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Adenocarcinoma and carcinoid developing spontaneously in the stomach of mutant strains of Mastomys natalensis

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Summary. Neoplastic and nonneoplastic lesions developing spontaneously in antral and fundic mucosae of stomachs of mutant chamois-coloured Z (130 animals) and Y (67 animals) strains of Mastomys aged 18 to 24 months were examined histologically and histochemically. The Z strain developed both antral lesions (hyperplasia 29.2%; dysplasia 23.8%; adenocarcinoma 17.7%) and fundic carcinoid(s) (72.3%). The antral lesions were limited to the lesser curvature near the pyloric ring. Macroscopically, adenocarcinomas resembled human gastric carcinomas of either Borrmann's type I or II. Histochemically, adenocarcinoma cells were characterised by marked reduction of total mucins produced and predominance of mucins with both periodic acid-Schiff and Alcian blue reactivities (neutral and sialated class II mucins). An infiltrating adenocarcinoma was successfully transplanted into nude mice, reaching the 7th generation of transplantations over 4 years, and retained histological features of the primary tumour. The ultrastructural appearance of growing transplanted tumours supported the reduced production of mucins by adenocarcinoma cells with scarcity of mucin granules and intracellular cysts.

However, the Y strain never developed antral lesions like the wild strain, developing fundic carcinoid(s) only. Microscopically, these carcinoids contained argyrophilic nonargentaffin granules, and biochemically produced histamine consistently but no 5-hydroxytryptamine (5-HT, serotonin) like those of the wild strain. Since we found unexpectedly that a line of F_2 but not F_1 hybrids between wild and Z strains developed the same antral lesions as Z strain, a preliminary experiment was performed to confirm the development of antral lesions in F_2 hybrids newly produced by brother-sister mating. Among 41 surviving F_2 offspring, 4 (9.8%) developed hyperplasia, 2 (4.9%) dysplasia and none adenocarcinoma. The numbers (6) of animals observed with these lesions approximated to their expected numbers (7.3).

Key words: Mastomys – Stomach – Adenocarcinoma – Carcinoid – Histogenesis

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Introduction

Mastomys natalensis is an African rodent, intermediate in size and several other respects between the mouse and rat. The original wild strain has a dark gray coat with a light gray belly (agouti) and black eyes. Two mutants, called Y and Z strains, have appeared in the long process of inbreeding of non-agouti-coloured Mastomys colonies (Randeria 1980a, b). Both strains of Mastomys have a yellowish coat with a pale gray belly (chamois) and pink eyes. Mastomys of original strain develops a high incidence of an argyrophilic carcinoid in the fundus of glandular stomach spontaneously (Snell and Stewart 1969a, b). It has been reported that both Y and Z strains of Mastomys developed carcinoid in the fundus and adenocarcinoma along the lesser curvature of antrum spontaneously, the predilection site of human gastric carcinoma (Randeria 1979, 1980c). To the best of our knowledge, these mutant strains are the only mammals other than humans to develop gastric carcinoma spontaneously. The exact incidence and histogenesis of their tumours, however, cannot be fully elucidated, since the original reports have described some morphological features of the developing tumours based on the observation of only a small number of tumour-bearing animals. It seems important to delineate these uncertain as-

pects of the gastric tumours found more precisely in order to establish these 2 strains of Mastomys as an animal model suitable for the study of spontaneous gastric carcinoma in man. In this paper, we extended our observation to a larger group of both Y and Z strains of Mastomys. More studies were added including the histological grading and the histochemical features of antral lesions, light and electron microscopic observations of adenocarcinoma that were successfully transplanted into nude mice, and statistical analysis of antral lesions developing in various hybrid strains between the wild agouti and mutant chamois Mastomys. Our results showed that the Z but not the Y strain and F_2 but not F_1 offspring between Z strain and wild strains developed antral lesions which proceeded along the hyperplasia-dysplasia-adenocarcinoma sequence. Recently, Tielemans et al. (1987) examined mucosal glycoprotein and cell kinetic patterns of antral lesions of Z strain at different ages and pointed out the proliferative mucosal changes preceding the onset of spontaneous gastric adenocarcinoma. Their observations correspond rather well to the findings in the present study.

Materials and methods

Mastomys of Z strain were descendants of 2 inbred pairs provided in 1981 by Dr. Jer D. Randeria. One hundred and thirty animals (62 males and 68 females) were necropsied when they reached 18 months of age. Mastomys of Y strain were descendants of 6 inbred pairs provided in 1986 by J.C. Austin. Sixty-seven animals (29 males and 38 females) were necropsied between 18 and 24 months of age. The stomachs were opened along the greater curvature, stretched on a small soft wooden board with pins, the mucosa upward, and rinsed in neutral buffered formalin for more than 24 h at room temperature. After washing resected stomachs in tap water, all gastric lesions noted with a binocular loupe ($\times 6.3$) were excised with the surrounding normal mucosal area by a surgical blade, and embedded in paraffin. Four μm-thick sections were cut and routinely stained with haematoxylin and eosin. Special stains included periodic acid Schiff (PAS)-Alcian blue (AB) at pH 2.5, high iron diamine (HID)-AB at pH. 2.5, periodic acid (PA)-concanavalin A (Con A)-horseradish peroxidase (HRP) and PA-reduction (Red)-Con A-HRP (Katsuyama and Spicer 1978), and Masson-Fontana's argentaffin and Grimelius' argyrophil procedures.

For the tumour transplantations into nude mice, the tumour tissues from a primary infiltrating adenocarcinoma measuring 12×12 mm in diameter were minced with scissors, and small pieces were loaded into a steel trocar. They were subcutaneously transplanted into 2-month-old nude mice (KSN-nu/nu, Shizuoka Laboratory Animal Center, Japan) in July, 1985.

For ultrastructural observation of adenocarcinoma cells, the portions of the growing transplants at the 2nd and 3rd generations were fixed in 2% paraformaldehyde-2.5% glutaraldehyde phosphate buffer (pH 7.4), and examined with the electron microscope.

For histamine and 5-hydroxyindole-3-acetic acid (5-HIAA) in the urine, urine was collected over a 24-h period in a special metabolic cage (Nakamura et al. 1971). These substances were determined as described previously (Hosoda et al. 1979).

Immunohistochemical demonstration of 5-HT in primary carcinoids was performed as described previously (Suyama et al. 1984).

The production of hybrid strains between mutant (Z) and wild strains was initiated by mating a male of Z strain and a female of wild strain in 1982. From this pair, more than 40 agouti-coloured F_1 hybrids were obtained. Twenty females and 20 males were maintained for 18 to 26 months for possible development of gastric tumour. A F_1 pair was chosen for producing F_2 offspring. A F_2 male and female with chamois coat and pink eyes called A line, and a F_2 male and female with brownish gray coat (intermediate between dark and yellow colours) and black eyes called B line, were mated. After 3 generations of successive brother-sister matings of pairs with the same coat and eye colours, 57 offspring (28 males and 29 females) of the A line and 47 (26 males and 21 females) of the B line that survived for 18 months were sacrificed for examination of antral lesions. Since the antral lesions including carcinoma were found to develop in animals of A line, the mating experiment was started again with a male of Z strain and a female of wild strain. F_1 hybrids were a male and 2 females with wild agouti coat and eye colours. They were mated for the production of F_2 offspring. Fifty-eight offspring (31 males and 27 females) born were segregated into 4 phenotypes using coat and eye colours; dark gray coat and black eyes (B/-, P/-), dark brownish gray coat and dark red eyes (B/-, p/p), brownish gray coat and black eyes (b/b, P/-) and yellowish coat and pink eyes (b/b, p/p). These phenotypes may accord well with those established in mice which were expressed by 2 independent genetic loci, b-locus and p-locus affecting their coat colours (Silvers 1979). Table 1 shows the expected and observed numbers of 4 phenotypes in 58 F_2 offspring at 6 months of age. The fit of χ^2 tests on the observed and expected numbers of 4 phenotypes showed that the observed numbers closely approximated the expected numbers ($\chi^2 = 1.99$, df $= 3$, $p = 0.55$).

Table 1. F_2 offsprings segregated into 4 phenotypes of coat and eye colours

Phenotype		Probability	Number	
Coat	Eye		Expected	Observed
B/-	P/-	9/16	32.5	29
B/-	p/p	3/16	11.0	15
b/b	P/-	3/16	11.0	10
b/b	p/p	1/16	3.5	4
Total		16/16	58	58

Results

The tumours developing in glandular stomach of Mastomys of Z strain were situated consistently either in the lesser curvature of antrum near pyloric ring or along the the greater curvature extending towards anterior and posterior walls of fundus. The antral tumours were single sessile polypoid growths measuring 2 to 12 mm in diameter (Fig. 1). The large tumours were often centrally ulcerated (Fig. 1 d). The fundic tumours were ses-

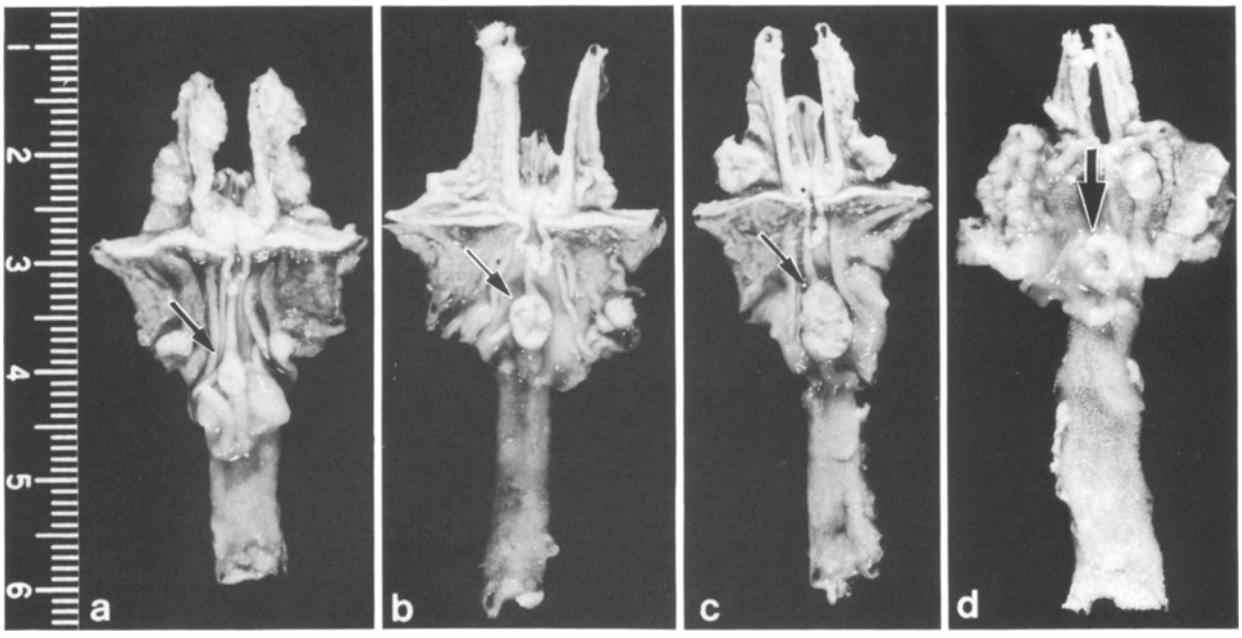

Fig. 1. Antral lesions (*arrows*) in glandular stomachs after opening along greater curvature (stomach) and posterior wall (duodenum). (**a**) Hyperplasia. (**b**) Dysplasia. (**c**) Adenocarcinoma. (**d**) Infiltrating adenocarcinoma that is centrally ulcerated. There is a carcinoid with a polypoid appearance in the anterior wall of the fundus

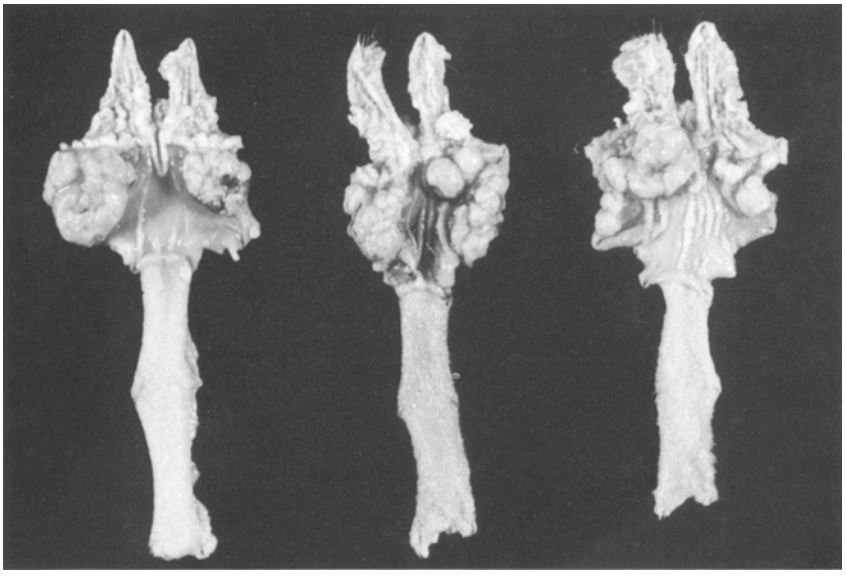

Fig. 2. Multiple carcinoids developing in the fundus of Y strain of Mastomys. Stomach and duodenum are opened along greater curvature and posterior wall, respectively. Note smoothness of antral mucosa above pyloric ring

sile polypoid and often multiple, measuring 2 to 20 mm in diameter. All Mastomys of Y strain, however, developed only fundic tumours similar to those found in Z strain (Fig. 2). When the fundic tumours of both strains reached more than 10 mm in diameter, their duodena were moderately distended, occasionally forming small ulcers. The antral tumours of Mastomys of A line and F_2 offspring were essentially similar to those developing in Z strain.

The normal antral mucosa of aged Mastomys consisted of surface mucous cells, foveola mucous cells and pyloric gland cells. Histochemically, surface mucous cells contained neutral class II mucins (PAS and PA-Con A-HRP positive), foveola mucous cells acid class II mucins (AB and HID positive), and pyloric gland cells acid class III mucins (AB, HID and PA-Red-Con A-HRP positive). Mucous neck cells that contained neural class III mucins (PAS and PA-Red-Con A-HRP positive)

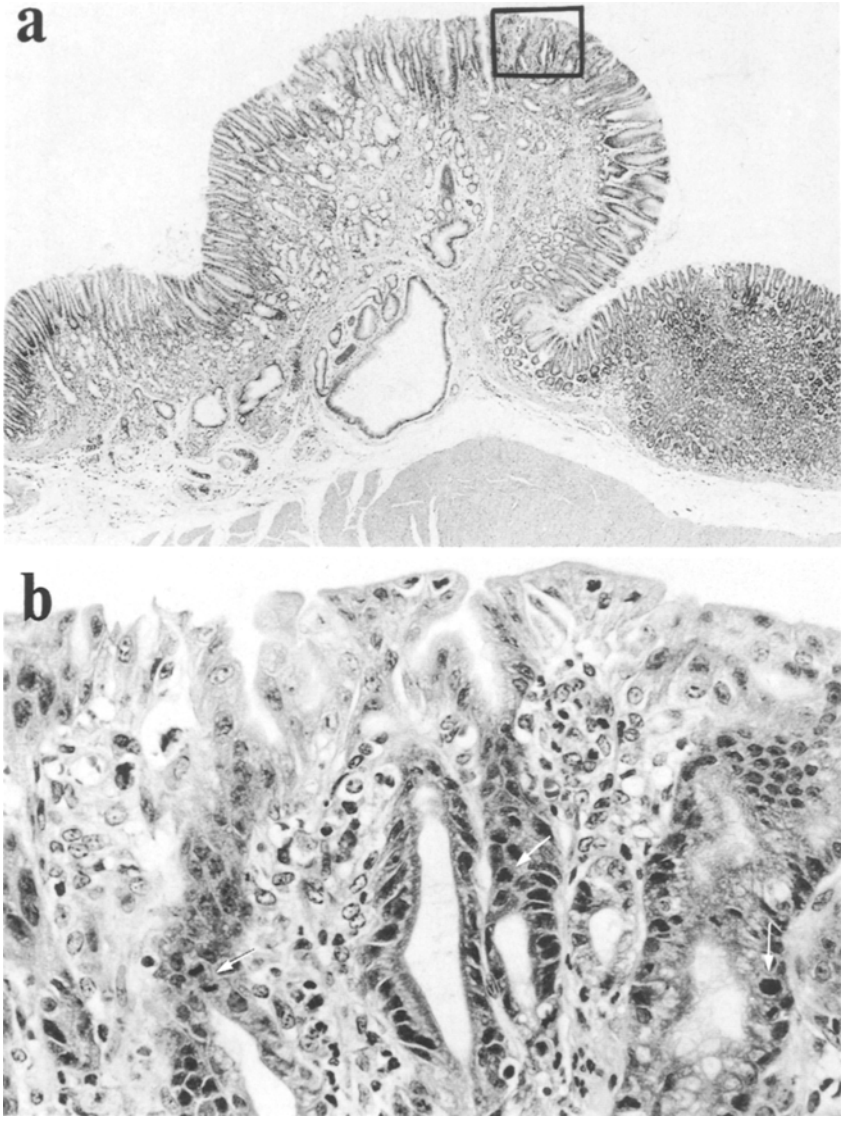

Fig. 3. Hyperplasia. (**a**) Polypoid growth is simple proliferation of whole cell types constituting antral mucosa as a whole. Several cystically dilatated glands are formed in the lamina propria and submucosa. HE (×35); (**b**) Magnification of apical part of polypoid growth rimmed by rectangular black line in (**a**). Several glands in the center are slightly deformed. The nuclear polarity of glandular cells becomes irregular and several mitoses are seen (*white arrows*). HE (×175)

were inconspicuous. We could demonstrate neither a definite atrophic gastritis nor intestinal metaplasia in stomachs irrespective of the presence or absence of antral lesions of aged Mastomys.

Histologically, the antral lesions were graded into hyperplasia, dysplasia and adenocarcinoma, though there were proliferative lesions difficult to grade into a single histological type because of the presence of unclassifiable lesions admixed with hyperplastic and dysplastic glands, and dysplastic glands and adenocarcinoma in a single growth. Hyperplasia was defined as the lesion in which the proliferating epithelial cells consisted of surface mucous cells, foveola mucous cells and pyloric gland cells, and retained normal glandular architecture as a whole, even if less than 10 dysplastic glands were present in its central part, and very occasionally cystically dilated well-differentiated glands were formed in the deep part of elevated mucosa (Fig. 3). Histochemically, the upper part of hyperplastic mucosae mainly consisted of surface mucous cells. Their lower part, however, consisted of various glandular ducts containing foveola mucuos cells, pyloric gland cells and unclassifiable mucous cells that showed the reactivity of both neutral and acid class II mucins. The cystically dilated mucous glands in the deep portion consisted of surface mucous cells, foveola mucous cells and unclassifiable mucous cells. Hyperplasia was usually in the form of small polypoid lesions, and was found in 21.0% of males and 36.8% of females (Table 2).

Dysplasia consisted of crowded tall, columnar epithelial cells with a large vesicular nucleus and

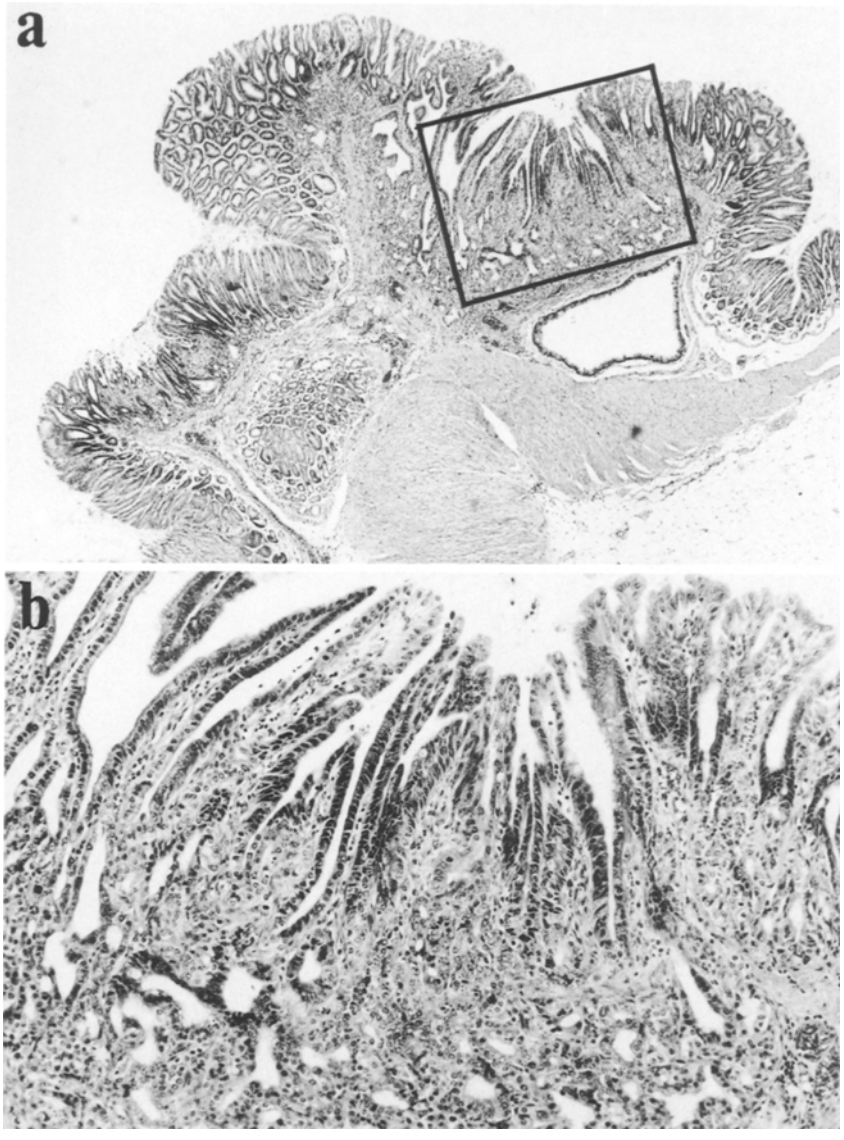

Fig. 4. Dysplasia. (**a**) Centrally depressed polypoid growth is bilaterally surrounded by hyperplastic glands. A cystically dilated large gland is formed in the submucosa. HE (×35); (**b**) Magnification of central part of dysplastic lesion rimmed by rectangular black line in (**a**). Dysplastic tubules consisting of columnar epithelial cells with irregular nuclear polarity proliferate in the upper part. Their tubular structure becomes indistinct in the deep part. HE (×113)

Table 2. Spontaneous gastric lesions of 18-month-old Mastomys of Z strain

Sex	Number of animals	Number of animals with gastric lesions (%)			
		Hyperplasia	Dysplasia	Adenocarcinoma	Carcinoid
Male	62	13 (21.0)	18 (29.0)	14 (22.6)	50 (80.6)
Female	68	25 (36.8)	13 (19.1)	9 (13.2)	44 (64.7)
Total	130	38 (29.2)	31 (23.8)	23 (17.7)	94 (72.3)

a prominent nucleolus. Their nuclear polarity was not regularly oriented, and mitoses were frequent. These cells formed disorganized tubules of varying sizes. The distortion, branching and budding of tubules were often present. In the propria and submucosa, cystically dilated large glands admixed with either differentiated or atypical mucous cells were often observed. Dysplastic lesions usually accompanied the surrounding elevated hyperplastic area (Fig. 4). For the definition of dysplasia, therefore, we adopted histological criteria by which the area should contain more than 10 dysplastic glands, and the depth of growth must be limited to the upper half of submucosa. Histochemically, the amounts of mucins produced in dysplastic epithelia were generally reduced, and the dissociation of reactions and types of mucins occurred even in a single gland. In the relatively well-organized dysplastic glands, the mucous cells in their apical parts contained neutral class II mucins like surface mucous cells, and those in their middle and basal parts had both neutral and acid class II mucins

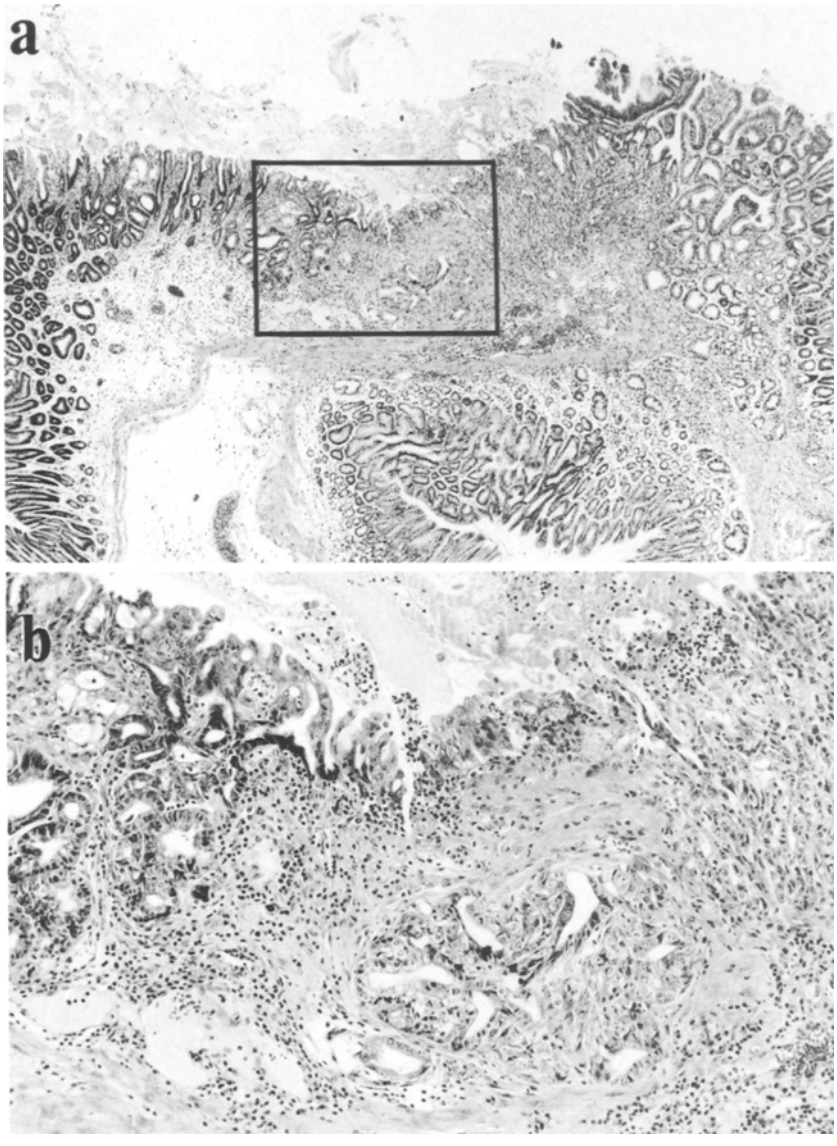

Fig. 5. Adenocarcinoma. (**a**) Flatly elevated growth is centrally ulcerated. HE (×35); (**b**) Magnification of left half of adenocarcinoma rimmed by rectangular black line in (**a**). HE (×35); Dysplastic tubules consisting of columnar cells on the left laterally surround cancer tissues which invade submucosa losing tubular structure on the right. HE (×113)

along the cell border. With the distortion and branching of glandular structure, the distribution pattern of these mucins became uneven, and the cells that contained both neutral and sialated but less sulfated class II mucins predominated. Cystically dilatated large glands in propria and submucosa were also composed of mixed cell types. The main cell types in differentiated glands showed the histochemical features of surface mucous cells, foveola mucous cells and pyloric gland cells, and main cell type in less differentiated ones had the histochemical features of unclassifiable mucous cells. Accumulation of mucins within these glands was slight. The mucous cells with histochemical features of pyloric gland cells were decreased in dysplastic lesions. Dysplastic glands did not contain argyrophil endocrine cells with Grimelius' procedure. Dysplasias were polypoid or flatly elevated, often centrally depressed lesions larger than hyperplasia, and were found in 29.0% of males and 19.1% of females (Table 2).

Adenocarcinoma consisted of atypical epithelial cells essentially similar to those found in dysplasia, but cellular atypia such as loss of nuclear polarity and increase in nucleo-cytoplasmic ratio, and mitoses were more marked than dysplasia, and the deformed tubules invaded the surrounding tissues. The dysplastic epithelia usually overlaid and surrounded adenocarcinoma (Fig. 5). Therefore, we defined adenocarcinoma as growth in which the tubular structure was lost at least in part, and the proliferating cells reached the muscularis propria or further infiltrated the muscularis propria. In 4 of 23 adenocarcinomas thus diagnosed, adenocar-

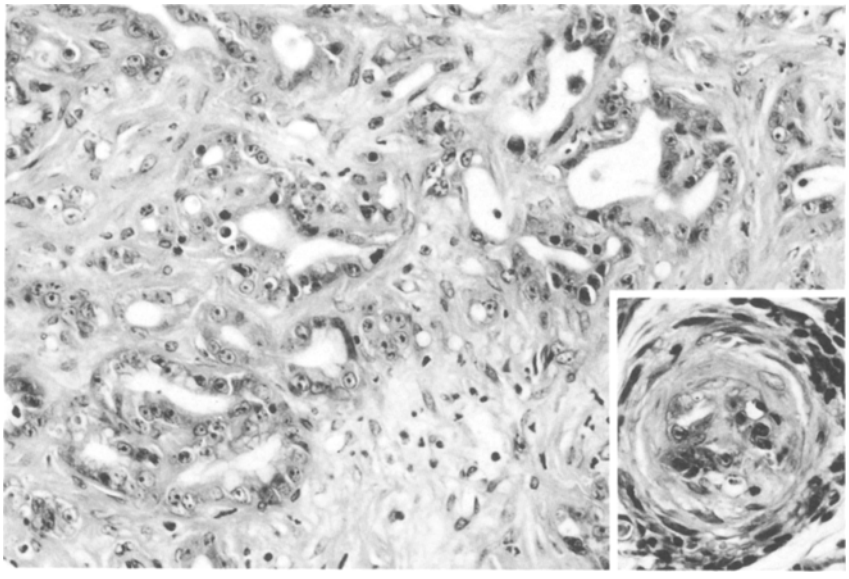

Fig. 6. Adenocarcinoma cells losing nuclear polarity and infiltrating the muscularis propria. They consist of cuboidal epithelial cells and proliferate in the tubular pattern. Note the marked proliferation of fibrous tissues surrounding tubular adenocarcinoma. HE (×540) *Inset*: An intraarteriolar cancer deposit in serosal surface of the same tumor. HE (×640)

cinoma cells completely penetrated the muscularis propria in the desmoplastic pattern, reaching the serosal surface with the formation of an intraarteriolar cancer deposit (Fig. 6). There was, however, no metastasis of adenocarcinoma cells to the regional lymph nodes or other organs. No typical signet-ring cell was present in adenocarcinomas. In adenocarcinomas, cystically dilated glands were often found in the submucosa and occasionally even in the muscularis propria. These glands consisted mainly of cuboidal epithelial cells with slightly irregular nuclear polarity. Histochemically, the amounts of mucins in adenocarcinoma cells of both mucosal and deeply invasive parts were markedly reduced. A rather thin line of mucins showing both neutral and sialated, partially sulfated class II mucins was recognized along the cell border. Only rarely were there any acid class III mucins in this line. Cystically dilatated glands in submucosa and muscularis propria showed histochemical features similar to those observed in less differentiated glands in dysplasia. As in dysplastic epithelial cells, no adenocarcinoma cells, either superficially or deeply situated, intermingled with argyrophil endocrine cells with Grimelius' procedure. Adenocarcinomas were either large polypoid or centrally ulcerated lesions, and were found in 22.6% of males and in 13.2% of females (Table 2).

The histological appearances of fundic tumours in Mastomys of Y and Z strains were essentially similar to those of argyrophilic carcinoids developing in the original wild strain. Briefly, the carcinoid cells were uniform in size, and round or cuboidal in shape. Their cytoplasm was usually eosinophilic and finely granular. The nuclei were ovoid spherical, and contained prominent nucleoli. Mitoses were frequent. The neoplastic cells were arranged in cords and solid nests surrounded by thin vascular connective tissues. They consistently contained fine argyrophilic, nonargentaffin granules with Grimelius' and Masson-Fontana's procedures. The carcinoids developed 100% of Y strain and 72.3% of Z strain (Table 2).

The histamine excreted in the urine of 15 animals (8 males and 7 females) of Y strain that reached 24 months of age amounted to 1.55 ± 0.84 μg (mean ± SD)/day (range 0.52 to 3.05 μg), while that of 21 animals (10 males and 11 females) of 7 months of age that did not develop carcinoid was 0.42 ± 0.22 μg/day (range, 0.17 to 0.76 μg) ($p < 0.001$). Among 15 animals, 5 bearing carcinoids less than 10 mm in the largest dimension (7.9 ± 2.3 mm) excreted 0.78 ± 0.34 μg/day of histamine in the urine and the remaining 10 bearing tumours more than 10 mm in the largest dimension (14.0 ± 1.25 mm) excreted 1.79 ± 0.87 μg/day of histamine in the urine ($p < 0.01$). The amounts of 5-HIAA excreted in the urine were not increased in the carcinoid-bearing group (6.5 ± 3.7 μg/day and 6.9 ± 2.1 μg/day in old and young animals, respectively). Twenty-two carcinoids of Y strain were examined immunohistochemically for 5-HT, but only 2 contained scattered cells that gave positive reaction to this amine in sections of the whole tumour.

The transplantation of an infiltrating adenocarcinoma into nude mice was successful. The transplanted tumours were usually visible with the naked eye 3 months after transplantation, reached 3 to 5 mm in diameter 2 months later, and trans-

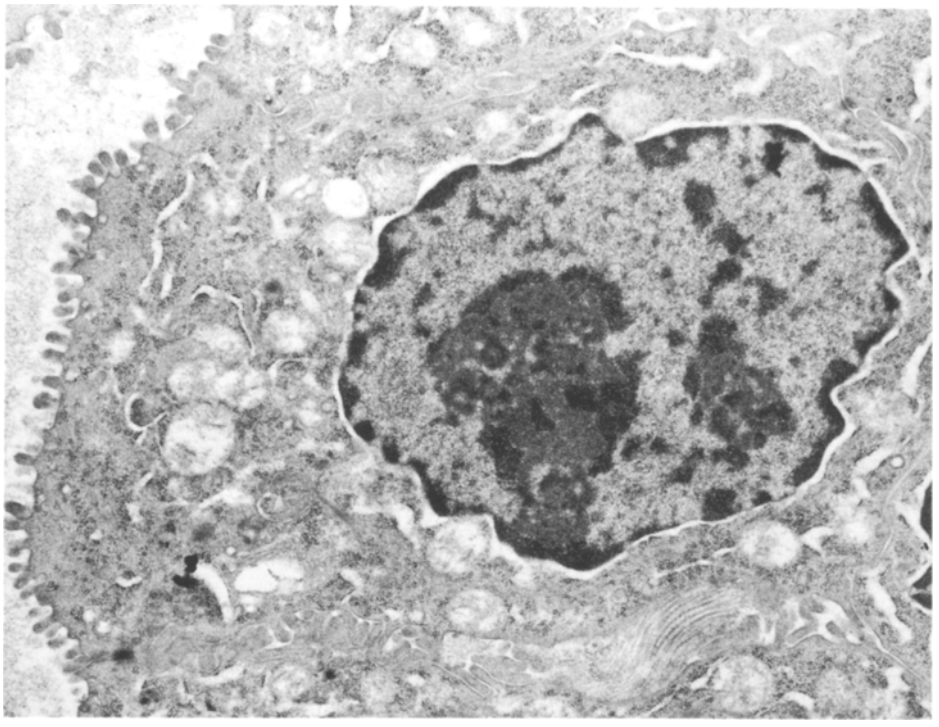

Fig. 7. Adenocarcinoma cell of cuboidal shape forms stumpy microvilli not only on luminal surface but also on bilateral sides. Several desmosomes are unevenly distributed. Nucleus has prominent nucleolus and aggregated chromatin, and its margin is irregular. Note scarcity of intracytoplasmic mucin granules as well as cysts and collagen fibres intervening in the intercellular space. ×18000

planted into nude mice of the same kind. By the end of 1988, the serial transplantations reached the 7th generation at an interval of about 6 months. The growing transplants retained the general histological features of primary tumour through 7 successive transplantations over 4 years. The adenocarcinoma cells were low columnar or cuboidal and had ovoid nuclei with prominent nucleoli. Mitoses were readily observable. They formed tubules of varying sizes. In some places, adenocarcinoma cells proliferated in desmoplastic pattern, as seen in primary tumours. No metastasis occurred in other organs. Histochemically, both neutral and sialated but not sulfated mucins faintly outlined the inner cell border of cancer tubules. The cystically dilatated cancer tubules contained large amounts of both neutral and sialated class II mucins in which small amounts of sulfated class II mucins were mixed. The acid class III mucins were not demonstrated either on the inner cell border of cancer tubules or in tubular lumens. Ultrastructurally, neoplastic cells were low columnar or cuboidal, and the stumpy microvilli were formed not only on the luminal border, but also in the intercellular space (Fig. 7). Desmosomes were unevenly distributed. The formation of basal lamina was inconspicuous. The collagen fibres were numerous around cancer tubules, often intervening in the intercellular space. The nuclei had prominent nucleoli, aggregated chromatin and irregular margins. Mitochondria were often swollen. The endoplasmic reticulum was also distended and ribosomes were scattered throughout the cytoplasm. Typical mucin granules and intracellular cysts were seldom seen (Fig. 7).

Of the 2 hybrid lines, the genotype of the A line was b/b and p/p, because all descendants had chamois coat and pink eyes, and that of the B line was b/b and P/- or b/b and p/p, because its descendants had brownish gray coat and black eyes or chamois coat and pink eyes. Forty F_1 hybrids (20 males and 20 females) did not develop any antral lesion at the age of 18 months. Among 57 animals of A line, hyperplasia developed in 2 males and 4 females (10.5%), dysplasia in 5 males and 4 females (15.8%), and adenocarcinomas in 7 males and 2 females (15.8%). However, only 1 male of the 47 animals of the B line developed dysplasia. For further evaluation of possible participation of some genetic factor in the development of antral lesions, we reproduced F_2 offspring by brother-sister mating of F_1 hybrids between wild and Z strains. Among 58 F_2 offspring, 41 (19 males and 22 females) that survived from 18 to 22 months were sacrificed for examination of antral lesions. Hyperplasia developed in 1 male and 3 females (9.8%), and dysplasia in 2 females (4.9%). No adenocarcinoma developed in these animals. From the coat and eye colours, the genotypes of all animals with hyperplasia were B/- and

P/-, and those of 2 animals with dysplasia b/b and p/p, and b/b and P/-.

Discussion

The main aim of this study was to elucidate, histologically and histochemically, the relationships between hyperplasia, dysplasia and adenocarcinoma developing spontaneously in the gastric antrum of aged Mastomys of Z strain.

Macroscopically, antral lesions were restricted to the lesser curvature near the pyloric ring consistently; the predilection site for human gastric carcinoma. Their appearance was of either a single sessile polypoid or of centrally ulcerated growths. The former corresponded to human gastric carcinoma of Borrmann's type I. The latter resembled the human counterpart of Borrmann's type II. We never encountered any abnormal lesion in 67 Mastomys of Y strain. In our long experience in over 1000 autopsies of wild agouti-coloured Mastomys, the antral mucosae of their stomachs were entirely smooth and retained 1 or 2 regular folds. Therefore, we conclude that mutant Z strain of Mastomys is the only mammal other than human to develop gastric carcinoma spontaneously.

Microscopically, we graded antral lesions into 3 types, hyperplasia, dysplasia and adenocarcinoma, according to the histological classification of proliferative lesions in the human stomach (Morson et al. 1980; Ming et al. 1984).

Typical hyperplastic glands were usually a simple proliferation of all the cell types of the antral mucosa, though the ratio of constituent cell types varied from one lesion to another. Histochemically, however, these glands often contained unclassifiable mucous cells with both neutral and acid class II mucins. Furthermore, they were occasionally admixed with several, centrally situated dysplastic glands that produced fewer mucins and showed histochemical features similar to those of unclassifiable mucous cells. These findings suggest that at least some hyperplastic glands became dysplastic in aged Mastomys.

Dysplasia was usually surrounded by hyperplastic areas, and cystically dilatated large glands were often formed in the propria and submucosa. Histochemically, the production of mucins by dysplastic cells was reduced, and the reactivities of mucins became confined to the inner cell border. With increasing cellular and structural atypism of dysplastic glands, the dissociated reactivities of mucins occurred, and the population of unclassifiable mucous cells predominated. These findings suggest that the poorly differentiated portion of dysplastic glands might be precancerous.

As described before, the lesions that we regarded as adenocarcinomas consisted usually of dysplastic and cancerous tissues. Adenocarcinoma cells reached the muscularis propria, and some further infiltrated the entire gastric wall in desmoplastic fashion. The histochemical findings of mucins revealed the markedly reduced production of total mucins and convergence into mainly both neutral and sialated class II mucins similar to those produced by unclassifiable mucous cells. These observations strongly suggest that adenocarcinomas might develop from the preexisting dysplastic epithelium.

An infiltrating adenocarcinoma was successfully transplanted into nude mice. The growing transplanted tumours retained the histological features of tubular, partially cystotubular adenocarcinoma relatively rich in stroma, similar to that of primary tumour, throughout all generations of transplantation. The ultrastructural appearance of cancer cells further supports the reduced production of mucins by cancer cells because of the scarcity of mucin granules and intracellular cysts.

From these results, we believe that the initial lesions developing in gastric antrum of Z strain of Mastomys may be mucosal hyperplasia and that some hyperplastic glands transformed into dysplastic ones, from which adenocarcinomas eventually develop. The histogenesis of the antral lesions may reasonably be explained by the so-called hyperplasia-dysplasia-carcinoma sequence, though a causal relationship between them remains uncertain.

In wild agouti-coloured Mastomys, the fundic carcinoid occur in approximately two-thirds of old males and one-third of old females (Snell and Stewart 1969a, b). In the present mutant chamois-coloured Z strain of Mastomys, their incidence was higher than in the wild strain (male: 80.6% and female: 64.7%). In addition, all Mastomys of Y strain of both sexes in our series developed fundic carcinoid(s) in spite of the complete absence of antral lesions. They were argyrophilic, nonargentaffin carcinoids. Since the amounts of histamine but not 5-HIAA excreted in urine of aged Mastomys of Y strain were significantly increased compared with those of younger ones and paralleled the tumour sizes, and immunohistochemically detectable 5-HT positive cells were rare within tumour tissues, the carcinoids of Mastomys of Y and probably Z strains produced histamine like those of the wild strain (Hosoda et al. 1970, 1971). Thus, Mastomys of Y strain may be a good model to

study genetic aspects of the carcinogenesis of human gastric carcinoid.

Since both wild and 2 mutant strains were used after more than 20 successive generations of brother-sister matings, they were inbred strains, thus retaining the homozygous state predominantly at every genetic locus. Only mutant Z strain developed antral lesions with hyperplasia-dysplasia-carcinoma sequence with 70.7% penetrance (Table 2). The animals of this strain also developed cataract and obesity when they reached 12 months of age (Randeria and Randeria 1980), suggesting some metabolic disturbances including diabetes in the hosts. Since animals of wild strain were not associated with such abnormal conditions, we initially made F_1 and F_2 hybrids between wild and Z strains to evaluate hereditary aspects of these abnormal conditions. Thus, the A and B lines were isolated as described before. Apart from our initial aim in this animal experiment, we unexpectedly found that old animals of the A line developed the same antral lesions with the hyperplasia-dysplasia-carcinoma sequence (incidence of 42.1%) as those developing in original Z strain. The fact that none of the F_1 hybrids (20 males and 20 females) and only 1 of the B line developed antral lesion implies that the offspring of the A line still carry some genetic factor from Z strain which is expressed in the homozygous state.

To confirm this fact, we reproduced F_1 and F_2 hybrids by brother-sister mating, and found the development of antral lesions in F_2 offspring. If we presume that the presence of tumour gene designated T affecting Mastomys stomach is like B and P affecting coat and eye colours in mouse, respectively, the genotype of wild strain may be expressed as T/T and that of Z strain t/t because of the lack of tumour development in F_1 hybrids (T/t). In F_2 offspring, T/T (25%), T/t (50%) and t/t (25%) must be segregated. Based on the incidences of hyerplasia (29.2%), dysplasia (23.8%) and adenocarcinoma (17.7%) in 130 animals of the original Z strain, we can calculate the numbers of these 3 lesions expected to develop in F_2 offspring with t/t genotype by multiplying the incidence of each antral lesion (%), the segregation rate of t/t in F_2 (0.25) and the effective numbers of F_2, divided by 100 (0.41). The expected numbers of hyperplasia, dysplasia and adenocarcinoma thus calculated were 3.0, 2.4 and 1.8, respectively. The total observed numbers (6.0; 4 hyperplasias and 2 dysplasias) were not significantly different from the total expected numbers (7.2), though no antral lesion identifiable with adenocarcinoma could be found in the our present series.

Our results also showed that the development of antral lesions seemed independent of the phenotypes of coat and eye colours, because animals with such lesions consisted of 3 different colour phenotypes (B/- and P/-, b/b and p/p, and b/b and P/-). These results may suggest that a single gene which is recessively mutated is responsible for the development of antral lesions, and are very consistent with the increasingly recognized current view that the recessive mutations play an important role in the pathogenesis of various forms of human neoplasms such as hereditary retinoblastoma, Wilms' tumour and familial adenomatous polyposis (Knudson 1985; Green 1988).

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