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The turnover and shedding of epithelial cells

Part I The turnover in the gastro-intestinal tract

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SYNOPSIS This paper confirms that the epithelial lining of the small intestine is in a state of continuous replacement and demonstrates that the whole of the gastrointestinal epithelium has a similar dynamic equilibrium.

The intestinal epithelial cells proliferate and turn over quickly. The cells divide in the basal or crypt areas and migrate up the surface of the villi from which they appear to be shed into the intestinal lumen. A number of workers have confirmed this fact in the small intestine and made observations in the stomach mucosa (Friedman, 1945; Leblond and Stevens, 1948; Stevens and Leblond, 1953). The purpose of this work is to review the epithelial turnover of the whole gastro-intestinal tract in the mouse.

The methods used for measuring the turnover of a cell population fall into two categories. Either the rate of production or destruction can be measured, since in a steady state these must be equal, or the mean cell life of the population can be determined. There is no method available for measuring the rate of destruction of the epithelial cells in the intestinal tract but the rate of production can be inferred from the number of mitoses relative to the total number of cells in the epithelium (mitotic index). Further, if the mitotic duration is known, the turnover time, *i.e.*, the time taken for replacement of the whole population of epithelial cells, can be calculated. This method has been used by Leblond and others for the small intestine.

The mean cell life can only be measured if cells can be marked with a permanent label and then followed throughout their life until they leave the population. Such labelling has been made possible by the use of radioactive substances that are specifically incorporated into deoxyribonucleic acid and therefore become a permanent nuclear label. Tritiated thymidine has been used in this method and with autoradiography it gives a visual picture of cell turnover (Leblond and Messier, 1958). The radio-labelled substance is taken up by the nuclei of cells in premitotic state (Taylor, Woods, and Hughes, 1957) and subsequently these cells, and their descendants, remain detectable by autoradiographic techniques. It is assumed in this experiment that the labelled cells behave in the same way as normal cells, although it is possible that the introduction of tritium-labelled components may result in modifications of the deoxyribonucleic acid molecule (Krause and Plaut, 1960).

Both methods indicate that the turnover of cells is extremely rapid but there is some discrepancy between the published results. The mitotic index gives a faster rate than the labelling of cells, probably due to an assumed mitotic duration which is too short. Thus Leblond and Stevens (1948), using the mitotic index, calculated a renewal rate of 1·57 days in the duodenum and 1·35 days in the ileum of the rat, whereas Leblond and Messier (1958) showed a renewal rate of three days in the small intestine of the mouse by labelling with tritiated thymidine.

The present experiments were performed on mice using labelling with tritiated thymidine.

METHODS

Eighteen-day old albino male mice were used in all experiments. Twenty-three mice were each injected intraperitoneally with 100 μc. of tritiated thymidine (A.E.R.E. Harwell: 100 mc./mM.) in 0·2 ml. saline. Animals were sacrificed at one, two, four, and six hours, and one, two, three, and five days after injection. In some the peritoneal and thoracic cavities were opened and the whole mouse fixed in Bouin's solution so that complete sagittal sections of a half mouse could be made. In the rest the gastro-intestinal tract was removed and specimens were taken from various parts of the gut and fixed in Bouin's solution. Autoradiographs were set up using stripping film (Kodak Ario), and exposed for one month. The sections were stained with neutral red after developing.

RESULTS

The labelled cells were clearly apparent with positive grains clustered over and sharply confined to the nucleus. There was no evidence of labelling of the cytoplasm.

RATE OF UPTAKE OF TRITIATED THYMIDINE Maximal uptake had occurred in one hour in all the tissues examined. No further increase in density or distribution was seen up to six hours.

DISTRIBUTION OF UPTAKE The site and number of cells labelled up to six hours indicate the cell 'production zones' and the relative amount of division occurring in them. Throughout the gut three patterns were seen.

1 In the organs lined by stratified epithelium, *i.e.*, the tongue, oesophagus, and gastric fundus, a few cells in the basal layer were labelled (Fig. 1).

2 The body of the stomach showed a specific pattern of labelling over the middle third of the glands (Fig. 2). These glands are lined by parietal

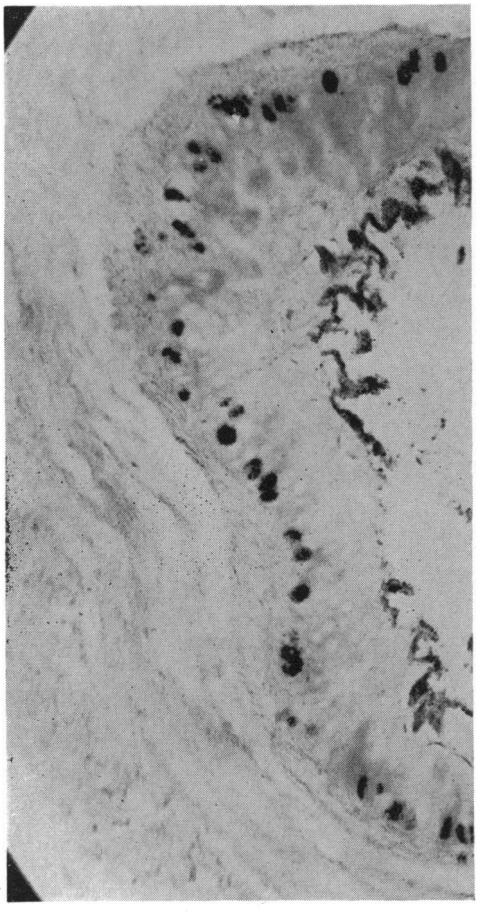

FIG. 1

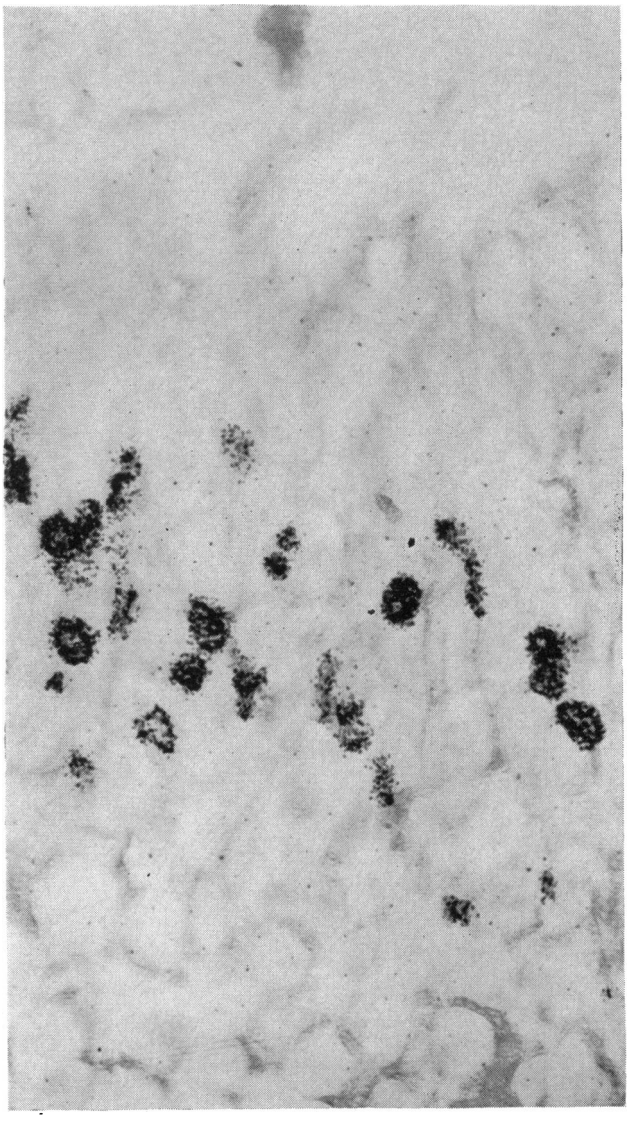

FIG. 2

FIG. 1. *Oesophagus showing labelling of nuclei in the basal layer two hours after injection 100 μc. of tritium-labelled thymidine.*

FIG. 2. *Body of stomach showing labelling of nuclei in the mid-third of the glands two hours after injection.*

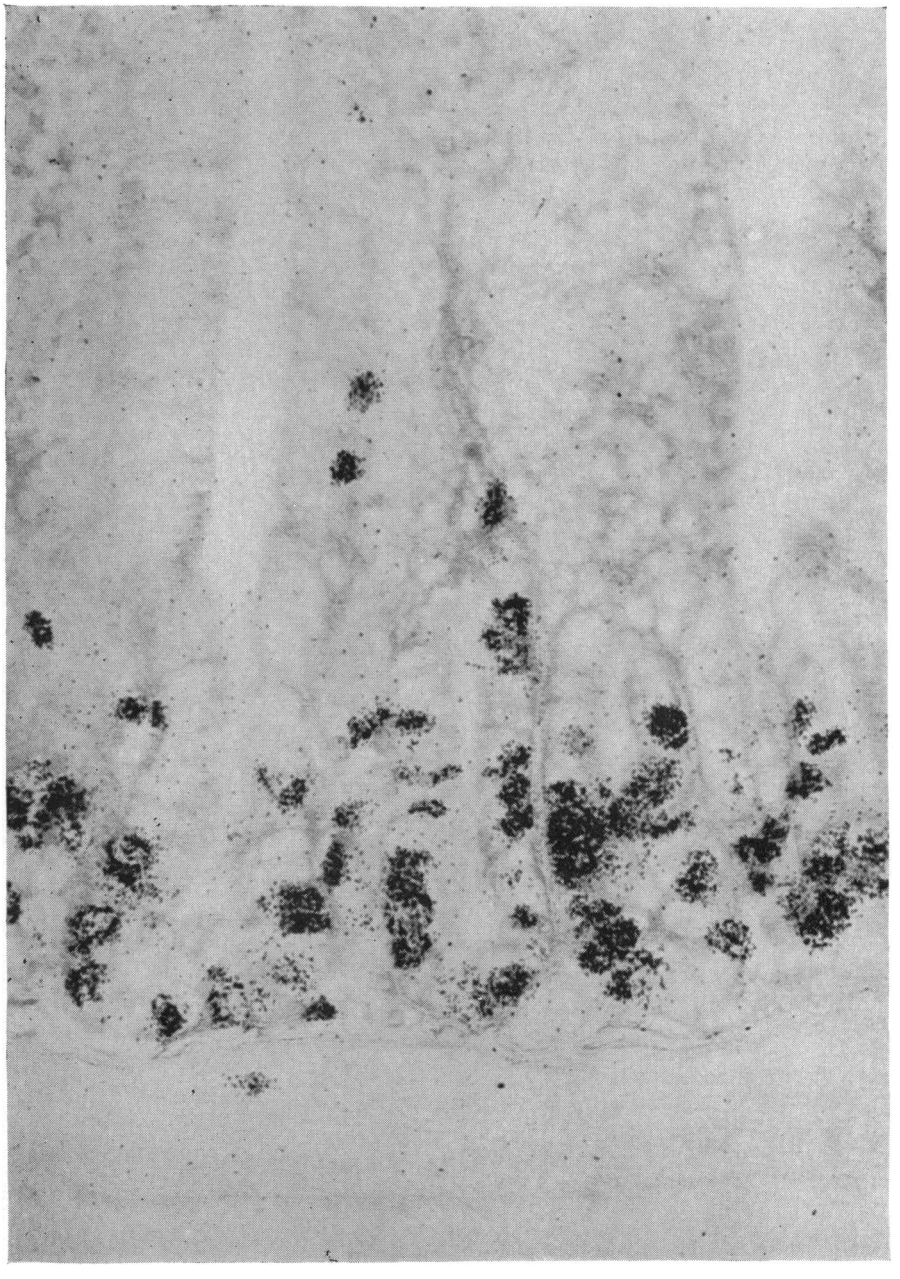

FIG. 3. *Jejunum showing labelling of nuclei in the crypts two hours after injection. Labelled lymphocytes are present in the villi.*

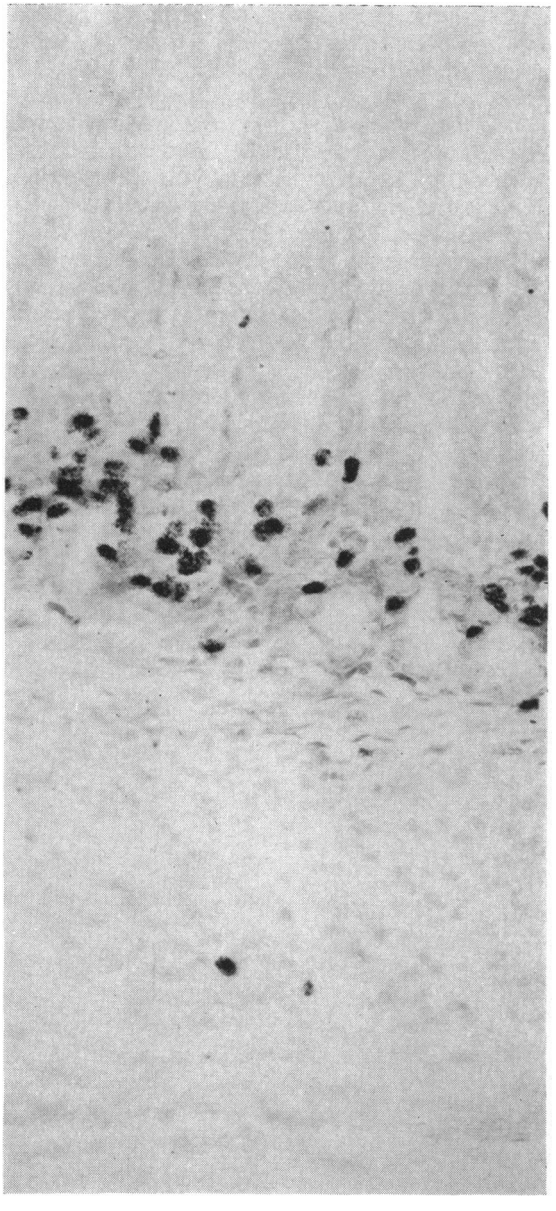

FIG. 4. *Gastric antrum showing labelling of nuclei in lower third of glands two hours after injection.*

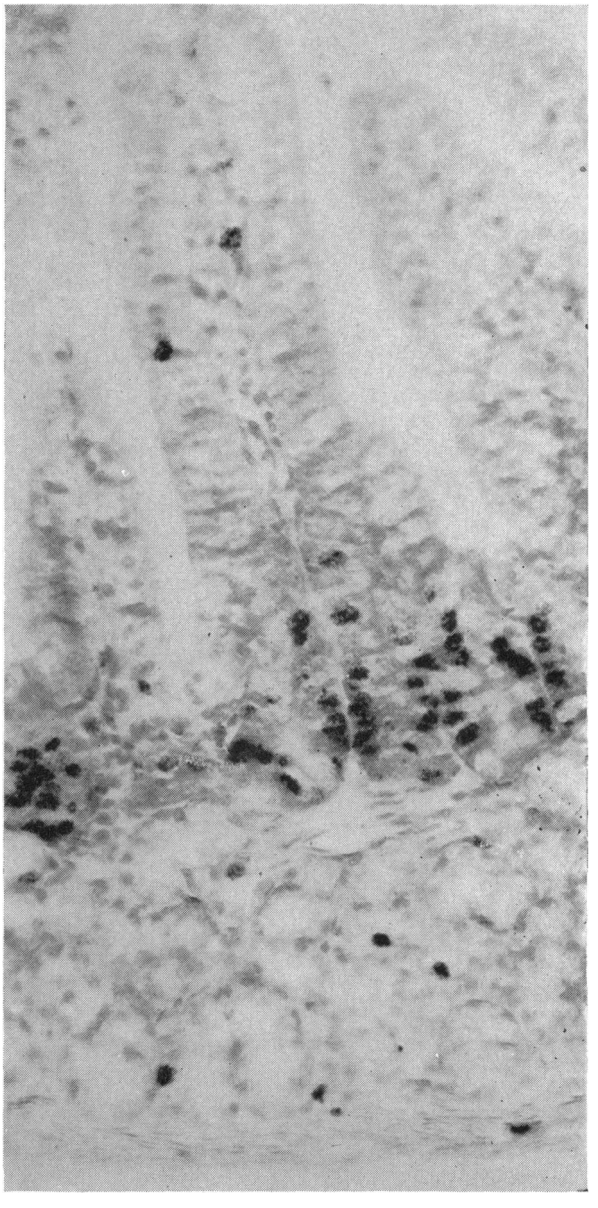

FIG. 5. *Duodenum showing labelled nuclei in crypts (above) but very scanty labelling in Brunner's glands (below) two hours after injection.*

and chief cells over the lower half and epithelial and goblet cells over the upper half but with considerable overlapping and mixing of these elements in the middle third. In the middle third a number of epithelial and goblet cells were labelled but there was no labelling of the parietal cells. In the lower third occasional cells were labelled, and these appeared to be chief rather than parietal cells.

3 The rest of the mucous epithelium of the gut showed a common pattern. In the duodenum and small intestine the cells lining the crypts were labelled (Fig. 3) and in the simple glandular epithelium of the gastric antrum and colon the cells lining the basal quarter or third of the glands were labelled (Fig. 4). In these areas most of the cells showed labelling. A few cells in the bottom of the crypts were unlabelled and these may have been Paneth or argentaffin cells but it was impossible to identify them. Brunner's glands were unlabelled except for a very occasional cell (Fig. 5).

Throughout the gut the lymphoid follicles showed the presence of labelled cells at one hour, and labelled lymphocytes were present in the mucosa in all specimens examined up to five days.

EVIDENCE OF MULTIPLICATION OF CELLS The observations at 24 and 48 hours showed an increased number of labelled cells in the gastric antrum, small intestine, and colon. For example, in the duodenum almost the entire villus was labelled at 48 hours though the crypt was nearly free of labelled cells. In contrast this increase of labelled cells was not apparent in stratified epithelium, reflecting the smaller number of mitoses.

MIGRATION OF LABELLED CELLS From the production zones the labelled cells were seen to move towards the surface of the epithelium or the tips of the villi. As has already been described a large number of cells are produced bearing the label, so it was assumed that the time taken for the first cell to reach the surface was representative of the migration time. The migration was a fairly constant phenomenon in any given tissue though there were minor variations between villi in the small intestine. The migration time of the stratified epithelia was about five days for the oesophagus and gastric fundus and somewhat over five days for the tongue (Fig. 6). In the body of the stomach labelled cells migrated from the middle third to near the surface in 24 hours, so the migration time is probably just over one day. The gastric antrum and colon showed a migration time of about one day. In the duodenum and jejunum the labelled cells travelled up the surface of the villi and the tips were reached sometime between 48 and 72 hours (Fig. 7). In the ileum, however, the tips were reached in 24 hours and it is possible that this difference is attributable to the shorter length of the villi in this part of the small intestine. Once the column of migrating cells had reached the epithelial surface or villous tip the number of labelled cells steadily diminished, although

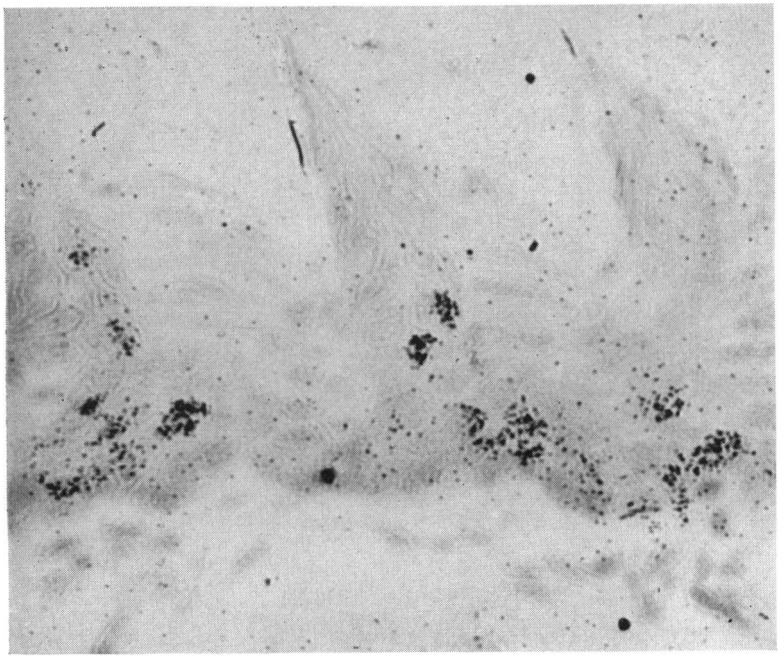

FIG. 6. *Tongue showing migration of labelled nuclei away from the basal layer two days after injection.*

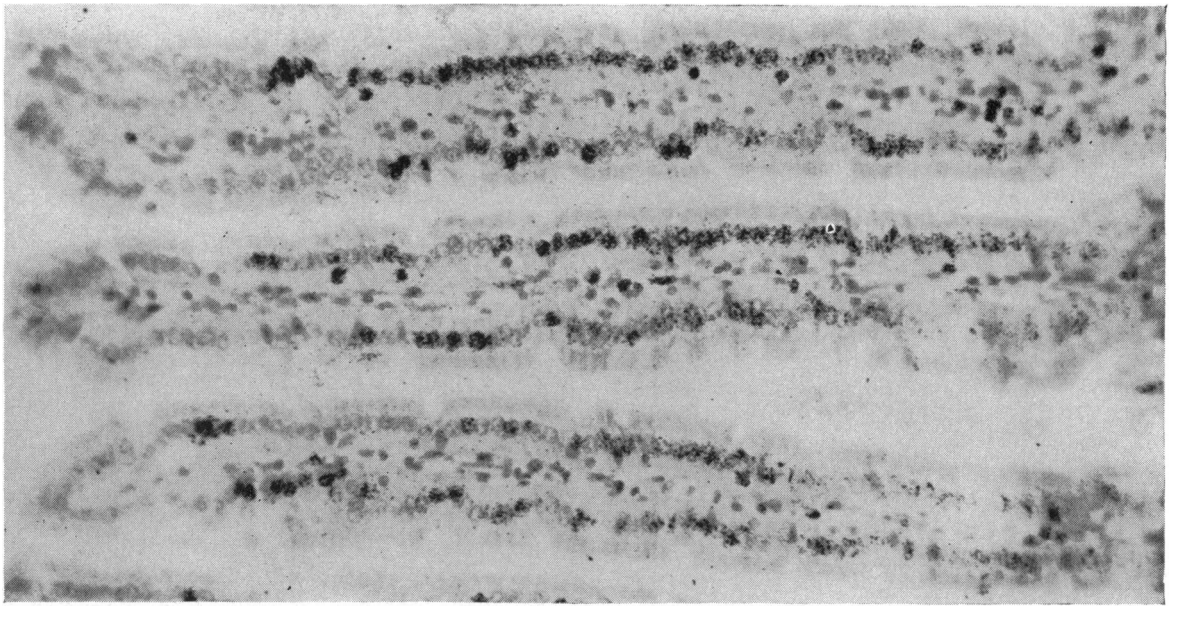

FIG. 7. *Jejunum showing labelled nuclei having migrated almost to the tips of the villi 48 hours after injection.*

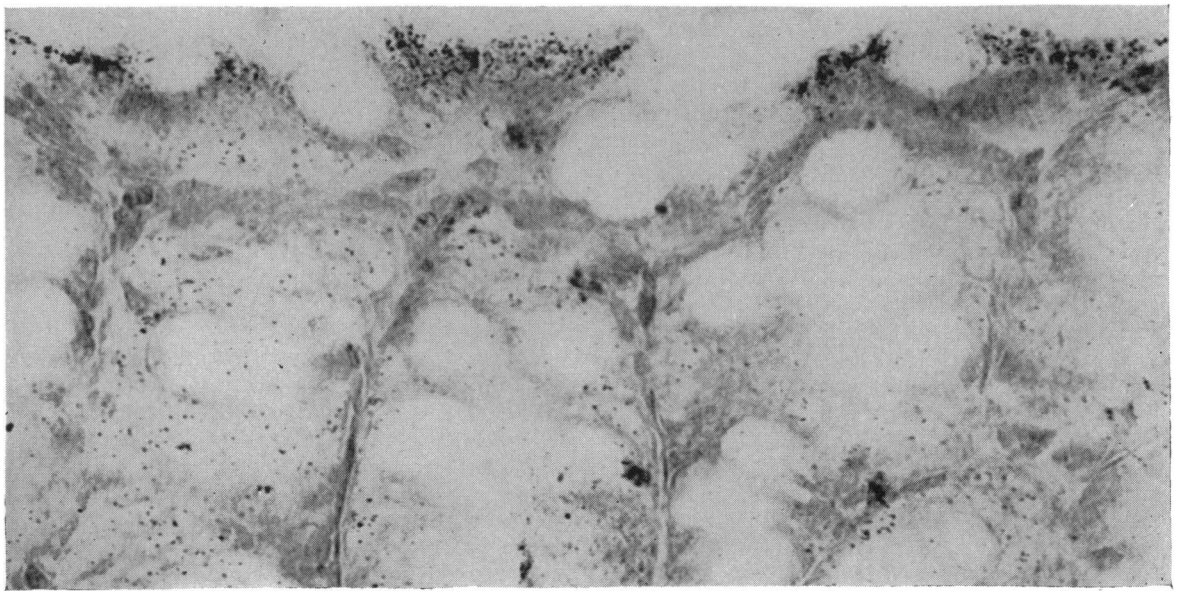

FIG. 8. *Colon showing fringe of positive grains in the cytoplasm of surface cells five days after injection.*

in the production zones a few cells remained with some labelling up to five days.

EVIDENCE OF CELL LOSS The diminution in number of labelled cells is, in itself, evidence of loss of epithelial cells. In the autoradiographs, however, it was rare to see evidence of individual cells shedding from the epithelial surface, and it was also rare to see clumps of cells in the lumen of the gut, although occasional disintegrated cells were evident with the nuclear label spilled about.

EVIDENCE OF REABSORPTION OF THE LABEL In the early stages, and during the migration of the epithelial cells, the positive grains in the autoradiographs were concentrated over the nuclei. During the period in which the labelled nuclei were disappearing, the epithelial cells were searched for the reappearance of radio-activity. This was seen only in the colon where, in the surface cells, a fringe of positive grains was present in the outer part of the cytoplasm (Fig. 8). This was evident from two to five days when all the labelled nuclei had disappeared from the colonic epithelium, but labelled cells were still disappearing from the duodenum and jejunum.

DISCUSSION

This work has confirmed the extremely rapid turnover of the gastro-intestinal epithelial cells. Although the observations were made in weanling mice the magnitude of cell proliferation was vastly in excess of that required for growth, and, in addition, the results agree well with those of other workers using adult animals (Friedman, 1945; McMinn, 1954) and the duodenal mucosa in man (Bertalanffy and Nagy, 1958).

The overall pattern that emerges is one of intense and constant proliferation in the production zones, and a steady migration of adult epithelial and goblet cells towards the lumen. The life of these cells seems to depend on the distance to be covered, the longest life being in the jejunum where the distance up the villi to the lumen is longest. In areas covered by stratified epithelium division and migration are similar but slower. The mucosa of the body of the stomach is a special case for, in this situation, the glands contain non-dividing parietal and chief cells in the lower half and actively dividing epithelial and goblet cells in the upper half. The 'production zone' is in the middle third of the glands and most of the cells appear to migrate to the surface. However, dividing and non-dividing elements are mixed in the middle third of the gland and some of these epithelial cells may be unable to migrate and may be lost into the neck region of the gland pit, as Stevens and Leblond (1953) suggest.

All workers in this field have suggested that the cells are shed into the lumen of the gut after they have reached the surface of the mucosa or the tip of the villus. This is considered in Part II. All that can be said here is that it is rare for cells to be seen in the act of shedding, and cells in the lumen are difficult to find in our preparations. This points to the swift dissolution of shed cells. It is highly probable that many of the constituents of these cells are reabsorbed, but direct evidence is lacking in the small intestine. However, in the colon the label was seen to reaccumulate in the periphery of the cytoplasm of the surface cells and was interpreted as indicating reabsorption of tritium, perhaps still incorporated with thymidine, into the epithelial cells. This appearance suggests that the colonic cells cannot handle it and that the substance piles up in the cytoplasm. In contrast, in the small intestine the tritium-labelled molecule may be rapidly absorbed so that it is not seen in the epithelial cells.

The reason for this intense division and rapid turnover of epithelial cells is not clear, but it involves the body in a vast exchange of cell mass every day. Indeed Leblond and Walker (1956) have calculated that man may lose half a pound of cells into the intestinal lumen in 24 hours. The only cells not participating in this process are the secretory cells of the stomach, Brunner's glands in the duodenum, and also the cells of the salivary glands and pancreas (Bamforth, Creamer, and Shorter, 1960).

SUMMARY

The turnover of gastro-intestinal epithelium in the mouse has been studied, using tritiated thymidine as a nuclear label and autoradiography. In the tongue, oesophagus, and fundus of the stomach the turnover time is about five days. In the body of the stomach the epithelial cells originate in the mid-third of the glands and migrate to the surface, turning over in one day. In the gastric antrum and colon the epithelial cells originate in the basal part of the glands and migrate to the surface, taking about one day for the turnover cycle. In the duodenum and small intestine the epithelial cells arise in the crypts and migrate up the villi to the tips, taking from one to about two and a half days depending on the length of the villi. Parietal and chief cells in the stomach and Brunner's glands in the duodenum do not turn over in this manner.