Three-dimensional structure of the rat small intestinal mucosa related to mucosal dynamics

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General Introduction

In other communications (Loehry and Creamer, 1969; Loehry, Croft, Singh, and Creamer, 1969) we have established that the three-dimensional structure of the small intestinal mucosa is more complex than has been supposed, with a preponderance of crypts over villi, and with many small inter villous ridges which probably represent the migration lines of epithelial cells that are channelled round from crypts onto villi. We have demonstrated how the evolution of morphological variations in villous architecture in disease is due to the hypertrophy of these inter villous ridges as villi shorten, to form leaves, convolutions, and a flat mucosa. The question that remained is why there should occur this hypertrophy of the inter villous ridges in disease, and how these structural changes were related to the dynamic state of the mucosa. As a working hypothesis we considered that this hypertrophy of the inter villous ridges was due to an increased turnover state, when cells emerging from the crypts at a faster rate than normal caused hypertrophy of normal migration lines. This situation, together with an increase of cell loss causing shortening of the villi, could be responsible for the typical abnormal mucosal patterns in disease. In order to test this hypothesis we have related the three-dimensional mucosal structure to the dynamic state in various experimental conditions in rats. For practical purposes three basic processes were considered for altering the dynamic state of the mucosa: (1) Decreased cell production produced by the administration of methotrexate; (2) increased cell production studied in the lactating rat; (3) increased cell loss was produced by infesting rats with the nematode Nippostrongylus brasiliensis.

Part I Mucosal structure and dynamics in the rat after the administration of methotrexate

The therapeutic value of the folic acid antagonists is related to their action as mitotic inhibitors, and it is therefore not surprising that they produce pathological changes in the small intestinal mucosa with its high index of mitotic activity. Histological studies after a single dose of an antimetotic drug in experimental animals (Dustin, 1950; Vitale, Zamcheck, DiGiorgio, and Hegsted, 1954; Millington, Finean, Forbes, and Frazer, 1962) and in man (Trier, 1962) have demonstrated a period of mitotic inhibition in the small intestinal mucosa for one to two days followed by a period of mitotic regeneration. Trier (1962) has performed mitotic counts on serial jejunal biopsies in patients after a single dose of methotrexate, and has demonstrated an overshoot in the mitotic index above normal levels in the recovery phase. From these studies it seems likely that the effect of methotrexate on the small intestinal mucosa is to produce a period of decreased cell output from the crypts due to mitotic arrest, followed by a period of regeneration when possibly an increased turnover state is present. The purpose of the present experiment was therefore to study the effects of a single dose of methotrexate on the dynamic state of the rat small intestinal mucosa, and to relate this to the changes in the three-dimensional mucosal structure in both the aplastic and recovery phases.

METHODS

Methotrexate sodium, 7.5 mg, was injected intramuscularly into 14 male albino rats of similar age and weight at 10 am on day 0. Pairs of animals were killed at the same time on each of the following seven days (days 1 to 7) and the small intestine was removed.
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DYNAMIC STATE This was assessed by the following methods.

Mitotic counting Counts were done at a magnification of ×270 with a binocular microscope. Three thousand crypt cells were counted in specimens of mucosa from days 1 to 7 and in normal controls, and the mitotic activity was assessed by the number of mitoses present per 1,000 crypt cells.

Villus height This was determined on days 1 to 7 and in normal controls by counting the villous cell column in at least 70 well orientated villi.

Labelling Two rats on day 5 after methotrexate and two normal controls were injected intraperitoneally with 100 μc of tritium-labelled thymidine. One methotrexate and one normal rat were killed after eight hours and the other pair 24 hours after the injection, and autoradiographs performed on sections of the small intestinal mucosa. In order to obtain some objective assessment of the comparative rates of turnover, the villous cell column from the base of the villi to the highest labelled cell was compared in at least 30 well orientated villi from the normal and methotrexate rats in the 24-hour period.

STRUCTURAL CHANGES The three-dimensional changes in mucosal structure were studied and photographed through the dissecting microscope, in autolysed and unautolysed mucosa from days 1 to 7 after methotrexate.

RESULTS

GENERAL OBSERVATIONS Animals that had received methotrexate showed obvious malaise from the day following injection. Anorexia, weight loss, diminished activity, ruffling of the fur, pigmentation of the mouth and eyes, and diarrhoea were a prominent feature. By day 7 most animals appeared normal again.

DYNAMIC STATE Mitotic counts Figure 1 is an illustration. There is a marked reduction in the mitotic activity on days 1 and 2 after methotrexate with a few mitoses reappearing on day 3. A marked increase in mitotic activity is present on days 4 to 6 as the mucosa regenerates, returning to normal on day 7. Villous height (Fig. 1) The mean villous height becomes progressively shorter until on day 3 an almost 'flat' appearance is seen. After this there is a progressive increase in villous height over the next three days. By day 7 villi are back to normal again.

Labelling At eight hours after injection of the tritiated thymidine both the control and day 5 methotrexate rats showed labelling of crypt cells, and in the latter group there was occasional labelling at the base of villi. At 24 hours the control rats showed labelling approximately one third of the way up the villi, whereas in the methotrexate-treated rats, by this time on day 6, the lower two-thirds was labelled. The height of the labelled villous cell column at 24 hours in the control rats was a mean of 22 cells (standard deviation 4·3) compared with a mean of 41 cells (standard deviation 4·3) in the methotrexate-treated group. The difference between the two groups is highly significant (p < 0·001).

STRUCTURAL CHANGES On day 1 after methotrexate in both unautolysed and autolysed material (Figs. 2a and b) the mucosa appears morphologically normal, though in fact villous height is already reduced. Over the next two days the villi progressively shrink, till on day 3 the mucosa appears 'flat' (Figs. 3a and b). However, although this 'flat' appearance is seen in the unautolysed specimen on that day (Fig. 3a), the changes in the autolysed mucosa (Fig. 3b) illustrate quite different changes from the flat mucosa in coeliac disease, and demonstrate that the flattening produced in a situation of cell aplasia is merely due to shrinkage of villi. A most striking change, however, is seen on day 4. Throughout the autolysed specimen (Fig. 4b) there is generalized hypertrophy of the inter villous ridges, in parallel with the mucosal regeneration, mitotic activity, and increased turnover state at this stage. These prominent inter villous ridges are responsible for the convoluted appearance of the mucosa at this stage which is illustrated in the unautolysed specimen (Fig. 4a). These hypertrophied inter villous ridges and convolutions persist throughout day 5 and then the mucosa gradually returns to normal again on day 7.
FIG. 2a and b. Unautolysed and autolysed jejunal mucosa from rats on day after methotrexate. × 60.

FIG. 3a and b. Unautolysed and autolysed jejunal mucosa from rats on day 3 after methotrexate. Although the mucosa appears 'flat' in the unautolysed specimen autolysis shows that the changes are merely due to shrinkage of villi. × 60.
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**DISCUSSION**

These experiments demonstrate that the administration of a single dose of methotrexate to rats produces a period of diminished cell production in the small intestinal mucosa for the first three days after injections, and the effect of this on mucosal structure is to produce short hypoplastic villi. As the mucosa regenerates on days 4 to 6 after methotrexate there is a temporary increased turnover state as the villi are reconstituted. The most prominent structural changes in the mucosa at this stage is the hypertrophy and prominence of the intervillous ridges, presumably as a response to the increased output of cells from the crypts. Wiernik (1966a, b, c) has shown a somewhat similar sequence of events in the human small intestinal mucosa after irradiation, with a period of aplasia followed by an ‘overshoot’ and noted progressive stunting of villi in the aplastic period.

The present experiments demonstrate the necessity of studying the three-dimensional changes in the small intestinal mucosa as well as the histological appearances. Previous authors have produced histological flattening of the mucosa by experimentally inducing mitotic inhibition (Clark and Harland, 1963) and have considered that these changes are sufficiently similar to the appearances in coeliac disease to suggest that the cause of the flat mucosa in this condition is due to cell aplasia. These experiments demonstrate that the three-dimensional mucosal structure in cell aplasia is quite different from coeliac disease, with short, stubby villi appearing as the response to diminished cell output. In the recovery period, however, a convoluted mucosa was seen for a while, related to

**FIG. 4a and b.** Unautolysed and autolysed jejunal mucosa from rats on day 4 after methotrexate. Villi are regenerating, and the increased cell production at this stage has produced generalized hypertrophy of the intervillous ridges. × 60.
the increased output of epithelial cells and hypertrophy of the intervillos ridges.

SUMMARY

The injection of methotrexate in rats produced a period of epithelial cell aplasia in the small intestinal mucosa for the first three days followed by a period of increased cell production for three days as the mucosa regenerated. Studies of the three-dimensional mucosal structure showed that the period of cell aplasia produced merely stunted villi, whereas in the recovery phase there was hypertrophy of intervillos ridges and a convoluted mucosa.

REFERENCES


Part II Mucosal structure and dynamics in the lactating rat

It is now well established that the small intestine along with many other organs is considerably hypertrophied in the lactating rat (Souders and Morgan, 1957; Fell, Smith, and Cambell, 1963; Cambell and Fell, 1964). There is considerable hyperplasia of villi to almost twice their normal size, and these changes progress to a maximum at weaning, after which there is a gradual return to normal. Cambell and Fell (1964) have demonstrated how these intestinal changes are dependent on the increased food consumption during lactation.

Boyne, Fell, and Robb (1966), measuring mucosal and serosal surface areas in lactating and virgin rats, noted considerable ‘branching and fusion’ of villi in histological sections from the upper small intestine of the lactating group as well as increased height and surface area. From these studies it seemed possible to us that in the small intestinal mucosa of the lactating rat there might be a change in the dynamic state producing a primary increase in cell production to form the hypertrophied mucosa, and that the branching of villi noted by Boyne et al (1966) might in fact be due to hypertrophy of intervillos ridges, which had become prominent at the base of the tall villi in response to the increased cell output. The aim of the present experiments therefore was to study the dynamic state of the small intestinal mucosa in the lactating rat, and to relate this to the three-dimensional mucosal structure.

METHODS

DYNAMIC STATE Four albino rats in the sixteenth day of lactation and four virgin controls of similar age and weight were injected intraperitoneally with 100 µg of tritium-labelled thymidine at the same time. Two lactating and two control rats were killed at eight hours, and the remaining rats at 24 hours after the injection. Sections were taken from the upper jejunum, and processed by autoradiography. The comparative rates of cell migration in the two groups were assessed by counting the cell column from the base of the villi to the uppermost labelled cell in 50 well orientated villi at the 24-hour period after injection of the isotope.

STRUCTURAL CHANGES These were assessed on the sixteenth day of lactation in the jejunum (1) after autolysis and (2) by serial horizontal cross sections (Loehry and Creamer, 1969).

RESULTS

DYNAMIC STATE At eight hours after injection of the tritium-labelled thymidine the crypt cells showed labelling in both the lactating and virgin rats, but in the lactating group labelling was often seen at the base of the villi. At 24 hours the lower one third of villi in the virgin rats was labelled whereas in the lactating group the lower half of the considerably taller villi showed labelling. The mean height of the