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A MORPHOLOGICAL AND HISTOCHEMICAL STUDY OF A DRUG-INDUCED ENTEROPATHY IN THE ALDERLEY PARK RAT

L. B. MURGATROYD

From Imperial Chemical Industries Ltd, Pharmaceutical Division, Department of Pathology, Safety of Medicines, Alderley Park, Macclesfield, Cheshire

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Summary.—Two experiments were devised to produce an experimental enteropathy. In Experiment I, male Alderley Park rats were dosed daily by gavage with 20 mg/kg and 60 mg/kg of an antibacterial compound ICI 17,363.

Animals were killed sequentially at daily intervals up to and including Day 9 to study the development of the enteropathy.

In Experiment II rats were dosed daily with 60 mg/kg of the same compound. All animals were killed on Day 5 owing to a rapid development of the enteropathic condition.

The duodenum was examined histologically and histochemically. Duodenal changes included vacuolation of columnar epithelial cells and villus stunting. There were marked reductions in mitotic activity in the crypt epithelial cells from Day 7 onwards (Experiment I) and almost total loss of hydrolytic and oxidative enzyme activity.

In Experiment II the changes were more severe and haemorrhage and erosion of the duodenal mucosa were observed.

The development of the enteropathic lesion appears to be due largely to the antimitotic effect of the compound, although a direct toxic effect upon the intestinal mucosa cannot be ruled out.

THE INTESTINAL TRACT is constantly subjected to numerous forms of chemical insult and may transform, absorb or reject the substances coming into contact with it. Intestinal epithelium is in a constant state of turnover and replacement which usually compensates for cell loss at the villus tips. This rapid turnover of cells may reflect the effect of continuous variable insults to which the intestinal tract is subjected.

Certain substances may have a toxic effect on the intestinal mucosa resulting in a loss of surface epithelium followed by villus atrophy accompanied by a change in the rate of cell turnover. This inevitably results in a flattened mucosa.

Several experimental enteropathies have been investigated in animals. After studies of a drug-induced enteropathy of the jejunum of patients following treatment with Triparanol, McPherson and Shorter (1965) dosed Sprague–Dawley rats with Triparanol (25–50 mg/kg/day). They observed flattening of the villi and cellular infiltration of the lamina propria in the jejunal mucosa after a period of 2 weeks. On withdrawal of the drug, the enteropathy disappeared. A drug-induced enteropathy characterized by lipid in macrophages was reported by Gray *et al.* (1974), who dosed Sprague–Dawley rats with a synthetic 2-dodecylglutaramide ester of erythromycin. The rats were given 100 mg/kg/day for 1 week. No gross lesions were observed but lipid located in the macrophages was reported. Braide and Aronson (1974) induced enteropathy of the small intestine by infusing eqimolar quantities of calcium chloride and ethylene diamino tetra acetic acid for up to 72 h. Light microscopy showed degenerative changes in the crypt cells of the duodenal

mucosa. Electron microscopy showed a high degree of mitochondrial swelling and loss of villi was observed after 24–36 h had elapsed.

The effects of folic acid antagonists upon the intestinal mucosa were first investigated in detail by Woll and Oleson (1951). They dosed albino rats with aminopterin (25–100 μg/kg orally). They described intestinal changes including stunting of villi and flattening of mucosa. From the results, the authors postulated a pathogenesis for sprue. Vitale *et al.* (1954) dosed rats with aminopterin and observed reduced mitotic activity in the crypts. At a later stage degeneration and vacuolation of the villus tips was observed. Robinson, Antonioli and Vanatti (1966) reported similar changes and Bowring and May (1976), in their studies on the uptake of salicylates following dosing with aminopterin, reported the presence of flattened epithelial cells in the intestinal mucosa.

A recent subacute toxicity study of a novel antibacterial compound (subsequently referred to as ICI 17,363) in rats produced a marked enteropathy of the duodenal mucosa.

This paper reports a more detailed morphological and histochemical investigation of the enteropathy in an attempt to elucidate a possible mechanism for the development of the lesion.

MATERIALS AND METHODS

Animals

A total of 52 male Wistar-derived Alderley Park rats weighing approximately 100–120 g were used in 2 experiments.

Animals were housed in Wilmslow racks on mesh floors, 5 animals per cage in Experiment I and 6 animals per cage in Experiment II. ICI diet and tap water were freely available. Environmental temperature was maintained at 70 ± 2°F. Animals were exposed to a mixture of artificial light and daylight with a 12h light/dark cycle.

Administration of compound

Before dosing the compound was made up as an aqueous suspension in 0·5% Tween 80 and ball-milled continuously for 24 h. Rats were dosed daily by gastric lavage.

Experiment I.—Ten rats were allocated to each of 4 groups; Group I was the undosed control, Group II received a daily oral dose of 0·5% Tween 80 in distilled water, Group III received a daily oral dose of antibacterial ICI 17,363 (20 mg/kg) in 0·5% Tween 80 and Group IV received a daily oral dose of antibacterial ICI 17,363 (60 mg/kg) in 0·5% Tween 80.

Experiment II.—Six rats were allocated to each of 2 groups; Group I was the undosed control and Group II received a daily oral dose of ICI 17,363 (60 mg/kg) in 0·5% Tween 80. The body weights of this group of animals were less than those of animals in Experiment I.

Experiment I was designed to study the development of the enteropathy and animals were killed daily.

Experiment II was designed to confirm the major changes observed in Experiment I. In an effort to eliminate biological variation often encountered in animals, a larger number of animals were studied at a selected time period. The time period selected was dependent upon the health of the dosed animals.

Necropsy

All rats were killed with an overdose of ether.

Experiment I.—One animal from each group was necropsied daily up to and including Day 8. On Day 9, 2 animals from each group were necropsied owing to excessive progression of the enteropathy in Groups III and IV.

Experiment II.—All animals were killed on Day 5 owing to the rapid progression of the enteropathic condition in Group II.

Body weight

This was recorded on Day 0 and terminally in all cases.

Clinical changes and macroscopic appearance of the intestinal tract of all animals were recorded.

Histology

2cm sections of duodenum were removed from each animal distal to the pyloric sphincter in both experiments.

Each block of tissue was divided equally. One half was fixed in 10% neutral buffered formaldehyde (Culling, 1974) and processed routinely for paraffin sections. The remaining half of each block of tissue was quenched in liquid N_2 and sectioned unfixed on a Slee rotary cryostat at approximately 6 μm. In all cases, sections in transverse plane were prepared.

Staining procedures.—Paraffin-embedded sections were stained with haematoxylin and eosin, periodic-acid–Schiff for neutral mucosubstances (Culling, 1974), 1% Alcian Blue (Steedman, 1950) for acidic mucosubstances, 1% Toluidine Blue for sulphated mucosubstances, Feulgen reaction for DNA using a 30-min hydrolysis time in 5N HCl at room temperature (Murga-

troyd, 1966), Diazo reaction for argentaffin granules (Culling, 1974) and the methyl-green-pyronin procedure for RNA and DNA (Murgatroyd, 1963).

Histochemical procedures.—Cryostat sections were incubated to demonstrate acid phosphatase (Barka and Anderson, 1962), alkaline phosphatase (Culling, 1974), non-specific esterase (Pearse, 1972), adenosine triphosphatase (Wachstein, Meisel and Niedwietz, 1960), glucose-6-phosphatase (Wachstein and Meisel, 1956) and succinic dehydrogenase activity (Nachlas *et al.*, 1957).

Neutral lipid was demonstrated by staining with Oil Red O (Lillie and Ashburn, 1943).

Mitotic counts

A semi-quantitative indication of the mitotic index was achieved by counting the number of mitotic figures present in 30 crypts of similar proportion seen in Feulgen-stained sections from all groups in both experiments. A similar technique was used by Braide and Aronson (1974). Only clear mitoses were counted and the mitotic index was expressed as the average number of mitoses per crypt.

Photomicrography

This was carried out on a Vickers photoplan M.41 microscope fitted with automatic camera and exposure unit. Black and white photomicrographs were prepared using Panatomic X and Ilford Pan F.

RESULTS

Clinical condition

Experiment I.—All animals in Groups I and II remained healthy. Animals in Groups III and IV showed considerable deterioration on Days 7, 8 and 9. Piloerection and diarrhoea were observed and there was little weight gain in Group IV. On Day 9 a net loss of weight was recorded.

The weight gain or loss of every animal in the 4 groups was obtained by subtracting the initial body weight from the terminal body weight and is shown in Fig. 1a.

Experiment II.—No changes were observed in Group I. All animals remained healthy and gained weight.

In Group II very marked deterioration was observed on Day 5. All animals showed piloerection, evidence of wasting and diarrhoea. Several animals lost weight and the remaining animals showed no weight gain over the 5-day period. The weight gain or loss of these animals is shown in Fig. 1b.

Gross pathology

Experiment I.—No changes were observed in Groups I or II. A marked reddening of the duodenal mucosa and swelling of the caecum was observed in Groups III and IV on Days 8 and 9.

Experiment II.—No changes were observed in Group I. A very marked reddening of the duodenal mucosa was observed in all animals in Group II. In one animal

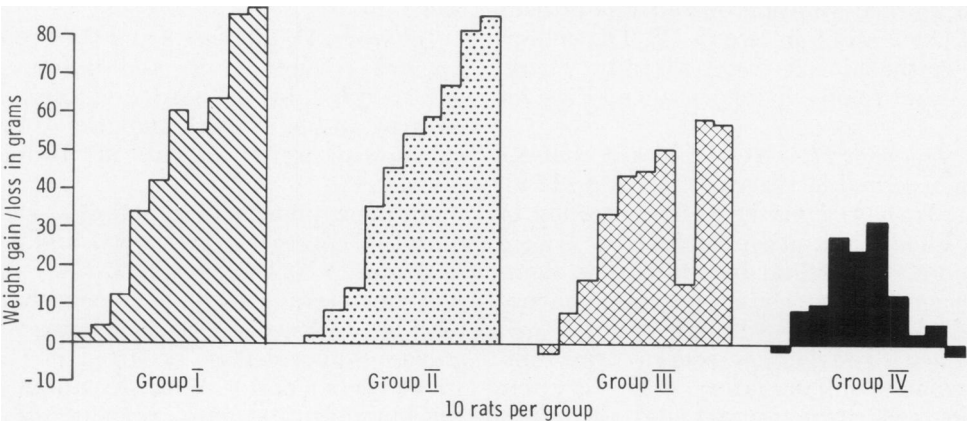


FIG. 1a.—Weight gain or loss in rats after multiple dosing with the antibacterial compound ICI 17,363. Experiment I: Group I—Undosed control. Group II—Dosed control (0·5% Tween 80). Group III—20 mg/kg antibacterial ICI 17,363 daily. Group IV—60 mg/kg antibacterial ICI 17,363 daily. One animal from each group killed daily.

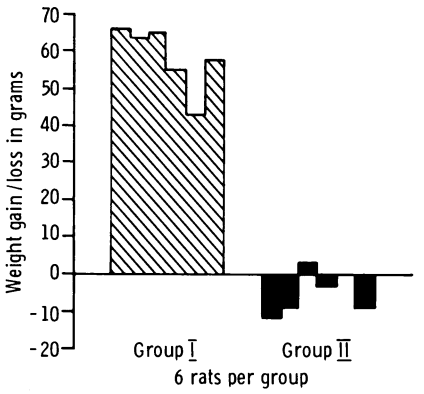


FIG. 1b.—Weight gain or loss in rats after multiple dosing with the antibacterial ICI 17,363. Experiment II: Group I—Undosed control. Group II—60 mg/kg antibacterial ICI 17,363 daily. All animals killed on Day 5.

there was evidence of haemorrhage into the duodenum and the intestinal tract was grossly distended with fluid.

The overall gross changes seen in this experiment were more severe than those observed in Experiment I.

Histopathology

Experiment I.—No significant changes were observed in Groups I or II. Cytoplasmic vacuolation and necrosis of the columnar epithelial cells in the duodenal mucosa was observed in Group III on Day 9. Necrosis of the tips of villi together with marked stunting of villi was present on Days 8 and 9 in Group IV. The columnar epithelial cells were severely vacuolated and foamy in appearance (Figs 2a, b).

Experiment II.—No significant changes were observed in Group I. In Group II all animals showed marked villus atrophy of the duodenum accompanied by severe columnar epithelial dysplasia. The damaged surface epithelial cells were vacuolated. In some regions the mucosa was flattened. The lamina propria was infiltrated with inflammatory cells, very few goblet cells were present and the crypt epithelial cells showed severe dysplasia and dilatation. Lymphatic dilatation was also observed (Figs 3 and 4). In one animal marked erosion of the duodenal mucosa with haemorrhage into the lumen was noted.

Histochemistry

Experiment I.—In Groups I and II the distribution of goblet cells present in the columnar epithelium lining the villi of the duodenum was normal. Goblet cells were also clearly visible in the epithelial crypts. They were intensely stained with periodic-acid–Schiff and Alcian Blue and weakly metachromatic with Toluidine Blue. The brush border was clearly stained with PAS, as were mucosubstances in Brunner's glands. Intense reactions for alkaline phosphatase, glucose-6-phosphatase, and adenosine triphosphatase were observed in columnar epithelial cells and in the brush border lining the villi. Intense succinic dehydrogenase activity was present in the crypt epithelial cells. No other enzyme activity was recorded in these cells (Figs 5a and 6a). A weak pyroninophilia of columnar epithelial cells and an intense pyroninophilia of crypt epithelial cells was observed.

Neutral lipid was present in numerous mononuclear cells scattered throughout the lamina propria. Very little lipid was present in the columnar epithelial cells or in the crypt epithelial cells.

Only occasional argentaffin cells were seen.

In Group IV at Days 8 and 9 there were marked reductions in the numbers of goblet cells, brush border staining was reduced and only small amounts of mucosubstances were present in Brunner's glands.

Alkaline phosphatase activity was reduced in Group III at Days 7 and 8 and in Group IV at Days 8 and 9.

Almost total loss of acid phosphatase, esterase, adenosine triphosphatase and glucose-6-phosphatase occurred in Group IV at Days 8 and 9. Marked reductions in succinic-dehydrogenase activity in the crypt epithelial cells were also observed (Figs 5b and 6b).

Increased pyroninophilia of columnar

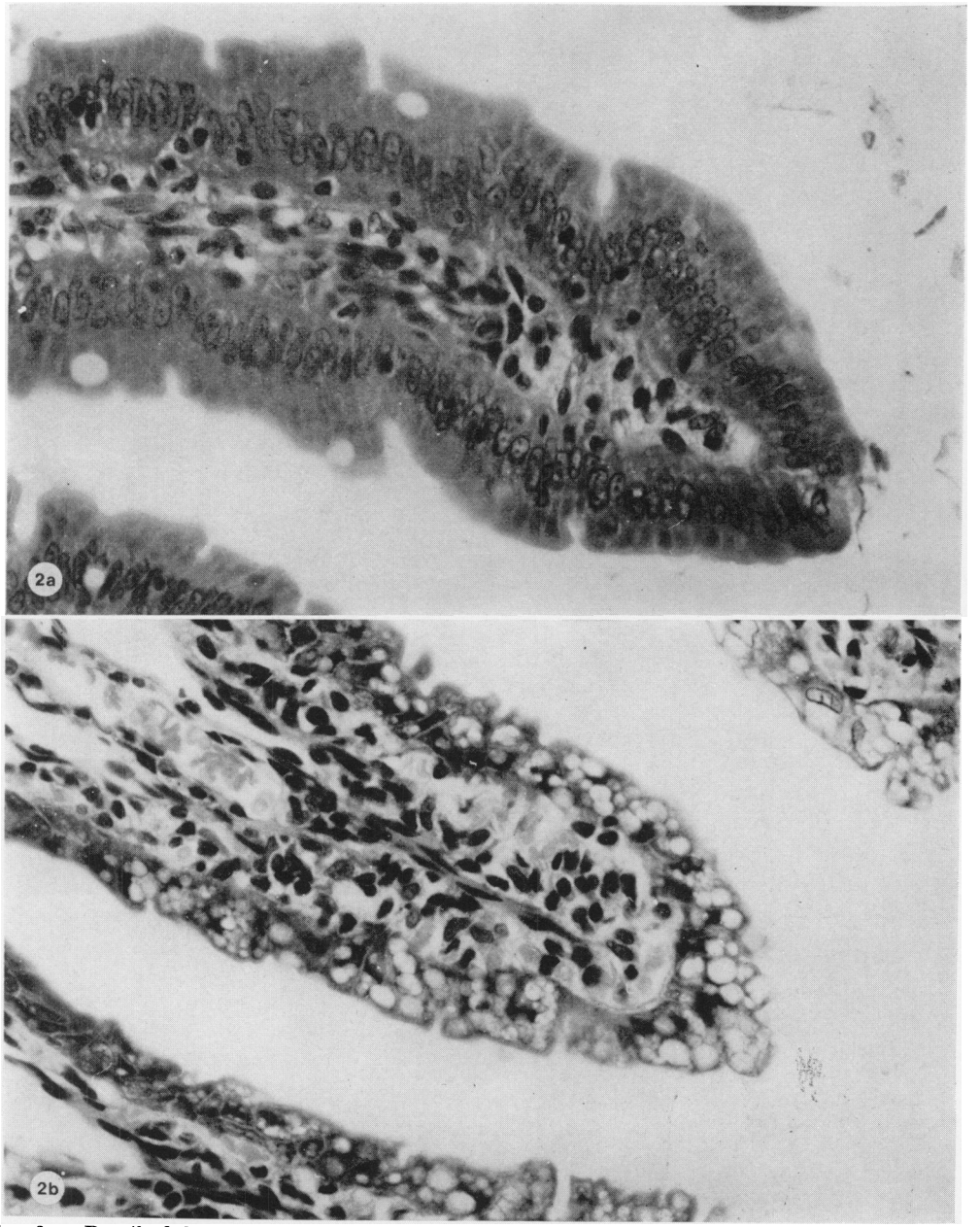
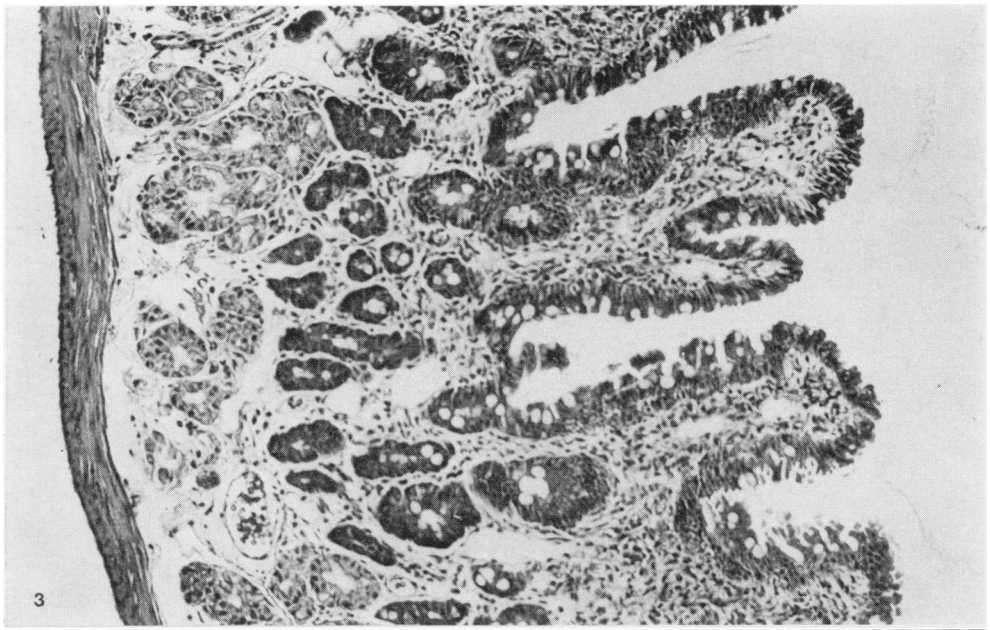


FIG. 2a.—Detail of the columnar epithelial cells in the duodenum of the rat from Group II (Experiment I, Tween-dosed only). Animal killed Day 9. Normal morphology. H. & E. × 510.

FIG. 2b.—Detail of columnar epithelial cells in the duodenum of the rat from Group IV (60 mg/kg daily ICI 17,363). Animal killed on Day 9. Note severe vacuolation and severe dysplasia of epithelial cells. H. & E. × 510.



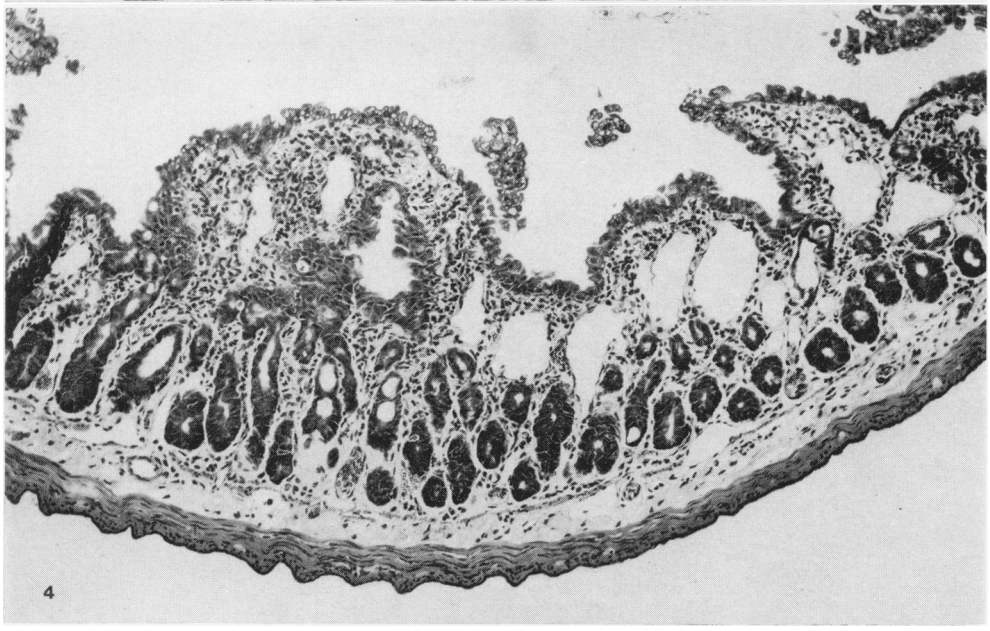


FIG. 3.—Duodenum from rat of Group II (Experiment II, 60 mg/kg daily ICI 17,363. Animal killed on Day 5. Note severe epithelial dysplasia. H. & E. × 85.

FIG. 4.—Duodenum from rat of Group II (Experiment II, 60 mg/kg daily ICI 17,363). Animal killed on Day 5. Note flattened mucosa, lymphatic dilatation and crypt dilation. Very few goblet cells were present. H. & E. × 85.

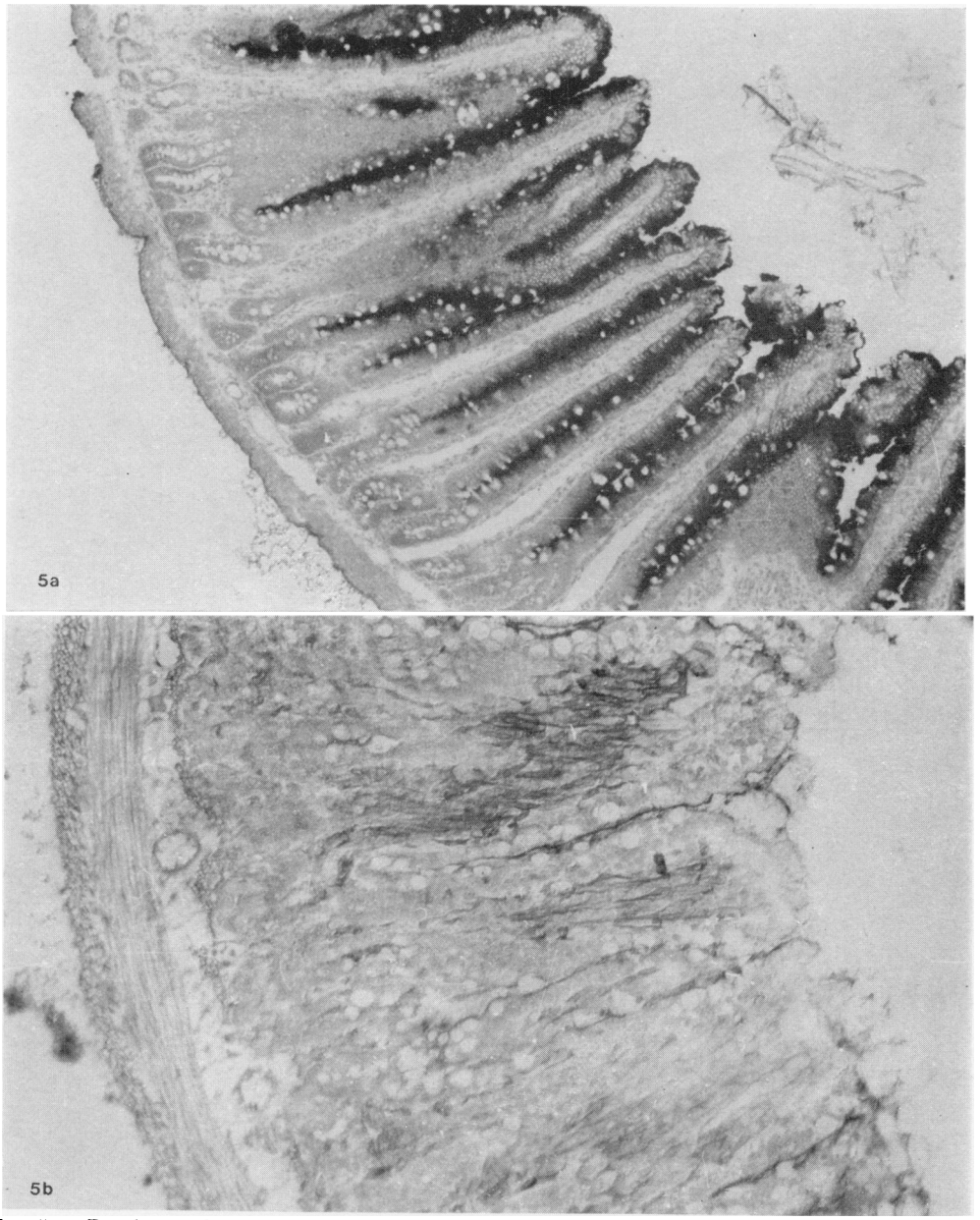


FIG. 5a.—Duodenum from rat in Group II. (Experiment I, Tween-dosed only). Note strong esterase activity in the columnar epithelial cells. × 213.

FIG. 5b.—Duodenum from rat of Group IV (Experiment I, 60 mg/kg daily ICI 17,363). Animal killed on Day 9. Almost total loss of esterase activity from the columnar epithelial cells. Note also the marked villus stunting. × 213.

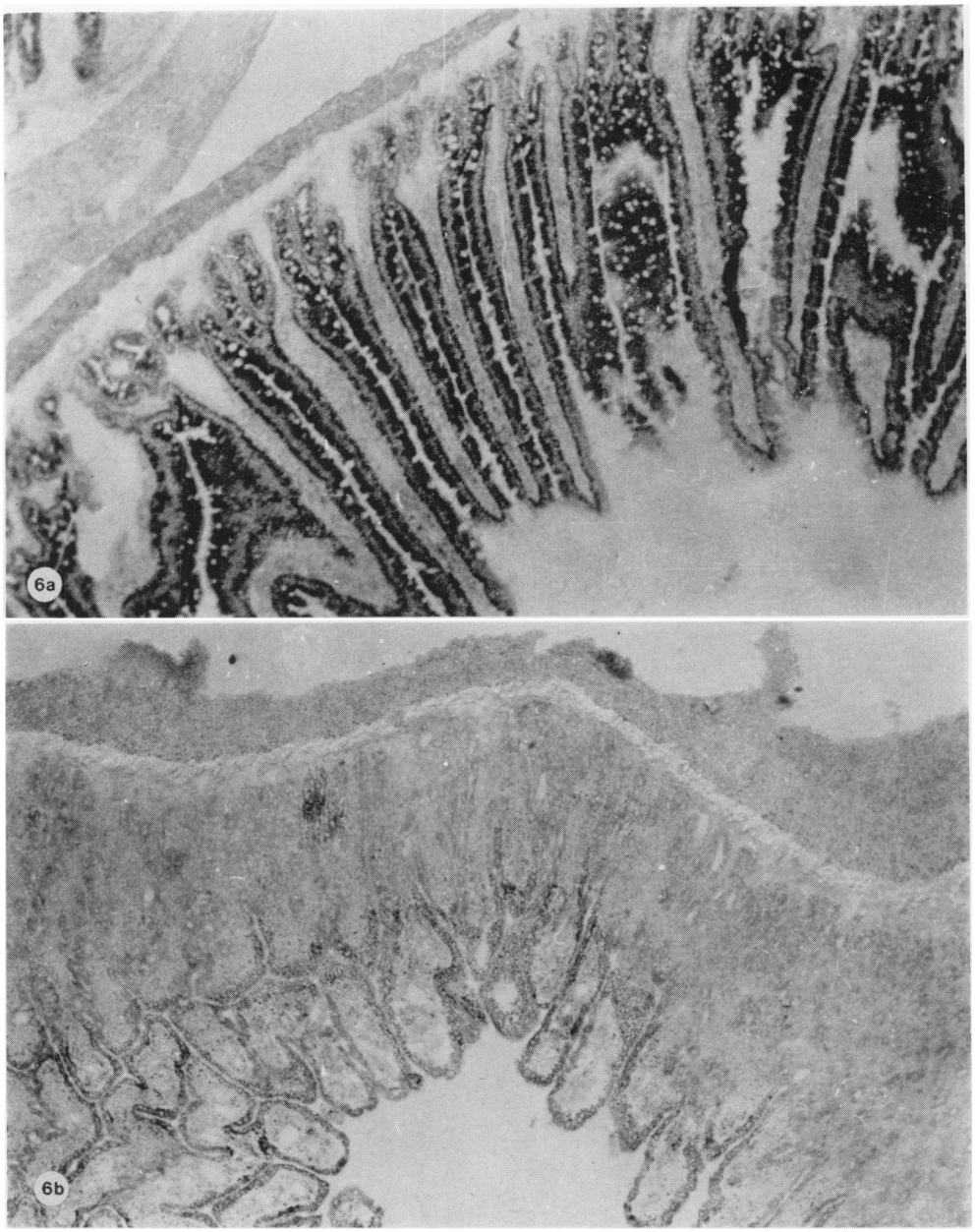


FIG. 6a.—Duodenum from rat in Group II (Experiment I, Tween-dosed only). Strong succinic-dehydrogenase activity in the columnar and in the crypt epithelial cells. Animal killed on Day 9. × 213.

FIG. 6b.—Duodenum from rat in Group IV (Experiment I, 60 mg/kg daily ICI 17,363). Note the marked loss of enzyme activity from the columnar and particularly the crypt epithelial cells. × 213.

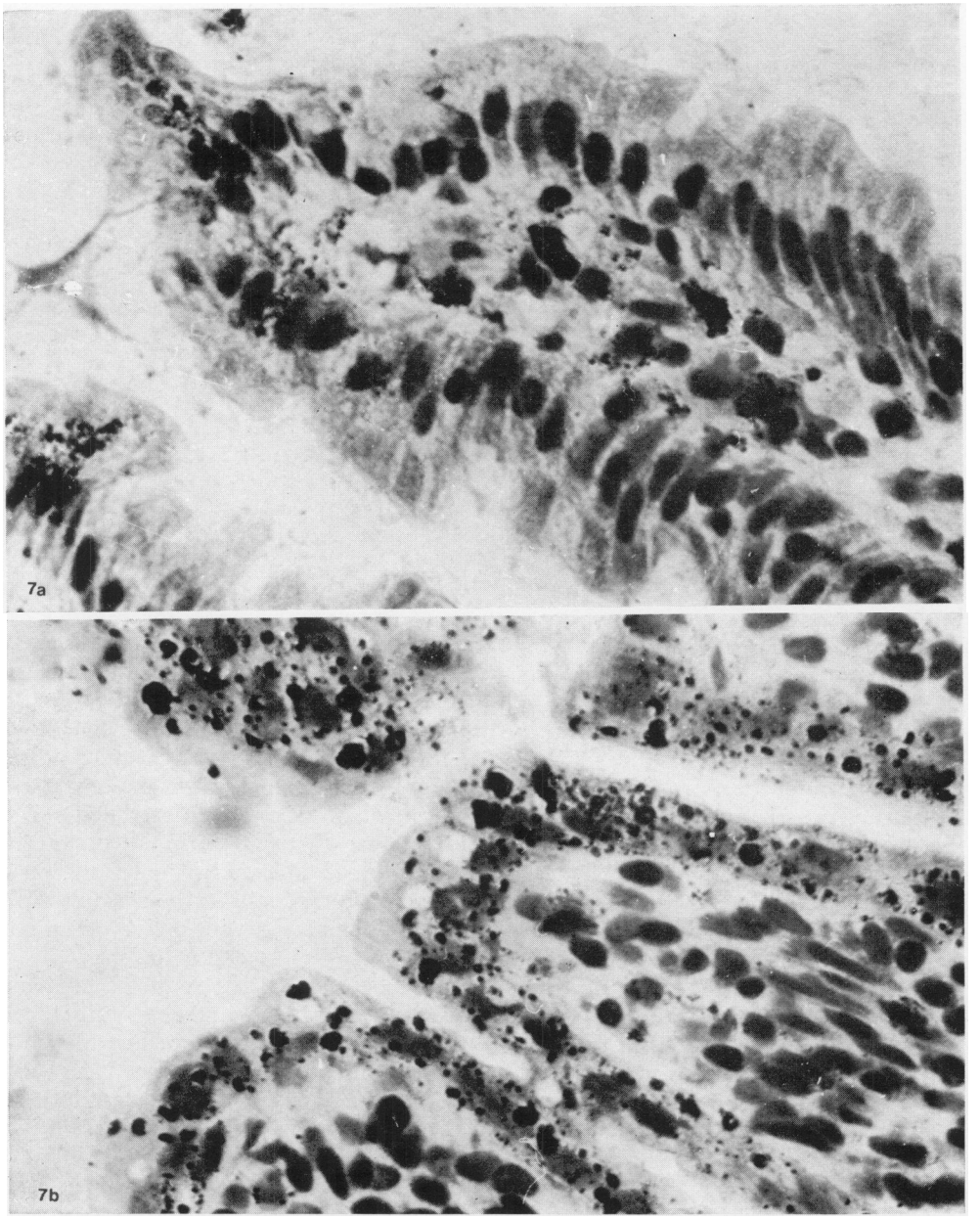


FIG. 7a.—Detail of columnar epithelial cells in the rat duodenum from Group I (Experiment II, undosed control). Animal killed on Day 5. Cryostat section stained with Oil red O. Only small amounts of lipid present in the lamina propria and very little lipid present in the columnar epithelial cells. Counterstained with haematoxylin. × 850.

FIG. 7b.—Detail of columnar epithelial cells in the rat duodenum from Group II (Experiment II, 60 mg/kg daily ICI 17,363). Animal killed on Day 5. Cryostat section stained with Oil red O. Note the marked accumulation of lipid droplets within the columnar epithelial cells. Counterstained with haematoxylin. × 850.

TABLE.—*Average number of mitoses per epithelial crypt*

	Number of crypts counted	Average number of mitoses per epithelial crypt on Days 7, 8 and 9		
		7	8	9
Experiment I				
10 Animals per group				
Group I	30	1·83	1·56	1·96
Group II	30	1·95	2·01	1·79
Group III	30	0·9	0·63	1·36
Group IV	30	0·99	0·76	0·56

		Average number of mitoses per epithelial crypt for each animal on Day 5					
		1	2	3	4	5	6
Experiment II							
6 Animals per group							
Group I	30	1·5	1·23	1·9	1·66	1·79	1·95
Group II	30	0·46	0·43	0·46	0·96	0·39	0·51

epithelial cells was observed in Group III at Days 7 and 8 and markedly increased in Group IV at Days 8 and 9. Lipid droplets were observed in several vacuolated columnar epithelial cells. There was little change in the distribution and numbers of argentaffin cells observed.

Experiment II.—A similar distribution of mucosubstances and enzyme activities seen in Experiment I (Groups I and II) was recorded in all animals in Group I. In Group II, losses of goblet cells and enzyme activity were more marked. All animals showed a marked increase in pyroninophilia of columnar epithelial cells lining atrophied villi and flattened mucosa. Large numbers of lipid droplets were observed in the vacuolated columnar epithelial cells from all animals in Group II (Figs 7a and b).

Mitotic index

Sections stained with Feulgen reaction from both experiments revealed mitotic figures in the epithelial crypts.

There were marked reductions in mitotic activity in the crypt epithelial cells in Groups III and IV (Experiment I) and in Group II (Experiment II) summarized in the Table.

DISCUSSION

From the results of both experiments in this study, a definite sequence of events is seen to emerge during the development of the enteropathy. Initially the columnar epithelial cells of the duodenum exhibit cytoplasmic vacuolation and fenestration, and villus height appears normal. Villus atrophy then follows as a result of reduced mitotic activity in the epithelial crypts. Vitale *et al.* (1954) and Robinson *et al.* (1966) reported similar changes to the ileum when rats were dosed with a sublethal dose of aminopterin. Mitotic activity was reduced followed by vacuolation and degeneration of cells in the tips of villi.

Histochemically the persistance of RNA-rich cytoplasm in the columnar epithelial cells together with reduced hydrolytic enzyme activity is a strong indicator that the cells reaching the surface of the villi are immature. Increased RNA content was observed in the cytoplasm of immature absorptive epithelial cells by Padykula *et al.* (1961) in their studies on idiopathic steatorrhoea in man. Rubin *et al.* (1962) confirmed by electron microscopy that there were increases in free ribosomes in the immature absorptive cells.

Vacuolation of epithelial cells observed in cryostat sections and paraffin sections was shown to be due partly to lipid accumulation and partly to hydropic change, which could be a direct toxic effect of ICI 17,363. Gray *et al.* (1974) reported the

presence of vacuolated surface epithelial cells containing lipid droplets after rats had been dosed with erythromycin esters.

Marked epithelial dysplasia and distention of the crypts would increase impaired maturation of epithelial cells. Further evidence of this is the reduced number of goblet cells and the loss in succinic dehydrogenase activity from the crypt epithelial cells. In the later stages of the enteropathy a flattened mucosa develops devoid of goblet cells. Numbers of goblet cells in the jejunum were seen by Padykula *et al.* (1961) to vary in idiopathic steatorrhoea, reduced numbers being found in areas of flattened mucosa.

The retention of fluid in the intestinal tract observed in both experiments may be a direct result of damage to the surface epithelial cells. Loss of PAS staining within the brush border suggests damage to the microvilli resulting in impaired absorption. A decrease in surface area caused by villus atrophy would further impair absorption. Water is absorbed along an osmotic gradient created by normal absorption of sodium and amino acids. Decreased absorption of these substances may lead to a breakdown of the osmotic gradient, resulting in the retention of fluid in the intestinal tract.

The role of alkaline-phosphatase activity in the intestinal tract is not fully understood. It is thought to play some part in the absorption process and it has been suggested by Fernley (1971) that its role may be in lipid transport.

Acid-phosphatase and esterase activity was almost abolished from the surface epithelial cells of the duodenum in both experiments. The presence of hydrolytic enzymes in the surface epithelial cells indicate their importance in normal absorption. In addition, both enzymes are lysosomal and release of such enzymes may be an important factor in the initial stages of epithelial necrosis.

Severe losses of adenosine-triphosphatase activity from the surface epithelial cells and the brush border are a strong indication of a breakdown in active ion transport mechanisms essential to the normal absorption processes carried out in these cells. Hydropic change in cells is a further indication that this mechanism is not functioning normally. The pathological changes described are comparable with similar changes reported by other workers studying enteropathic conditions in man, namely sprue (Baker and Mathon, 1968; Klipstein and Baker, 1970) and idiopathic steatorrhoea (Padykula *et al.*, 1961).

In both experiments reduction in villus height was roughly proportional to the loss in body weight and to the loss of enzyme activity from the surface epithelial cells.

The marked differences in the drug-induced enteropathy lay in the ultimate severity of the lesion produced and in the marked reduction in mitotic activity within the epithelial cells of the crypts.

The compound is probably producing a villus atrophy by a combination of two mechanisms. There is interference with cell division in the crypts, reducing the number of replacement epithelial cells produced. The cells which are produced are incapable of maturing to true absorptive epithelial cells. In addition, there may be a direct toxic effect upon the surface epithelial cells leading to increased cell loss. Reduced cell replacement together with increased cell loss may ultimately lead to erosion and ulceration.

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