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Nuclear binding of tritiated actinomycin in surface epithelial cells from normal stomach and atrophic gastritis

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SUMMARY Tritiated actinomycin binding to DNA is closely linked to the degree of repression in chromatin. 3H-AM binding to DNA is the most pronounced in nuclei of cells committed into cycle. Inversely, in cells in the last steps of their differentiation or (and) in the resting state (non-dividing cells), 3H-AM binding for DNA is diminished down to a baseline since it is limited by the deoxynucleoproteins. Epithelial cells of stomach mucosa and duodenum demonstrate an increased cell uptake of tritiated actinomycin from the surface to the bottom of the pits. In severe gastritis and in intestinalysed metaplasia this was abolished: with a uniform enhancement of 3H-AM binding. These findings seem to indicate that these cells are derepressed.

Atrophic gastritis is a pathological condition that may predispose to the development of gastric cancer (Schindler, 1965; Siurala *et al.*, 1966; Walker *et al.*, 1971). Numerous authors have investigated cell proliferation kinetics in normal and atrophic stomach mucosa. An increased proliferation of the epithelial cells in the latter has been described (Deschner and Lipkin, 1976), while in cases of intestinalisation of the gastric mucosa, which is found in over 90% of gastric cancers (Morson, 1959), the same authors have described an impairment of epithelial cell maturation.

Earlier work has reported an increased nuclear fixation of tritiated actinomycin (3H-AM) in cancer cells as compared to the corresponding normal cells (Brachet and Hulin, 1970; Pileri *et al.*, 1972; Heenen *et al.*, 1973). This technique has been shown to be a valid method for the rough evaluation of the number of free DNA sites: the fixation of labelled actinomycin by the nuclei is closely linked to the degree of repression in chromatin (Brachet and Ficq, 1965; Brachet, 1971).

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In this study, we have compared the binding of 3H-AM to chromatin in epithelial cells in normal stomachs and in atrophic gastritis by autoradiographs. Significant increases of 3H-AM binding would suggest that these cells are in a depressed state.

Methods

Twenty-five patients were studied; eight had a normal gastric mucosa and 17 had a atrophic gastritis as defined by the histological criteria described by Whitehead *et al.* (1972).

The grades of gastritis were (1) superficial, and (2) atrophic with three grades: mild, moderate, and severe. Superficial gastritis is characterised by mononuclear and polymorphonuclear cell infiltration located in the surface epithelium and gastric pit region. In mild atrophic gastritis the infiltration occurs into all layers of lamina propria with a slight reduction of parietal and chief cells. Moderate and severe atrophic gastritis includes all the above signs, in addition to a greater reduction in parietal and chief cells, and the presence of intestinal metaplasia and regenerative nodules.

It must be remembered that in this classification 'active atrophic gastritis' signifies the presence of

regenerative nodules where 'both degenerative and regenerative processes are seen'. The mean age of the two groups was 58 years ± 16. Among the 17 patients with gastritis, four had a concomitant benign gastric ulcer, three a subtotal gastrectomy, two cirrhosis, one uraemia, one a pyloroplasty, and six had unrelated diseases. Biopsies (four for each patient) were always taken from the fundus of the stomach at endoscopy and in the middle part of the anterior face of the lesser curvature.

Tissue samples were fixed for 48 hours in pure methanol. After paraffin embedding, the tissue was sectioned in 3 μ thicknesses. A sample for routine histology was stained with haematoxylin erythrosine. Histological slides were then treated with 3H-AM as previously described (Brachet *et al.*, 1970) and submitted to the autoradiographic process. Samples of normal and atrophic mucosa were placed on the same slide; eight slides were performed for each case. Grain count was assessed in all cells where the nucleus was covered with more than two grains and a minimum of 100 cells were scored in each case. For example, when cells of surface epithelium are compared to cells of the bottom of the pits all cells covered by more than two grains of each region are counted.

When intestinal metaplasia was observed histologically, samples of gastric mucosa were compared with normal biopsies from the fourth part of the duodenum, taken from five other normal patients under endoscopic guidance. Intestinal metaplasia was observed in seven patients and coexisted always with severe atrophic gastritis. The biopsies were first assessed for atrophic gastritis and intestinalisation—when there is intestinal metaplasia, epithelial cells of small intestinal type (absorptive cells, goblet cells etc.) can be recognised.

Results

NORMAL GASTRIC MUCOSA PITS

Nearly all the cells are labelled. Table 1 shows that the highest number of grains occurs in cells at the bottom of the pits (neck and isthmus region).

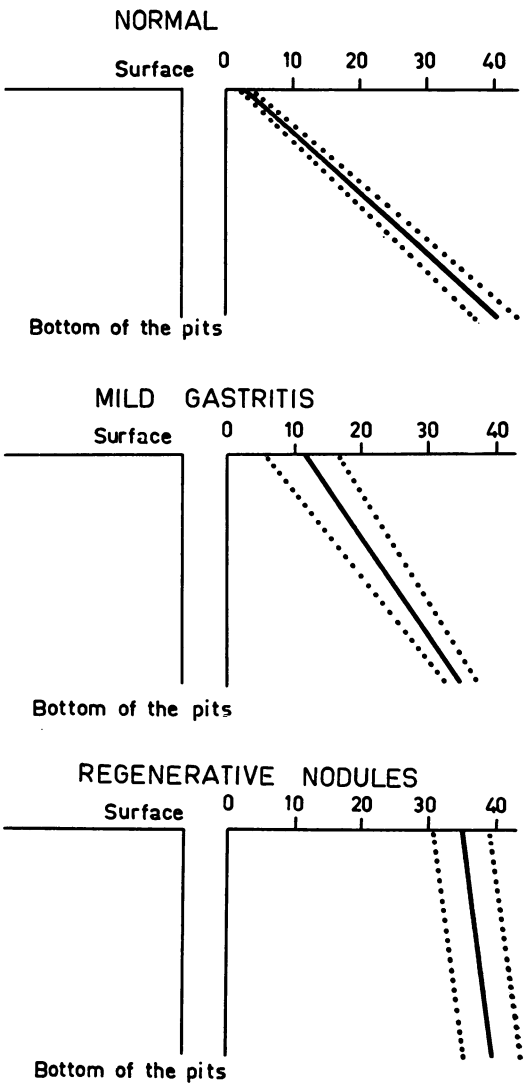

Fig. 1 *Comparison of the nucleus grains count of tritiated actinomycin from the bottom of the pits to the surface. The gradient of labelling is very well marked in normal mucosa.*

Table 1 *Binding of 3H actinomycin in nuclei of surface epithelial cells and undifferentiated neck cells in normal stomach and in cases of atrophic gastritis (average number of silver grains/nucleus)*

	Normal (*A*)	*Mild atrophic gastritis* (*B*)	*Regenerative nodules* (*C*)
Surface epithelial cells	3 ± 2	10 ± 11	36 ± 9
Undifferentiated neck cells	40 ± 7	32 ± 5	41 ± 9
± : Standard deviation			
Statistical differences in surface epithelial cells	(A) vs (B)	$P < 0{\cdot}0001$	Number of cells: 100
	(B) vs (C)	$P < 0{\cdot}0001$	
Statistical differences in surface epithelial vs undifferentiated neck cells	(A) $P < 0{\cdot}0001$	Number of cells : 500	
	(B) $P < 0{\cdot}0001$	: 500	
	(C) NS	: 100	

Moreover, the data indicate a gradient of labelling across the pits from stem cells to differentiated luminal cell layer (Fig. 1).

PATHOLOGICAL GASTRIC MUCOSA PITS

Superficial or mild gastritis (eight cases)
A gradient of labelling similar to that seen in normal mucosa is observed, but luminal cells are more heavily labelled than their counterparts in normal mucosa (Fig. 1).

However, this increased grain number in superficial pathological mucosa cells remains significantly lower ($P < 0{\cdot}0001$) than that found for the proliferating bottom cells (Table 1).

Severe gastritis (9 cases)
Regenerative nodules The gradient described above is abolished: cells of the different layers show a uniform enhancement of 3H-AM binding, the grain numbers being similar from the bottom to the surface (Fig. 1).

Intestinal metaplasia In some areas differentiated glands are observed. In such cases, there is also a gradient of labelled cells from the surface to the bottom, as observed in normal fourth duodenum, but the surface epithelial cells are more heavily labelled than in normal intestinal epithelium (Figs 2 and 3). In most cases, the surface epithelial cells of intestinal metaplasia are as intensely labelled as those at the bottom of the glands (Fig. 3) (Table 2).

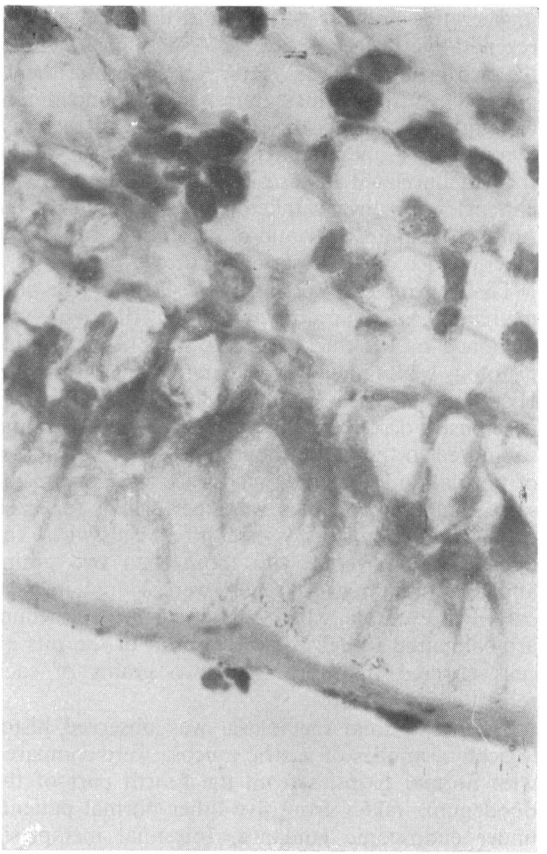

Fig. 2 *Normal gastric surface epithelium: cells are either not labelled or are poorly labelled. Note some lymphocytes heavily labelled.* × *630 (original magnification).*

Discussion

NORMAL MUCOSA
Maximal grain count is observed in the lower part of pits where cells are proliferating and depict great genetic activity as demonstrated by high incorporation of thymidine H_3 (Deschner *et al.*, 1972; Willems *et al.*, 1972). By contrast, epithelial cells of lumen area known to have no mitotic activity show the minimal 3H-AM binding. Results of these authors and ours are in agreement with the fact that cells of the luminal area are completely differentiated and become non-dividing. This gradient of 3H-AM binding also agrees with previous observations of Brachet and his colleagues. In fact, these authors have described that, in amphibian embryo cells undergoing differentiation, actinomycin binding decreases in cells which differentiate and is very low in fully differentiated cells, such as spermatozoa or lens cells (Brachet and Hulin, 1970).

Moreover, other authors have assumed that the capacity of this intercalating ligand to bind to DNA is limited by the presence of chromosomal proteins during transcription and replication (Ringertz and Bolund, 1969; Beato *et al.*, 1970; Pederson and Robbins, 1972). It has also been shown, when resting and cycling cells are compared, that the capacity to bind 3H-AM by the nuclei is increased in the latter (Pederson, 1972; Preumont *et al.*, 1977).

ATROPHIC MUCOSA
In atrophic mucosa, the hypothesis that an orderly sequence in the progression of atrophic gastritis to gastric carcinoma exists has been proposed by Deschner *et al.* (1972).

In keeping with this proposition, they have demonstrated an abnormal pattern in the incorporation of 3H-thymidine, 3H-uridine, and 3H-leucine in the epithelial cells of gastric pits in this condition.

Other studies performed by Croft *et al.* (1966) and Castrup (1977) show that the cell cycle becomes

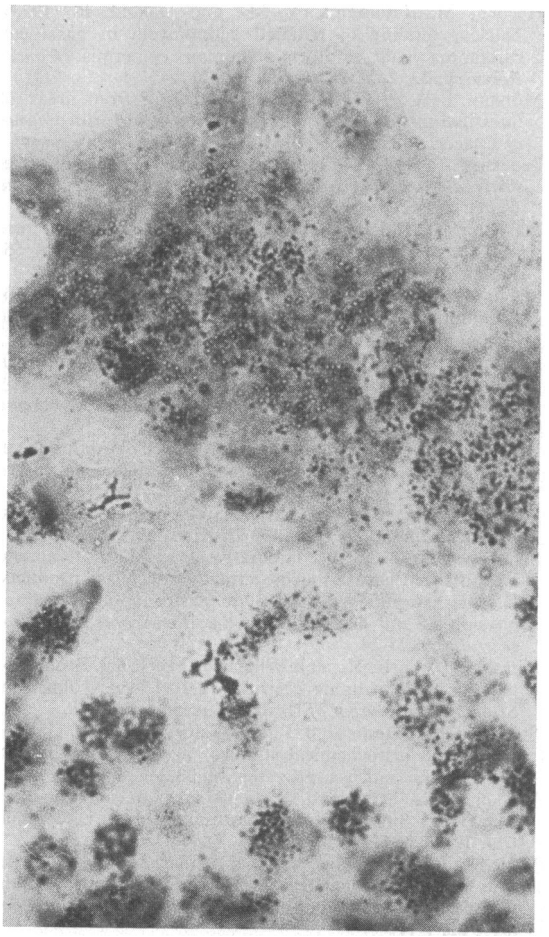

Fig. 3 *Intestinal metaplasia: surface epithelial cells very heavily labelled (as much as in the bottom of glands).* × *630 (original magnification).*

shorter and shorter in the different forms of atrophic gastritis from the mild form of the disease to the severe one. Our results agree well with this, in that the mean number of grains per cell of surface epithelium is more raised in cases of atrophic gastritis and can be correlated with the severity of the gastric lesion.

The maximal mean number of grains is counted in the epithelial cells of the regenerative nodules. This increased 3H-AM binding might be explained by the fact that cells remain engaged in a different cellular cycle in cases of gastritis. In some cases of intestinal metaplasia surface epithelial cells are as intensely labelled as those at the bottom of the glands. This could reflect a derepression of the genome in the cells, and the expression of different genes. Genes are associated with histone and non-histone proteins to form a nucleoprotein complex called chromatin. Chromatin consist of a transcriptionally active open fraction (euchromatin) and a condensed fraction (heterochromatin) containing the genes non-expressed in specific cells. There is a higher binding of tritiated actinomycin in euchromatin (derepressed) than in heterochromatin (repressed) (Berlowitz *et al.*, 1969). In agreement with this view, we have demonstrated (Stoffels *et al.*, 1972) that intestinal metaplasia is not a homogeneous change.

When it is studied by histoenzymology, marked differences are observed among individual cases, in the location and the intensity of the following enzymatic activity: succinic dehydrogenase, β-hydroxybutyric dehydrogenase, lactic dehydrogenase, isocitric dehydrogenase, glucose-6 phosphate dehydrogenase, α-glycerophosphate dehydrogenase, adenosine triphoshatase, non-specific esterase, alkaline phosphatase, and acide phosphatase.

Moreover, Schrager (1977) has pointed out that the intestinalised gastric mucosa provide glycoproteins with changed blood group specificity. The limits of sensitivity of our method do not permit differences at the level of the stem cells to be demonstrated. Nevertheless, progeny cells demonstrated difference in the capacity of their chromatin to fix 3H-AM which could be interpreted as the result of expression of different genes or of a somatic mutation.

Table 2 *Binding of 3H actinomycin in nuclei of surface epithelial cells and bottom of glands in normal fourth duodenum and intestinal metaplasia (average number of silver grains/nucleus)*

			Intestinal metaplasia		
	Normal duodenum	P	*Well differentiated*	P	*Less differentiated*†
Surface epithelium*	14 ± 9	< 0·0001	30 ± 12	< 0·0001	> 80
Bottom of glands	60 ± 16		70 ± 16		> 80

* Number of cells: 100.
† >80: the number of grains is so raised that it is technically not possible to count them.
±: Standard deviation.

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