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Histochemical and proliferative changes preceding the onset of spontaneous gastric adenocarcinoma in Mastomys natalensis

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Summary. The histochemical and proliferative changes preceding spontaneously developing gastric adenocarcinoma were examined in Mastomys natalensis. The stomachs of 86 inbred animals from the DWZ strain were examined between 1 and 24 months of age. Two thirds of the animals had a small solitary ulcer in the middle of the lesser curvature which was observed by the first month of age. With increasing age, more and more dysplastic glands invading the submucosa were seen around the ulcer and eventually adenocarcinoma was observed after 12 month. Histochemical alterations included increased intracellular peanut lectin and Concanavalin A binding sites in the gastric pits and the development of lectin binding sites and of acid glycoproteins in the surface epithelium. Using ^{3}H thymidine and autoradiography increased labelling and mitotic indices with extension of the progenitor area to the mucosal surface were observed around the ulcer. The coexistence of these histochemical and histokinetic alterations may represent an early sign of malignant potential in the gastric mucosa.

Key words: Gastric carcinoma – Gastric ulcer – Precancerous – Glycoprotein – Cell kinetics

Introduction

There is increasing evidence that alterations in epithelial mucin composition may characterize precancerous conditions in the gut. A tendency of epithelial sulfomucins to be replaced by sialomucins has been described in dysplastic colon mucosa (Chabot and Colacchio 1985; Shamsuddin and Trump 1981) and colon carcinoma (Montero and Segura 1980). Similar changes are found also in normal appearing colonic mucosa of patients with colon cancer (Montero and Segura 1980; Dawson and Filipe 1976).

In the normal human gastric mucosa sulfomucins are virtually absent (Montero and Segura 1980) while these compounds are found in most gastric adenocarcinoma (Montero and Segura 1980; Jass and Filipe 1980; Häkkinen et al. 1984). Sulfated mucins have also been observed in gastric adenomatous polyps (Jass and Filipe 1980) and in the intestinalized gastric mucosa surrounding gastric carcinoma (Montero and Segura 1980; Jass and Filipe 1980). However, sulfomucins are absent from gastric cancers which do not involve intestinalization (Montero and Segura 1980). Since sulfomucins are also characterizing normal intestinal and colonic glands, the presence of these mucosubstances might be due to intestinal metaplasia without being directly related to cancer. Recently, lectins have been used to demonstrate several terminal saccharides in glycoprotein molecules (Sharon 1977). Significant alterations in lectin binding sites have been observed in colorectal adenoma and carcinoma (Boland et al. 1982; Yonezawa et al. 1982; Cooper 1982). There is also some evidence that lectin staining can be modified in several gastric lesions in man (Fischer et al. 1984a). Besides histochemical alterations, variations in epithelial cell kinetics including an extension of the proliferative area in the glands have been described in a series of precancerous lesions of the colon like ulcerative colitis (Bleiberg et al. 1970; Eastwood and Trier 1973), villous adenomas or familial polyposis (Bleiberg et al. 1972; Lipkin and Deschner 1976). Similar observations have been made in the stomach, namely in atrophic gastritis (Deschner et al. 1972; Zhang et al. 1983) in the gastric stump after antrectomy (Assad and Eastwood 1980) and also in rodents receiving gastric carcinogens (Deschner et al. 1979; Hattori et al. 1984). Whether such prolifera-

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tion disorders represent constant indicators of premalignant conditions remains to be established.

In the present study an as yet unexplored experimental lesion of the stomach known to precede experimental carcinoma has been investigated. Variations in mucosal glycoprotein and cell kinetic patterns have been compared with those described by others in precancerous conditions of the gut mucosa.

Materials and methods

Male inbred Mastomys natalensis originating from the DWZ strain (Randeria 1979) were used. 86 animals of 1 month, 6 month, 12 month and 18 month to 24 month were kept fasted for 24 h and killed between 9 a.m. and 11 a.m. The stomach was opened along the greater curvature, rinsed in saline and pinned on paraffin blocs, the mucosa upward. The total gastric mucosa was inspected with a binocular loupe (× 6.3) and macroscopic lesions were noted. After fixation in neutral buffered formalin (4%) for 18 h at 4° C, gastric samples were taken from three standardized areas in the antrum, one in the middle between oesophagus and pylorus on the lesser curvature, one from the anterior and one from the posterior antral surface. The specimens were embedded in paraffin. Sections, 4 µm to 6 µm thick, were carried out perpendicular to the mucosal surface and routinely stained with haematoxylin and eosin (HE). For describing the histochemical staining characteristics, the antral gland was subdivided into the basal part, isthmus and pit. Because this division was not possible in abnormal glands they were subdivided into lower, middle and upper thirds. For mucin histochemistry, periodic acid Schiff (PAS), Alcian blue at pH 2.5 (AB-2.5), Alcian blue at pH 0.5 (AB-0.5), high iron diamine (HID), PAS-AB-2.5 and AB2.5-HID reactions were used.

Concanavaline A (ConA) and peanut lectin (PL) labeled with horseradish peroxidase (HRP) and fluorescein isothyocyanate (FITC) labeled Ulex europaeus agglutinin I (UEA I) were used to visualize selectively terminal mono or disaccharides (Sharon 1977) (Table 1).

Tissue sections were covered 45′ with 10 µg/ml PBS buffer solution of PL, 10 µg/ml tris buffer solution of Con A or 100 µg/ml PBS buffer solution of UEAI. Fluorescent sections were examined immediately with a Leitz fluorescence microscope. HRP conjugated lectins were stained with 3-3′diaminobenzidine. Non labelled lectins or the addition of the appropriate sugar to the labelled lectin buffer solution were used for control sections.

The Grimelius technique was used for staining the entero-endocrine cells.

All chemicals were purchased from Sigma, Sigma, Chemical Company, St. Louis, MO, USA.

Autoradiography. Ten randomly selected 6 month old animals were given one IP injection of 1 mCi/kg of ^{3}H-thymidine (spec. act. 6 mCi/mmol, IRE, Fleurus, Belgium) one hour before sacrifice. The mucosal sections were covered with nuclear emulsion (Ilford K5) for 18 days and stained with haematoxylin and eosine. Cells were considered labelled when more than 4 silver granules were seen over the nucleus. Both sides of 25 normal pyloric glands sectioned longitudinally were examined in each animal. At least 10 glandular columns per animal were counted in the abnormal mucosa surrounding the lesion. The most superficial epithelial cell in the glandular tube was referred to as n° 1, the cell beneath it as n° 2 etc. until the most basally located glandular cell was reached. The height of the glandular tube (H.G.T.) was expressed as the mean number of cells between the most superficial and the deepest epithelial cell in one column of the glandular tube. The proliferation zone (P.Z.) was defined as the area between the most superficial and the deepest labelled epithelial cells in the tubes. The labelling index (L.I.) was expressed as the percentage of labelled cells on the total epithelial cell population of the P.Z. The mitotic index (M.I.) was counted in the same population. Statistical analysis on the proliferative parameters were carried out using the student *t* test after arc. sin. transformation of the proportions. The Kolmogorov-Smirnov test for 2 samples was used for testing variations in proliferation zone.

Table 1

Lectin	Major sugar specificity	Inhibiting sugar
Concanavalin A (ConA)	α D mannose α D glucose N-acetyl-glucosamine	Mannose
Peanut lectin (PL)	β-galactose-(1–3)-N-acetylgalactosamine	Galactose
Ulex europaeus agglutinin I (UEAI)	α L fucose	Fucose

Results

A small sessile mucosal elevation, 1 mm to 5 mm in diameter was observed in the middle of the lesser curvature in 58% of the animals. Diffuse gastric bleeding was present in 6.7% of the animals beyond 12 month of age. A central ulceration was visible without magnification in the largest tumours. No extragastric tumours were found in any animal.

The microscopic lesions in the animals before one year of age consisted of a solitary ulcer, surrounded by an important polynuclear and lymphocytic infiltrate (Fig. 1a). The mucosa around the ulcer showed the cytologic and architectural characteristics of "minor dysplasia" described by Ming et al. (1984). Epithelial cells were pleiomorph with large nuclei, prominent nucleoli and a basophilic cytoplasm. Their mucus content was reduced. Architectural changes with glandular branching and folding and intraluminal cellular budding and crowding were observed (Fig. 1b). There was no intestinal metaplasia. After the age of 12 month an increasing number of lesions appeared to break through the muscularis mucosa and to extend into the submucosa (Fig. 2a). This heterotopic tissue consisted mainly of well differentiated mucosal cells forming glandular structures. The latter seemed to originate from the glands at the base of the mucosa thus resembling "submucosal glan-

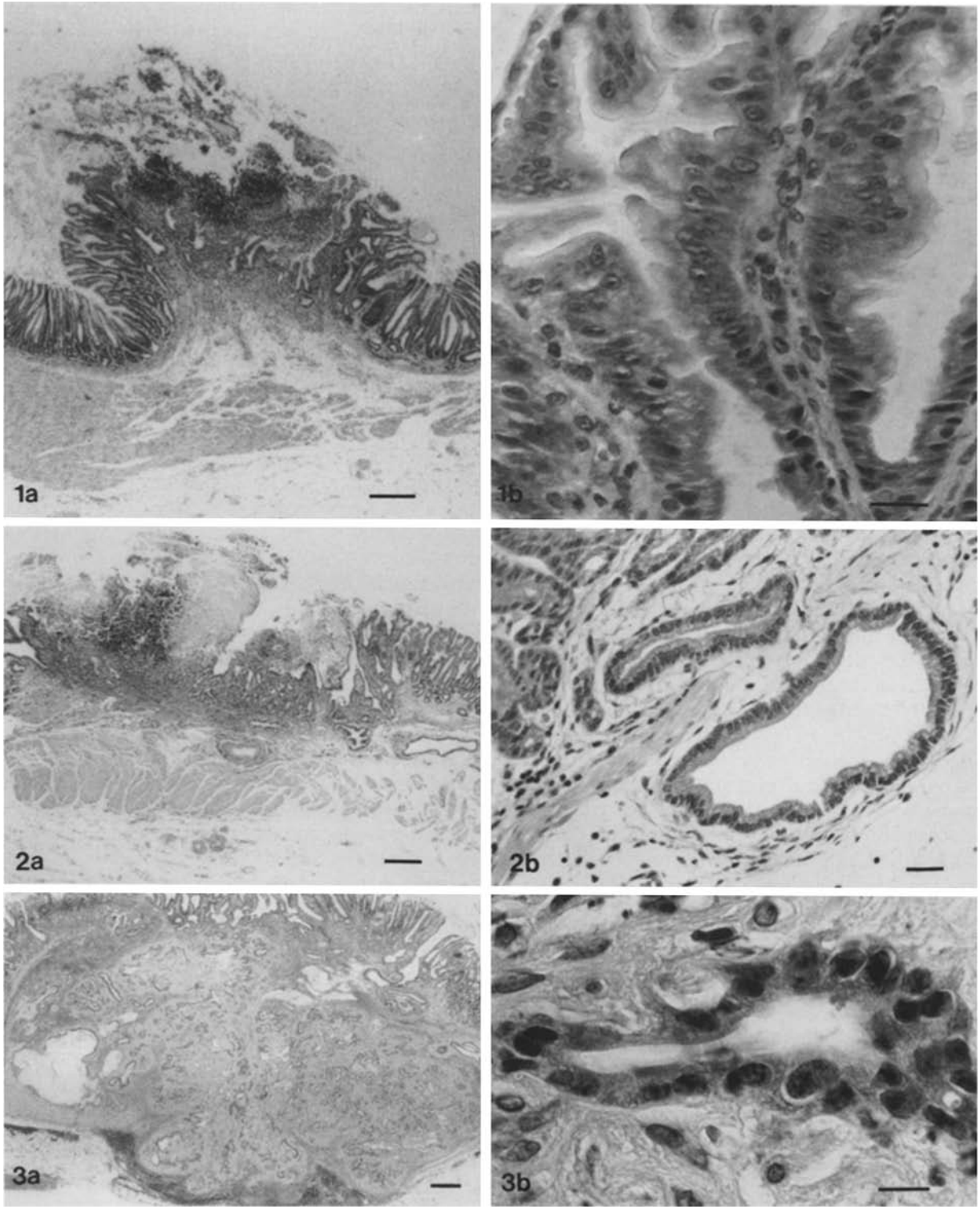

Fig. 1. Antral lesion in a 6 month old Mastomys. Central erosion (**a**) surrounded by dysplastic epithelium (**b**). **a** H.E. × 40; scale bar = 0.2 mm; **b** H.E. × 500 scale bar = 20 μm

Fig. 2. Adenomatous hyperplasia of the glandular stomach in a 12 month old Mastomys. Mucosal epithelium extending into the submucosa. **a** H.E. × 30; scale bar = 0.2 mm; **b** H.E. × 300; scale bar = 20 μm

Fig. 3. Invasive carcinoma in a 24 month old Mastomys, invading the serosa. **a** H.E. × 10; scale bar = 0.5 mm; **b** H.E. × 800; scale bar = 10 μm

Table 2

Age month	*n*	Lesion		Adenomatous Hyperplasia		Cancer	
		n	(%)	*n*	(%)	*n*	(%)
1	21	13	(62)	0	(0)	0	(0)
6	20	14	(70)	3	(15)	0	(0)
12	28	14	(50)	10	(36)	2	(7)
18/24	17	13	(76)	8	(47)	4	(24)

Overall antral lesion frequency, adenomatous hyperplasia frequency and cancer frequency in relation to the age of the animals

dular proliferation" or "adenomatous hyperplasia" (Fig. 3b) (Kunze et al. 1979; Sugimura and Kawachi 1973). Two animals of 12 month of age and 4 animals of 24 month had antral adenocarcinoma including anarchic clusters of intensely proliferating neoplastic cells which were invading the entire gastric wall (Fig. 2a, b). Both "adenomatous hyperplasias" and cancers became more frequent with age (Table 2).

Grimelius staining reaction was consistantly negative in the antral cancers while being normally positive in the entero-endocrine cells elsewhere in the stomach. In the normal mucosa of the antrum, mucins in the basal glands and in the isthmus were of the acid type with sulfated acid mucosubstances predominating. Neutral mucins predominated in the pit but sulfated and non sulfated acid mucosubstances were also present. The surface mucous cells contained neutral glycoproteins exclusively (Fig. 4a). In the dysplastic mucosa, both sulfated and non sulfated acid mucosubstances were observed throughout the entire glandular tubes, as well as in the surface epithelium (Fig. 4b).

In the normal antral mucosa UEAI binding sites were equally distributed over the entire glandular tubes. PL and ConA binding sites were seen mainly in the basal glands and isthmus. None of the superficial pit or surface mucous cells were stained with PL (Fig. 5a). ConA positive cells were observed occasionally in the superficial pits. In the precancerous area mucosal cells with PL and ConA binding sites were observed over the entire height of the glandular tube (Fig. 5b). In the malignant lesions, occasional cells staining with UEAI, ConA and PL could be observed.

The proliferative zone was neatly enlarged in the abnormal glandular tubes surrounding the ulcer and a few labelled cells were also observed at the mucosal surface (Figs. 6a, 6b, 7). The mean height of the glandular tubes in this area was nearly doubled ($p<0.001$). Both labelling and mitotic indices were higher ($p<0.05$) than those values in the normal pyloric glands (Table 3).

Discussion

The present animal model offered the advantage that single gastric adenocarcinomas arise in a predictable site of the gastric mucosa. The mucosal lesions were always located in the antrum and in the middle of the lesser curvature. This uniform characteristic of the cancer made it possible to investigate the local modifications which precede the development of cancer in the mucosa. In approximately two thirds of the animals a small ulcer surrounded by dysplastic glands was observed from the earliest stages of life. With increasing age, an increasing proportion of lesions was found to extent into the submucosa and, eventually, to transform into typical adenocarcinoma. A similar sequency has been described previously in the glandular stomach of rats treated with N-Methyl-N′-nitro-N-nitrosoguanidine (MNNG) (Sugimura and Kawachi 1973; Kunze et al. 1979). Confusion with gastric carcinoids, which arise in other strains of Mastomys (Randeria 1979; Håkanson et al. 1979) was excluded by the morphological and histochemical characteristics of the tumour. No entero-endocrine cells were observed in the present cancers.

Histochemical changes around the ulcer included increasing concentrations of intracellular acid mucins and lectin binding sites in the glands, and also the appearance of sulfomucins in the surface epithelium. Similar modifications have been observed in human pathology namely in normal appearing gastric mucosa adjacent to cancer (Montero and Segura 1980; Jass and Filipe 1980). Sulfomucins might be comparable to fetal antigens (Häkkinen et al. 1985) of which the reappearance may occur in the process of cancerogenesis. Peanut lectin binding sites have been described in human gastric cancer (Fisher et al. 1984a; Kahn and Baumal 1983) and in the mucosa around it. But an increase in PL and ConA receptors was also observed in hyperplastic and regenerating gastric mucosa around quite benign lesions (Fisher et al. 1984a). They could, therefore, represent a non specific characteristic of inflammation. However, in our present experimental model not only a diffuse increase in concentration in the glands but also the appearance of new PL binding sites in the apical cytoplasm of the superficial epithelium was observed.

Expression of lectin binding sites is probably

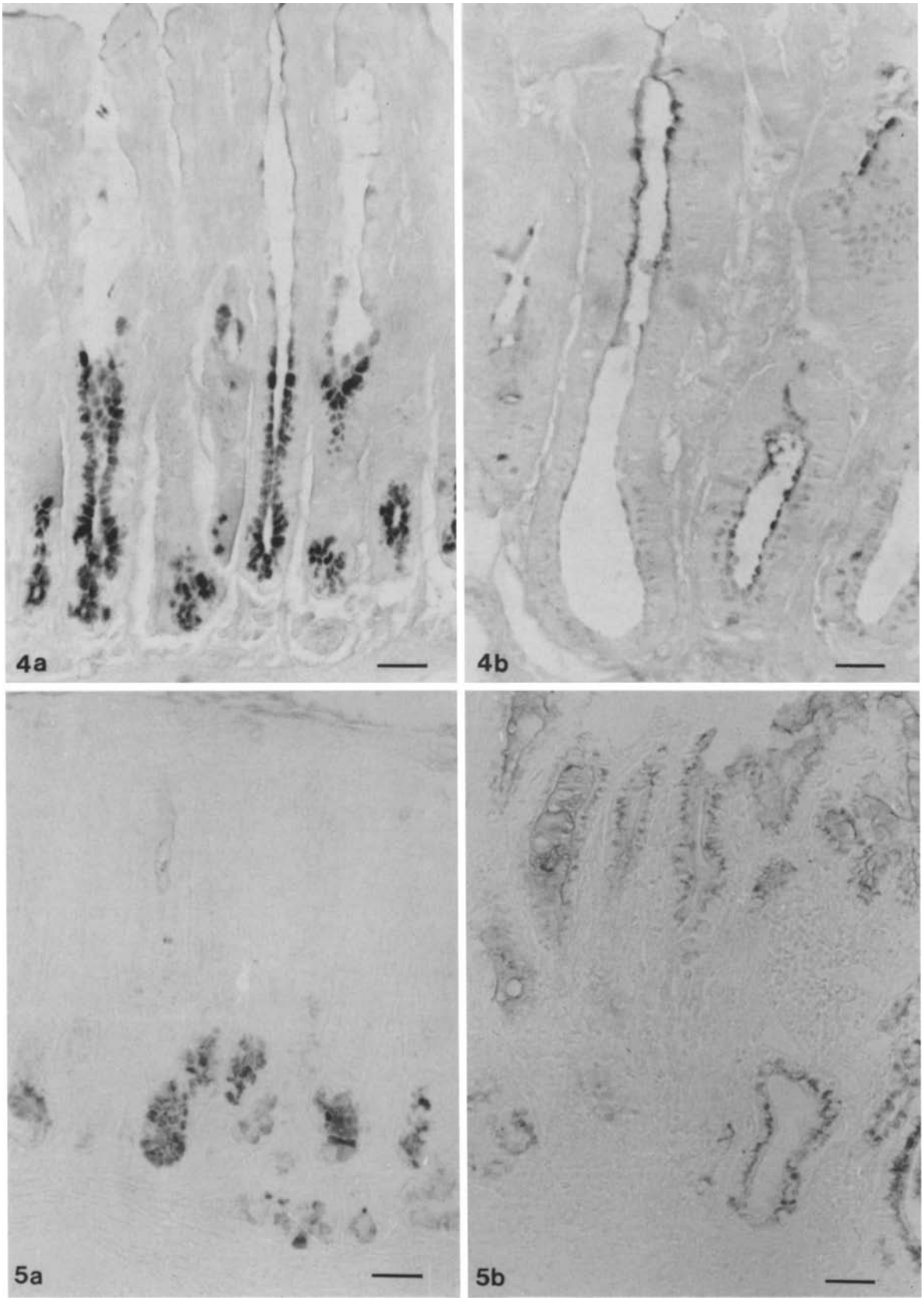

Fig. 4. HID staining of normal (**a**) and dysplastic (**b**) antral mucosa. Sulfated mucins being restricted to the basal glands and isthmus in the normal mucosa (**a**) are present throughout the dysplastic mucosa (**b**). H.E. × 400; scale bar: 20 μm

Fig. 5. Peanut lectin HRP of normal (**a**) and dysplastic (**b**) antral mucosa. PL binding sites being restricted to the basal glands and isthmus in the normal mucosa (**a**) are present throughout the dysplastic mucosa (**b**). H.E. × 400; scale bar: 20 μm

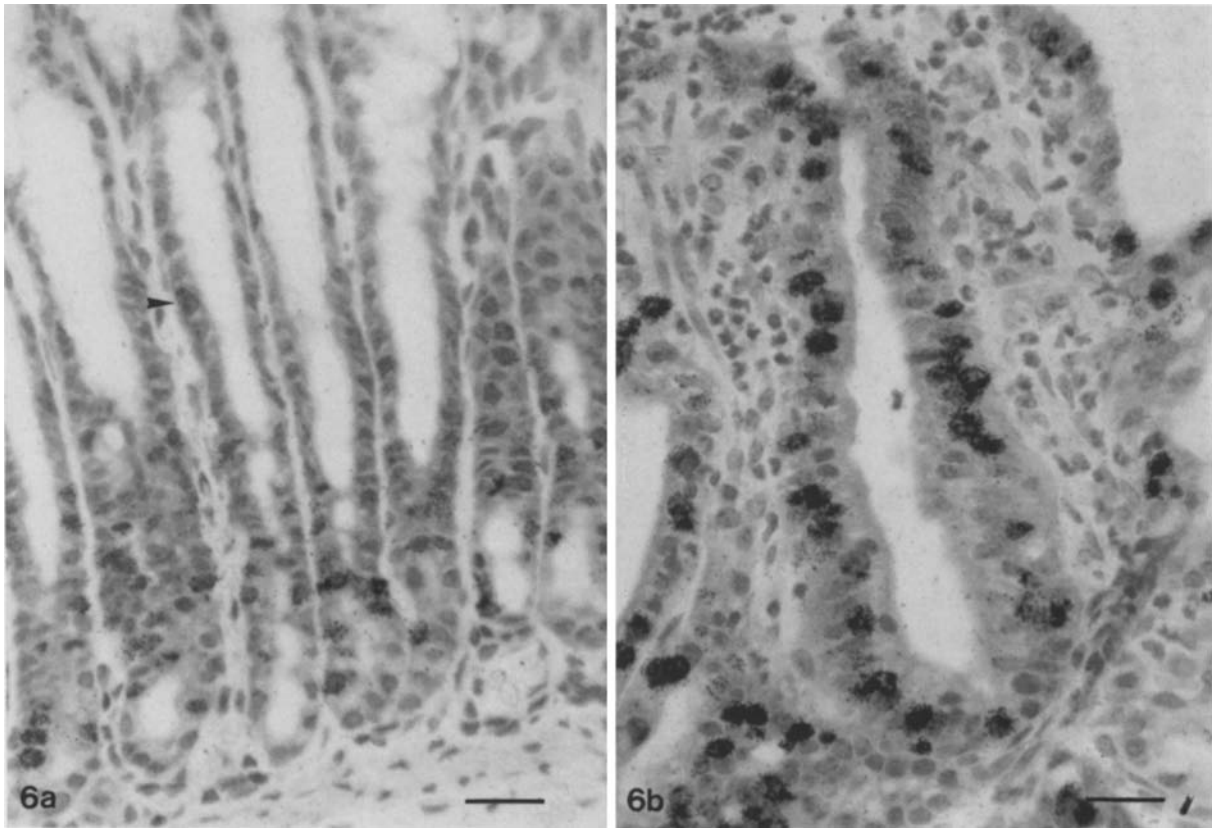

Fig. 6. Autoradiography of normal (**a**) and dysplastic (**b**) antral mucosa. *Arrowhead*: uppermost labelled cell in the normal mucosa. H.E. × 500; scale bar: 20 μm

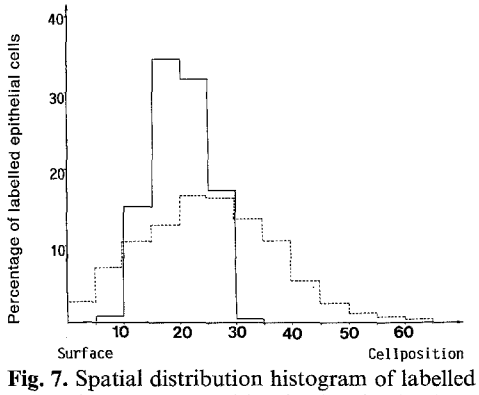

Fig. 7. Spatial distribution histogram of labelled epithelial cells in relation to their position in the gland. The most superficial epithelial cell in the glandular tube was referred to as n° 1, the cell beneath it as n° 2, etc. until the most basally located glandular cell was reached. Note the wider distribution in the dysplastic mucosa. — Normal mucosa; – – – dysplastic mucosa. ($p < 0.001$, Kolmogorov-Smirnov test)

caused by a disturbed elongation of the oligosaccharide in the glycoprotein molecules (Fisher et al. 1984b). Such alterations in cell surface glycoprotein, namely of galactose-(1–3)-N-acetyl-galactosamine are believed to be important in making a distinction between malignant or premalignant lesions and normal or simply reactive epithelium,

Table 3

	L.I. (%)	M.I. (%)	H.G.T. (*n*)
Normal	16±1*	1.6±0.2*	27.8±0.6**
Dysplasia	23±3*	2.7±0.5*	58.3±4.1**

Labelling index (L.I.), mitotic index (M.I.) and height of the glandular tube (H.G.T.) in normal and dysplastic antral mucosa. Mean values±SEM.
*$P < 0.05$; **$p < 0.01$

at least in the large intestine (Shamsuddin 1986; Boland and Auhren 1985).

However, alterations in the pattern of gastric cell proliferation were also observed in our animals. These alterations closely resembled those found in human atrophic gastritis (Deschner et al. 1972) around gastric ulcers and cancers (Zhang et al. 1983) as well as in lesions preceding the appearance of chemically induced gastric cancers in rats (Deschner et al. 1979; Hattori et al. 1984). Increased proliferation activity with extension of the proliferation zone towards the mucosal surface is a frequent observation in gut lesions known to precede cancer with a high frequency. It has been attributed to "derepression" of cell proliferation and

is considered to characterize precancerous maturation disorders in the digestive glands (Lipkin and Deschner 1976).

In conclusion characteristic histochemical as well as cytokinetic alterations were observed at the site where the spontaneous gastric cancer is known to arise in this animal model. These alterations were not observed elsewhere in the glandular stomach of the animals. We cannot exclude the possibility that the observed modifications represent nonspecific inflammatory phenomena secondary to the existence of an ulceration but the coincidence would be remarkable. Their resemblance to alterations in the human gastric mucosa in conditions where cancer is known to appear is an additional argument to strengthen the hypothesis that a series of easily detectable histochemical and cell kinetic changes precedes the onset of cancer in the gastric mucosa.

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