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Changes in structure and peptidase activity of rat small intestine induced by prednisolone

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SUMMARY Chronic dietary administration of prednisolone to the adult rat caused alteration in morphology and enzyme activity of the small intestinal mucosa. Reduction of villous height, crypt depth, and surface epithelial cell height was accompanied by a significant increase in the specific activity of several jejunal peptidases.

The epithelial damage seen in the small intestine of patients with coeliac disease is related to the dietary ingestion of gluten. A deficiency of a peptidase capable of detoxifying gluten may be the underlying defect (Frazer, 1956). The intestinal mucosal damage responds to the dietary exclusion of gluten. Wall, Douglas, Booth, and Pearse (1970a) successfully used prednisolone to treat adults with coeliac disease, and furthermore showed a return of peptidase activity to normal in the regenerated mucosa despite the continued ingestion of gluten. This study describes the morphological and enzymic effect of prednisolone in pharmacological doses on the small intestine of the normal adult rat.

Methods

Eight female Wistar albino rats weighing 180-200 g were fed prednisolone-21-phosphate in a daily dose of 0·15 mg mixed with a standard diet (41B, E. Dixon and Sons, Ltd, Middlesex, England) taken *ad libitum*. Control animals were of similar weight and received the same diet. After four weeks animals were fasted overnight, killed by cervical dislocation, and the small intestine was removed.

HISTOLOGY

A 1 cm segment immediately distal to the duodenojejunal junction and immediately proximal to the ileocaecal junction was opened, orientated on flat paper, fixed in buffered 10% formal saline, dehydrated, and embedded in paraffin. Sections 4μ thick were stained with both haematoxylin and eosin and PAS. Crypt depth (muscularis mucosae to crypt mouth) and villous height (crypt mouth to villous tip) were measured in 20 consecutive well orientated areas as previously described (Wall, Middleton, Pearse, and Booth, 1970b). Epithelial cell height was assessed by measuring five adjacent cells on each side of the middle third of 10 consecutive well orientated villi.

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ELECTRON MICROSCOPY

Specimens of intestine, approximately 1 cm in size, were immediately fixed for one hour in ice-cold phosphate-buffered osmium tetroxide, dehydrated, and embedded in Araldite epoxy resin. Epithelium from the middle third of the villus was sectioned at 600 Å, stained with uranyl acetate and lead citrate, and examined with an EM 6B electron microscope.

HISTOCHEMISTRY

Pieces of upper jejunum and lower ileum were orientated against a cube of rat liver, snap frozen, and stored in liquid nitrogen. Further pieces were fixed in buffered formol calcium at 4°C for 12 hours, washed and stored in gum sucrose at 4°C. The activity of the following enzyme reactions was compared by simultaneous incubation of 6 μ cryostat sections from control and treated rats: alkaline phosphatase, L-leucyl-βnaphthylamidase, E-600 resistant esterase, succinic dehydrogenase, NAD diaphorase, monoamine oxidase, glucose-6-phosphatase, and non-specific esterase by methods

described by Pearse (1968). The intensity of the reaction product was visually compared by two observers in control and treated tissues.

ENZYMOLOGY

Immediately after sacrifice the upper 15 cm of jejunum was washed with ice-cold 0·15 M NaCl and everted over a metal rod. The mucosa was scraped off with a glass slide and homogenized for 30 seconds in 10 volumes of ice-cold distilled water in an MSE homogenizer. The homogenate was then centrifuged at 3,000 *g* for 30 min and aliquots of the supernatant were stored at −25°C.

Peptidase activity was assayed by the automated kinetic method of Lenard, Johnson, Hyman, and Hess (1965) modified by Peters (1969), at a substrate concentration of 0·2 mM in 0·067M phosphate buffer at 37°C. Details of *p*H optima and appropriate cofactors are listed in Table I. The hydrolysis of 1 micromole peptide per hour represents 1 unit of activity. Activities of the lysosomal enzymes aryl sulphatase and pteroylpolyglutamate hydrolase were measured by the methods of Roy (1953) and Hoffbrand and Peters (1969) respectively. The activity of the brush border enzyme L-leucyl-βnaphthylamidase was measured by the method of Goldbarg and Rutenburg (1958). Protein estimations were by the method of Lowry, Rosebrough, Farr, and Randell (1951). Bovine serum albumin (Armour) was the standard.

Peptide	*pH Optimum*	*Activator*
Glycl-glycine	7·4	0·2mM Co^{2+}
L leucyl-l leucine	8·0	0·2mM Mn^{2+}
L prolyl-glycine	8·4[1]	0·2mM Mn^{2+}
Glycyl-glycyl-glycine	7·4	0·1mM EDTA
L leucyl-glycyl-glycine	7·4	0·1mM EDTA

Table I *Conditions for peptidase assay*

[1]*p*H optimum > 9·0

Results

HISTOLOGY

The results are shown in Table II and Figure 1. A significant decrease of villous height and crypt depth was found in both jejunum and ileum of treated rats. Surface cell height was reduced significantly in the ileum and to a borderline degree of statistical significance in the jejunum. The thickness of the muscle coat was not quantified.

ELECTRON MICROSCOPY

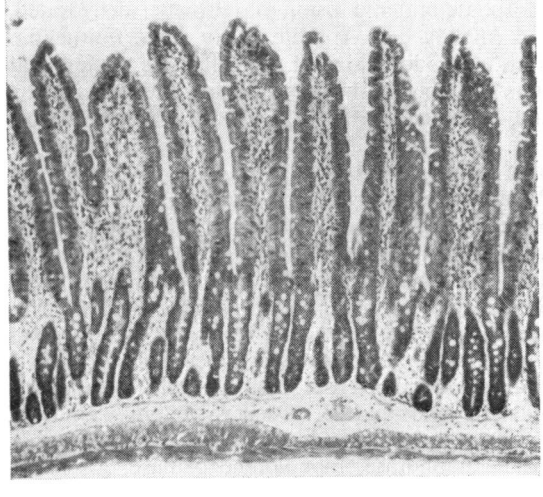
No abnormalities were observed, and in particular no increase of endoplasmic reticulum or of microvillous size was seen.

HISTOCHEMISTRY

Only the leucyl-βnaphthylamidase reaction showed a change. A clear-cut increase in activity was demonstrated in both jejunum and ileum of the treated animals, although the spatial distribution of the enzyme was normal. The change was best seen in postfixed tissues, but was also observed in formalin prefixed tissues.

ENZYME STUDIES

A significant increase of both brush-border and soluble peptidase activity was seen in the treated



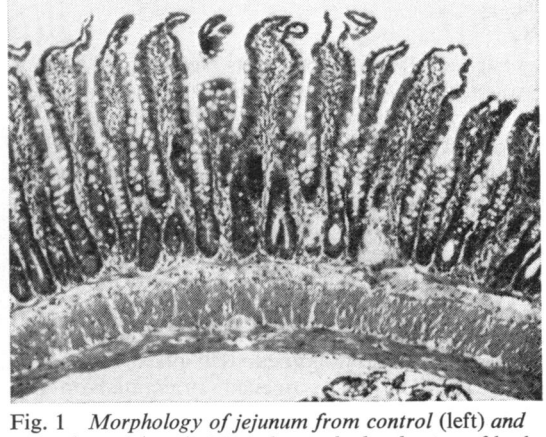


Fig. 1 *Morphology of jejunum from control* (left) *and treated rats* (above). *Note the marked reduction of both villous height and crypt depth. Haematoxylin and eosin. Original magnification* × 75.

	Total Mucosal Thickness	*Villous Height*	*Crypt Depth*	*Epithelial Cell Height*
Jejunum				
Control	728 ± 26	475 ± 26	254 ± 20	32·1 ± 1·1
Treated	466 ± 24	291 ± 17	175 ± 16	28·3 ± 1·3
Statistical significance	$P < 0.001$	$P < 0.001$	$P = 0.01$	$P = 0.05$
Ileum				
Control	624 ± 24	335 ± 14	289 ± 20	24·9 ± 0·7
Treated	339 ± 30	154 ± 10	189 ± 32	19·2 ± 0·5
Statistical significance	$P = 0.001$	$P = 0.001$	$P < 0.01$	$P < 0.001$

Table II *Histological measurements of jejunum and ileum*[1]

[1]Results are expressed in microns and are the means ± SEM

Substrate	*Controls*	*Treated*	*Statistical Significance*
Glycyl-glycine	1·91 ± 0·10	3·52 ± 0·30	$P = 0.002$
L leucyl-leucine	22·1 ± 1·2	47·0 ± 4·4	$P < 0.001$
L prolyl-glycine	0·54 ± 0·02	0·99 ± 0·04	$P = 0.001$
Glycyl-glycyl-glycine	0·25 ± 0·03	0·39 ± 0·03	$P = 0.01$
L leucyl-glycyl-glycine	5·25 ± 0·34	6·25 ± 0·94	$P = 0.01$
L leucyl-βnaphthylamide	0·24 ± 0·02	0·49 ± 0·02	$P < 0.001$

Table III *Peptidase activity of jejunal homogenates from treated rats and controls*[1]

[1]Results are expressed in units/mg protein and are the means ± SEM

Enzyme	*Controls*	*Treated*	*P*
Aryl sulphatase	0·457 ± 0·003	0·0503 ± 0·006	$P = 0.48$
Pteroyl-polyglutamate hydrolase	77·4 ± 13·4	75·4 ± 10·2	$P = 0.91$

Table IV *Activity of lysosomal enzymes in jejunal homogenates from treated rats and controls*[1]

[1]Results are expressed in units/mg protein and are the means ± SEM

animals for all substrates tested (Table III). In contrast, there was no significant change in the activity of the two lysosomal enzymes tested (Table IV).

Discussion

The chronic dietary administration of prednisolone to the normal adult rat has here been shown to cause mucosal atrophy and a reduction in the height of intestinal epithelial cells. Corticosteroids in high dosage inhibit mitosis (Bucher, 1963; Hechter and Halkerston, 1965; Ragins and Wincze, 1969) and this effect may explain the mucosal atrophy. The eduction of epithelial surface cell height may also be due to the protein catabolic effect of corticosteroids (Izzo and Glasser, 1961).

In contrast to these morphological changes, significant increases of several jejunal peptidases were observed with both biochemical and histochemical methods. Such enzyme changes have not previously been described, although Talanti and Hopsu (1961) noted decreased activity of leucyl-βnaphthylamidase activity in rat gastric mucosa following adrenalectomy, and Schwartz, Robertson, and Holmes (1956) showed that glycyl-glycyl-glycine-peptidase activity in rat diaphragm was inhibited by adrenalectomy and restored to normal by cortisone. Pina Hamabata, and Laguna (1962) claimed that hydrocortisone stimulated the activity of this peptidase *in vitro*. However, Peters (1968) could not show this effect when a jejunal homogenate was incubated with hydrocortisone, but demonstrated inhibition of enzyme activity at pharmacological concentrations (10^{-3}M), an observation reported for other proteolytic enzymes by Bellamy and Leonard (1966).

The activity of other intestinal enzymes has been shown to be hormone sensitive. Moog and Grey (1968) demonstrated the induction of alkaline phosphatase in the duodenum of weanling mice by hydrocortisone, and Stifel, Herman, and Rosenweig (1969) demonstrated increased activity of several glycolytic enzymes in the jejunum of adult rats treated with oestrogen, progesterone, or testosterone. Wall *et al* (1970b) described increased histochemical activity of several brush border enzymes in the jejunum of rats treated with pharmacological doses of thyroid extract.

At first sight there is some apparent incongruity in our results. Although there was some evidence of impaired protein metabolism, reflected by structural changes in the mucosa, there was an increase in the activity of certain enzymes. Similar findings have, however, been noted in the regenerating rat liver, when the administration of hydrocortisone caused

inhibition of mitosis and increased synthesis of RNA (Bucher, 1963).

Our study may have clinical implications. Inflammatory diseases of the gastrointestinal tract, such as pernicious anaemia, coeliac disease, regional enteritis, and chronic ulcerative colitis, are known to be responsive to corticosteroids. The beneficial effects of these hormones have been attributed to anti-inflammatory or anti-immune mechanisms. It is, however, possible that corticosteroids may induce peptidase activity in the intestinal cell, thereby facilitating proteolysis of toxic dietary or bacterial proteins. Further study of the mode of action of corticosteroids in human inflammatory intestinal diseases seems to be indicated.

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