Enhancement of ileal adaptation by prednisolone after proximal small bowel resection in the rat

J. SCOTT¹, R. M. BATT, AND T. J. PETERS

From the Department of Medicine, Royal Postgraduate Medical School, London, and Division of Clinical Cell Biology, Clinical Research Centre, Harrow

SUMMARY The effect of prednisolone on the adapted ileum of the rat after jejunal resection was examined. Three weeks after 50% proximal small bowel resection animals were fed pharmacological doses of soluble prednisolone (0.75 mg/kg/day) over a one week period, and killed at four weeks. Animals treated with prednisolone showed significant increases in brush border α -glucosidase, leucyl-2-naphthylamidase and γ -glutamyl transferase ($P < 0.01$) per unit length of intestine compared with resection alone and transection reanastomosis control groups. This increase was the result of a significant enhancement ($P < 0.01$) of brush border digestive enzyme activity per milligram of epithelial cell DNA—that is, per enterocyte—and was associated with a similar increase in enterocyte RNA content. In contrast, the activities of lysosomal and mitochondrial marker enzymes per milligram of DNA were similar in each group. Cell proliferation was not further stimulated by prednisolone. Thus prednisolone can selectively enhance brush border digestive capacity after intestinal resection without increasing cell proliferation. The increase in enterocyte RNA suggests that enzyme induction may be the mechanism of this effect.

In a number of species, including man, after partial resection of the small bowel the remaining small intestine undergoes an adaptive response (Booth *et al.*, 1959; Porus, 1965; Dowling and Booth, 1967; Weinstein *et al.*, 1969; Weser and Hernandez, 1971). The dominant component of this response consists of a large increase in the number of absorptive cells. This response results in small bowel dilatation, villus hypertrophy, more rapid cell migration, and, despite relative immaturity of the individual enterocyte, enhanced digestive enzyme activity and absorption per unit length of intestine. The mechanism of this response is imperfectly understood, but exogenous nutrients, endogenous pancreatic and biliary secretions, and the trophic effects of enteric and possibly other hormones are probably important (Gleeson *et al.*, 1971; Gleeson *et al.*, 1972b; Altman, 1974; Riecken *et al.*, 1974; Menge *et al.*, 1975; Jacobs *et al.*, 1976; Barros D'Sa *et al.*, 1977; Muller *et al.*, 1977; Hughes *et al.*, 1978; Weser, 1978; Williamson *et al.*, 1978). Factors of subsidiary importance may include

mechanical, bacterial, and neurovascular influences (Williamson, 1978).

Glucocorticoids have several different actions on the intestine, including enhancement of the digestive and absorptive function of both the normal jejunum and ileum, without increasing the number of absorptive cells (Clark, 1959; Banerjee and Varma, 1966; Charney *et al.*, 1975; Batt and Peters, 1976b; Loeb, 1976; Lebenthal, 1977; Field, 1978; Scott *et al.*, 1978). Indeed, depending on the glucocorticoid used, the dose and length of administration, the predominant effect of glucocorticoids on cell proliferation in the rat small intestine appears to be inhibitory (Wall and Peters, 1971; Wright *et al.*, 1978; Scott *et al.*, 1978). Thus glucocorticoids enhance the digestive and absorptive capacity of the rat small intestine by increasing brush border enzyme activity and absorptive capacity of the existing population of enterocytes.

In the present study the effect of prednisolone on the adapted ileum after jejunal resection in the rat has been examined. The results indicate that prednisolone enhances the adaptive response to small intestinal resection in the rat by adding a functional hypertrophy to an adaptive hyperplasia.

¹Address for correspondence: Dr. J. Scott, RPMS, Du Cane Road, London W12 0HS.

Methods

EXPERIMENTAL DESIGN; ANIMALS AND DIET

Adult male Wistar rats of mean body weight 218 ± 30 g ($\pm$ SD) were maintained in individual cages and fed *ad libitum* on a no. 41B diet (Oxoid Ltd., London) with free access to water. Only animals showing an increase in weight over the next four days were used in the experiment. The rats were randomised into the following three groups of eight animals each: (1) 50% proximal small intestinal resection; (2) 50% proximal small intestinal resection for prednisolone feeding; (3) transection, reanastomosis controls. Control groups which received prednisolone or received prednisolone and had a transection, reanastomosis performed were not studied. Transection, reanastomosis does not alter net intestinal function from normal (Hanson *et al.*, 1977); and we have previously shown that prednisolone fed for one week in the same dose as here enhances the function of the normal rat ileum (Batt and Peters, 1976b).

SURGICAL PROCEDURE

Animals were starved overnight before surgery and anaesthetised with ether. Resected animals had 50 cm of proximal small intestine removed from a point 5 cm distal to the ligament of Treitz. In the control animals the small intestine was transected and reanastomosed at 55 cm distal to the ligament of Treitz.

POST-OPERATIVE FEEDING

After surgery the animals were maintained for 36 hours on 5% glucose solution containing penicillin (1 g/l) and streptomycin (0.6 g/l). Animals were pair-fed down to the transection, reanastomosis control group: all animals received and ate about 25g per day of a 41B mash diet mixed with an equal volume of water. Free access to water was allowed. Prednisolone-21-phosphate (Merck, Sharp and Dohme Ltd., Hoddesdon, Herts., UK) was started in the designated group three weeks after surgery in a daily dose of 0.75 mg/kg body weight and continued until the animals were killed seven days later. The drug was dissolved in water and thoroughly mixed with the no. 41B mash. Feeding was at 6.00 p.m. each evening and animals were not starved before being killed. A 12 hour light/dark pattern was maintained with a room temperature of 21°C.

PREPARATION OF SPECIMENS

Animals were killed at 28 days. The remaining small bowel was rapidly removed, patency of the anastomosis verified, and the lumen washed in ice-cold Krebs Ringer bicarbonate buffer. The length of the small intestine was measured with a 5g weight tied to

the distal end. A 22 cm segment was taken from a point 5cm distal to the anastomosis. From each end of the excised segment a 1 cm segment was taken, cut open longitudinally, pinned flat on cork, and fixed in buffered 10% formal saline for histology and autoradiography. The remaining 20 cm was blotted and weighed. Epithelial cells from the villi — that is, enterocytes — were prepared from this segment: its remnant was homogenised so that total enzyme activity could be calculated and expressed per length of intestine as well as per mg of DNA in the epithelial cell preparation. From this data the percentage yield of epithelial cells was determined, so that the total amount of RNA, protein, and DNA in the total villus epithelial cell population could be calculated and expressed per cm intestine (Batt and Peters, 1976a). Specimens were stored at -20°C .

BIOCHEMICAL STUDIES; ENZYME ACTIVITIES

Brush border α -glucosidase and leucyl-2-naphthylamidase, lysosomal N-acetyl- β -glucosaminidase and mitochondrial cytochrome oxidase were assayed in the epithelial cell preparations as described by Batt and Peters (1976b). γ -Glutamyl transferase was assayed by the method of Seymour and Peters (1977). Protein was determined by the method of Schacterle and Pollack (1973), and DNA and RNA by the method of Prasad *et al.* (1972) with calf thymus DNA (Sigma type V) and calf liver RNA (Sigma type IV) used as standards.

INTESTINAL STRUCTURE;

LIGHT MICROSCOPY

After fixation specimens for histology were prepared, and villus height, crypt depth, and epithelial cell density measured as previously described (Batt and Peters, 1976b).

CELL POPULATION KINETICS

Rats were injected with $125 \mu\text{Ci } ^3\text{H}$ methylthymidine (15,000 – 30,000 mCi/mmol; The Radiochemical Centre) and killed at four or 23 hours. Enterocyte migration rate and cell turnover time were determined as described previously by Gleeson *et al.* (1972a) and modified by Batt and Peters (1976b).

STATISTICAL METHODS

Two-way analysis of variance and Duncan's multiple range test were applied to the data (Edwards, 1968).

Results

NUTRITIONAL STATE

(Table 1) Calorie intake was similar in the three groups of rats during each of the four weeks after surgery. Gain in total body weight revealed no

Table 1 Calorie intake, weight gain, and bowel weight (mean $\pm$ SEM) in rats with resection plus prednisolone, resection alone, and transection, reanastomosis controls.

	Control		Resection alone		Resection + prednisolone
Calorie intake (MJ) per month	11.5 $\pm$ 2		11.3 $\pm$ 1		11.7 $\pm$ 2
Calorie intake (MJ) fourth week	2.9 $\pm$ 1	NS	2.9 $\pm$ 1	NS	3.0 $\pm$ 2
Weight gain total (g)	63 $\pm$ 8	NS	86 $\pm$ 6	NS	51 $\pm$ 6
Weight gain (g) fourth week	16 $\pm$ 4	NS	13 $\pm$ 5	NS	12 $\pm$ 2
Gut weight (mg/cm)	113 $\pm$ 5		167 $\pm$ 8		183 $\pm$ 9
		P < 0.01		NS	

NS: Not significant.

differences between the groups over the four weeks. The weight of the ileal remnant was similar in both resected groups of rats, but for each group was significantly greater than in the sham operated controls.

BIOCHEMICAL STUDIES

Brush border enzymes (Fig. 1)

Both resected groups had significantly enhanced activities of brush border enzymes per unit length of intestine compared with the transection, reanastomosis control group. Prednisolone, however, produced an additional increase in the activities of these brush border enzymes per length of intestine. The increase produced by prednisolone was due to a significant increase in brush border enzyme activity expressed per milligram of epithelial cell DNA—that is, per enterocyte—compared with the other two groups. Resection alone, in contrast, produced no alteration in the activities of brush border enzymes per epithelial cell.

Lysosomal and mitochondrial enzymes (Fig. 2)

In contrast with the brush border enzymes, the activities of lysosomal and mitochondrial enzymes were similar per unit length of intestine in both resected groups, with the expected increase in activity compared with the transection, reanastomosis control group. Enzyme activity per milligram of epithelial cell DNA was similar in all three groups.

RNA, DNA, and protein (Fig. 3)

As with the brush border enzyme activities the RNA per unit length of intestine was increased in both resected groups, and, similarly, prednisolone had produced an additional significant increase in RNA per length of intestine. The RNA content in the prednisolone treated group per milligram epithelial cell DNA was greater than either of the other groups.

In contrast, the total enterocyte protein content per length of intestine was similar in both resected groups, but increased compared with the control group. The enterocyte protein content per milligram

Fig 1 Brush border enzyme activity expressed per cm of intestine and per mg of enterocyte DNA. Data from eight rats expressed as mean $\pm$ SEM. Control group unshaded, resection alone stippled, and resection plus prednisolone hatched. a = not significant; b = P < 0.01

Fig. 2 Mitochondrial cytochrome oxidase and lysosomal N-acetyl- β -glucosaminidase activity expressed per cm of intestine and per mg enterocyte DNA. Data, shading, and symbols as in Fig. 1

of epithelial cell DNA was similar in all three groups. The total epithelial cell DNA per length of intestine was similar in both resected groups, but, as expected, increased compared with the control group.

INTESTINAL STRUCTURE

Macroscopic appearances

Both the prednisolone treated resected group and group with resection alone showed obvious adaption with enlargement and dilatation compared with the transection, reanastomosis controls.

Light microscopy (Table 2)

Villus height and crypt depth were significantly increased in both resection groups compared with the transection, reanastomosis control group; the increase was greatest in the resection group which had not received prednisolone, although the differences between the resection groups were not significant. The epithelial cell density (number of cells per 200 μ m length mid-villus) was slightly greater in both resection groups, but not significantly different from the control group.

Fig. 3 Enterocyte RNA, DNA, and protein per cm gut; RNA and protein per mg enterocyte DNA. Data, shading, and symbols as in Fig. 1.

Table 2 *Histological measurement (mean $\pm$ SEM) of villus height, crypt depth, and epithelial cell density in rats with resection plus prednisolone, resection alone, and transection, reanastomosis controls*

	Control		Resection alone		Resection + prednisolone
Villus height (m μ)	278 $\pm$ 12		478 $\pm$ 12	NS	467 $\pm$ 6
		P < 0.01			
Crypt depth (m μ)	142 $\pm$ 4		173 $\pm$ 3	NS	167 $\pm$ 5
		P < 0.01			
Epithelial cell density (no./200 m μ length midvillus)	36 $\pm$ 0.6	NS	38 $\pm$ 0.5	NS	38 $\pm$ 0.4

NS: Not significant.

Cell population kinetics (Table 3)

The time for a cell to migrate the length of an adapted villus of increased height (epithelial cell turnover time) was significantly increased in both resection groups. This time was slightly but significantly greater in the prednisolone treated group. The migration rate of epithelial cells along the villus was significantly increased in both resection groups. The rate in the resected group which had not received prednisolone was marginally, but significantly faster than in the prednisolone treated group.

Discussion

In this study the effects of pharmacological doses of soluble prednisolone on the adapted ileum after jejunal resection have been examined. The results show that the enhanced digestive capacity of the small intestine remaining after jejunal resection can be additionally enhanced by prednisolone. This increase was the result of a selective increase in the activity of the brush border digestive enzymes of the enterocytes. It was not associated with a further increase in cell proliferation and is therefore likely to be because of a direct effect of prednisolone on the adult absorptive cell.

In previous studies we have shown that oral prednisolone administered for one week and one month selectively enhances brush border α -glucosidase and leucyl-2-naphthylamidase activity and increases D-galactose absorption in the jejunum and ileum of the normal rat (Batt and Peters, 1976b; Scott *et al.*, 1978). This was an effect on the individual enterocyte and not due to increased cellularity. In the present study prednisolone similarly enhanced the digestive function of the adapted ileum without further

stimulating cell proliferation. Indeed, the large dose of prednisolone used (equivalent to more than 40mg per day in 70 kg. man) produced a small decrease in the adaptive hyperplasia. This inhibitory effect of glucocorticoids on cell proliferation can be dissociated from the stimulatory effect on cell function; it appears to depend on the glucocorticoid used, the dose and period of administration (Wall and Peters, 1971; Scott *et al.*, 1978; Wright *et al.*, 1978). In contrast, the adapted intestine has increased function due to cellular hyperplasia (Gleeson *et al.*, 1972a; b; McCarthy and Kim, 1973; Weser and Hernandez, 1971). Thus it can be said that glucocorticoids add a functional hypertrophy to an adaptive hyperplasia.

Glucocorticoids act principally by binding to specific cytoplasmic receptor proteins which are present in the enterocyte (Pressley and Funder, 1975; Gelehrter, 1976a b; Yamamoto and Alberts, 1976). The activated steroid and receptor complex is then rapidly translocated to the nucleus where binding to the chromatin occurs. Template activity is enhanced and specific messenger RNAs for inducible enzymes are produced, thus leading to enhanced enzyme synthesis. In the present study the selective increase in brush border, but not of lysosomal or mitochondrial enzymes or of total enterocyte protein, and the large increase in epithelial cell RNA suggest that such a mechanism may have been operative in increasing the digestive capacity of the increased enterocyte population. This view is additionally supported by the relationship between brush border enzyme activity and epithelial cell RNA—that is, brush border enzyme activity: RNA content—which was similar in the three groups. Furthermore, in the rat intestine prednisolone significantly increases RNA associated with the rough endoplasmic

Table 3 *Migration rate and villus transit time of epithelial cells (mean $\pm$ SEM) in rats with resection plus prednisolone, resection alone, and transection, reanastomosis controls*

	Control		Resection alone		Resection + prednisolone
Migration rate (m μ /h)	11 $\pm$ 0.4		17 $\pm$ 0.2	P < 0.05	14 $\pm$ 0.4
		P < 0.05			
Villus transit time (h)	23 $\pm$ 0.7		29 $\pm$ 0.4	P < 0.05	31 $\pm$ 0.4
		P < 0.05			

reticulum—that is, membrane bound ribosomes and associated RNA—and significantly increases ^{14}C -tryosine labelling of brush border proteins (Scott *et al.*, 1977; Batt *et al.*, 1978). It therefore appears likely that prednisolone is inducing the synthesis of brush border enzymes and transporters and could be acting by harnessing an underlying physiological mechanism. Other mechanisms such as alteration in RNA and enzyme degradation cannot, however, be excluded at present.

In conclusion, prednisolone enhances the adaptive response to intestinal resection by increasing the functional capacity of the already markedly increased enterocyte population. If these observations can be extended to man, prednisolone may be of short-term value in the short bowel syndrome.

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