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Stimulation of Enzyme, Protein, and DNA Output from a Jejunal Fistula in the Rat (39648)

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We have modified the classical Thiry-Vella jejunal fistula, originally devised for dogs, for use in rats. This preparation has two main advantages over the use of dogs for studying the intestine: (i) The rat recovers rapidly from surgery and (ii) animal and maintenance costs are low. With this preparation we demonstrate the stimulatory effect of duodenal infusion of raw egg white on the output of DNA, protein, and enzyme activity from the fistula.

Methods. Surgical procedure. Food but not water is withheld for 16–18 hr before surgery. During the operation, maintenance of methoxyflurane anesthesia (Metafane, Pitman-Moore, Washington Crossing, New Jersey) is controlled by a vaporizing stream of O_2/CO_2 (95/5). A stainless steel rod with a small hole near one end is passed subcutaneously from a midline abdominal incision to a small dorsal incision through the skin on the rat's back. One end of both intestinal cannulae is passed through the hole in the rod and then pulled with the rod subcutaneously through to the abdominal incision. Intestinal cannulae are 25-cm lengths of Silastic medical grade tubing (0.64 × 1.19 mm) with a collar (1.02 × 2.16 mm) 1 cm from the beveled end (Fig. 1a). One cannula is inserted into the fistula and one into the duodenum. The free ends of the cannulae emerge through the skin dorsally out of the rat's reach. Sutures are placed at the small skin incision on the rat's back to secure the cannulae at their exit.

One centimeter above the ampulla of Vater a purse string suture is made with 5-0 suture and an intestinal cannula inserted through the gut wall and secured with ties above and below the collar. To form the fistula the gut is first transected between forceps about 2 cm distal to the ligament of Treitz and both cut ends are closed with an inverting suture and a second row of a purse string suture. About 0.5 cm from the closed end of the prospective fistula another intestinal cannula is inserted through the gut wall as described above for the duodenum (Fig. 1b). At the second transection, about 8 cm distal from the first, the distal end is closed and the proximal end is left open for isoperistaltic drainage of the fistula. Continuity of the gut is reestablished by side to side anastomosis. The open end of the fistula is brought out through an abdominal stab wound near the midline to protrude 0.5 cm from the skin. It is anchored by sutures to the peritoneum and subcutaneous fascia (Fig. 1c). A 0.5-cm segment of 1.0 × 0.80-cm Tygon tubing is fastened with Eastman 910 adhesive to the skin to surround the stoma of the fistula.

The animal is kept in a modified Bollman restraint cage. For the first 24 hr it has 5% dextrose solution to drink, after which time it has water and a powdered semisynthetic diet.[1] The rats eat readily and maintain their weight throughout the experimental period. The animals are usable after 2–3 days. In some fistulous animals, we have cannulated the jugular vein for infusion of drugs, and the common bile–pancreatic duct for collection of bile–pancreatic juice. These procedures were described in detail elsewhere (1, 2).

Experimental procedure. The fistula was perfused with saline at a rate of 10 ml/hr to clear the fistula of accumulated mucus and cellular debris. Samples were collected over ice for 30-min intervals. After 4 hr of basal perfusion, 350 mg of raw egg white (spray-dried, Nutritional Biochemical Co., Cleveland, Ohio) dissolved in 3 ml of saline, was

[1] Diet composition: Casein 24%, Jones and Foster salt mix, Nutritional Biochemical Co., 4%; vitamin mix, Nutritional Biochemical Co., 2%; corn oil, 6%; dextrin, 59%; Celluflour, 5%.

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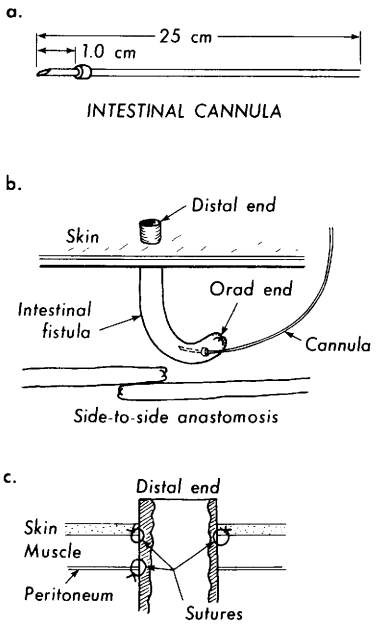

FIG. 1. (a) Cannula for insertion into intestine, or fistula, made of Silastic tubing. (b) Diagram of intestinal fistula and side-to-side anastomosis in the rat. (c) Details of suturing distal opening of fistula into the belly wall.

infused for a 25-min period into the duodenum or 3 ml of saline was infused as a control. The volume of the collected perfusate was measured, brought to 5 ml with distilled water, and homogenized in a ground-glass Duall tissue homogenizer with 12 up–down strokes. Three milliliters of the perfusate was treated for subsequent DNA analysis by the diphenylamine method of Goldsmith (3). The remaining homogenate was frozen and analyzed within 14 days for total protein (Lowry *et al.*, 4), peptidase activity (L-leucyl-β-napthylamide substrate, 5), and alkaline phosphatase activity (*o*-carboxyphenyl phosphate substrate, 6).

To test whether the mucosa of the fistula degenerated relative to the intestinal mucosa exposed to chyme during the time of the perfusion experiments, the isolated segment and two 10-cm intestinal segments orad and caudad from the anastomosis were removed 9–11 days postoperative. Each segment was rinsed three times with 2 ml of saline and slit longitudinally. The mucosae were examined macroscopically for any gross abnormality then scraped off with a glass slide, frozen on dry ice, lyophylized, and stored in a vacuum desiccator. The dried powder was ground with mortar and pestle and a weighed amount was homogenized in 10 ml of glass-distilled water for 1 min. The samples were kept at 4° during and after homogenization. The homogenates were analyzed as described previously for DNA, protein, peptidase activity, and alkaline phosphatase activity.

Results. As shown in Fig. 2, duodenal infusion of raw egg white, compared to saline infusion, increased the output of protein, DNA, peptidase activity, and alkaline phosphatase activity from an intestinal fistula ($P < 0.05$). The DNA output in the perfusate was threefold higher as a result of egg white infusion, which was the largest increase of the parameters measured. Protein, peptidase activity, and alkaline phosphatase activity were about 1.5-, 2.0-, and 2.5-fold higher, respectively ($N = 5$).

Table I summarizes the results of analyzing the mucosa from rats that were used in perfusion experiments. The protein and DNA content of the fistula mucosa was slightly greater than that of either adjacent intestinal segment ($P < 0.05$). Enzyme activity in the fistula was similar to that in the intestinal segment caudad to the anastomosis.

Discussion. The jejunal fistula described above allowed easy perfusion of the lumen and quantitative collection of the perfusate. Perfusion was facilitated by a small diameter cannula inserted into the orad end of the

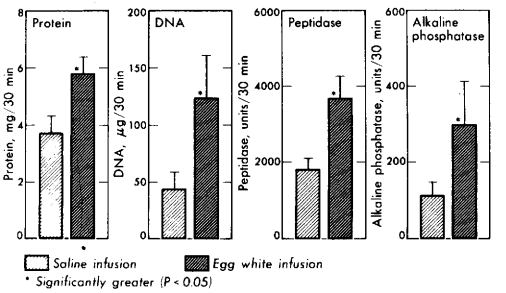

FIG. 2. Output of protein, DNA, peptidase, and alkaline phosphatase activity from a perfused (10 ml of saline/hr) intestinal fistula during infusion of saline or 350 mg of raw egg white into the duodenum in rats. Output of all parameters of secretion was significantly higher during egg white infusion ($P < 0.05$) ($n = 5$).

TABLE I. ANALYSIS OF MUCOSA FROM THE INTESTINE AND JEJUNAL FISTULA IN RATS.[a]

	Intestine, orad from anastomosis	Jejunal fistula	Intestine, caudad from anastomosis
Protein (mg)[b]	0.73 ± 0.06[c]	0.85 ± 0.06	0.76 ± 0.06
DNA (μg)[b]	39 ± 3.6	48 ± 2.9	36 ± 2.1
Peptidase (units)[b]	408 ± 37	735 ± 116	835 ± 128
Alkaline phosphatase (units)	56.3 ± 15.0	18.3 ± 2.8	14.5 ± 2.8

[a] $N = 5$
[b] Each value is expressed as the amount per milligram of lyophilized mucosa.
[c] Each value is mean ± SEM.

isolated segment (Fig. 1b). Infusion of substances into the intact duodenum was accomplished by a similar cannula. Cramer and Couch (7) developed a suturable stainless steel gastrointestinal cannula for use in fistulae in dogs, rabbits, and rats. These cannulae were inserted into each end of the gut segment and protruded through the belly wall; tubing was attached to the orad cannula for perfusion. We tried the method of Cramer and Couch on the distal end of a fistula with indifferent success. The cannula was awkward to secure, and the stainless steel exit tubing often became clogged with mucus. Simply allowing the distal end of the fistula to protrude 0.5 cm through the belly wall avoided clogging and prevented leakage of fistula contents into the abdominal cavity.

Animals were used for perfusion experiments for up to a week beginning on the 4th postoperative day after which they were killed for autopsy. The fistula often appeared slightly smaller in diameter than the intact intestine but the mucosal surface appeared macroscopically normal. Analysis of the mucosa showed a slight increase in the amount of protein and DNA per milligram of dried mucosa in the isolated segment (Table I). Several investigators have determined changes in morphology and function that occur in an isolated segment relative to the intact intestine (8, 9). After an intestinal resection, hypertrophic changes in the intact intestine near the anastomosis take place, but these changes are not significant until 4 weeks after operation (8, 9). At this time macroscopic atrophic changes and decreases in protein per centimeter of intestine and cell turnover time in the fistula are noticeable. Our rat preparation functioned normally for at least 1–2 weeks after operation.

The variations in peptidase and alkaline phosphatase activities among the three sections (Table I) probably reflected the distribution of enzyme activity along the intestine found normally. Saito *et al.* (10) reported that alkaline phosphatase activity was highest in the proximal intestine and decreased in the jejunum and upper ileum. Peptidase activity was highest in the jejunum and upper ileum and lower in the proximal intestine and distal ileum. This corresponds with our observation that peptidase activity was highest in the jejunal fistula dn the intestinal segment caudad to the anastomosis and that alkaline phosphatase activity was highest in the orad segment of the intestine.

Research on the intestinal secretory response to foods has been done primarily with dogs bearing Thiry-Vella fistulae. Fluid volume was often the only response measured and, hence, very little information is available on enzyme or cell release. Feeding sugar, fat, or meat stimulated fluid secretion (11, 12). Nasset *et al.* (13) measured volume and enzyme responses of gut transplants to a fed meal and found that innervated transplants in 7 dogs gave equivocal results. After denervation, 6 of 7 dogs gave positive responses to feeding. In our rat preparation it was possible to infuse diet components directly into the duodenum and to measure the DNA as well as enzyme responses in the fistula. Figure 2 shows that in rats an innervated intestinal segment gave a positive response to infusion of egg white into the duodenum, indicating a neural or humoral control of intestinal secretion or a combination of both. Nasset (14) presented evidence of the existence of a hormone, enterocrinin, that stimulates intestinal secretion. Enterocrinin activity was shown to be present in extracts of small gut mucosa in

seven species, including man. This work was done before the announcement of pancreozymin activity by Harper and Raper (15). Fink's fractionation procedures resulted in a 300-fold concentration of enterocrinin activity so that as little as 2 μg/kg iv in a dog would give a measurable volume response (16). Unfortunately, no other laboratory has attempted to repeat the work on enterocrinin and hence there is no other evidence to confirm or confute the results briefly referred to above. Several investigators (17–19) have shown however, that cholecystokinin (CCK) stimulates secretion of intestinal enzyme activity. Protein in the intestine stimulates the release of CCK from the intestine, as evidenced by an increased pancreatic enzyme secretion (1). Therefore, CCK may have stimulated the positive intestinal response to egg white. In humans CCK stimulates intestinal motor activity (20). Increased motility could increase desquamation rate in the intestine which would be reflected in the increased enzyme and DNA output.

Summary. A new technique for preparing intestinal fistulae in rats is presented. Perfusion of the fistula is facilitated by inserting small-diameter tubing into the closed orad end of the fistula. The distal end protrudes a 0.5 cm through the belly wall for quantitative collection of perfusate or secretion. Continuity of the gut is reestablished by a side-to-side anastomosis. Analysis of the mucosa from the fistula and from the intact intestine demonstrated that the mucosae are comparable during the week the animals are used for perfusion experiments. The results also demonstrate that duodenal infusion of raw egg white stimulates the output of protein, DNA, peptidase and alkaline phosphatase activity in the jejunal fistula perfusate.

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