findings are frequently seen in alcoholic liver disease and, more recently, similar pathologic findings have been noted in patients with nonalcoholic steatohepatitis (NASH). Patients with alcoholic liver disease may present with a wide range of clinical and histologic findings, whereas patients with NASH have been described typically as middle-aged women with obesity, diabetes mellitus, and asymptomatic increases in liver enzymes.[3-5] NASH has also been seen in patients following intestinal bypass surgery for clinically severe obesity, who are receiving long-term total parenteral nutrition and are deficient in choline and are taking a variety of medications including corticosteroids, estrogens, and amiodarone.[2]

Recently, severe hepatic steatosis in donor allograft livers has been associated with primary nonfunction of the graft.[6-8] In addition to graft failure, hepatic steatosis is associated with decreased patient survival and bile output and increased postoperative transaminase concentrations and intraoperative bleeding.[7] Suspicion of fatty infiltration of the donor liver is an indication for allograft biopsy, and livers with severe (>60% hepatocytes) steatosis are not used for transplantation.[7] The risk of major hepatic resection in fatty infiltrated livers, however, is unknown.

The primary purpose of this study was to determine the safety of major hepatic resection in patients with steatotic livers. Additionally, we wished to assess the prognostic value of clinical and biochemical parameters that may predict steatosis preoperatively.

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PATIENTS AND METHODS

The medical records of all patients undergoing hepatic resection for benign or malignant disease were identified by a computer-based indexing system. From 1990 through 1993, 135 patients had undergone a major hepatic resection (≥4 hepatic segments), and their medical records were analyzed in detail for potential preoperative predictors of steatosis and perioperative complications. Aspartate aminotransferase (AST), alkaline phosphatase, and bilirubin concentrations were determined before and after hepatic resection and are expressed as percentages above normal institutional values. Because this study was retrospective in design and an accurate history of alcohol use was unreliable, we made no attempt to differentiate alcohol-induced steatosis from nonalcoholic steatohepatitis.

Histopathology of hepatic parenchyma from the resection margin was reviewed for the presence of macro- or microvesicular steatosis. The extent of steatosis on hematoxylin and eosin–stained slides was determined and graded as none (group 1), mild with less than 30% of hepatocytes involved (group 2), and moderate to severe when at least 30% of the hepatocytes demonstrated steatosis (group 3). Histologic sections were not specifically examined for features of alcohol-induced injury such as hyalin.

Operative mortality and morbidity were defined as death or a complication within 30 days of resection or prior to hospital discharge. Hepatic failure was determined on the basis of clinical findings including increased serum bilirubin or ammonia concentrations, decreased hepatic synthetic function with increased prothrombin time and decreased albumin, jaundice, and the development of ascites or encephalopathy.

Data are summarized as mean values ± standard error of the mean. Statistical analysis of within-group comparisons was performed with a two-tailed unpaired *t* test. The Kruskal-Wallis test was used for between-group comparisons. A significance level of 0.05 was used for both the *t* test and the Kruskal-Wallis test.

RESULTS

Extent of Steatosis

Histopathologic examination showed that 72 (53%), 56 (42%), and seven (5%) of the patients demonstrated no, mild, or moderate-to-severe steatosis, respectively. No patients had an inflammatory infiltrate suggestive of steatohepatitis, fibrosis, or cirrhosis. Fig. 1 compares the histopathology of a patient with normal hepatic parenchyma at the margin of resection *(A)* with that of a typical patient with moderate-to-severe steatosis *(B)*.

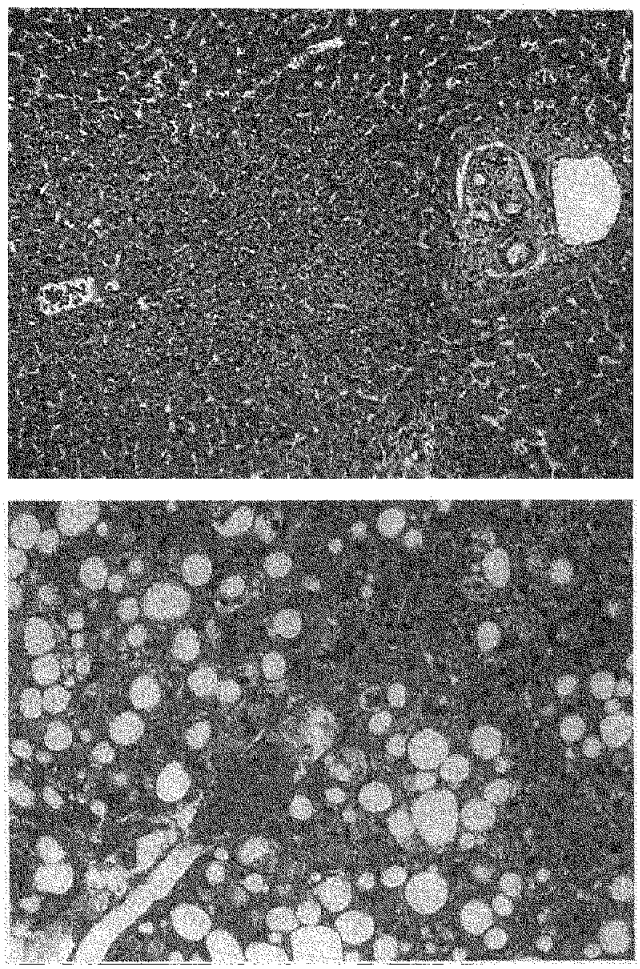

Fig. 1. Hematoxylin and eosin–stained sections from patients with normal liver **(A)** and moderate-to-severe steatosis **(B)** at the hepatic resection margin (medium-power magnification).

Patient Characteristics

Patient characteristics are presented in Table I. Patients in group 1 were younger than patients in groups 2 and 3, but no difference in distribution of the sexes was noted. The vast majority of patients in all groups underwent hepatectomy for malignant disease, and most of them had hepatic resection for colorectal metastases. The preoperative performance status did not differ among the groups. The body mass index was increased in patients with moderate-to-severe steatosis compared to patients in groups 1 and 2 (33.4 ± 2.9 vs. 25.8 ± 0.5 vs. 26.5 ± 1.0; $P < 0.05$).

Intraoperative Data

Intraoperative data are presented in Table II. The number of hepatic segments resected in each group was similar, and the proportion of patients who un-

Table I. Patient characteristics

	Group 1 (N = 72)	Group 2 (N = 56)	Group 3 (N = 7)
Age (yr)	55 ± 2*	64 ± 1	61 ± 3
Males/Females	56/44	59/41	57/93
Benign disease (%)	11	5	0
Malignant disease (%)	89	95	100
Colorectal metastases (%)	46	54	57
Performance status†	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.3
Body mass index	25.8 ± 0.5	26.5 ± 1.0	33.4 ± 2.9‡

*P <0.05 vs. groups 2 and 3.
†Based on Eastern Cooperative Oncology Group performance status.
‡P <0.05 vs. groups 1 and 2.

Table II. Intraoperative data

	Group 1 (N = 72)	Group 2 (N = 56)	Group 3 (N = 7)
Segments resected	4.3 ± 0.1	4.2 ± 0.1	4.6 ± 0.3
Inflow occlusion (min)	27 ± 5	31 ± 4	19 ± 1
Operative time (min)	290 ± 9	287 ± 13	355 ± 24*
Patients transfused (%)	51	52	71
Hepatectomy—right/left (%)	64/36	79/21	100/0

*P <0.05 vs. groups 1 and 2.

derwent either a right or left hepatectomy did not differ among the groups, although all patients in group 3 had a right hepatectomy. Inflow occlusion time was not significantly different between groups, but mean clamp time for patients in group 3 (19 ± 1 minutes) was one third less than clamp times in groups 1 (27 ± 5 minutes) and 2 (31 ± 4 minutes; P >0.05). Operative time was substantially longer in patients with moderate-to-severe hepatic steatosis, and the percentage of patients requiring intraoperative blood transfusion was increased in this group, although this difference was not statistically significant.

Outcome

Patient outcome for all groups is presented in Fig. 2. Mortality was 14% in group 3 compared to 3% in group 1 and 7% in group 2. The death in group 3 was related to hepatic failure following a right hepatectomy for colorectal metastases in a patient with a preoperative bilirubin value of 1.7 mg/dl. Although hepatic insufficiency was noted in patients in groups 1 and 2, the deaths in these groups were unrelated to hepatic failure. The rate of postoperative biliary leakage was 3%, 9%, and 14% in groups 1, 2, and 3, respectively. Overall complications in patients with moderate-to-severe steatosis was 29% compared to 10% for those with no steatosis or 14% for those with mild steatosis. The length of hospital stay was not different among the groups.

Biochemical Profile

Preoperative assessment included liver function tests and parameters of hepatic synthetic function including the serum albumin concentration and the prothrombin time. Synthetic function was normal preoperatively in each group. Serum AST concentrations were increased from 8% to 72% (Fig. 3) above the normal preoperative range in all groups and serum bilirubin was increased preoperatively in groups 1 (4.7% ± 23.6%) and 3 (96.1% ± 119.8%) (Fig. 4). The mean preoperative serum bilirubin concentrations in group 3 patients was 2.2 ± 1.3 mg/dl.

Changes in AST and bilirubin concentrations after hepatic resection were determined and compared to preoperative values. A significant increase in AST was seen postoperatively within groups 2 and 3 (see Fig. 3). Similarly, preoperative and postoperative bilirubin concentrations were compared, and a significant postoperative increase was noted within group 2 (see Fig. 4). Although an apparent difference was

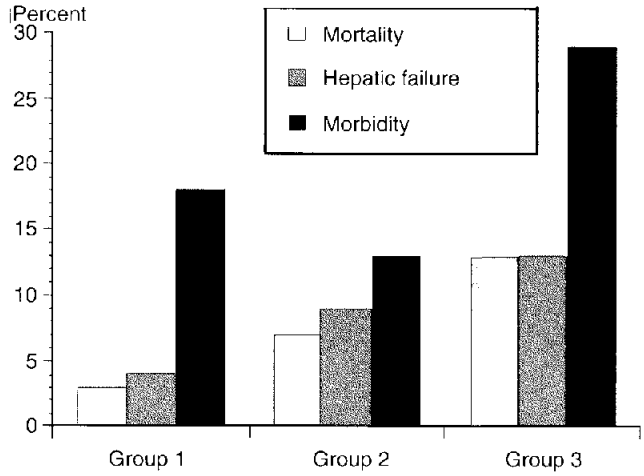

Fig. 2. Mortality, hepatic failure, and overall morbidity after major hepatic resection in patients with no steatosis (group 1), mild steatosis (group 2), or moderate-to-severe steatosis (group 3).

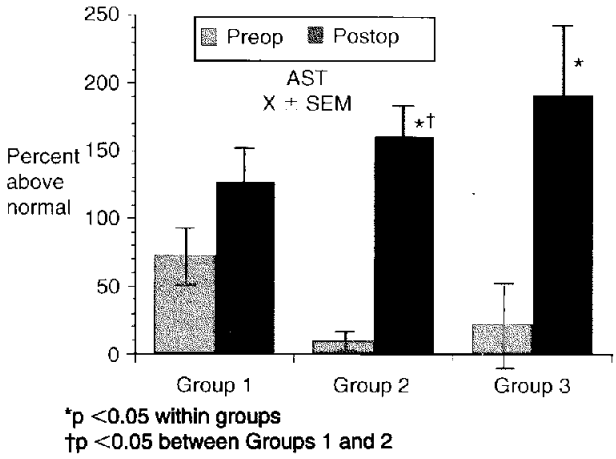

Fig. 3. Changes (percentage above normal) in AST concentrations in patients with no steatosis (group 1), mild steatosis (group 2), or moderate-to-severe steatosis (group 3) before and after major hepatic resection.

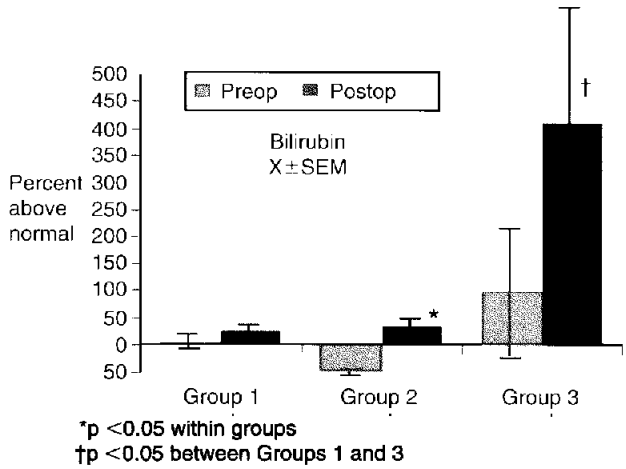

Fig. 4. Changes (percentage above normal) in bilirubin concentrations in patients with no steatosis (group 1), mild steatosis (group 2), or moderate-to-severe steatosis (group 3) before and after hepatic resection.

also present in group 3, this change was not statistically significant. Intergroup differences were noted between groups 1 and 2 for the change between preoperative and postoperative AST concentrations (see Fig. 3). Postoperative bilirubin concentrations and changes in bilirubin concentrations (preoperative vs. postoperative) were increased in group 3 compared to group 1 (see Fig. 4).

DISCUSSION

The aim of this study was to determine the safety of major hepatectomy in patients with hepatic steatosis. Our study is the first study in the English literature to examine the risk of major hepatectomy in fatty infiltrated liver. Although the number of patients with moderate-to-severe steatosis was small, we showed that moderate-to-severe steatosis is associated with a trend toward increased postoperative mortality, morbidity, risk of blood transfusion, and longer operative time. Preoperative assessment showed that patients with moderate-to-severe steatosis were obese with a body mass index greater than 30. Additionally, these patients had an increased preoperative bilirubin concentration suggestive of hepatic dysfunction, and postoperative changes in bilirubin and AST were increased in patients with moderate-to-severe hepatic steatosis.

Death following major hepatectomy is associated with increased intraoperative blood loss resulting from loss of control of a major hepatic artery or vein or inability to achieve hemostasis at the resection margin.[9] Hepatic steatosis results in a soft, friable liver that makes identification and control of hepatic vessels difficult during parenchymal division. We prefer to transect the hepatic parenchyma with selective inflow occlusion and the use of the ultrasonic aspirator for careful identification and ligation of bile ducts and vessels. With this technique transfusions have been necessary in less than 50% of all patients.[10] In this study, however, approximately 70% of patients with moderate-to-severe steatosis required red blood cell transfusion because of increased blood loss during parenchymal transsection. The increased operative time in patients with moderate-to-severe steatosis is further evidence that hepatectomy is technically challenging in this group of patients.

Perioperative mortality may also be related to postoperative liver failure following major resection in patients with insufficient functional hepatic reserve. The effect of steatosis on hepatocyte function in the liver remnant is unknown, but several potential mechanisms of further hepatic dysfunction may occur. Obese patients with diabetes mellitus have peripheral insulin resistance that results in increased free fatty acid delivery to the remnant liver.[11,12] These free fatty acids may impair membrane integrity, depress enzyme activity, and cause mitochondrial swelling and lysosomal fragility, all of which further exacerbate hepatic insufficiency.[13] Free fatty acids may also result in the production of free radicals that cause further hepatocyte injury and initiate cytokine cascades.[14,15] Hepatic steatosis may also be associated with impaired hepatic microcirculation.[16] Enlarged hepatocytes from intracellular lipid may result in narrowing of the hepatic sinusoids with impaired delivery of oxygen and nutrients. Finally, the ability of the steatotic liver to regenerate is unknown but may be inadequate to provide sufficient functional hepatic reserve. The mechanisms of postoperative hepatic failure in patients with hepatic steatosis remain speculative and were not addressed in this study but warrant further investigation.

Clinically severe obesity is defined as a body mass index greater than 30. Obesity is a risk factor for nonalcoholic steatosis. In this study, the mean body mass index in patients with moderate-to-severe steatosis was 33. Hepatic steatosis is present in 60% to 90% of obese patients and one third of patients have steatosis of 50% of hepatocytes.[17] An autopsy series demonstrated that the extent of both steatosis and steatohepatitis was correlated with the severity of obesity.[18] The development of cirrhosis as a result of fatty infiltration has been debated, but Adler and Schaffner[19] showed that in addition to steatosis and steatohepatitis, both fibrosis and cirrhosis can result from fatty infiltration in obese patients.[19] None of our patients exhibited steatohepatitis, fibrosis, or cirrhosis from fatty infiltration. These findings suggest that obese patients who are candidates for major hepatic resection are at increased risk for complications including death and liver failure. The role of preoperative biopsy of the hepatic parenchyma in these patients has not been examined but should be considered in these high-risk patients.

Many patients with hepatic steatosis are asymptomatic, but underlying liver disease is suspected based on increases in liver-associated enzymes such as aspartate and alanine aminotransferases. Most patients with asymptomatic increases in transaminases have chronic active hepatitis with or without cirrhosis. In approximately 20% of patients, however, steatosis is responsible for the biochemical abnormalities.[20] In the study by Hay et al.,[20] all patients with chronic increases in aminotransferases due to steatosis were women, most of whom were obese and did not have diabetes mellitus. This finding suggests that altered insulin, carbohydrate, and lipid metabolism from diabetes mellitus is not necessary for the development of hepatic steatosis. In our study, preoperative concen-

trations of AST ranged from 8% to 72% above the normal institutional range and did not correlate with the extent of steatosis. Therefore preoperative concentrations of AST are not helpful in discriminating patients at risk for hepatic steatosis and possible postoperative complications. In contrast, in this study patients with moderate-to-severe steatosis had preoperative bilirubin concentrations that were approximately twice the normal level, but wide variation existed in the preoperative bilirubin concentrations. These findings make it unlikely that preoperative serum bilirubin concentrations will be helpful in defining patients with moderate-to-severe steatosis.

This study demonstrates that accurate preoperative identification of moderate-to-severe steatosis should be sought. However, recognition will be difficult because of the lack of sensitivity of clinical and biochemical parameters. Radiologic studies such as ultrasonography, computed tomography, and magnetic resonance imaging can identify fatty infiltration of the liver, but the extent of steatosis from histologic specimens has not been correlated with the radiologic findings.[21] Radiologic findings were not included in this study because of the lack of uniformity of preoperative imaging studies obtained. Furthermore, the frequency and accuracy of reporting this finding by the radiologist is difficult to determine in a retrospective study. Prospective evaluation of a single radiographic technique in the identification of fatty liver and correlation with the extent of steatosis on histologic review is warranted for potential accurate preoperative identification of moderate-to-severe steatosis.

CONCLUSION

This study demonstrates that hepatic steatosis may be associated with increased perioperative morbidity and mortality. Patients with clinically severe obesity likely have significant fatty infiltration of the liver and appear to be at increased risk for perioperative complications after major hepatectomy. Biochemical identification of moderate-to-severe steatosis is unreliable, and further efforts at preoperative identification of steatosis should be directed at quantifying steatosis radiologically. In patients who are likely to have a steatotic liver and require a major hepatic resection, preoperative biopsy of the normal-appearing hepatic parenchyma may aid in quantifying the risk of hepatic resection.

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Discussion

Dr. M. Choti (Baltimore, Md.). You did not report the breakdown of the diagnosis; in particular, what percentage of patients underwent major resection for hepatocellular carcinoma? As you know, hepatocellular cancer is associated with increased morbidity and possibly an increased incidence of steatohepatitis and/or cirrhosis. Second, often there can be a localized tumor effect around tumors, and fatty infiltration can sometimes be patchy. Did you have an opportunity to determine whether this steatosis was uniform throughout the specimen, or did you look at any areas distant from the margin of the tumor?

Dr. K. E. Behrns. We did look at the breakdown of benign vs. malignant disease. Nearly 90% of the resections in all three groups were for malignant disease. About 50% of those (between 45% and 60%) were for colorectal metastases. The rest represented other malignant processes. We did not look at remote areas of the liver for evidence of steatosis; these specimens were all near the margins of the resection and we did not biopsy areas that were remote to look for evidence of steatosis, but your comment about localization or local fatty infiltration is very important.

Dr. J. Kral (Brooklyn, N.Y.). Gastrointestinal surgeons need to be aware that obesity is a devastating disease. I want you to clarify something though. You did discuss nonalcoholic steatohepatitis (NASH). Time and time again, however, you are discussing steatosis. Steatosis and NASH are two different entities. You are demonstrating steatosis but are you not really discussing the inflammatory responses?

Dr. Behrns. You are correct in pointing out that steatosis is much different from steatohepatitis and that these patients essentially all had steatosis. There was no evidence of an inflammatory response, and this represents a different group from NASH patients. We did not stratify our patients preoperatively as to the cause of steatosis because we could not obtain a reliable history of alcohol use from retrospective data.

Dr. S.M. Strasberg (St. Louis, Mo.). In the area of liver preservation, steatosis is a known risk factor. Severe steatosis is an absolute risk factor for poor function and nonfunction. Mild or moderate steatosis is a relative risk factor, that is, it is a risk factor when associated with other risk factors such as age or prolonged preservation time. In the 14% of patients who died, were there other associated risk factors such as advanced age or extended resection? My second question is, what were the causes of death in the 14% of patients who died? Did they die of liver failure or other causes?

Dr. Behrns. Among the patients who died, there were no differences in demographics such as age or other comorbid conditions. The cause of their deaths was liver failure accompanied by other end-organ failure, but liver failure was the primary cause of death in these patients.

Dr. P.D. Greig (Toronto, Ontario, Canada). You alluded to the fact that all groups had the same incidence of inflow occlusion, but you did not tell us exactly how many of the seven patients who had steatosis had inflow occlusion. I wonder if what you are telling us is that the fatty liver does not tolerate warm ischemia as well as the nonfatty liver.

Dr. Behrns. I think that is an important observation. The mean inflow occlusion time for the patients in group 3 was 19 minutes. It was relatively short and that occurred in approximately half of the patients. This value was used selectively, so there may be a bias that the fatty infiltrated liver will not tolerate prolonged inflow occlusion time.

Dr. W. Meyers (Worchester, Mass.). Did you say that you had normalized these patients for obesity? How do you separate obesity from steatosis?

Dr. Behrns. Obesity was examined just by calculating the body mass index and that was the sole basis for judging patients as obese and with a body mass index greater than 30 indicating obesity.

Dr. R.E. Condon (Milwaukee, Wis.). Fatty liver follows somewhat prolonged and exclusively parenteral nutrition in a reasonable proportion of patients so treated. It is thought to be due to a lack of insulin following food intake stimulus. Fatty liver in that clinical setting is an insulinopenic response. Did your patients have their insulin levels measured? Were they treated preoperatively with parenteral nutrition for any prolonged period of time?

Dr. Behrns. Insulin concentrations were not determined perioperatively in these patients, and none had been receiving total parenteral nutrition preoperatively. The etiology of the steatosis in these patients is really unknown because we could not reliably obtain an alcohol use history, and it may have been related to alcohol or it may be that some patients had nonalcoholic steatosis.

The "Three Mayos" Photograph: Its Origin and Significance

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Until recently, little was known regarding the famous "Three Mayos" photograph. New findings are herein described, which demonstrate that this photograph was taken by Dr. Harvey Cushing in 1905 at the site of Dr. Henry Plummer's first Rochester, Minnesota home. These new findings make this photograph even more historically significant as a tangible symbol of the friendship that existed between four of this country's greatest surgeons. (J GASTROINTEST SURG 1998;2:299-303.)

The Doctors Mayo—William J., Charles H., and their father William W.—are tremendously important figures in the history of American gastroenterologic surgery. Perhaps the most historically significant photograph we possess of them is a casual outdoors shot, traditionally thought to have been taken around 1910 (Fig. 1).

This photograph is important for several reasons. It is one of only two extant photos showing all three men together. What is more, we know the Mayos themselves thought highly of it, as they had several copies enlarged and inscribed. Three of these copies are still in existence, one of which, according to Charles Mayo, was displayed in the library of the Mayo Clinic as early as 1916.[1] Whether the Mayos sent additional copies to their friends and colleagues we do not know.

Despite its importance, until recently very little was known about this photograph. Neither the photographer nor the site at which the Mayos were standing had been identified. Recent research has provided these missing bits of information and has, in the process, bestowed upon this photograph an even greater historical significance. This new information is the subject of this report.

Fig. 1. Dr. Harvey Cushing's copy of the "Doctors Mayo" photograph.

A FAMOUS PHOTOGRAPHER

To address the question of who shot the "three Mayos" photograph, some background information is necessary. In the year 1990, while attending the Thirteenth International Medical Congress in Paris, William J. Mayo first met Dr. Harvey Cushing.[2] This encounter was to begin a 39-year, lifelong friendship between Dr. Cushing and the Mayo brothers.

William Mayo was 39 years old at the time and had already gained some degree of national prominence as a general surgeon.[3] Cushing, at 31, had just completed his surgical residency at Johns Hopkins Hospital under Dr. William Halsted and had not yet embarked on his great neurosurgical career.[4]

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It was during this first meeting that the two men, along with William Mayo's friend, the prominent Chicago surgeon Albert Ochsner, devised the idea of a traveling surgical club. The three were tired of merely hearing of new procedures at the meetings they attended and agreed that a club of surgeons should be formed for the purpose of visiting the clinics of its members in order to view first hand the techniques used there.[2] Mayo and Ochsner were well aware of the importance of such visits, as they had already been conducting them on their own for some time.[5]

When the three informed their colleagues of the idea, many immediately embraced the concept. Thus the Society of Clinical Surgery, initially composed of 36 of this country's most promising younger surgeons, officially became a reality in 1903.[3] The Society continues as a vital force in international surgery to this day.

As planned from its inception, Society meetings took the form of visits to the various members' clinics. The first three of many such meetings to be held in Rochester, Minnesota, at the Mayo Clinic were the fifth in 1905 (only day 2 of this 3-day meeting was held in Rochester), the thirteenth in 1909, and the twenty-first in 1913.[6] Cushing attended all three of them.

It is a little-known fact that Harvey Cushing was an avid amateur photographer. As a matter of fact, by the time he met William Mayo he had already taken numerous photographs (some now quite famous) of his colleagues at Johns Hopkins.[4] We have discovered that he took several photographs with his Kodak box camera during his visits to Rochester as well. One of these was that of the three Mayos. Conclusive evidence exists linking the "three Mayos" photograph to Cushing, primarily in the form of written correspondence to and from the brothers. The original negative and first photograph to be developed from it have also been located.

Regarding the written evidence, copies still exist of more than 100 of the letters written between Cushing and the Mayos. The photograph in question is referred to in no fewer than five of these[1,7-10] including a mention of "that Kodak I once took of you, Will and your father" in a letter written by Cushing to Charles Mayo in 1934.[8] He speaks in another letter of his attempt to find the negative so that he might send it to Charles.[7] This search was apparently successful, as the original negative can now be found in the photographic archives of the Mayo Clinic.

The first photograph to have been developed from Cushing's negative is to be found in his Clinical Society scrapbook, now housed in the Sterling Library of Yale University. In the same section of the scrapbook may also be found additional photographs taken by Cushing while he was in Rochester. These include a snapshot of several of the Society's members posing on the steps of the old Mayo Library and another of William J. Mayo with his son-in-law, Dr. Donald Balfour.

Cushing's inscribed enlargement of the photograph, a twin to that which formerly hung in the library of the Mayo Clinic, may also be found in Yale's Cushing Library. Letters make it clear that the Mayos send this copy of the photograph he took to Cushing at his request. His previous copy had apparently been misplaced during his move to Yale from Peter Bent Brigham Hospital.[8,9]

It is no surprise that Cushing chose to photograph all three of the Doctors Mayo, as he is known to have been a great admirer of this country's pioneer physicians, and of William W. Mayo in particular.[11-14] It was characteristic of him to also include a mention of the elder Mayo in an obituary of the two brothers that he wrote in 1939, soon before his own death.[15]

A NOTEWORTHY LOCATION

A content analysis of the photograph was aided by the fact that the building seen in the distance behind the three Mayos possesses a rather unique pattern of brickwork. This pattern appears to have been a feature of only one Rochester building of the period: the former courthouse.

An old city map provides the building's shape, and thus allows an exact determination of the location of the three men (Fig. 2). From the configuration of the walls behind them, the men may be seen to be facing south. Using the courthouse as a point of reference, we may draw a line of sight from it to the men. Doing so locates them at 514 West Zumbro (the street separating them from the courthouse being West Zumbro Street, since renamed Second Street Southwest). (see Fig. 2, *insert*) This is an important address, as it was the site of the first Rochester home of Dr. Henry Plummer, built in 1904.[16]

Thus the Mayos are seen to be standing on the north side of Henry Plummer's home, to the west of his front door. What appears in the picture to be a sudden loss of sidewalk below Charles Mayo's feet results from the fact that they are standing on a landscaped rise of ground in front of the house. This landscaping may also be seen in a period photograph of the house (Fig. 3).

Plummer, a famous internist and a key figure in the development of the Mayo Clinic, often entertained colleagues at his home.[16] It was probably during a lunchtime visit that this snapshot was taken. Cushing provides the year of the photograph in a letter written to William J. Mayo in 1935. He states the "Kodak"

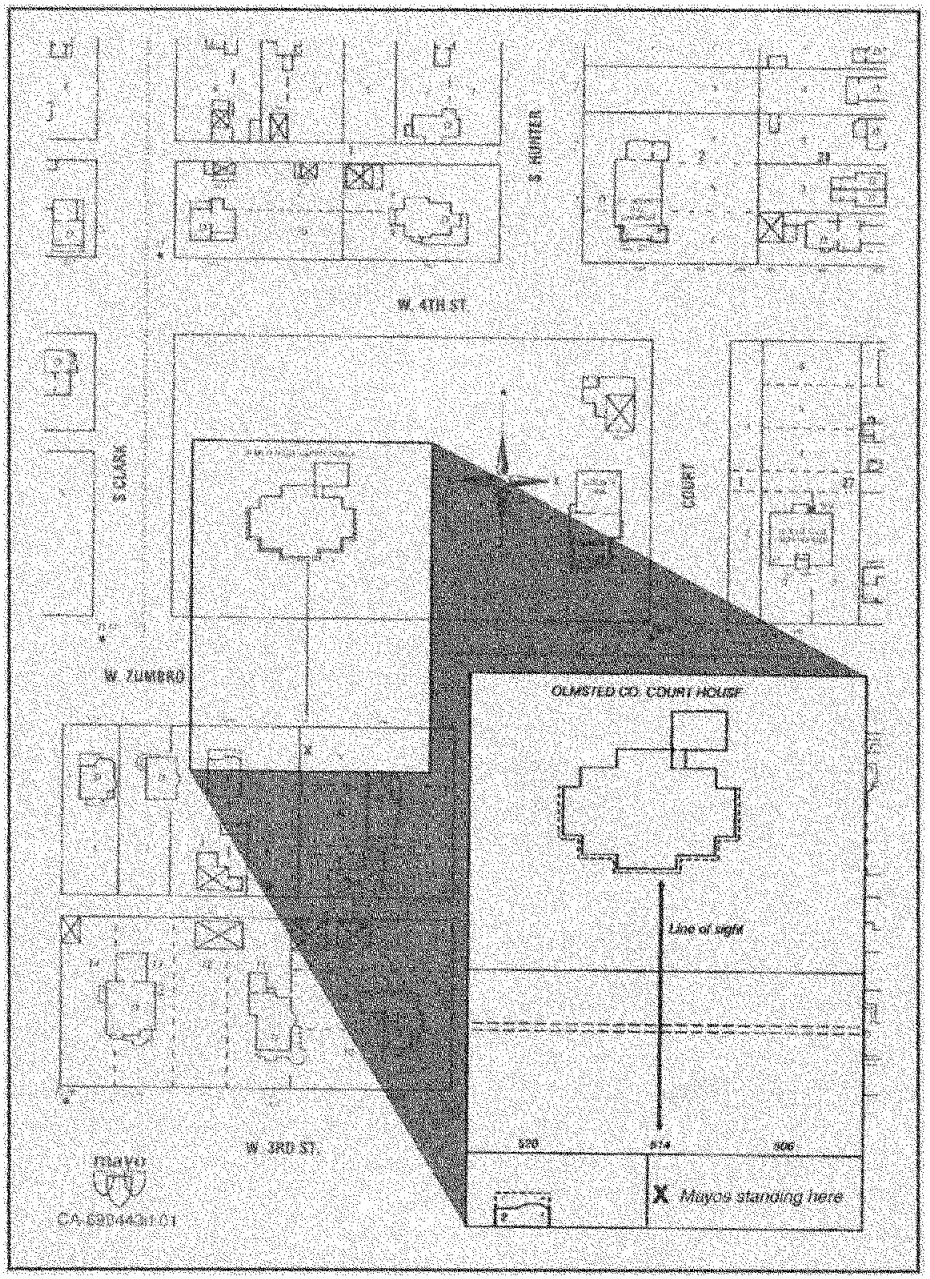

Fig. 2. Segment of turn-of-the-century Rochester, Minnesota map with insert showing location of the Mayos at 514 West Zumbro Street, based on a "line of sight" analysis from the courthouse wall.

Fig. 3. Dr. Henry Plummer's home at 514 West Zumbro Street, taken circa 1910. (From Willius FA. Henry Stanley Plummer: A Diversified Genius. Springfield: Charles C Thomas, Publisher, 1960, pp 10-18. Reproduced with permission.)

was taken "the first time the Clinical Society met in Rochester,"[10] which was October 6, 1905.

CONCLUSION

The photograph of the three Mayos discussed herein has always been known to possess great historical importance. Not only is it one of only two in the Clinic's collection featuring the three Mayo physicians together, but it was also almost certainly their favorite, as it was this photo that they chose to autograph and enlarge. The additional information presented herein demonstrates its added importance as a symbol of the friendship that existed between four of the greatest surgeons of our modern era: Harvey Cushing and the three Doctors Mayo.

I would like to thank the following persons for their invaluable assistance on this project: at the Mayo Clinic, Ms. Caroline Beck of the History of Medicine section and Ms. Kristi Ostrom of the Photographic Archives department; at the Olmsted County Historical Society, Ms. Sherry Sweetman; and at Yale University, Ms. Toby Appel and Ms. Amy Sharon of the Cushing Library and Ms. Diane Kaplan of the Sterling Library. Finally, I would like to also thank my son John for his excellent microfilm reviews.

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Letter to the Editors

Treatment of Hepatic Metastases From Colorectal Carcinoma

To the Editors:

I write to commend the excellent review on hepatic metastases from colorectal carcinoma published in the September/October 1997 issue of the JOURNAL OF GASTROINTESTINAL SURGERY.[1] I have addressed this problem in several previous publications.[2-5] The authors are to be commended for the log transformation of various data. The basic growth equation is:

$$N = N_0 e^{bt},$$

where N equals the number of cancer cells at time "t", N_0 is the initial number of cancer cells, e is the base of the natural logarithms, b is the growth constant, and t is the time elapsing between N_0 and N in days.

This transforms to:

$$DT_{(pot)} = \frac{\ln 2}{b}$$

for the potential doubling time ($DT_{(pot)}$).

As Gaddum[6] reported, a random variation in the magnitude of the growth constant, b, will produce a lognormal frequency distribution in $DT_{(pot)}$. The Weber-Fechner law predicts a lognormal variation in tumor sizes at discovery.[7]

In actuality, the $DT_{(pot)}$ is never reached because of cell death from apoptosis and, in the case of primary colon cancers, surface desquamation. As a result, the actual doubling time (DT_{act}) will become progressively longer with steady deceleration. The Gompertz equation is a special case of decelerating growth but is not the only case. Even with deceleration, the lognormal distribution of actual growth rates and survivorship persists. By using the lognormal distribution, the so-call "outliers" lie within the confines of the frequency distribution most likely attributable to the random variation in the magnitude of the growth constant, b. By moving the decimal point three places to the right for b, one gets an estimate of the potential number of new cells per thousand cells per day.

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To the Editors:

Congratulations on your publication of the "Consensus Statement on Treatment of Hepatic Metastases From Colorectal Cancer" in the September/October 1997 issue of JOURNAL OF GASTROINTESTINAL SURGERY. Your editorial asks for feedback. I wholeheartedly support your approach of publishing consensus statements. I look forward to similar statements on the treatment of other diseases of the alimentary tract that you may choose to highlight.

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