Analysis of the effects of food and of digestive secretions on the small intestine of the rat

1. Mucosal morphology and epithelial replacement

R. M. CLARKE¹, R. ECKNAUER, AND G. FEYERABEND

From the Departments of Clinical Chemistry and Surgery, University of Ulm, West Germany

SUMMARY A modified Roux-en-Y repositioning of rat small intestine was performed so that the proximal segment of bowel (A) received only bile and pancreatic secretions, the second (B) received food direct from the stomach, and these two segments drained into a third (C). Four to five weeks after operation, cell production was assessed by injection of vincristine into operated, sham-operated and unoperated rats, and counts of blocked metaphases were made on isolated microdissected crypts. Villus height, crypt depth, and the number of crypts per villus (crypt/villus ratio) were also measured. Most of segment A showed no significant differences from sham-operated intestine, although the normal proximo-distal gradient of villus height was abolished. At the distal end (near the anastomosis with segments B and C), crypt depth and cell production were increased. The villus height gradient in segment B was also abolished, although crypt depth and cell production were significantly increased, especially at the proximal end. Crypt/villus ratio was also increased. Segment C showed all the characteristics of small bowel promoted to a more proximal position: increased villus height, crypt depth and cell production. Increased crypt/villus ratio was also observed. These results are discussed in terms of the role of food and of digestive secretions in the control of mucosal morphology and epithelial replacement.

There have been numerous previous investigations of the influence of individual factors on small intestinal function and structure (Levin, 1969; Tilson and Wright, 1972; Dowling and Riecken, 1974), but these factors have usually been considered in isolation; there have been few attempts to identify the separate contributions of biliary and pancreatic secretions, of the bacterial flora, and of food, on intestinal morphology, bile salt metabolism and intestinal function. The experimental model was the rat, in which surgical rearrangement of the small intestine resulted in different parts of the bowel being exposed to bile and pancreatic secretions, to food, and to the combined action of both. This paper describes the morphological and epithelial cell kinetic changes which occur in this experimental model, and the findings will be used as a basis for the discussion of intestinal function in subsequent publications.

¹Present address and for correspondence: Professor Rufus Clarke, Faculty of Medicine, University of Newcastle, Newcastle, 2308 N.S.W., Australia.

Methods

Twenty-one male specified-pathogen-free rats (CHBB strain) weighing 180-200 g were caged in seven groups of three. They were allowed food (Altromin from Altrogge, Lage-Lippe, Germany) and drinking water *ad libitum* until 20 hours before operation, when food was withdrawn. They were anaesthetised with an intraperitoneal injection of 60 mg pentobarbitone sodium (Nembutal, Abbott)/kg body weight. One animal in each group was unoperated, while the second (sham-operated) underwent median laparotomy, transection, and reanastomosis of the bowel, 1 cm distal to the pylorus, and 20 cm distal to the ligament of Treitz. The operated animal had a median laparotomy and a modified Roux-en-Y operation in which the duodenum was transected 1 cm distal to the pylorus, and the distal cut end of the duodenum was closed off. The bowel was transected again 20 cm distal to the ligament of Treitz, and the distal end brought up and anastomosed to the proximal cut end of the duodenum. This segment of small bowel, which received gastric effluent, was

labelled segment B. A further 20 cm distally, the distal end of the segment which received biliary and pancreatic secretions (segment A) was anastomosed end-to-side, and the gut distal to this, which received the effluent from segments A and B, was labelled segment C (Fig. 1).

Sixteen hours after operation the animals were allowed access to 10% glucose solution for 24 hours, and thereafter received their accustomed diet. The animals in each cage were kept together for four to five weeks after operation, until they were investigated on the same day. To assess cell production, each rat was injected intraperitoneally with a solution of 1 mg vincristine sulphate (Oncovin, Lilly)/kg body weight between 08 30 and 09 30. Under pentobarbitone sodium anaesthesia, the small intestine was flushed out with a solution of 9 g sodium chloride/l, and then distended with fixative (absolute ethanol and glacial acetic acid, 3:1 v/v) to a pressure of 350

had unformed stools, and in two the stool was only partly formed. All other rats had stools of normal consistency. The rats with loose stools showed no other differences from the other operated rats. At necropsy, two rats (one operated and one sham-operated) were found to have partial intestinal obstruction at the site of the anastomosis, and measurements from these two rats were not included.

POSTOPERATIVE WEIGHT GAIN

Operated rats gained 113 ± 15 g (mean $\pm$ SD) post-operatively, significantly ($P < 0.001$) less than sham-operated (157 ± 17 g), which in turn gained significantly less ($P < 0.05$) than the unoperated rats (193 ± 36 g).

VILLUS HEIGHT

Villus height declined steadily along the length of the gut in unoperated rats (Fig. 2), and was not significantly different in sham-operated rats, except at

Fig. 1 Disposition of the small intestine. Black spots indicate the sites at which intestinal wall was examined.

mm of fixative (Clarke, 1974) two hours after injection of vincristine. After fixation for 24 hours, the intestine was stored in 70% ethanol, and specimens of gut wall from the sites illustrated in Fig. 1 were treated in bulk by the Feulgen reaction. Measurements of villus height and crypt depth (10 per specimen), crypt/villus ratio, and vincristine-metaphases per crypt (20 crypts per specimen) were made (Clarke, 1974). Student's *t* test was used to assess the significance of differences between groups.

Results

CONDITION ONE MONTH AFTER OPERATION

All the animals appeared healthy. One operated rat

Fig. 2 Mucosal morphology and epithelial cell production in unoperated and sham-operated rats. Abscissa: distance along intestine (expressed as a percentage of the total small intestinal length); ordinate (from above downwards): villus height (μ m); crypt depth (μ m); crypt/villus ratio; counts of vincristine-metaphases/crypt 2 h. The interrupted vertical lines represent the sites of intestinal anastomoses in the sham-operated rats. Points represent the mean values from six rats in each group; bars represent 1 standard error of the mean; $\circ$ unoperated; $\bullet$ sham operated. Significant differences between groups: + $P < 0.05$; ++ $P < 0.01$.

the site 3% (approx. 3 cm) below the second anastomosis, where villus height was significantly greater ($P < 0.05$). In the operated rats (Fig. 3), in segment A (exposed to bile and pancreatic secretions), villus height was not significantly altered but the normal proximo-distal gradient of villus height appeared to be abolished. Similarly in segment B (food and gastric juice) there were no significant differences, and the gradient was again abolished. In segment C (food and digestive juices) villus height was significantly greater in operated intestines than in sham-operated ones ($P < 0.05$).

CRYPT DEPTH

There were no significant differences between unoperated and sham-operated intestines (Fig. 2). In the operated intestines (Fig. 3), crypt depth was significantly increased in the last specimen from segment A, and at all sites distal to this.

CRYPT/VILLUS RATIO

Crypt/villus ratio (the number of crypts per villus)

Fig 3 Mucosal morphology and epithelial cell production in operated rats. Abscissa and ordinate: as Fig. 2. The interrupted vertical lines represent the sites of intestinal anastomoses in the operated rats. Points represent the mean values from five operated rats; bars represent 1 standard error of the mean. Stippled area represents range of ± 1 SEM in sham-operated rats (from Fig. 2). Significant differences between operated and sham-operated groups: + $P < 0.05$; ++ $P < 0.01$; +++ $P < 0.001$.

was significantly different in unoperated and sham-operated intestines at only two sites (Fig. 2), and the higher ratios in the unoperated animals were perhaps associated with their greater body weight (Clarke, 1972). In operated guts, in spite of lower body weights, crypt/villus ratios were significantly higher than in sham-operated guts at two of the three sites examined in segment B (Fig. 3), although the differences between operated and unoperated guts were not significant. At three of the four sites examined in segment C, crypt/villus ratios were significantly higher than in sham-operated or unoperated animals.

VINCRI-STINE-METAPHASE COUNTS PER CRYPT

Vincristine failed to block mitosis in two animals, which were excluded. There were no significant differences between unoperated and sham-operated intestines, whose crypts contained a mean of 40-50 vincristine-metaphases after treatment for two hours with vincristine (Fig. 2). In the operated intestines, the counts were not different from those in sham-operated crypts at the upper end of segment A, but showed a rise at the most distal site in this segment, 3% (approx. 3 cm) above the anastomosis with the food-containing gut. In segment B, the counts of vincristine-metaphases were significantly higher ($P < 0.001$), particularly at the upper end where this segment received the gastric effluent. In segment C the counts were also significantly higher than those in sham-operated intestines, all the way to the terminal ileum ($P < 0.01$ for each of the four sites).

Discussion

The method of assessing cell production differs from that in previous investigations (Clarke, 1974, 1976) in that counts were made of the number of blocked metaphases which had accumulated after a two hour treatment with vincristine, instead of counts of metaphases after different durations of treatment with vincristine. It is thus no longer strictly accurate to speak of 'cell production rate', although the counts of vincristine-metaphases in unoperated animals are consistent with those in the earlier work, in which the regression line drawn through the counts of blocked metaphases plotted against time was always found to pass near the origin. In this investigation, emphasis has been placed on comparison of counts from crypts from corresponding sites in the guts of animals subjected to different surgical treatments, and it is thus valid to make comparisons between the groups of animals, remembering that, as in most other published work in this field, the rate of cell production is not being measured.

In segment A, which was deprived of luminal food, but retained biliary and pancreatic secretions, villus height was maintained at normal levels, which confirms the observations of Altmann and Leblond (1970). The maintenance of vincristine-metaphase counts at control levels in the absence of food implies that bile and pancreatic secretions may exert a stimulatory effect, although, if this were true, one might expect the effect to diminish as distance from the duodenal papilla increased, whereas in fact the opposite occurred. The increased epithelial replacement observed near the anastomosis with segments B and C was probably due to reflux of gut contents.

Segment C showed all the characteristics of gut which has been advanced to a more proximal position (increased villus height, crypt depth, and cell production—Dowling and Booth, 1967; Hanson and Osborne, 1971; Gleeson *et al.*, 1972), and it is clear that these changes are due not to removal of gut tissue, but to an alteration of the functional status of the affected part of the gut. The crypt/villus ratio was also increased; this is the first report of such an observation after surgical interference with the gut. It was not observed 10 days after a similar operation (Clarke, 1974), when an increase in crypt cell production rate was already clearly evident. Presumably, these are both adaptations which increase the rate of production of cells per villus, the more rapid response being an increase in cell production per unit (crypt), followed by a slower increase in the number of units themselves. The increase in crypt number is probably real; it has been shown to occur in ageing (Clarke, 1972), and the alternative explanation, that villi decrease in number, is not supported by the observations of Forrester (1972).

Segment B did not behave in the same way, since, although there was a considerable increase in cell production and crypt depth, the villus hypertrophy characteristic of gut which has been advanced to a more proximal location was absent. This absence could be ascribed to lack of biliary or pancreatic secretions, or (if the upper part of the segment was perhaps presented with molecules too large for the mucosal enzymes to digest) to reduced 'luminal nutrition' of 'functional demand', but this is unlikely in view of the increased cell production. A more likely explanation is that the low pH of the gastric effluent damaged the intestinal mucosa in the same way as near a gastroenterostomy (Creamer, 1964), and this might also explain the absence of villus hypertrophy, since the tendency in cases of epithelial damage is towards villus atrophy (Townley *et al.*, 1964; Philipson, 1975). Villus epithelial damage at the upper end of segment B could also explain the counts of vincristine-metaphases seen at this site,

which are strikingly higher than those elsewhere in segments B and C.

Some of the changes could be due to alterations in output of gastrointestinal polypeptide hormones, but our present inadequate knowledge of the physiological role of these hormones does not allow useful discussion of this point; in any case, these hormones are more likely to cause systemic effects rather than the local changes described here.

The original aim of the experiment was to attempt to separate bile and pancreatic secretions, and food, as influences on mucosal morphology and epithelial replacement. The defect of the model is the possibility that gastric acid damaged the intestinal mucosa, and thus the observations in segment B cannot necessarily be ascribed to food alone. The relationship of these morphological and cell kinetic changes to luminal enzyme activity and bile salt concentrations, activity of so-called brush border enzymes, and intestinal absorption, will be described elsewhere.

This work was performed in the 'Sonderforschungsbereich 112' (Cell system physiology, project E4) financed by the Deutsche Forschungsgemeinschaft, Bad Godesberg, whose support is gratefully acknowledged. We thank Frau I. Silbereis for technical assistance.

References

- Altmann, G. G., and Leblond, C. P. (1970). Factors influencing villus size in the small intestine of adult rats as revealed by transposition of intestinal segments. *American Journal of Anatomy*, **127**, 15-36.
- Clarke, R. M. (1972). The effect of growth and of fasting on the number of villi and crypts in the small intestine of the albino rat. *Journal of Anatomy*, **112**, 27-33.
- Clarke, R. M. (1974). Control of intestinal epithelial replacement: lack of evidence for a tissue-specific blood-borne factor. *Cell and Tissue Kinetics*, **7**, 241-250.
- Clarke, R. M. (1976). Evidence for both luminal and systemic factors in the control of rat intestinal epithelial replacement. *Clinical Science and Molecular Medicine*, **50**, 139-144.
- Creamer, B. (1964). Small-intestinal mucosal dynamics and the environment. *British Medical Journal*, **2**, 1373-1375.
- Dowling, R. H., and Booth, C. C. (1967). Structural and functional changes following small intestinal resection in the rat. *Clinical Science*, **32**, 139-149.
- Dowling, R. H., and Riecken, E. O. (eds) (1974). *Intestinal Adaptation: Proceedings of an International Conference on the Anatomy, Physiology and Biochemistry of Intestinal Absorption*. Schattauer: Stuttgart.
- Forrester, J. M. (1972). The number of villi in rat's jejunum and ileum; effect of normal growth, partial enterectomy, and tube feeding. *Journal of Anatomy*, **111**, 283-291.
- Gleeson, M. H., Cullen, J., and Dowling, R. H. (1972). Intestinal structure and function after small bowel by-pass in the rat. *Clinical Science*, **43**, 731-742.
- Hanson, W. R., and Osborne, J. W. (1971). Epithelial cell

- kinetics in the small intestine of the rat 60 days after resection of 70% of the ileum and jejunum. *Gastroenterology*, **60**, 1087-1097.
- Levin, R. J. (1969). The effects of hormones on the absorptive, metabolic and digestive functions of the small intestine. *Journal of Endocrinology*, **45**, 315-348.
- Philipson, B. (1975). Morphology in the cat ileal mucosa following construction of an ileal reservoir or transposition of patches to different locations. *Scandinavian Journal of Gastroenterology*, **10**, 369-377.
- Tilson, M. D., and Wright, H. K. (1972). Adaptational changes in the ileum following jejunectomy. In *Regulation of Organ and Tissue Growth*, pp. 257-270. Edited by R. J. Goss. Academic Press: New York.
- Townley, R. R. W., Cass, M. H., and Anderson, C. M. (1964). Small intestinal mucosal patterns of coeliac disease and idiopathic steatorrhoea seen in other situations. *Gut*, **5**, 51-55.

The October 1976 Issue

THE OCTOBER 1976 ISSUE CONTAINS THE FOLLOWING PAPERS

- Mucosal gastrin concentration, molecular forms of gastrin, number and ultrastructure of G-cells in patients with duodenal ulcer W. CREUTZFELDT, R. ARNOLD, C. CREUTZFELDT, AND N. S. TRACK
- Identification of the D₁-cell as the source of human pancreatic polypeptide (HPP) PH. HEITZ, JULIA M. POLAK, S. R. BLOOM, AND A. G. E. PEARSE
- Coeliac disease and dermatitis herpetiformis: further studies of their relationship B. B. SCOTT, SANDRA YOUNG, S. M. RAJAH, JANET MARKS, AND M. S. LOSOWSKY
- Blood pH: a test for assessment of severity in proctocolitis R. CAPRILLI, P. VERNIA, O. COLANERI, AND A. TORSOLI
- Analysis of the lympho-plasmacytic infiltrate in Crohn's disease with special reference to identification of lymphocyte-subpopulations S. G. M. MEUWISSEN, THEA M. FELTKAMP-VROOM, A. BRUTEL DE LA RIVIÈRE, A. E. G. KR. VON DEM BORNE, AND G. N. TYTGAT
- Contrasting features and responses to treatment of severe chronic active liver disease with and without hepatitis B_s antigen S. W. SCHALM, W. H. J. SUMMERSKILL, G. L. GITNICK, AND LILA R. ELVEBACK
- Demonstration of an intracellular copper-binding protein by orcein staining in long-standing cholestatic liver diseases M. SALASPURO AND P. SIPONEN
- Morphological types of hepatocellular hyalin in Indian childhood cirrhosis: an ultrastructural study N. C. NAYAK AND S. ROY
- Arterial blood gas tensions during upper gastrointestinal endoscopy P. J. WHORWELL, C. L. SMITH, AND K. J. FOSTER
- Progress report* Cholangiography in the jaundiced patient ELWYN ELIAS
- The British Society of Gastroenterology
- British Society for Digestive Endoscopy
- Notes and activities
- Books

Copies are still available and may be obtained from the PUBLISHING MANAGER,
BRITISH MEDICAL ASSOCIATION, TAVISTOCK SQUARE, LONDON, WC1H 9JR, price £2.00, including postage