PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 158, 101–104 (1978)

Postresectional Hyperplasia of the Small Intestine without Bile and Pancreatic Juice[1] (40149)

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Partial small bowel resection causes mucosal hyperplasia of the intestinal remnant (1–3). Proximal enterectomy exposes the distal small bowel more directly to bile and pancreatic secretions, which have been implicated as stimulators of the postresectional response (4).

To test whether pancreaticobiliary secretions mediate postresectional distal hyperplasia and to obtain direct corroboration of experiments in which distal hyperplasia was observed after pancreaticobiliary diversion to the midgut (5), we examined the rat ileum 48 hr after jejunectomy, with and without ligation of the pancreaticobiliary (PB) duct. Amounts of nucleic acid in the bowel at that time anticipate those at longer intervals (3). Compensatory hyperplasia occured despite a ligated PB duct, but the presence of bile and pancreatic juice seemed necessary for a full hyperplastic response.

Experimental design. Male Sprague–Dawley rats (Charles River Breeding Laboratories, Inc., Wilmington, MA) weighing 200–230 g were randomly assigned to four experimental groups:

Ligation and transection (LT). Ligation of the PB duct immediately proximal to the papilla of Vater, with transection and end-to-end anastomosis of the jejunum 2 cm distal to the ligament of Treitz.

Ligation and resection (LR). Ligation of the PB duct as above, with proximal resection of 50% (45 cm) of the small bowel distal to the ligament of Treitz, followed by end-to-end anastomosis.

Control and transection (CT). Transection of the small bowel as above, with operative exposure of the PB duct.

Control and resection (CR). Resection of the small bowel, with exposure of the PB duct.

Operations were carried out between 0830 and 1300 hr, under light ether anesthesia. After transection or resection, continuity was restored by single layer anastomosis with 6–0 Tevdek. The PB duct was divided between two 5–0 silk ties.

Animals were fed Purina Laboratory Chow until the time of operation. Afterward, food but not water was withheld for 24 hr.

Rats were sacrificed 48 hr after operation by cervical dislocation between 0830 and 1300 hr. One hr before death, each rat was given a subcutaneous injection of 50 μCi ^{3}H-methyl-thymidine (6.7 Ci/mmole, New England Nuclear Corp., Boston, MA).

Immediately after death, the small bowel was removed and flushed with ice cold isotonic NaCl solution. A 5-cm segment of bowel, measured under a constant tension of 3 g over a single pulley, was cut from the center of the ileum. The segment was opened longitudinally over an ice cold plate, and the mucosa was scraped from the muscularis with a glass slide. The mucosa was then frozen in liquid nitrogen within 10 min of death. Scrapings were preserved at −70°, until subsequent analysis. When examined by light microscopy, representative specimens of intestinal wall revealed no crypts remaining after removal of the mucosa.

Content of RNA and DNA in mucosal samples was determined by the method of Scott *et al.* (6) as modified by Hinrichs *et al.* (7). Radioactivity in DNA was assayed from aliquots of DNA containing supernatant counted by liquid scintillation spectrometry with an efficiency of 29%, corrected by internal standardization.

Statistics. Significance was tested by Student's *t* test for unpaired data.

Animals. Rats with ligation of the PB duct

[1] This study was supported by Public Health Service Grant No. 2R 26 CA-17324 from the National Cancer Institute through the National Large Bowel Cancer Project.

0037-9727/78/1581-0101$01.00/0

tolerated the procedure well for 48 hr, although they became slightly icteric. Mortality rates were: 2/30 after ligation and transection (LT), 4/35 after ligation and resection (LR), 0/27 after control and transection (CT), and 1/28 after control and resection (CR). To see whether analysis of variance would identify relations not disclosed by Student's *t* test, results from random animals were discarded so that N for all groups would be identical.

The cause of death was peritonitis from leakage at either the site of anastomosis or the point of ligation of the PB duct. In the first 24 hr after operation, all animals lost about 11% of body weight. During the second 24 hr, rats with PB duct ligations lost another 3% of initial body weight, while rats in the control groups regained 3% of initial body weight.

Nucleic acid content. The rise in nucleic acids after proximal small bowel resection was less in animals with PB duct ligation than in those without. Compared with jejunal transection, resection increased midgut content of RNA 12% with PB duct ligation, but 44% without it, while DNA content rose 13% with PB duct ligation but 25% without it (Table I). Although ligation of the PB duct did not influence DNA or RNA contents after transection, ligation prevented much of the expected increase in mucosal RNA and DNA contents after resection.

The RNA/DNA ratio rose after resection in control animals, but not in rats with PB duct ligation. The ratio of the content of RNA to DNA is an estimate of the amount of RNA per average diploid cell (8).

DNA radioactivity. When corrected for the radioactivity of the acid soluble pool (DNA dpm per mg DNA per acid soluble dpm), DNA specific activity did not change after resection in either rats with PB duct ligation or those without it. Uncorrected measurements of specific activity paralleled those of DNA content. The amount of radioactivity in DNA, expressed per mg of DNA present (DNA specific activity), is an estimate of the proportion of proliferating cells (9).

Discussion. Some degree of hyperplasia in the rat ileum 48 hr after jejunal resection can occur without bile and pancreatic juice in the lumen of the bowel. Increased height of ileal villi is also observed after proximal intestinal bypass in the absence of pancreatic and biliary secretions (10).

Exocrine secretions seem to be necessary for the optimal proliferative response of the small bowel after proximal resection, however. Adaptive hyperplasia is reduced in rats with ligated ducts compared with animals with normal ducts. Both the average cellular content of RNA (RNA/DNA) and the number of cells (DNA content) are diminished. When pancreaticobiliary secretions are supplied to ectopic portions of the small intestine by transplantation or by infusion, they produce distal hyperplasia (4). Furthermore, ileal hyperplasia after jejunectomy is enhanced by distal transplantation of the papilla of Vater (11, 12). As this enhanced response is also

TABLE I. EFFECT OF JEJUNAL RESECTION AND PBD LIGATION UPON ILEAL MUCOSA NUCLEIC ACID CONTENT.

Experimental group		*N*	μg Nucleic acid/5 cm Mucosal Scraping ± SE[a] RNA	DNA	RNA/DNA[b]
PB Duct ligation and SB transection (LT)		27	2920 ± 95.5	2290 ± 59.1	1.28 ± 0.027
PB Duct ligation and Jejunectomy (LR)		27	3280 ± 99.4	2590 ± 76.5	1.27 ± 0.028
SB transection (CT)		27	2720 ± 89.6	2380 ± 80.3	1.16 ± 0.032
Jejunectomy (CR)		27	3910 ± 93.8	2970 ± 80.0	1.33 ± 0.030
Significance					
RNA	LT vs. LR: $P < 0.02$		DNA	LT vs. LR: $P < 0.01$	
	CT vs. CR: $P < 0.001$			CT vs. CR: $P < 0.001$	
	LR vs. CR: $P < 0.001$			LR vs. CR: $P < 0.01$	
	LT vs. CT: $P = 0.14$			LT vs. CT: $P = 0.39$	
RNA/DNA	LT vs. LR: $P = 0.94$				
	CT vs. CR: $P < 0.001$				
	LR vs. CR: $P = 0.20$				
	LT vs. CT: $P < 0.01$				

[a] All numbers are rounded to three significant digits.
[b] RNA/DNA are calculated from the original data, not the means given here.

observed in animals fed elemental diets, the stimulatory effect of bile and pancreatic juice probably is not due to their digestive action alone (11). The identity of the active component(s) of pancreaticobiliary secretions is disputable (1, 4, 13–16).

The diminished postresectional response in the absence of bile and pancreatic juice probably is not a result of jaundice and weight loss following PB duct ligation. In our experiments, tying the PB duct blunted the expected rise in nucleic acid content only among animals with small bowel resection; it did not influence the mucosal DNA and RNA of rats with jejunal transection. Unless there is an unrecognized nutritional problem peculiar to rats with both jejunectomy and PB duct ligation, differences in diet cannot explain the results. Alterations in the physical and chemical characteristics of chyme itself as a result of PB duct ligation may not be separable from the effects of withholding PB secretions from the mucosal cells. Suppressive effects of ligation of the common bile ducts in rats on angiogenesis and wound strength are not observable as early as 48 hr (17).

Small bowel resection does not change the corrected DNA specific activity. Specific activity in any event is a poor index of enteric proliferative activity in this experiment, because the DNA content is not constant. If in the formula for specific activity both the numerator (DNA radioactivity) and the denominator (DNA content) demonstrate a similar rise, the ratio is unchanged. If one adopts the common viewpoint that no corrections for acid soluble radioactivity should have been necessary because the pools were swamped, parallel changes in DNA specific activity and DNA content would make our evidence even stronger.

Since adaptive hyperplasia can occur without pancreatic and biliary secretions, at least one other factor must mediate the phenomenon. In addition to bile and pancreatic juice (14), previous investigations have most often emphasized the importance of "topical" or "luminal" nutrition (1, 10, 12, 18, 19), and hormones (2, 20) as mediators of the postresection response. It is likely that both bloodborne factors and local mediators are involved (1, 3, 13, 19–25).

Summary. To assess the role of pancreaticobiliary secretions in mediating adaptive hyperplasia of the small intestine, rats with ligation of the pancreaticobiliary duct were studied 48 hr after jejunectomy. After ligation, the rise in ileal RNA content was only 12% as compared with 44% control ($P < 0.02$) and the rise in DNA content was only 13% as compared with 25% control ($P < 0.01$). Some adaptive hyperplasia can occur after pancreaticobiliary ligation, but the hyperplastic response is potentiated by pancreaticobiliary exocrine secretion.

We thank Drs. Daniel Malamud and Robin C.N. Williamson for helpful discussions, Mrs. Rebecca R. Adjoyan for assistance, and Dr. Robert A. Lew for statistical advice.

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Received July 29, 1977. P.S.E.B.M. 1978, Vol. 158.