Ribonucleic acid and protein metabolism in the gut

Part I Observations in gastro-intestinal cells with rapid turnover

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EDITORIAL SYNOPSIS This paper reports the findings of an investigation of ribonucleic acid (R.N.A.) and protein metabolism in those epithelial cells of the gastrointestinal tract which have a rapid turnover, and correlates the results with the rate of production of new cells.

Ribonucleic acid (R.N.A.) is intimately concerned with the formation of protein within the cell. Its exact role is uncertain but there is evidence that it acts as an intermediary between the genetic 'memory' of the chromosomes and the protein-producing sites of the cell. Recent work suggests that much of the R.N.A. is formed in the nucleus and then migrates to the cytoplasm where most of the protein is formed (Zalokar, 1959; Goldstein and Micou, 1959; Caro and Forro, 1961).

Protein is manufactured for the structure of cells and also for enzymes which are required for cell function or secretion. Both these requirements for protein are in great demand in the gut. Most of the gastrointestinal epithelial cells are being replaced at an extremely fast rate for which protein synthesis is clearly necessary. Certain groups of epithelial cells, *i.e.*, those in the body of the stomach, in Brunner's glands of the duodenum, and in the pancreas, do not participate in this swift turnover but secrete large amounts of enzymes and electrolytes. However, these functions overlap to a considerable extent, for the epithelium of the small intestine is not only being actively replaced but also forms large amounts of enzymes.

This paper presents evidence on the site and intensity of R.N.A. and protein metabolism in those epithelial cells of the gut with a fast turnover. Similar studies of R.N.A. and protein metabolism in the fixed secretory cells will be considered in a separate communication. The observations have been made on mice after the injection of tritium-labelled uridine and tritium-labelled methionine using autoradiography to follow their concentration and movement. Uridine is incorporated into R.N.A. and methionine is an essential amino-acid which is commonly present in proteins. It has been assumed that the local irradiation from these substances has

no effect on the normal metabolic processes. The observations are mostly presented in tabular form. They are also correlated with data on desoxyribonucleic acid (D.N.A.) metabolism, presented in a previous communication, which has demonstrated the rate of cell turnover in each tissue in a comparable group of mice (Creamer, Shorter, and Bamforth, 1961).

METHODS

Fifty 18-day albino mice were used. Twenty-five were injected intraperitoneally with 100 μ c. of tritium-labelled uridine (A.E.R.E. Harwell; 1,000 mc./mM.) in 0.2 ml. of saline and the remaining 25 were injected intraperitoneally with 100 μ c. of tritium-labelled DL-methionine (A.E.R.E. Harwell; 150 mc./mM.) in a similar volume. The injections were carried out at 11 a.m. and the mice were kept in free-running cages with access to chow and water. Batches of five animals from each group were killed by ether anaesthesia at intervals of one, six, 24, 48, and 72 hours following injection and the tissues dissected immediately. The gastro-intestinal tract was removed and specimens from all areas were fixed in 10% formalin. Autoradiographs were set up using stripping film (Kodak, AR10) and exposed for one month. After developing the sections were stained with neutral red and examined by light microscopy.

RESULTS

The distribution and amount of label in the autoradiographs has been recorded using a grading of 0 to + + + +, where 0 = no label, + = light labelling, ++ = moderate labelling, +++ = heavy labelling, and + + + + = the heaviest labelling observed. There was usually no difficulty in interpreting the autoradiographs but, occasionally, two situations caused uncertainty: first, in some instances, where the label appeared to be

FIG. 1. Diagram of distribution of radioactive label in tongue. C = cytoplasm, N = nucleus. The hatched band indicates the position of D.N.A.-labelled cells following the injection of tritiated thymidine, taken from a previous study; 1, 6, 24, 48, and 72 indicate time in hours after injection of radioactive labels.

predominantly in the nucleus, it was impossible to be certain that it was not in the immediate perinuclear cytoplasm, and secondly, where the cytoplasm contained a large amount of the label, the extent of nuclear labelling was not always apparent.

SQUAMOUS EPITHELIUM Squamous epithelium forms the lining of the tongue, oesophagus, and fundus of the stomach. Only the results found in the tongue are presented here since there was no appreciable difference between these sites. The results are summarized in Fig. 1. Comparison with the rest of the gut showed that there was less activity in R.N.A. and protein formation than elsewhere. It will be seen that R.N.A. is formed in the nuclei of the cellular layer and then migrates to the cytoplasm. Protein is principally formed in the basal and lower half of the cellular layer.

Correlation with cell turnover The squamous epithelium of the gut has a cell life of just over five days. The movement of cells bearing the methionine label fits well with this and suggests that the cells

retain their protein as they migrate towards the keratin layer. However the uridine label never appears in the keratin and it is probable that R.N.A. is lost before cells enter this layer.

PYLORUS OF STOMACH The results are summarized in Fig. 2. Ribonucleic acid is formed principally in the basal area of the glands but in considerably smaller amounts than in the small intestine, as judged by the intensity of labelling. There was little change in the distribution of the uridine label between six and 24 hours but after 48 hours the surface cells, which had hitherto been empty, contained a moderate amount.

Protein is also formed in a moderate amount and mainly occurs in cells in the basal area and lower half of the glands. However, a striking finding was a big increase in the amount of methionine throughout almost the whole of the glands at six hours.

Correlation with cell turnover The epithelial cells of pyloric mucosa turn over in about one day which means that cells formed at the time of injection are

FIG. 2. Diagram of distribution of radioactive label in pylorus of stomach.

at, or near, the surface at 24 hours. However, the pattern of R.N.A. distribution is unchanged between six and 24 hours, indicating that R.N.A. is lost from the cells during their migration. At 48 hours the uridine label is present in the surface cells and this is most easily accounted for by reabsorption.

The big increase in the amount of methionine between one and six hours is of interest for in this time little movement of the cells has taken place. Thereafter, however, the amount of methionine label diminishes very much as would be expected from the migration of cells.

DUODENUM AND JEJUNUM These two areas are considered together as there was no appreciable difference between them. The results are summarized in Fig. 3. Brunner's glands do not share in the rapid cell turnover and are considered elsewhere.

In the duodenum and jejunum R.N.A. is being formed in the largest amounts seen anywhere in the epithelial of the gut. The sites of formation are the epithelial cells of the crypts and lower thirds of the villi and, strikingly, in some epithelial cells at the tips

of the villi, in which nuclear labelling is heavy at one hour. This correlated extremely well with the observations of protein formation at one hour since protein is formed in large amounts in cells of the crypts, the lower parts of the villi, and also at the villous tips.

At six hours there is a decrease in the amount of methionine label in cells at the tips but at 24 hours a marked change has occurred in that there is a large increase in the amount of methionine in cells covering the whole length of the villi.

The stroma of the villi also shows concentration of the labels. Uridine is present in about half the round cells at one hour after injection and these labelled cells are situated mainly in the middle of the core. At 24 hours these centrally situated cells contain appreciably less uridine but there are now many cells immediately beneath the basement membrane of the epithelium which show heavy concentration of the label. This is also found at 72 hours (Fig. 6).

Methionine is present in small amounts in most of the round cells in the core of villi at one hour after injection. At 24 hours there is an increase in the amount of methionine label in these cells and labelling is also diffusely present throughout the

FIG. 6

FIG. 6. *Jejunum; villus tip 72 hours after injection of 100 μ c. of tritium-labelled uridine. $\times 620$. Positions of heavily labelled round cells closely applied to basement membrane are indicated in the line drawing.*

Uridine

Methionine

FIG. 3. *Diagram of distribution of radioactive label in duodenum and jejunum.*

FIG. 4. Diagram of distribution of radioactive label in ileum.

interstitial spaces of the core. The same picture is seen at 48 hours but by 72 hours there is only light labelling of the round cells.

Correlation with cell turnover The cell life of the epithelium in this area is between two and three days. Ribonucleic acid and protein are predominantly formed in the production zones, as elsewhere. However, the striking finding in this part of the intestine is the formation of R.N.A. and protein in cells at the tips of the villi which are due to be lost within the next few hours. This observation is supported by the decrease in amounts of R.N.A. and protein in cells at the villous tips at six hours. Subsequently, as the cells formed in the crypts migrate up the villi, R.N.A. is lost and the increase in the uridine label in the cells of the outer part of villi, seen at 72 hours, is probably due to reabsorption.

The methionine studies present one anomaly in relation to cell turnover. Although at 24 hours the cells formed in crypts at the time of injection have only migrated to the lower third or half of the villi, there is at this time an increase in methionine in the cells of the outer half of the villi. This is accom-

panied by an increase in the amount of methionine in the villous cores. The subsequent movement of the labelled cells follows the pattern to be expected from the D.N.A. studies.

ILEUM The results are summarized in Fig. 4. Ribonucleic acid and protein are principally formed in the crypts and lower parts of villi, as indicated by the findings at one hour. At six hours the amount and distribution of R.N.A. is unchanged but the amount of methionine is increased in the outer half of the villi.

In the stroma of the villi some round cells were concentrating uridine at one and six hours. At 24 hours, and again at 72 hours, however, heavily labelled round cells were seen to be applied closely to the inner surface of the basement membrane of the epithelium. Round cells also showed labelling with methionine, lightly at six hours, moderately at 24 hours but, thereafter, only light labelling was present.

Correlation with cell turnover In the ileum epithelial cells migrate from the crypts to the tips of villi in 24 hours. Ribonucleic acid is lost as they

FIG. 5. Diagram of distribution of radioactive label in colon.

migrate and it seems likely that the increased amount of uridine occurring in cells lining the outer part of the villi at 48 and 72 hours is due to reabsorption. The increase in methionine noted at six hours, in epithelial cells over the outer half of the villi, cannot be due to migration, since D.N.A. studies have shown little change in the overall position of cells between one and six hours.

COLON The results are summarized in Fig. 5. Ribonucleic acid is formed principally in the basal and lower half of the glands. Protein is also formed in these sites with, in addition, some formation in the outer half of the glands and surface cells. No methionine labelling was seen in the mucus of goblet cells.

Correlation with cell turnover The colonic epithelium turns over in one day and R.N.A. is lost as the cells migrate to the surface. The accumulation of uridine in the surface cells at 72 hours is probably due to reabsorption.

The methionine studies indicate that the colonic epithelium is exceptional since no increase of the labelling occurs after one hour.

DISCUSSION

Several broad patterns emerge from this study. Synchronous R.N.A. and protein formation is actively occurring in the production zones, *i.e.*, the areas of mitosis from which cells migrate to the surface of the membranes. Generally the extent of this formation correlated well with the rate of production of new cells, being greatest in the upper small intestine and least in squamous epithelium, as might be expected. An unexpected finding is the 'burst' of R.N.A. and protein formation in cells at the tips of villi in both the duodenum and jejunum. This is occurring in cells that are about to be lost by desquamation into the lumen. It seems probable that this represents enzyme formation which may be necessary either for the partial dissolution of the cells before release, or for carriage by the cells into the lumen, or for both these activities.

The exact site of formation of R.N.A. is clear in squamous epithelium. It is formed in the nuclei and later migrates to the cytoplasm. Elsewhere, however, whereas R.N.A. is principally formed in nuclei,

there also appears to be some synthesis in cytoplasm, unless the speed of formation is so rapid that much has left the nuclei within one hour. These observations are too crude to give a more precise answer.

The subsequent behaviour of R.N.A. is consistent throughout the gut. As the cells migrate R.N.A. steadily disappears so that by the time the cells reach the surface little remains. This correlates well with the observation by Padykula, Strauss, Ladman, and Gardner (1961) that basophilia is prominent in the crypts but becomes less as the cells ascend the villi of the small intestine, since basophilia is predominantly due to the presence of ribonucleic acid. Some of this disappearance of R.N.A. may be related to the presence of ribonuclease in the small intestinal mucosa. It may also be significant that, in the small intestine, round cells lying immediately beneath the basement membrane take up the R.N.A. label strongly at the same time as the intensity of labelling is diminishing in epithelial cells. It is tempting to speculate that these round cells are transporting away some of the ribonucleic acid. In squamous epithelium this latter mechanism cannot be involved but, by whatever means R.N.A. is lost, none is seen to enter the keratin layer.

Protein is undoubtedly manufactured in dividing and young cells but after this initial concentration there is a further increase of methionine in mature cells of the pylorus and particularly of the small intestine. This is observed at six hours in the pylorus and ileum but in the duodenum and jejunum it does not reach maximal concentration until 24 hours. Although this might represent reabsorption of methionine the timing of the increase makes this unlikely. In the ileum the amount of methionine is decreasing at 48 and 72 hours with the migration of cells while, at the same time, heavily labelled cells are being lost into the jejunal lumen. If reabsorption were occurring an increased amount of methionine in the cells of the ileum might be expected at this time, but it is significant that this is not observed, and, furthermore, no increase of labelling is ever visible in the colon. It seems more likely, therefore, that this increase in methionine represents a build-up of protein in the mature cells. Coincident with this build-up there is an increase in methionine in round cells of the small intestinal villi and in the interstitial spaces of the villous cores. A reasonable explanation for this would be that the round cells bring protein moieties to the mature epithelial cells and these are

then built into enzymes. As this is occurring in areas at a time when there is no demonstrable R.N.A. formation, and not much of the original R.N.A. of the cell is left, it seems more likely that the protein is preformed elsewhere and then brought by round cells to the epithelial cells.

The reasons for the presence of round cells in large numbers in the gut has always been obscure. If the present findings are accepted, however, at least two functions can be assigned to them: the bringing of preformed protein particles to the epithelial cells and the removal of R.N.A. particles from them. With the techniques used it was impossible to identify the histological types of round cell participating in these functions.

SUMMARY

Ribonucleic acid and protein metabolism in the gastrointestinal epithelium with rapid cell turnover has been studied in mice using radioactive uridine and methionine with autoradiography. Ribonucleic acid and protein are formed synchronously in the production (mitotic) zones of the epithelium and also in the cells of the tips of the villi in the duodenum and jejunum. Migration of labelled R.N.A. and protein broadly follows the pattern of cell migration. During this period the amount of R.N.A. decreases in all epithelia while the amount of protein increases in mature cells of the pylorus and small intestine. There is evidence that R.N.A. may be removed by round cells in the epithelial stroma and that the increase in cellular protein may be due to the transport of protein particles to the epithelium by round cells. Finally, there is evidence of reabsorption of uridine and methionine from the lumen of the gut.

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