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Cell differentiation in human gastric gland as revealed by nuclear binding of tritiated actinomycin

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SUMMARY The nuclear binding of H^3 actinomycin, which is closely linked to the differentiation phenomenon, was studied in human normal gastric mucosa. Actinomycin binding decreases in cells which differentiate and becomes very low in fully differentiated cells. In the gastric pits, there is a decreasing gradient of labelling from the deeper stem cells to the well-differentiated superficial cells. This indicates that migration and renewal of the surface epithelium occurs following a 'pipe-line' system. All the undifferentiated stem cells are labelled. Where the parietal cells are concerned all degrees of labelling are observed at various levels with a decreasing proportion of labelling from the surface to the bottom of the gland. With mucous cells and chief cells in the upper part of the gland a great number of poorly labelled mucous neck cells is observed. In the middle and lower part of the gland there is a new growth of heavily labelled cells. This means that in the normal human stomach chief cells probably do not originate from mucous cells.

The introduction of H^3 thymidine autoradiography provided appreciable progress in the understanding of cell proliferation and has been extensively used on animal stomachs: the incorporation of H^3 thymidine occurs mainly at the isthmus region of gastric gland, where cell proliferation for the replacement of surface epithelial cells and glandular cells takes place (Messier and LeBlond, 1960; Hunt and Hunt, 1962; Willems and Lehy, 1975).

The migration of cells and the renewal of the surface epithelium appears to follow a 'pipe line' system—that is, 'first produced, first migrate'—from the depth of the pits up to the mucosal surface (MacDonald *et al.*, 1964, Willems *et al.*, 1971). The parietal cells are produced through transformation of the immature cells of the neck area (Ragins *et al.*, 1968; Willems *et al.*, 1972).

However, many questions remain to be definitively answered—for instance, what are the mechanisms of parietal cell migration (either pipe-line system or aleatory, stochastic system; are the mucous neck cells transformed into chief cells or is the turnover of chief cells assured by their own mitotic activity?

Several authors have suggested that the renewal of the chief cells is assured mainly by transformation of mucous neck cells (Creamer *et al.*, 1961; Lipkin *et al.*, 1964; MacDonald *et al.*, 1964; Matsuyama and Suzuki, 1970; Hattori and Fujita, 1976; Seeling *et al.*, 1978). Others consider that these cells are renewed by mitotic division (Myrhe, 1960; Hunt and Hunt, 1962; Willems *et al.*, 1972) occurring mainly during the night in rodents, on which the experiments were carried out. These points were investigated in the present study by observing the nuclear binding of H^3 actinomycin (3H.AM.), which is closely linked to the differentiation phenomenon in the various types of cells of human gastric mucosa.

This technique has been demonstrated to be a valid method for providing a rough evaluation of the number of free DNA sites capable of combining with 3H.AM. Actinomycin binding decreases in cells which differentiate and becomes very low in fully differentiated cells. Thus the surface epithelial cells are less labelled than the cells at the bottom of the pit, as previously demonstrated (Stoffels *et al.*, 1978).

Methods

Fourteen gastroscopically normal stomachs were biopsied in the fundus under endoscopic guidance between 09.00 and 11.00 A.M. hours after a 14 hours' fast. The average age of the patients was 55 years (SD ±11). They were gastroscoped for non-organic

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complaints. The stomach was gastroscopically normal and patients had no evidence of any systemic disease.

Biopsies (two for each patient) were always taken from the fundus, at endoscopy, and in the middle part of the anterior wall of the lesser curvature. The patient sample included five females and nine males. Tissue samples were fixed for 48 hours in 100% methanol. After paraffin embedding, the tissue was sectioned at 3μ thickness. A sample for routine histology was stained with haematoxylin erythrosine. The sections were then treated with 3H-actinomycin as previously described and submitted to the autoradiographic process (Brachet *et al.*, 1969; Preumont *et al.*, 1978). Twenty-four slides were prepared from the tissue biopsies obtained in each case. Grain count was assessed in all cells where the nucleus was covered with more than 2 grains. The total number of cells counted for all the stomachs was 1500 cells.

The gastric glands under the isthmus, from the region where parietal and mucous neck cells could be identified to the bottom of the glands, were subdivided into three parts: upper part, middle part, and lower part.

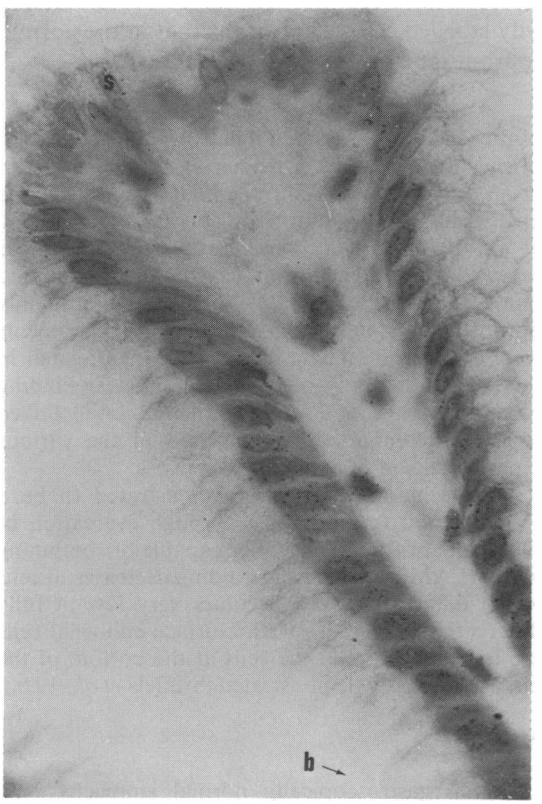

Fig. 1 *Surface epithelium and gastric pits. Gradient of labelling across the pits. s: surface, b: bottom of the pits (×250, original magnification). The absence of background can be observed.*

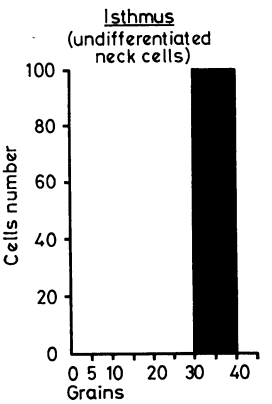

Fig. 2 *Grain count per cell distribution Each graph in Figs. 2, 3 and 4 represents the distribution of all cells counted.*

Results

SURFACE EPITHELIAL CELLS AND GASTRIC PITS

The surface epithelial cells were poorly labelled. A decreasing gradient of labelling from deeper stem cells to well-differentiated superficial cells was observed in the gastric pits (Fig. 1).

ISTHMUS

All the undifferentiated neck cells and mucous neck cells were heavily labelled (Fig. 2).

PARIETAL CELLS

The histogram of grain counts per cell over parietal cells indicated that all degrees of labelling may occur at the various levels of the gland. Each portion of gastric gland is composed of a mixture of differently labelled cells, but the proportion of heavily labelled cells decreases from the surface to the bottom of the gland (Fig. 3).

MUCOUS CELLS

In the upper part of the gland a great number of poorly labelled cells was observed. In the middle part of the gland heavily labelled cells were more frequent (Fig. 4).

CHIEF CELLS

In the lower part of the gland where most of the cells were chief cells and only a few mucous cells were observed, the grain count histogram showed a

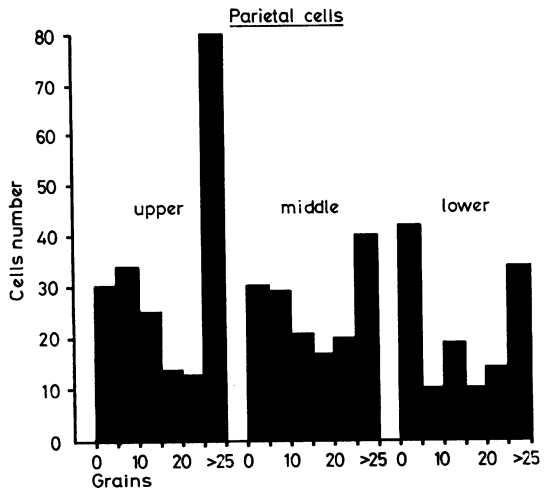

Fig. 3 *Grain count per cell distribution.*

greater number of poorly labelled cells, and a considerable number of heavily labelled cells.

Discussion

Little is known about the molecular events that occur during differentiation. DNA not only contains sequence information for structural genes, but also includes sequences important to the organisation of DNA into chromosomes and possibly for coordinated expression of many genes required to form differentiated cells. Genes are combined with histone and non-histone proteins to form a nucleoprotein complex called chromatin. Chromatin consists of a transcriptionally active open fraction (euchromatin) and a condensed fraction (heterochromatin) containing the genes not expressed in specific cells. There is a higher binding of tritiated actinomycin in euchromatin than in the repressed heterochromatin (Berlowitz *et al.*, 1969).

The capacity of this intercalating ligand to bind to DNA has been proved to be limited by the amount of chromosomal proteins (histone and non-histone proteins) (Ringertz and Bolund, 1969; Beato *et al.*, 1970; Pederson and Robbins, 1972). When resting and cycling cells are compared, the capacity to bind 3H-AM by the nuclei is higher in the latter (Pederson, 1972; Preumont *et al.*, 1978).

Histoautoradiography with H^3 thymidine is a useful method for determining the ability of cell types to divide and for calculating the rate of cell proliferation. It can also be used as a cellular tracer. Unfortunately, the results obtained differ greatly when experimental conditions are not exactly identical. For this reason many contradictory results have been published concerning gastric mucous neck cells, parietal and chief cells renewal. When cells are non-cycling (as indicated by studies using H^3 thymidine), the incorporation of H^3 actinomycin is closely linked to the differentiation phenomenon.

Brachet and Hulin (1969) have shown in amphibian embryos undergoing differentiation that actinomycin binding decreases when cells are differentiating and becomes very low in fully differentiated cells such as spermatozoa or lens cells.

Our results concerning the binding of H^3 actinomycin to human surface epithelium have already been discussed (Stoffels *et al.*, 1978) and agree with the results of Brachet and Hulin (1969). The maximal grain count was observed in the lower part, where cells are proliferating (high rate of 3H-thymidine uptake) (Willems *et al*, 1971; Willems and Lehy, 1975).

By contrast, epithelial cells of the glandular area (upper part) in which no mitotic activity occurs (Ragins *et al.*, 1968; Willems *et al.*, 1971, 1972; Willems and Lehy 1975; Hattori and Fujita, 1976) show a low 3H-AM binding. There is thus a gradient of labelling in the pits from stem cells to differentiated superficial cells (Stoffels *et al.*, 1978).

In a theoretical study of cellular migration in hamster stomachs Hattori *et al.* (1976) have plotted curves of distribution of labelled cells with H^3-thymidine. The shape of the curves appears similar to the frequency distribution of binomial or Poisson type.

Thus, the gastric gland seems to be composed of a mosaic of young and old parietal, chief, and neck cells. The migration process can be referred to as

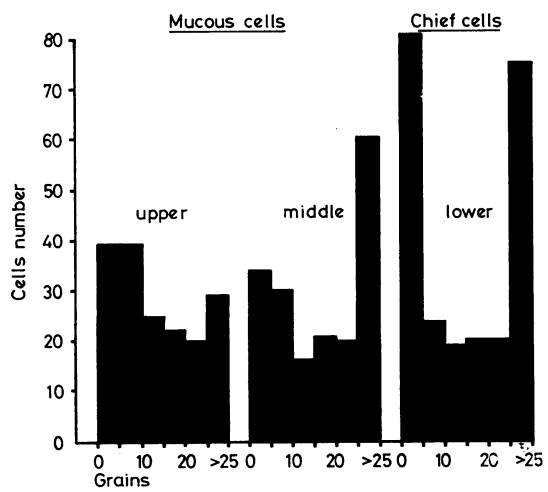

Fig. 4 *Grain count per cell distribution.*

the 'conjectural flow' system, with cellular movements perpendicular to the long axis of the glandular tubule, combined with downward cell migration. Our results partially corroborate this theory.

PARIETAL CELLS

There seems to be a mixture of well and slightly differentiated parietal cells in the full length of the gland, but the proportion of well-differentiated cells increases progressively after the migration process from the top to the bottom of the gland.

MUCOUS NECK CELLS

A mosaic of less and fully differentiated cells is observed in the upper and middle part of the gland, but there is a greater proportion of well-differentiated cells in the upper part, which could imply that the latter cells may have differentiated rapidly.

CHIEF CELLS

In the middle and lower part of the gland, a new growth of young chief cells is observed. Thus, the distribution of the chief cells does not correspond to a binomial frequency distribution, suggesting perhaps that some phenomenon other than progressive differentiation is taking place in this part of the gland. There is a new growth of chief cells, probably secondary to a mitotic activity of the chief cells in the inferior portion of the gland, independent of neck stem cell.

Willems (1977) has demonstrated that regeneration of chief cells in the normal rodent stomach is assured by the mitotic activity of other chief cells. Inferences from animal experiments to the human situation cannot be justified, but it is not possible to study DNA synthesis by injection of thymidine *in vivo* in human experiments. *In vitro* studies have been performed in man, but where the phenomenon of differentiation is concerned, studies *in vitro* and *in vivo* cannot be adequately compared. The observations of Willems could explain our results: if the mucous neck cells differentiated into chief cells in the normal human stomach and migrate as a 'conjectural flow system' the bottom of the glands would be composed of a mosaic of slightly and well-differentiated cells, with a greater number of well-differentiated cells. In the hypothesis that all the mucous neck cells progressively differentiate into chief cells, then all the chief cells would be less labelled by H_3 actinomycin than any mucous cell. We did not observe this. But this does not exclude the fact that under abnormal conditions chief cells might originate from mucous neck cells in animal stomachs. The molecular events that occur during differentiation of pluripotential stem cells are certainly complex and can be influenced by unusual conditions, such as neonatal development (Helander, 1969), long periods of fasting (Willems, 1971), vagotomy (Crean *et al.*, 1968), or unusual hormonal conditions (Crean, 1968; Crean *et al.*, 1971).

In conclusion, with parietal cells there is a mosaic of well- and less-differentiated cells throughout the length of the human stomach gland with an increase of well-differentiated cells when migration proceeds from the top to the bottom of the gland.

Mucous neck cells differentiate rapidly in the upper third of the gland, but our studies did not elucidate whether some of the mucous cells differentiate into chief cells.

There is a new growth of chief cells in the lower part of the gland.

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