Parenteral nutrition supplemented with short-chain fatty acids: effect on the small-bowel mucosa in normal rats[1-3]

Mark J Koruda, Rolando H Rolandelli, Donna Zimmaro Bliss, Jennifer Hastings, John L Rombeau, and R Gregg Settle

ABSTRACT When enteral nutrition is excluded from animals maintained solely with total parenteral nutrition (TPN), atrophy of the intestinal mucosa is observed. Short-chain fatty acids (SCFAs) are produced in the colon by the fermentation of dietary carbohydrates and fiber polysaccharides and have been shown to stimulate mucosal-cell mitotic activity in the intestine. This study compared the effects of an intravenous and an intracecal infusion of SCFAs on the small-bowel mucosa. Rats received standard TPN, TPN with SCFAs (sodium acetate, propionate, and butyrate), TPN with an intracecal infusion of SCFAs, or rat food. After 7 d jejunal and ileal mucosal weights, DNA, RNA, and protein were determined. Standard TPN produced significant atrophy of the jejunal and ileal mucosa. Both the intracecal and intravenous infusion of SCFAs significantly reduced the mucosal atrophy associated with TPN. The intravenous and intracolonic infusion of SCFAs were equally effective in inhibiting small-bowel mucosal atrophy. *Am J Clin Nutr* 1990;51:685–9.

KEY WORDS Short-chain fatty acids, acetate, propionate, butyrate, parenteral nutrition, small-bowel mucosa, mucosal adaptation

Introduction

Dietary manipulation has profound effects on the structure and function of the gastrointestinal tract. In particular, when enteral nutrition is excluded from animals maintained solely with total parenteral nutrition (TPN), atrophy of the intestinal mucosa is observed (1–4). The mucosal atrophy is characterized by a decrease in mucosal mass; DNA, RNA, and protein content; villus size; and mitotic index.

Short-chain fatty acids (SCFAs) are normally produced by the bacterial fermentation of dietary carbohydrates and fiber polysaccharides in the colon (5, 6). Three SCFAs account for 83% of all SCFAs produced and are distributed in a fairly constant ratio—60:25:15 (acetate:propionate:*n*-butyrate) (7). SCFAs are readily absorbed by the colonic mucosa (8–10). It has been shown that both the intracolonic infusion and the intraperitoneal injection of SCFAs stimulate the mucosal proliferation of the jejunum and ileum of normal rats (11–14). We demonstrated that the supplementation of TPN with SCFAs retarded the mucosal atrophy associated with TPN after massive bowel resection in rats (15).

This study examined the effect of TPN supplemented with SCFAs on the mucosa of the small bowel in normal rats. In addition, we compared the effect of an intravenous infusion of SCFAs with intracecal delivery on the small-bowel mucosa because SCFAs are normally produced and absorbed in the large bowel.

Materials and methods

Twenty-five adult male Sprague-Dawley rats (275–325 g) were housed in individual metabolic cages and fed a fiber-free liquid rat diet (Bioserv, Freehold, NJ) ad libitum for 3 d. This diet was given to minimize the effect of residual dietary fiber fermentation, and thus SCFA production, during the course of the experiment. On the fourth day all rats were anesthetized and underwent superior vena cava cannulation with swivel placement. In addition, five animals underwent insertion of a cecal catheter attached to a two-channel swivel to permit simultaneous intravenous and intracecal infusions. Sham laparotomies with cecal manipulation were performed on the 20 animals that did not receive cecal catheters.

Postoperatively the five animals with cecal catheters received standard TPN with an intracecal infusion of SCFAs (Cec-SCFA, $n = 5$). The remaining animals were randomly assigned to receive either standard TPN (Control, $n = 5$), TPN supplemented with intravenous SCFAs (IV-SCFA, $n = 8$), or 0.45% saline intravenously with standard rat food ad libitum (Food, $n = 7$). The TPN and intracecal solutions were prepared daily with the compositions outlined in **Table 1**. SCFAs (acetate, propionate, and *n*-butyrate) were added to the IV-SCFA TPN as the sodium salts (Sigma Chemical Co, St Louis) according to the proportions of Sakata and Englehardt (13). Sixty-two milliliters of the diets were infused daily to provide $\sim 1.5 \text{ g N} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ and 206 nonprotein $\text{kcal} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ (Table 1). All animals were offered water ad libitum during the feeding period.

[1] From the Departments of Surgery, Nursing, and Otorhinolaryngology, University of Pennsylvania and the VA Medical Center, Philadelphia.

[2] Supported in part by the Veterans Administration and the John Rhea Barton Surgical Associates.

[3] Address reprint requests to RG Settle, Medical Research (151), VA Medical Center, University and Woodland Avenues, Philadelphia, PA 19104.

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TABLE 1
Composition of infusion formulas*

	Control	IV-SCFA	Cec-SCFA
Glucose (mol/L)	1.5	1.5	—
Nitrogen (mmol/L)†	0.53	0.53	—
NaCl (mmol/L)	60	—	—
Sodium acetate (mmol/L)	—	36	75
Sodium propionate (mmol/L)	—	15	35
Sodium butyrate (mmol/L)	—	9	20
KCl (mmol/L)	20	20	—
KH_2PO_4 (mmol/L)	15	15	—
$CaCl_2$ (mmol/L)	8	8	—
$MgCl_2$ (mmol/L)	3	3	—
Vitamins (mL/L)‡	0.1	0.1	—

* Control, standard total parenteral nutrition; IV-SCFA, intravenous short-chain fatty acid; Cec-SCFA, intracecal short-chain fatty acid.
† Aminosyn 8.5% (Abbott, North Chicago, IL).
‡ MVC 9 + 3 (Lyphomed, Melrose Park, IL).

Urine and feces were collected from the three groups receiving TPN during diet infusion for total nitrogen determinations (16). Because of the inherent difficulties and inaccuracies associated with monitoring the actual ingestion of food by rats, we elected not to include the Food group in the nutrient balance studies.

The cecal infusion consisted of the sodium salts of acetate, propionate, and *n*-butyrate (Sigma Chemical Co) (Table 1). These concentrations were chosen to approximate the profile of SCFAs in normal rat colon (13). The pH of the solution was adjusted to 7.0 with concentrated HCl. Thirty milliliters per day were infused intracecally so that the Cec-SCFA and IV-SCFA groups received similar amounts of SCFAs.

On the seventh postoperative day animals were anesthetized and weighed and the intestine from the ligament of Trietz to the cecum was rapidly excised and rinsed in ice-cold saline. While the intestine was suspended with a 10-g weight, standardized 10-cm segments of jejunum (just distal to the ligament of Trietz) and ileum (just proximal to the ileocecal junction) were obtained. The mucosa from the intestinal segments was harvested as previously described (17) and was weighed, homogenized in water (20–40 mg/mL), and frozen at −25 °C until assayed for DNA (18, 19), RNA (20), and protein (21).

The entire liver from each animal was removed and weighed. Liver water content was determined gravimetrically by vacuum dessication at 90 °C. Liver fat content was determined by

TABLE 2
Cumulative nitrogen balance*

	Control	IV-SCFA	Cec-SCFA
		mmol/kg	
Intake	712 ± 19	733 ± 16	718 ± 4
Urine	303 ± 22	292 ± 24	369 ± 12
Feces	35 ± 5	32 ± 7	38 ± 7
Balance	374 ± 34	409 ± 15	311 ± 13

* $\bar{x}$ ± SEM. There were no significant differences by analysis of variance (ANOVA).

TABLE 3
Change in body weight*

	Control	IV-SCFA	Cec-SCFA	Food
Weight change (g)	11.6 ± 5.9	12.5 ± 5.4	5.0 ± 9.9	7.7 ± 13.6
Percent weight change (%)	5.1 ± 2.5	5.1 ± 2.2	2.5 ± 4.2	2.2 ± 3.8

* $\bar{x}$ ± SEM. There were no significant differences by ANOVA or by paired *t* test.

chloroform and methanol extraction (22) and liver nitrogen was determined by Kjeldahl analysis (16).

This experiment complied with the guidelines of the National Research Council for the care and use of laboratory animals.

Statistical analysis

All data are presented as the mean ± SEM. Paired *t* tests were used to evaluate the changes in body weight. Analysis of variance (ANOVA) was performed on each variable. When the ANOVA indicated a significant effect, *t* tests were used to make post hoc comparisons between dietary groups; $p < 0.05$ was considered significant (23).

Results

Nutritional variables

There were no significant differences in total dietary nitrogen intake, urinary and fecal nitrogen excretion, and nitrogen balance among the three groups that received TPN (**Table 2**). Similarly, there were no significant differences among the four groups for absolute change in body weight or for percent change in body weight (**Table 3**).

Mucosal variables

In general there were substantial decreases in mucosal weight and in DNA, RNA, and protein contents in the TPN group compared with the Food group (**Table 4**). In both the jejunum and ileum, the Control group had significantly lower mucos

TABLE 4
Change in means of mucosal variables in the TPN groups relative to the Food group

	Control	IV-SCFA	
		%	
Mucosal weight			
Jejunum	−68.2	-41.0	
Ileum	−69.1	-52.1	
Mucosal DNA			
Jejunum	−61.5	-27.5	-17.
Ileum	−63.9	-49.9	-46.
Mucosal RNA			
Jejunum	−64.9	-41.3	
Ileum	−74.1	-52.8	
Mucosal protein			
Jejunum	−64.9	-22.3	
Ileum	−64.3	-48.2	

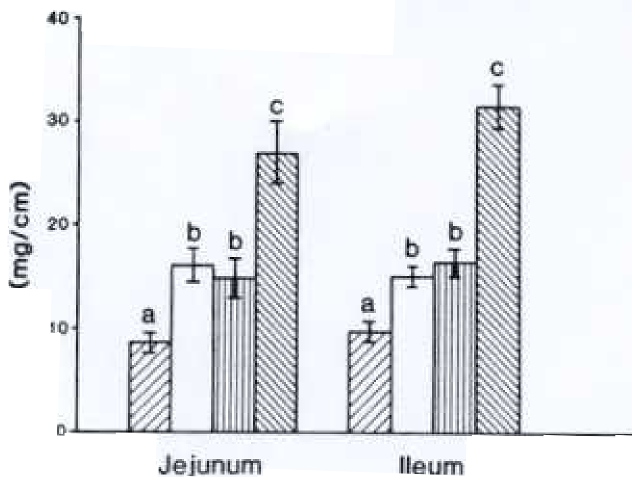

FIG 1. Mucosal weights for jejunal and ileal segments for the dietary groups. For each segment, a vs b, $p < 0.01$; a vs c, $p < 0.001$; and b vs c, $p < 0.001$. Control (□), standard total parenteral nutrition (TPN); IV-SCFA (□), standard TPN supplemented with intravenous short-chain fatty acids; Cec-SCFA (▥), standard TPN with intracecal infusion of SCFA; Food (□), rat chow ad libitum.

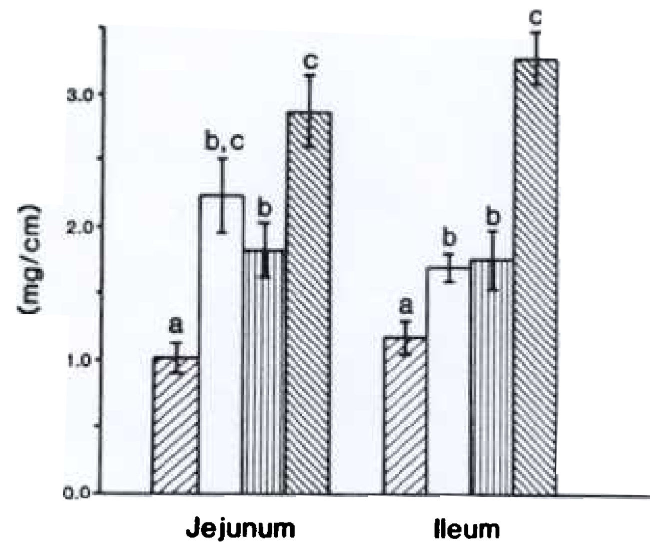

FIG 3. Mucosal protein for jejunal and ileal segments for the dietary groups. For each segment, a vs b, $p < 0.02$; a vs c, $p < 0.02$; a vs c, $p < 0.001$; and b vs c, $p < 0.01$.

weights than did the IV-SCFA, Cec-SCFA, and Food groups (**Fig 1**). The jejunal and ileal mucosal weights of the IV-SCFA and Cec-SCFA groups were significantly greater than those of the Control group and significantly less than those of the Food group but were not significantly different from each other. The same statistical relationship among groups was found for jejunal and ileal mucosal RNA (**Fig 2**), ileal mucosal protein content (**Fig 3**), and ileal mucosal DNA content (**Fig 4**).

Jejunal mucosal protein contents (Fig 3) of the Control and Cec-SCFA groups were significantly lower than the protein content of the Food group whereas the protein contents of the IV-SCFA and Food groups did not significantly differ. Jejunal DNA content (Fig 4) of the Control group was significantly lower than the mucosal DNA of the other three groups. However, in contrast to the other mucosal variables, the jejunal DNA for the IV-SCFA, Cec-SCFA, and Food groups did not differ significantly.

Changes in mucosal cell size are reflected by the ratio of RNA to DNA (24), particularly in early cell growth. The ratio of RNA to DNA did not differ significantly in the jejunum and ileum among the groups (**Table 5**).

Liver

There were no significant differences in total liver weight, percent water content, percent fat content, and nitrogen content among the groups (**Table 6**).

Discussion

These data demonstrate that compared with rat-food-fed rats, rats given standard TPN for 7 d showed significant muco-

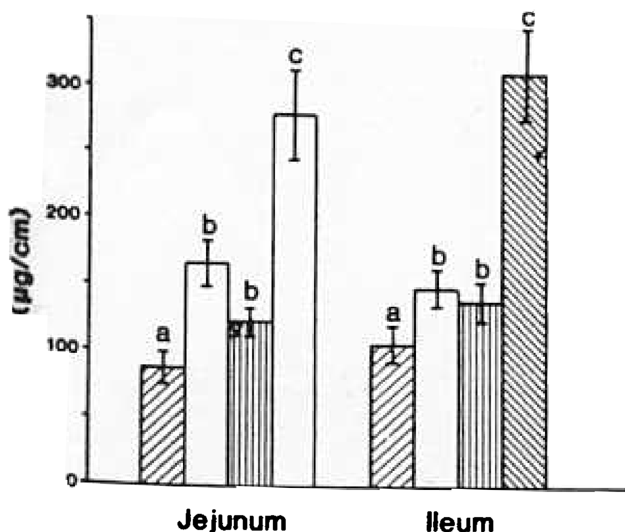

FIG 2. Mucosal RNA for jejunal and ileal segments for the dietary groups. For each segment, a vs b, $p < 0.05$; a vs c, $p < 0.005$; and b vs c, $p < 0.02$.

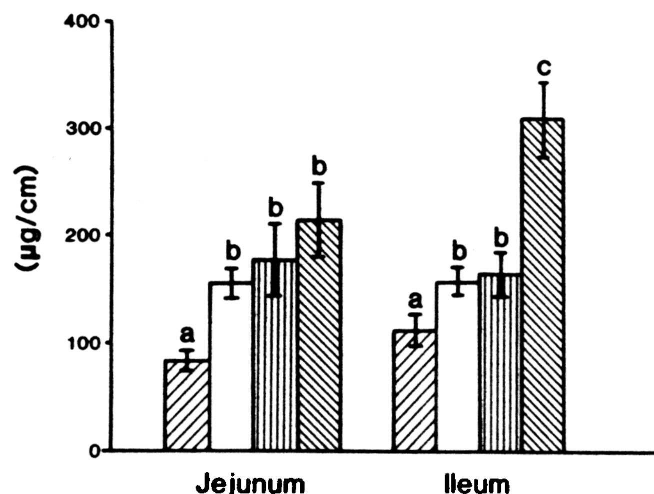

FIG 4. Mucosal DNA for jejunal and ileal segments for the dietary groups. For each segment, a vs b, $p < 0.05$; a vs c, $p < 0.005$; and b vs c, $p < 0.001$.

sal atrophy of the jejunum and ileum. This is indicated by substantial decreases in mucosal weight and in DNA, RNA, and protein contents (Table 4). This finding is consistent with previous observations that jejunal and ileal mucosal atrophy occurs when animals are maintained solely with TPN (1–4). More importantly, the present study demonstrates that the provision of SCFAs, either intravenously or intracecally, during TPN resulted in significantly less mucosal atrophy in the jejunum and ileum as indicated by smaller losses of mucosal weight and DNA, RNA, and protein contents (Table 4). The effects of the SCFAs on the small-bowel mucosa appear to be somewhat more pronounced in the jejunum than in the ileum. There is a tendency for the jejunal mucosa of the IV-SCFA and Cec-SCFA groups to experience a smaller decrease in the mucosal variables than the ileal mucosa.

Gastrointestinal mucosal growth is influenced by many factors such as the presence and type of luminal nutrients (25–28), pancreatic and biliary secretions (29–31), circulating enterohormones (32), and neurovascular reflexes (28). The atrophy of the intestinal mucosa that results from parenteral nutrition is thought to be mediated by one or more of these factors.

The mechanisms by which SCFAs may influence small-bowel mucosal proliferation is not clearly understood. Because SCFAs are normally produced in the colon by the bacterial fermentation of dietary carbohydrates and fiber polysaccharides (5, 6), the initial studies that demonstrated the effects of SCFAs on small-bowel mucosal proliferation were performed in a model where the SCFAs were directly infused into the colon (12, 13). These studies suggested that the intracolonic presence of SCFAs was necessary to exert their effects on the small bowel. However, the present study demonstrates that the intravenous and intracolonic infusion of SCFAs was equally effective in inhibiting small-bowel mucosal atrophy, indicating that the site of administration does not significantly influence the effects of SCFAs on the small-bowel mucosa.

SCFAs have several systemic effects that may influence intestinal mucosal proliferation. SCFAs, particularly acetate, increase intestinal blood flow (33), which may enhance mucosal proliferation (34). The autonomic nervous system has been shown to mediate some of the effects of SCFAs on the colonic mucosa (13). Pancreatic secretions are known to stimulate mucosal proliferation (29–31), and the intravenous infusion of SCFA, especially butyrate, was shown to increase pancreatic secretion (35). Circulating enterohormones play a significant role in intestinal mucosal proliferation. Although SCFAs stimulate the release of insulin (36), to our knowledge there have been no studies that have examined the effect of SCFAs on the secretion of humoral factors or gut hormones that mediate intestinal mucosal proliferation.

SCFAs, which are normally produced in the colon, are readily absorbed and metabolized by the colonic mucosa.

TABLE 5
Ratio of mucosal RNA to DNA*

	Control	IV-SCFA	Cec-SCFA	Food
Jejunum	1.07 ± 0.18	1.07 ± 0.01	0.77 ± 0.12	1.31 ± 0.09
Ileum	0.94 ± 0.07	0.95 ± 0.09	0.88 ± 0.10	0.99 ± 0.05

* $\bar{x}$ ± SEM. There were no significant differences by ANOVA.

TABLE 6
Liver composition*

	Control	IV-SCFA	Cec-SCFA	Food
Wet weight (g/kg)	36 ±	37 ± 1		
Water (%)	73 ±	73 ± 1		
Fat (%)	16 ±	17 ± 1		
Nitrogen (mg/g dry wt)	112 ± 1	112 ± 4	117 ±	117 ± 4

* $\bar{x}$ ± SEM. There were no significant differences by ANOVA.

Apart from the colonic mucosa, the liver is the main site of SCFA metabolism (37). Hepatic metabolism and clearance of SCFAs are substantial because portal concentrations of SCFAs are 150% greater than the concentrations of SCFAs simultaneously determined in the hepatic vein and 375% greater than systemic SCFA concentrations (38). This suggests that the effects of SCFAs on the small-bowel mucosa are indirect, perhaps produced by other mediators.

Products of SCFA metabolism may provide the mediators of SCFA mucosal trophism (39). The hepatic metabolism of both intraportally infused and intravenously administered SCFAs results in the production of the ketone bodies acetoacetate and β-hydroxybutyrate as well as the amino acids glutamine and glutamate (40, 41). Ketone bodies and glutamine are major respiratory fuels of the small intestine (42). Glutamine, β-hydroxybutyrate, and acetoacetate have been shown to be trophic to the small bowel and colon (39, 43). Therefore, it would appear that via common hepatic metabolism to the preferred intestinal fuel sources, both the portally delivered SCFAs (cecal infusion) and the intravenously infused SCFAs would have similar effects on the intestinal mucosa, as found in the present study.

The caloric contribution of the SCFAs should be considered as possibly influencing the effects on the small-bowel mucosa. Assuming the caloric densities of the SCFAs to be 3.4 kcal/g for acetate, 5.0 kcal/g for propionate, and 6.0 kcal/g for butyrate (44), the animals supplemented with intravenous or intracecal SCFAs would receive only 2% more nonprotein calories than the Control TPN group. It is unlikely that this minimal increase in caloric density for the SCFA groups would

References

1. Hughes CA, Dowling RH. Speed of onset of adaptive mucosal hypoplasia and hypofunction in the intestine of parenterally fed rats Clin Sci 1980:59:317–27.

2. Johnson LR, Copeland EM, Dudrick SJ, et al. Structural and hormonal alterations in the gastrointestinal tract of parenterally fed rats. Gastroenterology 1975;68:1177–83.
3. Levine GM, Deren JJ, Steiger E, et al. Role of oral intake in maintenance of gut mass and disaccharide activity. Gastroenterology 1974;67:975–82.
4. Goldstein RM, Hebiguchi T, Luk GD, et al. The effects of total parenteral nutrition on gastrointestinal growth and development. J Pediatr Surg 1985;20:785–91.
5. Nyman M, Asp NG. Fermentation of dietary fiber components in rat intestinal tract. Br J Nutr 1982;47:357–66.
6. Demigne C, Remesy C. Stimulation of absorption of volatile fatty acids and minerals in the cecum of rats adapted to a very high fiber diet. J Nutr 1985;115:53–60.
7. Cummings JH, Branch WJ. Fermentation and the production of short chain fatty acids in the human large intestine. In: Vahouny GB, Kritchevsky D, eds. Dietary fiber. Basic and clinical aspects. New York: Plenum Press, 1986:131–52.
8. McNeil NI, Cummings JH, James WPT. Short chain fatty acid absorption by the human large intestine. Gut 1978;19:919–22.
9. Ruppin H, Bar-Meir S, Soergel KH. Absorption of short chain fatty acids by the colon. Gastroenterology 1980;78:1500–7.
10. Roediger WEW, Moore P. Effect of short chain fatty acids on sodium absorption in isolated human colon perfused through the vascular bed. Dig Dis Sci 1981;26:100–6.
11. Tutton PJM, Barkla DH. Further studies on the effect of adenosine cyclic monophosphate derivatives on cell proliferation in jejunal crypts of rat. Clin Exp Pharmacol Physiol 1982;9:671–4.
12. Sakata T, Yajima T. Influence of short chain fatty acids on the epithelial cell division of the gastrointestinal tract. Q J Exp Physiol 1984;69:639–48.
13. Sakata T, Englehardt WV. Stimulating effect of short chain fatty acids on the epithelial cell proliferation in the rat large intestine. Comp Biochem Physiol 1983;74A:459–62.
14. Kripke SA, Fox AD, Berman JM, et al. Stimulation of intestinal mucosal growth with intracolonic infusion of short chain fatty acids. JPEN 1989;13:109–16.
15. Koruda MJ, Rolandelli RH, Settle RG, et al. The effect of parenteral nutrition supplemented with short chain fatty acids on adaptation to massive small bowel resection. Gastroenterology 1988;95:715–20.
16. Archibald PM. Nitrogen by the Kjeldahl method. In: Seligson D, ed. Standard methods of clinical chemistry. New York: Academic Press, 1959:91–2.
17. Koruda MJ, Rolandelli RH, Settle RG, et al. The effect of a pectin-supplemented elemental diet on intestinal adaptation to massive small bowel resection. JPEN 1986;10:343–50.
18. Burton K. A study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J 1965;62:315–23.
19. Giles KW, Myers A. An improved diphenylamine method for the estimation of deoxyribonucleic acid. Nature 1965;206:93.
20. Fleck A, Begg D. The estimation of ribonucleic acid using ultraviolet absorption measures. Biochim Biophys Acta 1965;108:333–9.
21. Lowry OH, Rosebrough NJ, Farr AL, et al. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265–75.
22. Floch J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipids from animal tissue. J Biol Chem 1957;226:497–509.
23. Armitage P. Statistical methods in medical research. New York: John Wiley, 1971.
24. Ford WDA, Boelhouwer RU, King WWK, et al. Total parenteral nutrition inhibits intestinal adaptive hyperplasia in young rats: reversal by feeding. Surgery 1984;96:527–34.
25. Morin CL, Ling V, Bornassa D. Small intestinal and colonic changes induced by a chemically defined diet. Dig Dis Sci 1980;25:123–8.
26. Jacobs LR. Effect of dietary fiber on mucosal growth and all proliferation in the small intestine of the rat: a comparison of oat, bran, pectin and guar with total fiber deprivation. Am J Clin Nutr 1983;37:954–60.
27. Tasman-Jones C, Owen RL, Jones AL. Semi-purified dietary fiber and small bowel morphology in rats. Dig Dis Sci 1982;27:519–24.
28. Bristol JB, Williamson RCN. Nutrition, operations and intestinal adaptation. JPEN 1988;12:299–309.
29. Altmann GG. Influence of bile and pancreatic secretions on the size of the intestinal villi in the rat. Am J Anat 1971;132:167–78.
30. Weser E, Heller R, Tawil T. Stimulation of mucosal growth in the rat ileum by bile and pancreatic secretions after jejunal resection. Gastroenterology 1977;73:524–9.
31. Al-Mukhtar MYT, Sagor GR, Ghati MA. The role of pancreatic secretico-biliary secretions in intestinal adaptation after resection and its relationship to enteroglucagon. Br J Surg 1983;70:398–400.
32. Bloom SR, Polak JM. The hormonal pattern of intestinal adaptation. Scand J Gastroenterol [Suppl] 1982;17:93–103.
33. Kvietys PR, Granger DN. Effects of volatile fatty acids on blood flow and oxygen uptake by the dog colon. Am J Physiol 1981;80:962–9.
34. Dowling RH. Small bowel adaptation and its regulation. Scand J Gastroenterol [Suppl] 1982;17:53–74.
35. Harada E, Kato S. Effect of short chain fatty acids on the secretory response of the ovine exocrine pancreas. Am J Physiol 1983;244:G284–90.
36. Holly DC, Evan JN. Secretion of insulin by the non-ruminant herbivore pancreas performed in vitro. J Anim Sci 1979;49:1021–9.
37. Remesy C, Demigne C, Chartier F. Origin and utilization of volatile fatty acids in the rat. Reprod Nutr Dev 1980;20:1339–49.
38. Cummings JH, Pomare EW, Branch WJ, et al. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut 1987;28:1221–7.
39. Kripke SA, Fox AD, Berman JM, et al. Inhibition of TPN-associated colonic atrophy with β-hydroxybutyrate. Surg Forum 1988;39:48–50.
40. Desmoulin F, Canioni P, Cozzone PJ. Glutamate-glutamine metabolism in the perfused rat liver: ^{13}C NMR study using 2-^{13}C-enriched acetate. FEBS Lett 1985;185:29–32.
41. Cross TA, Pahl C, Oberhansli R, et al. Ketogenesis in the living rat followed by ^{13}C NMR spectroscopy. Biochemistry 1984;23:6398–402.
42. Windmueller HG, Spaeth AE. Identification of ketone bodies and glutamine as the major respiratory fuels in vivo for postabsorptive rat small intestine. J Biol Chem 1978;253:69–76.
43. Hwang TL, O'Dwyer ST, Smith RJ, et al. Preservation of small bowel mucosa using glutamine-enriched parenteral nutrition. Surg Forum 1986;37:56–8.
44. Yang MG, Manoharen K, Mickelsen O. Nutritional contribution of volatile fatty acids from the cecum of rats. J Nutr 1970;100:545–50.