Caecal and Colonic Uptake of both Linoleic Acid and Cholesterol in Rats Following Intestinal Resection

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Caecal and colonic uptake of both linoleic acid and cholesterol were studied in rats after distal small bowel resection (DSBR). The results showed that the surgical operation increased the caecal and colonic uptake of linoleic acid. Supplementation with linoleic acid inhibited caecal and colonic uptake of linoleic acid. Experiments carried out in the presence of rotenone and ouabain suggest that facilitated diffusion is the predominant mechanism of caecal and colonic linoleic acid absorption, at least at low concentrations. An increase in caecal and colonic uptake of cholesterol was observed after the surgical operation. The study showed that facilitated diffusion seems to be the mechanism of linoleic acid absorption in the caecum and colon, and that both organ growth and changes in transport function of the epithelial cells of caecum and colon appear to be involved in the adaptive response of the bowel to intestinal resection.


The long-term outcome of massive small bowel resection depends mainly on the adaptive capacity of the remnant intestine. Both, morphological and functional adaptations of the remaining small intestine have been extensively studied (1). However, few studies have examined the effect of distal small bowel resection (DSBR) on the adaptive processes in the large bowel.

Linoleic acid is the most common essential fatty acid in the mammalian diet. Dietary deficiency of linoleic acid or its intestinal malabsorption can result in a wide variety of disorders (2). However, despite the biological importance of linoleic acid, its mode of absorption by the large intestine has not been studied previously. On the other hand, it is well known that DSBR affects cholesterol metabolism, including hepatic cholesterol synthesis (3,4).

The aim of the current study was to investigate the effect of DSBR on cholesterol and linoleic acid transport in the large intestine (caecum and colon). The mechanism of intestinal absorption of linoleic acid in the large intestine was evaluated.

MATERIALS AND METHODS

Chemicals. [14C]Linoleic acid and [14C]cholesterol (Amersham, U.K.) with specific activities of 50-60 mCi/mmol were used as tracers. [3H]Munin (Amersham, U.K.) with a specific activity of 1-5 mCi/mmol was used as an extracellular water marker. Cholesterol and linoleic acid (99% of purity) and all other chemicals were from Sigma Chemical Co. (St. Louis, MO).

Animals. Male Wistar rats weighing about 300 g were maintained on a standard pellet diet with free access to tap water. The rats were randomly assigned to one of three groups: sham-operated, 50% and 75% DSBR. The rats were anaesthetized with intraperitoneal sodium pentobarbitrol (4.5 mg/100 g body wt) after a 24-hr fast period. Laparotomy was performed, and rats assigned for DSBR underwent either 50% or 75% DSBR, as described by Murillo et al. (5). Briefly, the blood vessels of the resected intestinal segment were tied and sectioned, but the blood supply and innervation of the remaining intestine were carefully maintained. Intestinal continuity was reestablished by end-to-end anastomosis with Mersilene 3/0 thread. In another group, sham operation was performed, and the intestine was cut and reanastomosed without resection. In each instance, continuity of the gut was restored by end-to-end anastomosis. Six weeks after the surgical operation, animals were starved overnight (with access to water only) and killed by stunning and cervical dislocation. Both control and experimental groups were treated in the same manner to prevent effects that could mask differences between groups.

Tissue preparation. After the animals were killed, the abdomen was opened, caecum and colon were rapidly removed, gently rinsed free of intestinal contents with ice-cold saline (0.9% NaCl) solution and their weights (caecum and colon) and lengths (colon) recorded.

Intestinal sacs weighing about 100 mg were tied off and kept in cold saline solution until used. No fluid was placed in the serosal compartment. The intestinal sacs were preincubated for 10 min at 37°C in gassed Ringer's solution at pH 6.5 for linoleic acid or pH 7.4 for cholesterol (see below), to allow for equilibration at this temperature. In order to get intestinal tissue depleted of cellular adenosine triphosphate (ATP), in a separate experiment sacs were preincubated in the presence of 80 μM rotenone (metabolic inhibitor) and 20 μM ouabain (Na+,K+-ATPase inhibitor) for 15 min at 37°C (6). Sacs were then transferred to the incubation medium. Both, preincubation and incubation solutions were mixed at identical stirring rates of 750 revolutions/min in order to achieve low effective resistance of the intestinal unstirred water layer.

Preparation of incubation solution. Lipids were dissolved in 10 mM NaTC-Ringer's solution which contained (in mM): 140 NaCl; 10 KH2PO4; 2.4 K2HPO4 and 1.2 MgCl2 instead of 1.2 CaCl2 to avoid fatty acid soap formation.

Linoleic acid micellar solutions were prepared as previously described (7). Briefly, an appropriate amount of both 14C-labeled and unlabeled linoleic acid were dissolved in a small volume of ethanol. Ten mM NaTC-Ringer solution at pH 6.5 [to obtain maximum absorption (7,9), previously gassed with 5% O2, 5% CO2, was added to give a final linoleic acid concentration of 0.05 mM]. The micellar solution was prepared by ultrasonic irradiation for 10 min. If necessary, the pH was readjusted to 6.5. On separate occasions, linolenic acid was added to the linoleic acid solution at a concentration of 3 mM.

The technique used to prepare the cholesterol micellar solutions has been published previously (8). An

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Abbreviations: DSBR, distal small bowel resection; NaTC, sodium taurocholate.
appropriate amount of both 14C-labeled and unlabeled cholesterol was dissolved in an exact volume of chloroform/methanol (2:1, v/v). The chloroform/methanol phase was evaporated and the Ringer's solution (pH 7.4) previously gassed was added. The micellar solution was prepared by ultrasonic irradiation for 10 min. The solution was then further diluted by the addition of Ringer's solution with NaTC 10 mM and traces of [14C]cholesterol to give a final cholesterol concentration of 0.05 and 0.2 mM.

A trace amount of [3H]inulin as a radiolabeled volume marker was also added.

**Linoleic acid and cholesterol uptake.** After preincubation, sacs were transferred to the incubation medium containing [3H]inulin and either [14C]linoleic acid or [14C]cholesterol. Following incubation of intestinal sacs in labeled solutions for 6 min at 37°C, sacs were removed and quickly rinsed with 1 mM NaTC in order to remove some of the adherent incubation solution off the sac's surface. Sacs were then gently blotted on filter paper, weighed and dried overnight at 60°C. This temperature did not lead to any loss of linoleic acid. Dry sacs were weighed and saponified with 0.75 N NaOH. Scintillation fluid was added and radioactivity was determined.

**Expression of results.** The rate of uptake of solutes was calculated after correcting the total tissue 14C radioactivity for the mass of the probe molecule present in the adherent mucosal fluid, and these rates were expressed as the pmole of substrate taken up into the mucosal per mg wet intestinal tissue per 6 min incubation.

Values obtained are reported as the mean ± S.E. of results observed for a minimum of five separate experiments. Each experiment is a pool of two large intestine (caecum or colon) from two different rats.

The effect of DSBR on the intestinal uptake of substrate was examined by analysis of variance (ANOVA) procedures. The unpaired student's t-test was used to test the significance of the difference between the means for sham-operated and resected rats.

**RESULTS**

**Animal characteristics.** Postoperative mortality was 10% and 20% after 50% and 75% DSBR, respectively. Deaths occurred within the first 5 post-operative days and were attributed to the surgery. Initial body weights in each group of animals were the same. At the time of study, 6 wk after the surgical operation, mean body weights were significantly lower in both 50% and 75% resected rats, this decrease being higher after 75% than after 50% DSBR. Caecal tissue wet weights were significantly increased after both 50% and 75% DSBR, the increase being related to the extent of intestine removed. However, colonic tissue mass, expressed as mg/cm, only significantly increased after the massive resection (75%) (Table 1).

**Uptake of linoleic acid and effect of linolenic acid.** The absorption of 0.05 mM linoleic acid was studied in caecum and colon after DSBR. The results show that the surgical operation increased the caecal and colonic uptake of linoleic acid, the increase not being relative to the extent of the intestine removed (Fig. 1). Since the intestinal uptake of linoleic acid in jejunum appears to be mediated by a facilitated diffusion mechanism, and inhibited by linolenic acid (7,9), we also studied the effect of linolenic acid (3 mM) on caecal and colonic uptake of linoleic acid (0.05 mM). Results in Figure 1 indicate that the addition of linolenic acid inhibited the caecal and colonic uptake of linoleic acid in the three groups of animals, the rate of the inhibition being similar between sham and resected animals.

![FIG. 1. Effect of DSBR on caecal (C) and colonic (Co) uptake of linoleic acid (0.05mM). Influence of linolenic acid (3mM) (white diagonal fines). Results are given as means _S.E. of five separate experiments. Each experiment was done on 2 pooled large intestines (caecum or colon) from two rats. *p<0.05, **p<0.01, ***p<0.005, ****p<0.001 50% or 75% resected animals compared with corresponding sham animals.](image)

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<th>TABLE 1</th>
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<td><strong>Effect of DSBR on Body Weight and Intestinal Tissue</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Colon (mg/cm)</td>
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<sup>a</sup>Results are given as means ± S.E. of 10 animals in each group. *p<0.05, **p<0.01, ***p<0.005, ****p<0.001. 50% or 75% resected animals compared with sham animals.

<sup>b</sup>p<0.001. 75% compared with 50% resected animals.