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Adenomatous Changes and Adenocarcinoma of Glandular Stomach in Wistar Rats Induced by N-Methyl-N′-Nitro-N-Nitrosoguanidine

An Electron Microscopic and Histochemical Study

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Summary. Adenomatous changes, and early and invasive carcinomas of the glandular stomach in Wistar rats ingesting N-methyl-N′-nitro-N-nitrosoguanidine were studied. Almost all adenomatous changes and carcinomata were located near the midpoint of the lesser curvature. In electron microscopic and histochemical studies, both changes showed great cytological similarity. Electron microscopically, they were found to consist of predominantly undifferentiated cells with poorly developed cytoplasmic organelles, with some highly differentiated cells present. Histochemically, both showed strongly positive reactions for lysosomal enzymes. For tumor transplantation, five lesions were used and in all cases, the transplants were successful.

Key words: Experimental carcinoma – Rat stomach – Methyl-nitro-nitrosoguanidine (MNNG).

Introduction

In a previous study the authors attempted to clarify, by light and electron microscopy and by enzyme histochemistry, the evolution of a full-fledged gastric carcinoma induced in the glandular stomachs of rats by oral administration of N-methyl-N′-nitro-N-nitrosoguanidine (MNNG). It was observed that until the 20th week all 22 benign erosions were located near the midpoint of the lesser curvature. The epithelial cells at this site were identical to the so-called immature cells in the normal gastric epithelium and also to the regenerating epithelial cells induced by mechanical trauma. It was also observed that from about the 20th week 4 adenomatous changes, 2 early carcinomata and 5 invasive carcinomata appeared in exactly the same area as the erosions.

In this study the authors undertook the following; 1. to ascertain the positional and chronological relationship between the benign regenerative change,

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the adenomatous change, and carcinoma, using many more animals, 2. to analyse both the adenomatous change and carcinoma by light and electron microscopy and by histochemistry, in order to clarify the interrelationship between the two pathological events and 3. to transplant the gastric carcinoma to rats of the same strain. For these purposes, inbred Wistar rats were given drinking water with the same concentration of MNNG, for the same period as in the previous experiment, and left to live as long as possible.

Materials and Methods

Seven-week-old male Wistar strain rats weighing 155–220 gm were used in this investigation. They were obtained from the Institute of Experimental Gerontology in Basel and were since 1961 inbred in the Institute of Pathology, University of Bonn. MNNG[1] was diluted with distilled water at a concentration of 80 μg/ml. As described in our previous study (Kobori et al.), special care was taken to avoid the degradation of MNNG. Forty rats were allowed to drink only the MNNG solution "ad libitum" for 31 weeks (7 months). From the 32nd week, all the rats were given normal tap water to drink. Throughout this time, commercial feed[2] was given. All animals were weighed twice a week and when any rat lost more than 50 gm within approximately two weeks, it was sacrificed. The stomach was incised along the greater curvature from the pylorus to the cardia and was pinned out flat on a cork plate. After the removal of the specimens for the electron microscopic and histochemical studies, the stomach was put in fresh 4% formalin solution, embedded in paraffin, and stained with hematoxylin and eosin (HE), PAS, Grimelius (Grimelius, 1968) and Mallory-Azan.

The electron microscopic and histochemical studies were carried out as described in our previous report. The only difference in procedure is that the thick sections for electron microscopical studies were stained with HE and PAS after the removal of Epon (Lane and Europa, 1965), which provided much more information and better orientation during the study of ultrathin sections.

Five tumors were used for tumor transplantation. Each was divided into two portions, one of which was cut into pieces measuring 1 mm in diameters. These pieces were transplanted into the subcutaneous tissue of the back of young male Wistar rats, chosen from the same strain, while the other part of the tumor served for light and electron microscopical examination.

Results

Thirty-nine of the 40 rats were preserved longer than 7 months as the effective number at risk.

All 39 of these rats were killed at intervals until the 61st week. In this group 1 early (Fig. 2A) and 35 invasive carcinomata were found, i.e., 92 percent of animals developed cancer. Three of the 36 carcinomata showed double malignant foci, and were excluded from the subsequent studies. The remaining cases included 2 of adenomatous change and 1 of leiomyosarcoma.

The thirty-two invasive carcinomas were found to be at least 6–30 mm in size and mostly of the so-called Borrmann III type. Sixteen out of the 32 lesions were under 10 mm in size. 15 of 16 carcinomas whose accurate localisation could be determined were located near the midpoint of the lesser curvature. The remaining 16 neoplasms were too large to determine their origin accurately, however, their centers were located in this characteristic area (Fig. 1).

[1] Purchased from Aldrich Chemical Co. Inc., Milwaukee, U.S.A.

[2] Purchased from Höveler Kraftfutterwerke, Langenfeld-Immugrath, West Germany

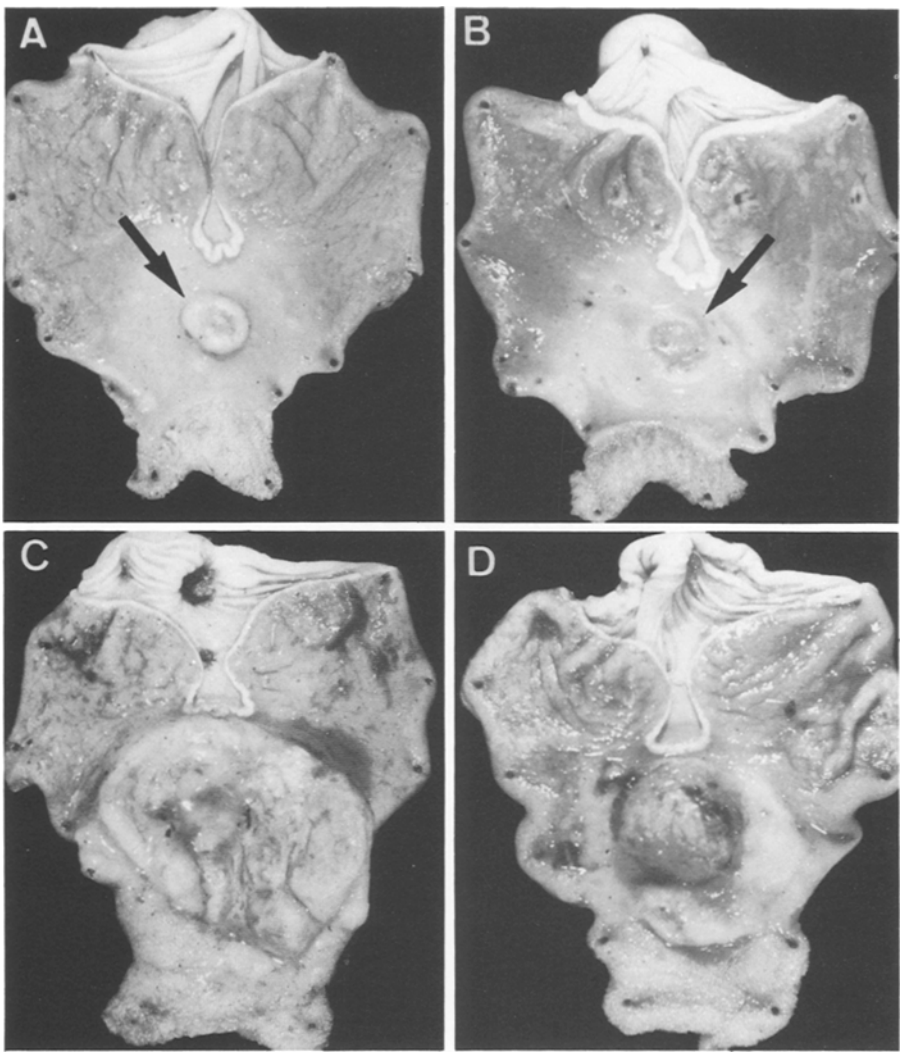


Fig. 1 A–D. Macroscopical pictures of the stomach of the rat. Early carcinoma (**A**) and invasive carcinomas (**B–D**). Even the large lesions appear to have arisen near the midpoint of the lesser curvature **C and D.** A, 45 w. B, 34 w. C, 54 w. D, 61 w

All 32 carcinomas were found in the pyloric gland area. Twenty-two out of 32 invasive carcinomas penetrated through the muscularis propria; in the remaining 10 cases, at least part of it was preserved, although the definite progressive invasion of the carcinoma reached near to its outer margin (Fig. 2B and C). In two cases, metastases to the perigastric lymphnodes were found.

In addition, atrophic gastritis was found in 14 cases, predominantly in the pyloric area. The changes were prominent in 2, and slight in 12 cases. A further 9 cases showed insignificant changes, while in the remaining 10 the degree

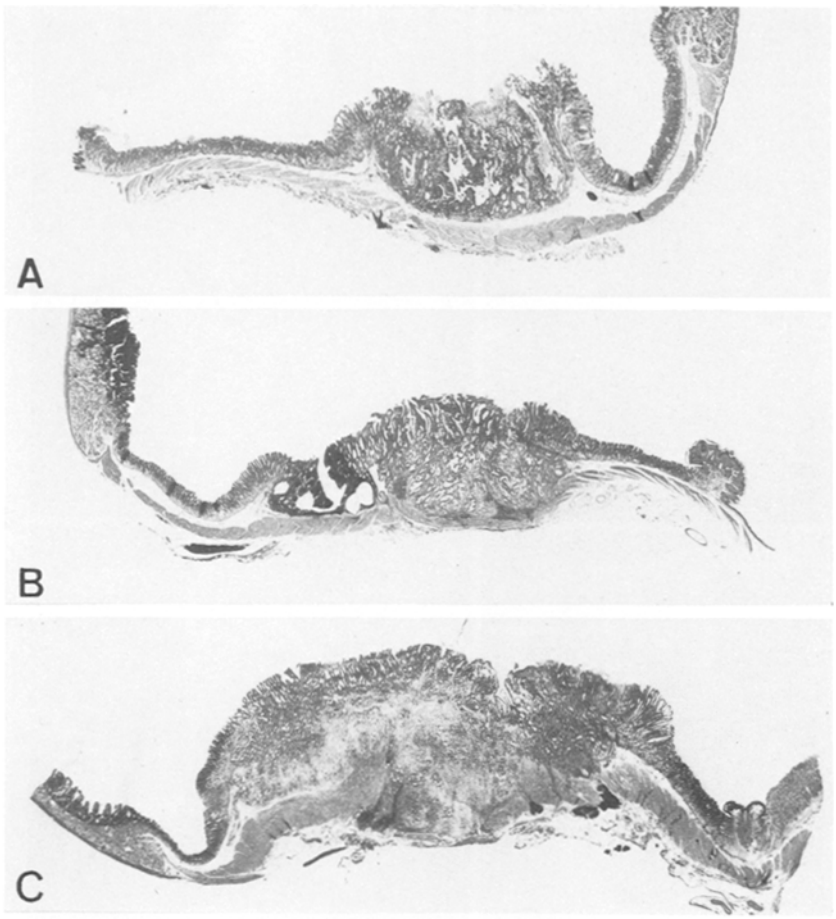


Fig. 2A–C. The survey of the early carcinoma of Figure 1 (**A**) and invasive carcinomas (**B and C**). The muscularis propria of one case is partly preserved (**B**) and of another, penetrated (**C**). A, 45 w. B, 34 w. C, 56 w

of atrophic gastritis could not be determined because of the extensive carcinomatous lesions. There was no marked change in the fundic gland area nor in the forestomach. Intestinal metaplasia was not seen.

Extragastric changes included 30 adenocarcinomas and 10 benign and malignant mesenchymal tumors found in the duodenum and in the first 10 cm of the small intestine. In most instances these seemed to be the main cause of the weight decrease of the rats. No significant change in the large intestine could be observed.

All 32 tumors were adenocarcinomata with a predominance of tubular structures; sometimes a papillary area was found (Fig. 3). Frequently the tubular carcinoma exhibited a cord-like pattern, which could be considered to be an undifferentiated carcinoma (Fig. 4). However, the typical diffuse carcinoma, which is so often observed in human material, was not seen. Carcinoma cells

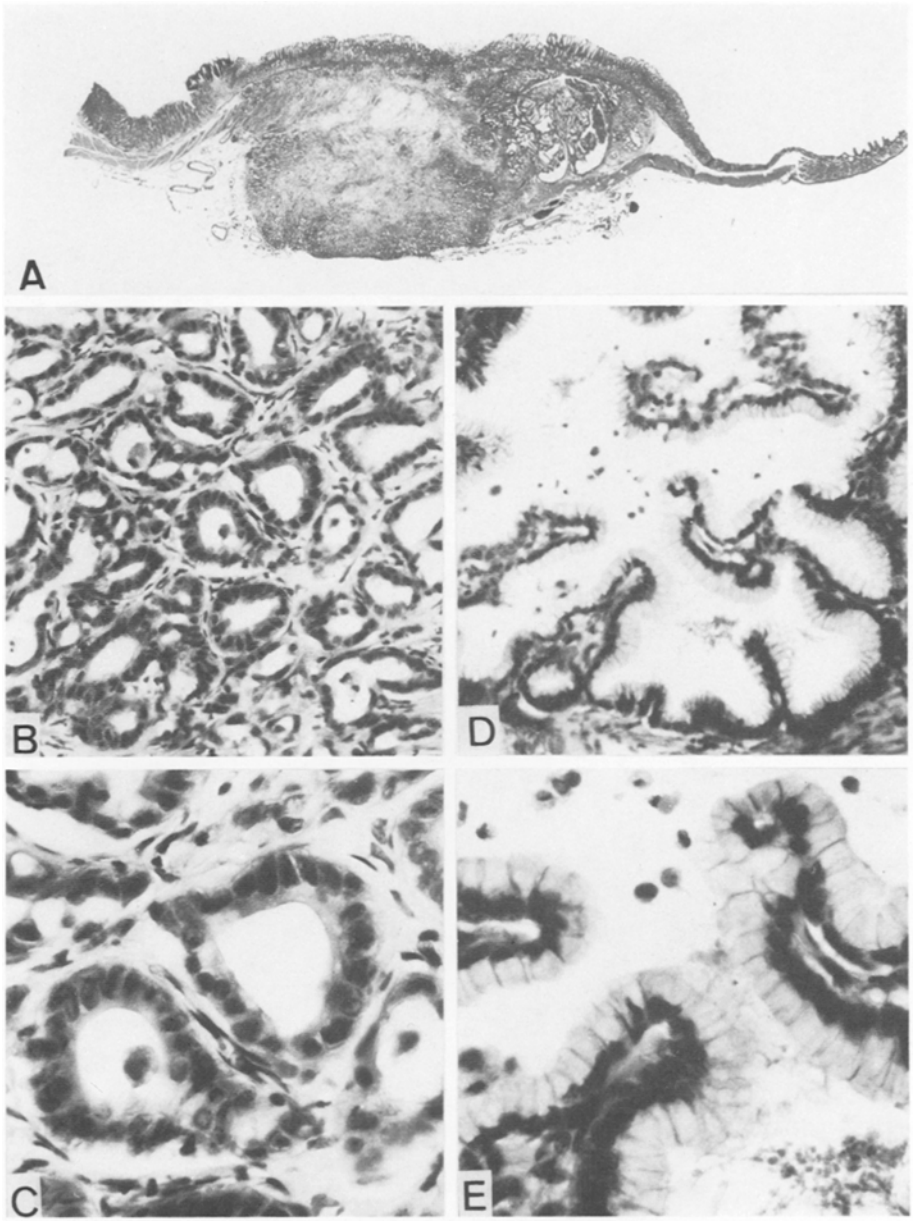


Fig. 3A–E. Light micrograph of a typical gastric carcinoma observed in the 55th week. Tubular carcinoma with proliferating stroma (**A**, left half, **B and C**) and papillary structure (**A**, right half, **D and E**). HE, B and D, ×100. C and E, ×250

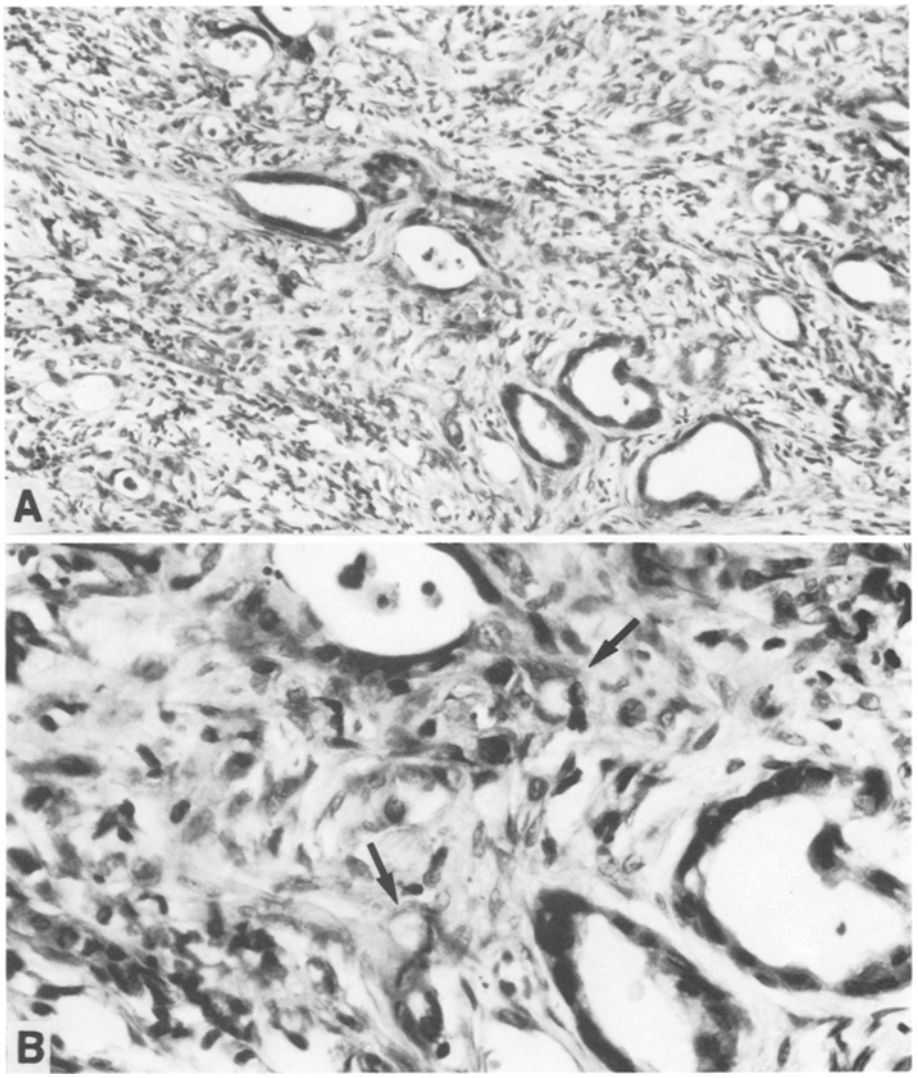


Fig. 4. Coexistence of tubular structure and undifferentiated carcinoma. Note the signet-ring cell carcinoma (arrow), which showed a strongly positive reaction to PAS staining. HE, A, ×100. B, ×250

showed large pleomorphic, vesicular nuclei with prominent nucleoli and their cytoplasm was scanty and basophilic. Mitoses were frequent. A positive PAS reaction was occasionally detected. Some of the tumor cells found in the papillary structure showed very limited cellular atypism and prominent mucous production (Fig. 3D and E).

Of 33 carcinomas, 23 contained Grimelius-positive argyrophil cells; 7 cases showed them diffusely, and 15 sporadically (Fig. 5A). In 6 carcinomas, tumor cells showed a tendency towards squamous differentiation (Fig. 5B).

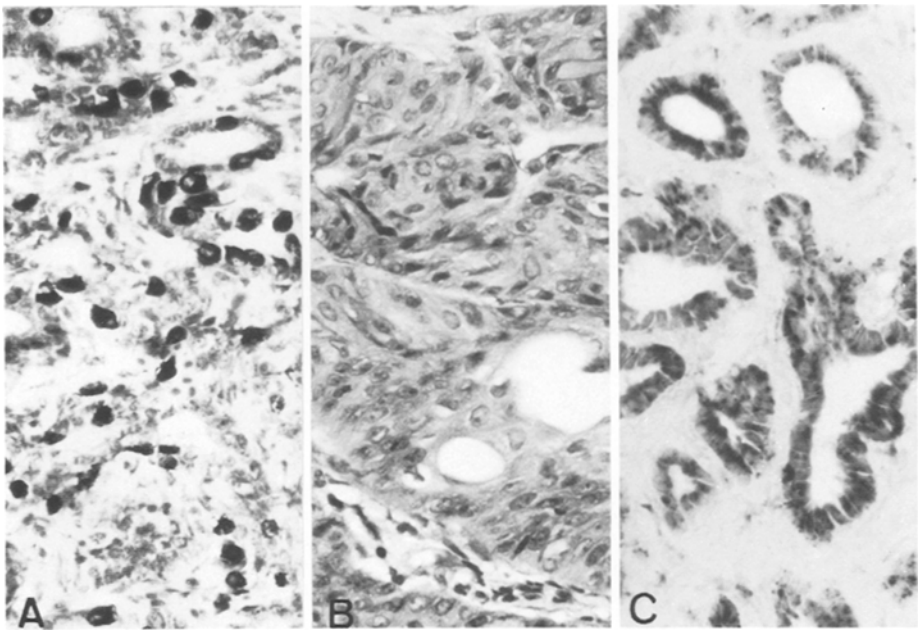


Fig. 5. A Argyrophil cells found diffusely in a carcinomatous lesion. **B** A small part showing a tendency towards squamous differentiation. **C** Carcinoma showing strongly positive reaction to non-specific esterase. A, Grimelius, ×250. B, HE, ×250. C, ×250

Histochemically, acid phosphatase and non-specific esterase (α-naphthyl acetate) were positive in all the examined 13 cases, always in the tubular part of the carcinoma, although the degree of activity varied (Fig. 5C). On the other hand, alkaline phosphatase and aminopeptidase, which have been recognized as characteristic of intestinal epithelium, were always negative.

Twenty-four carcinomas were studied by electron microscopy, 6 of which were obtained in the previous experiment. In each case, at least 3 blocks were examined. Almost all the carcinoma cells studied electron microscopically could be identified with those which were found in the thick sections stained with HE or PAS. Under the electron microscope most of the carcinoma cells were undifferentiated with poorly developed intracellular organelles (Fig. 6). This finding was independent of whether they were derived from the tubular, papillary or from undifferentiated part of the tumor. The cytoplasm of these cells was composed mainly of matrix, free ribosomes, and polysomes, whereas mitochondria and endoplasmic reticulum (much of which was of the rough type) were relatively scanty. Secretory granules were only occasionally seen. The Golgi apparatus was well developed. The nuclei were large with prominent nucleoli, the nuclear outline was very smooth in some cells, but conspicuously irregular in others. The plasmalemma also appeared in various forms; sometimes simple, and other times highly interdigitated. Poorly developed, stubby microvilli were frequently observed. Occasionally a projection from the base of an epithelial cell was found; these protrusions of the epithelial cell base ranged from minute

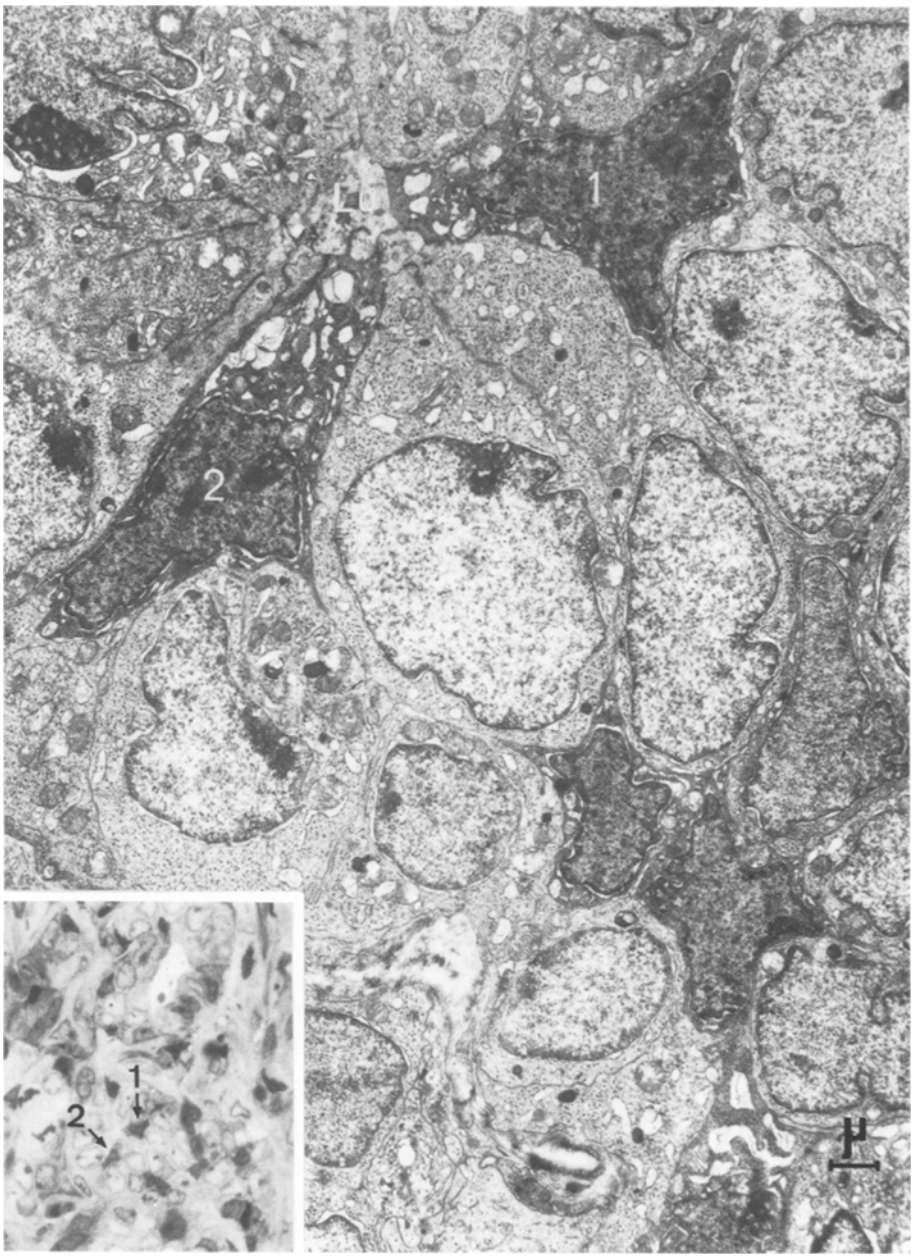


Fig. 6. Ultrastructure of the undifferentiated carcinoma cells obtained in the 48th week, which showed poorly developed organelles and numerous ribosomes. Note an irregular cellular arrangement. ×6300. *Lu* Lumen

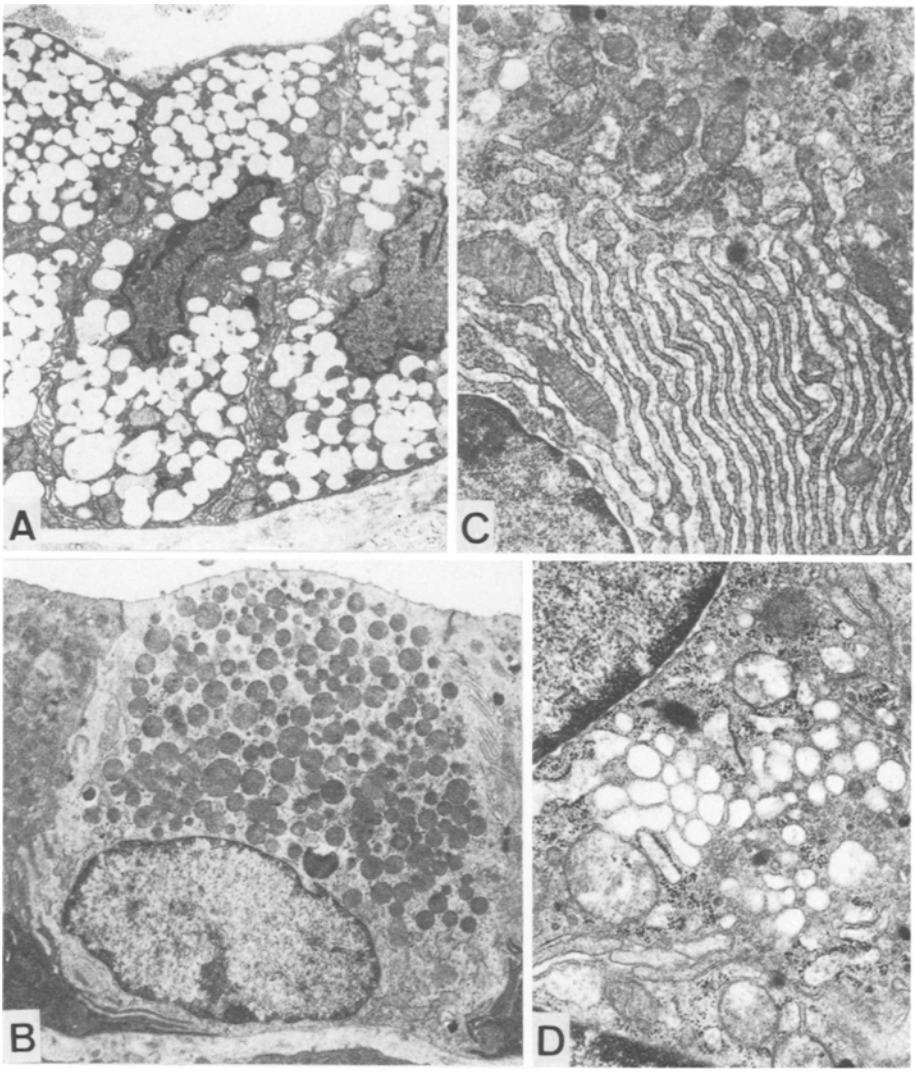


Fig. 7A–D. Ultrastructure of the carcinoma cells of gastric cell type. **A and B** Mucus neck cell type. The secretory granules of (**A**) are far less dense than the second category (**B**). They show a tendency to fuse with each other and frequently have opaque cores, while those of (**B**) are round, dense and separated from each other. **C** Chief cell type with elaborately developed granular endoplasmic reticulum. **D** Parietal cell type showing the smooth-surfaced tubovesicular system and large mitochondria. A, ×6000. B, ×5600. C, ×8500. D, 18,500

cytoplasmic tongues to extensive blebs which contained mitochondria and endoplasmic reticulum.

The remaining minority of the carcinoma cells consisted of those which showed a remarkable differentiation and frequently could not be distinguished from the corresponding cells found in normal gastrointestinal epithelium. All of these well-differentiated carcinoma cells were found within all parts of the carcinomata in the tubular, as well as in the papillary areas. In contrast the goblet cell type was observed only in the undifferentiated parts.

1. Gastric Cell Type

a) Mucus Neck Cell Type. Secretory granules in cells of this type could be roughly divided into two categories. One as homogeneous, far less dense than the second type. Secretory granules in the first category were scattered widely in the cytoplasm and showed a tendency to fuse with each other. Some had opaque cores (Fig. 7A). Granules of the second category were small, round and homogeneously dense, well separated from each other, and distributed in the supranuclear area (Fig. 7B).

b) Chief Cell Type. The most characteristic feature of these cells was an elaborately developed system of parallel, closely packed cisternae of the rough endoplasmic reticulum. An occasional mitochondrion was sandwiched between the cytoplasmic surfaces of adjacent cysternae (Fig. 7C).

c) Parietal Cell Type. Carcinoma cells resembling parietal cell were encountered very rarely. In the cytoplasm of a very few cells, a structure similar to that of the smooth-surfaced tubovesicular system of the parietal cell was observed (Fig. 7D).

2. Endocrine Cell Type

Carcinoma cells, which were similar to endocrine cells in the normal gastrointestinal tract, could be divided into two categories. a) The secretory granules showed various forms; round, oval, disc-shaped, and rod-shaped. They were distributed widely in the cytoplasm and their size was varied. These granules were similar to those of the classical enterochromaffin cell (cell "Type I") (Fig. 8A). b) The granules in this category were uniformly round and of varying density, i.e., some contained pale, fine granular material, others dense. These secretory granules were similar to those of the "Type V" cell which is the source of gastrin (Fig. 8B).

3. Intestinal Type

a) Absorptive Cell Type. Well-developed microvilli, which were observed in these cells, were more scattered and less organized than in the striated border

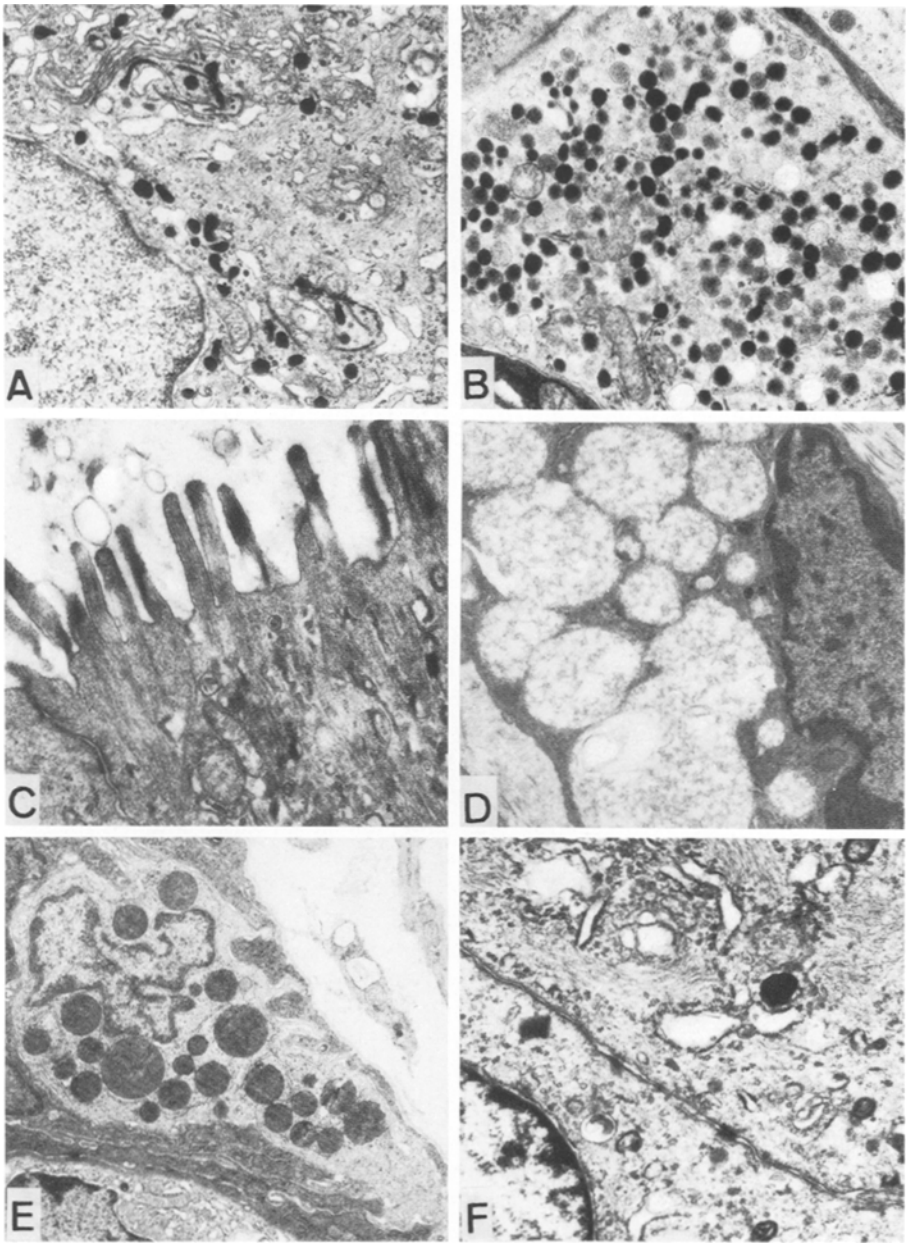


Fig. 8. A Carcinoma cells similar to the enterochromaffin cell with secretory granules of various shapes. **B** Secretory granules are round and of various density, which are similar to those of Type V cell. **C** Carcinoma cells of intestinal absorptive cell type. Note well developed microvilli with central cores stretching into the cytoplasm. **D** Goblet cell type carcinoma, whose mucus granules show characteristic reticular, honeycomb-like pattern. **E** Carcinoma cell similar to Paneth cell. Secretory granules are large, homogeneously dense and separated from each other. **F** Carcinoma cells showing a tendency towards squamous differentiation. Keratohyaline granules and many bundles of cytoplasmic filament are observed. A, ×11,300. B, ×12,500. C, ×17,500. D, ×6100. E, ×6300. F, ×12,000

of the normal intestinal absorptive cell. However, the central cores, which stretched deep into the cytoplasm, were clearly seen. A fuzzy coat was not observed (Fig. 8C).

b) Goblet Cell Type. Large secretory granules tended to produce a fused mass and showed the characteristic reticular or honeycomb-like structure surrounded by an unit membrane. The marked cytoplasmic density was noticed (Fig. 8D). The corresponding cells in the thick section revealed a granular positive reaction to the PAS staining. This type of carcinoma cell was observed in the undifferentiated area only.

c) Paneth Cell Type. The prominent features of these cells included large, electron-dense, homogeneous granules, which did not fuse like the mucous droplets of the goblet cell. They were distributed widely in the cytoplasm (Fig. 8E).

4. Squamous Cell Type

The cytoplasm of these cells was distinguished by the presence of large and dense keratohyaline granules and many bundles of cytoplasmic filaments, many of which were joined by desmosomes. The lateral cell surfaces were straight and had many well developed desmosomes (Fig. 8F).

All 6 adenomata, 4 of which were obtained from the previous experiment, were located near the midpoint of the lesser curvature. They were studied electron microscopically and histochemically and these findings showed their almost complete identity with those that were obtained in the study of the carcinomata. A moderate positive PAS reaction was observed in all cases and Grimelius-positive argyrophil cells were diffusely scattered in one lesion. Most of the cells from the adenomatous change were of the undifferentiated cell type with poorly developed organelles. Occasionally they showed cytoplasmic protrusions through basal membrane. Some cells showed a remarkable differentiation towards a mucous neck cell type, chief cell type, parietal cell type, endocrine cell type, and Paneth cell type, none of which could be distinguished from those found in the carcinomatous lesions.

Table 1. Data from the transplantation experiment

Donor rat no.	Age (W)	Period on MNNG (W)	Period of MNNG (W)	Histol. observations of donor rats			Week after transpl.	Recipient rats with lesion/ observed
				Muscularis propria	PAS	Lymphnode meta.		
1	59	31	22	Penetr.	±	−	13	1/1
2	59	31	22	Penetr.	++	−	8	1/1
3	60	31	23	Penetr.	±	−	12	1/2
4	61	31	24	Penetr.	±	−	11, 25	2/6
5	62	31	25	Penetr.	±	+	24	1/2

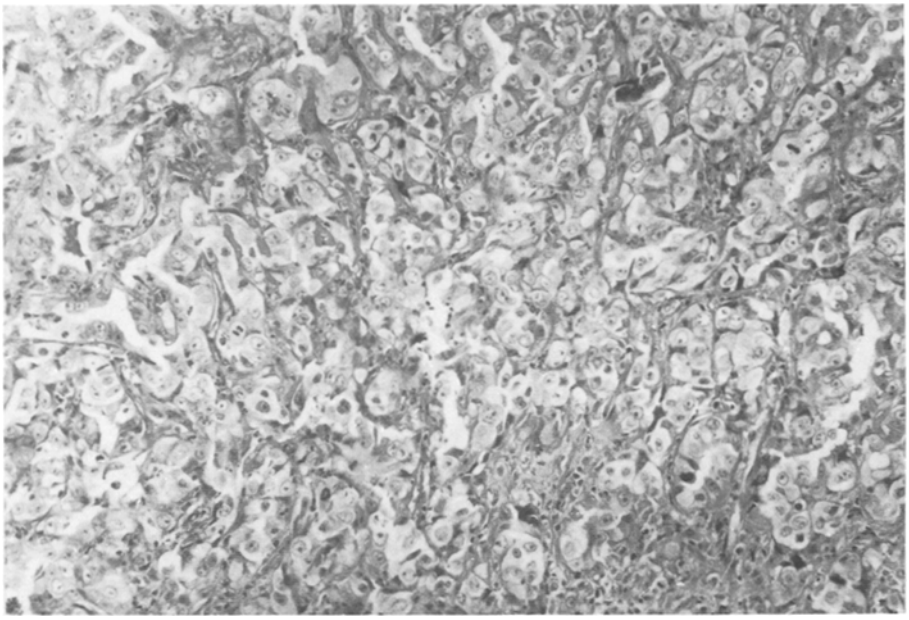


Fig. 9. Light micrograph of the transplanted gastric carcinoma (No. 1 in Table 1). The carcinoma cells are very large and show a tendency to form solid nests. HE, ×100

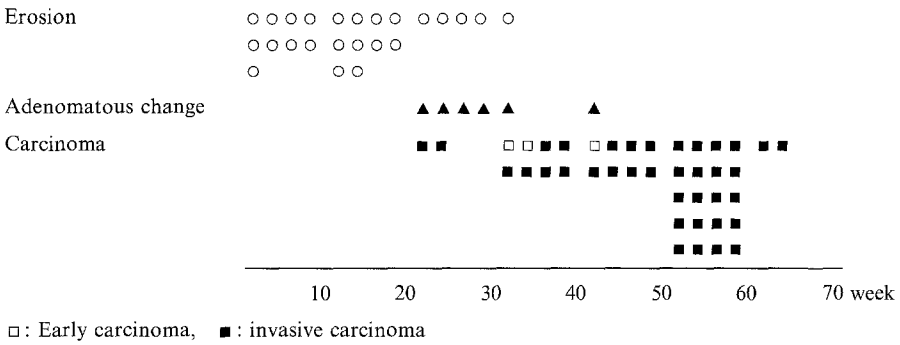
As stated in Table 1, transplantation of the carcinomata was successful in all 5 cases attempted. Light microscopically, 4 cases showed a histological pattern similar to that of the primary tumor. In one case, however, a part of the transplanted tumor had a definitely different appearance; the tumor cells were very large and formed a solid nest (Fig. 9). In 2 out of 5 cases, Grimelius positive argentaffin cells were found sporadically. Electron microscopically, most of the carcinoma cells consisted of undifferentiated cells, however, carcinoma cells of endocrine cell type were also found.

Discussion

1. Localization and Chronology of Benign and Malignant Lesions

A summary of changes observed in our previous and present studies reveals obvious chronological and positional rules in the appearance of the benign and malignant lesions (Table 2). Each of the 24 benign, regenerative erosions including 2 cases with ectopic glands, all 6 of the adenomatous changes, all 3 of the early carcinomas, and 20 out of 21 invasive carcinomas whose diameter were under 10 mm, were always located near the midpoint of the lesser curvature; while the center of the remaining 16 large invasive carcinomas also pointed to this area. These facts led us to suggest the following pattern of events: in the 5th week an erosion is chemically induced and persists for about 20

Table 2. Chronological relationship between erosion, adenomatous change and carcinoma



weeks with constant repetition of the erosive process and regeneration. This erosion progresses to adenomatous growth and then to an early carcinoma between the 25th and the 35th week. After the 35th week, the carcinoma begins to infiltrate deep into the muscularis propria and subserosa showing a definite morphological malignancy.

As we mentioned in the previous study, the fact that all these benign and malignant lesions showed exactly the same localization may depend on an optimal combination of strain of rats and concentration of MNNG. We used inbred Wistar rats and according to Bralow (1972), the incidence of gastric carcinoma in a group of Wistar-Mai-Furth rats after 39 generations of brother/sister mating was clearly lower when compared with that of the group of Wistar rats randomly-bred by the same breeder. Martin et al. (1974) also demonstrated that susceptibility to MNNG varied among the inbred strains and suggested that Bralow's result was due to the choice of a strain which was cancer-resistant.

2. Electron Microscopic Study

The findings of undifferentiated carcinoma cells in our experiments were consistent with those which were described by Onoe (1962), Goldman and Ming (1968), Sasano et al. (1967) in human gastric carcinoma. Many authors have documented carcinoma cells similar to the various normal gastrointestinal cells including endocrine cell and squamous cell (Hamperl, 1952; Seki, 1962; Azzopardi and Pollock, 1963; Onoe, 1963; Goldman and Ming, 1968; Sasano et al., 1967; Takayama et al., 1969; Kubo and Watanabe, 1971). Hananouchi et al. (1974) described electron microscopical findings of gastric carcinoma cells induced by MNNG but observed only four types of carcinoma cells, mucin-containing cells (goblet cell type and pit mucus cell type) intracellular microcyst cell, and poorly differentiated mucous cell. We found carcinoma cells similar to intestinal cells and considered these to be the intestinal type carcinoma of the stomach. Since Järvi and Lauren (1951) indicated from human material that metaplastic intestinal epithelium was possibly a precancerous lesion in some gastric carcinomata, the relationship between intestinal metaplasia and

intestinal type carcinoma of the stomach has been a subject of a long dispute (Nakamura et al., 1968; Planteydt and Willighagen, 1965). In our studies, the intestinal metaplasia could never be found, neither in a normal rat gastric epithelium nor in a benign regenerative one whether induced chemically or mechanically. Thus the view that intestinal type carcinoma derives exclusively from benign intestinal metaplasia cannot be supported. However, Sugimura et al. (1970), and Saito et al. (1970) observed light microscopically intestinal metaplasia in the non-neoplastic portion of the gastric epithelium in a few cases.

3. Differentiation of Carcinoma Cells

A particularly striking observation in our studies was the finding of highly differentiated epithelial cells in experimentally induced carcinomas. These cells were sometimes similar to the equivalent cells of normal gastro-intestinal epithelium. Thus it appears probable that they represent displaced portions of ordinary, non-neoplastic glands within the tumors. Other authors (Azzopardi and Pollock, 1963) have suggested the possibility that the differentiated epithelial cells are non-neoplastic colonisers within a malignant tumor. This theory seems to be improbable for the following reasons: Firstly, although in our study all lesions were located in the pyloric gland area, chief and parietal cell types were observed, and in addition, intestinal and squamous type carcinoma cells. Secondly, argyrophil cells were seen in the transplanted tumor. Hamperl (1952) had already described these cell types in metastatic lesions. Thirdly, various intermediate types of the above mentioned cells were found. Thus for example the cell shown in Figure 10 reveals massively bundled cytoplasmic filaments and dense keratohyaline granules which suggest a partial differentiation to a type of cell other than normal gastric epithelium, e.g. to squamous cell type. In contrast, the surface of those partially differentiating cells retains characteristics of glandular epithelium with developed microvilli, some of them with central cores stretching into the cytoplasm. Sasano et al. (1969) observed that some cells of human gastric carcinomas contain both types of secretory granules belonging to both the chief and mucous cell type. Similar findings also were reported by Ming et al. (1967) in benign metaplastic epithelial cells of human tissue. These findings support the view that highly differentiated carcinoma cells are not non-neoplastic colonisers but the result of differentiation of carcinoma cells in various directions.

4. Ascertainment of Malignancy

Various authors have used several criteria for the diagnosis of carcinoma of the glandular stomach in animals (Stewart et al., 1969; Bralow, 1972). Stewart suggested two practical approaches to ascertain the diagnosis of gastric carcinoma in rats: one depends upon the atypia of the tumor cells, the other depends upon the depth of infiltration. Thus, infiltration itself is not an absolute criterion

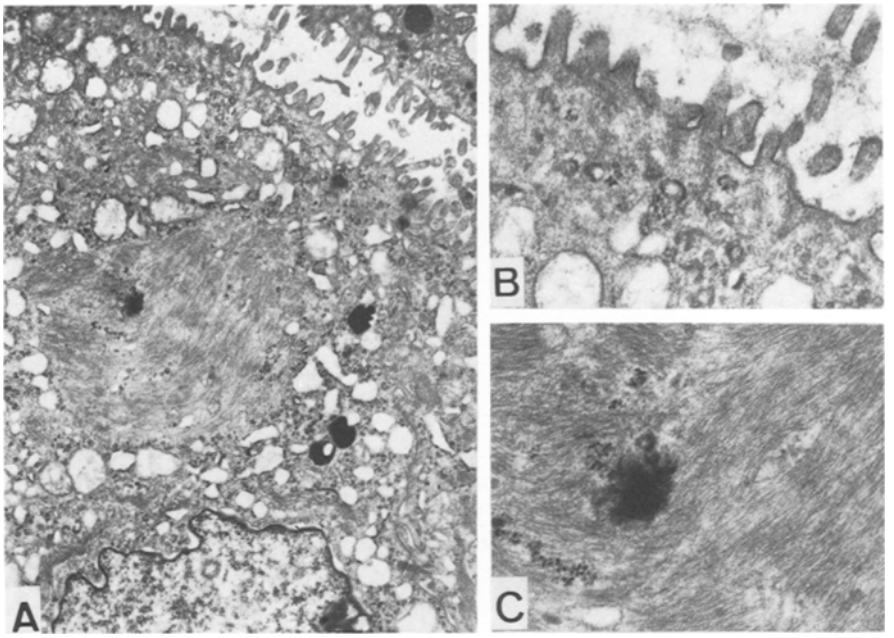


Fig. 10A–C. Carcinoma cell showing differentiation in two different directions. Note locally developed, massive cytoplasmic filament, keratohyaline granules and microvilli with central cores. A, ×8500. B, ×21,000. C, ×27,000

of malignancy. According to the modern concept of gastric carcinoma we consider the combination of several features as a proof of malignancy: the degree of cell atypia, the atypical or destroyed structure of proliferating gastric glands, and infiltration.

According to Toner et al. (1971) cytoplasmic protrusions of the epithelial cell are usually accompanied by lymphocytes crossing the basal membrane and are therefore, not necessarily a sign of malignancy. Other authors who have noticed this phenomenon in various carcinomatous lesions are more inclined to consider cytoplasmic protrusions through the basal membrane as an evidence of malignancy (Ashworth et al., 1961; Frei, 1962; Imai and Stein, 1963). Frei (1962) suggested two possible explanations: one is that the basement membrane of the epithelium has been penetrated and disrupted by the tumor cells, and the other is that neoplastic epithelial tissue grows faster than a basement membrane is formed beneath it. We did not observe penetration and disruption of the basal membrane in gastric epithelium of control animals or in chemically or mechanically induced regenerating epithelium. These observations lead us to the conclusion that the penetration and destruction of the basal membrane are signs of infiltration and invasive growth and therefore, another indication of malignancy.

The most important signs of malignancy of experimentally induced tumors include the capacity to metastasize and transplantability to other animals (Stew-

art et al., 1969). Both of these criteria were fulfilled in our experiments. Tumor transplantation was successful in contrast to experiments by other authors (Bralow et al., 1973; Martin et al., 1973). Our successful results seem to be dependent on the fact that all recipient rats were chosen from the same inbred strain which was susceptible to MNNG (Kobori, Gedigk u. Totović, 1976).

5. Relationship between Adenomatous Change and Carcinoma

The lesions with only mucosal spread or submucosal invasion could be devided into the two groups of adenomatous change and early carcinoma. Nearly all of the invasive carcinomas also showed a close positional and chronological relationship. Moreover, the adenomatous changes and the experimentally induced carcinomas were identical in regard to their cytological properties as revealed by electron microscopy and cytochemistry. We suggest therefore, that adenomatous changes induced by MNNG should be considered to be precancerous lesions.

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References

Ashworth, C.T., Stembridge, V.A., Luibel, F.J.: A study of basement membranes of normal epithelium, carcinoma in situ and invasive carcinoma of uterine cervix utilizing electron microscopy and histochemical methods. Acta cytol. (Philad.) **5**, 369–384 (1961)

Azzopardi, J.G., Pollock, D.J.: Argentaffin and argyrophil cells in gastric carcinoma. J. Path. Bact. **86**, 443–451 (1963)

Bralow, S.P.: Experimental gastric carcinogenesis. Digestion **5**, 290–310 (1972)

Bralow, S.P., Gruenstein, M., Meranze, D.R.: Host resistance to gastric adenocarcinomatosis in three strains of rats ingesting N-methyl-N′-nitro-N-nitrosoguanidine. Oncology **27**, 168–180 (1973)

Frei, J.V.: The fine structure of the basement membrane in epidermal tumors. J. Cell Biol. **15**, 335–342 (1962)

Goldman, H., Ming, S.C.: Fine structure of intestinal metaplasia and adenocarcinoma of the human stomach. Lab. Invest. **18**, 203–210 (1968)

Grimelius, L.: A silver nitrate stain for α-cells in human pancreatic islets. Acta Soc. Med. upsalien. **73**, 243–270 (1968)

Hamperl, H.: Über argyrophile Zelle. Virchows Arch. path. Anat. **321**, 482–507 (1952)

Hananouchi, M., Fukushima, S., Takahashi, M.: Electron microscopic studies on experimental undifferentiated adenocarcinomas of the glandular stomach in rats. Gann **65**, 323–330 (1974)

Imai, H., Stein, A.A.: Ultrastructure of adenocarcinoma of the colon. Gastroenterology **44**, 410–418 (1963)

Järvi, O., Lauren, P.: On the role of heterotopias of the intestinal epithelium in the pathogenesis of gastric cancer. Acta path. microbiol. scand. **29**, 26–44 (1951)

Kobori, O., Gedigk, P., Totović, V.: Early changes of glandular stomach in Wistar rats ingesting N-methyl-N′-nitro-N-nitrosoguanidine (MNNG). Z. Krebsforsch. (in press)

Kubo, T., Watanabe, H.: Neoplastic argentaffin cells in gastric and intestinal carcinomas. Cancer (Philad.) **27**, 447–454 (1971)

Lane, B.P., Europa, D.L.: Differential staining of ultrathin sections of epon-embedded tissues for light microscopy. J. Histochem. Cytochem. **13**, 579–583 (1965)

Martin, M.S., Bastien, H., Martin, F., Michiels, R., Martin, M.R., Justrabo, E.: Transplantation of intestinal carcinoma in inbred rats. Biomedicine **19**, 555–558 (1973)

Martin, M.S., Martin, F., Justrabo, E., Michiels, R., Bastien, H., Knobel, S.: Susceptibility of inbred rats to gastric and duodenal carcinomas induced by N-methyl-N′-nitro-N-nitrosoguanidine. J. nat. Cancer Inst. **53**, 837–840 (1974)

Ming, S.C., Goldman, H., Freiman, D.G.: Intestinal metaplasia and histogenesis of carcinoma in human stomach. Cancer (Philad.) **20**, 1418–1429 (1967)

Nakamura, K., Sugano, H., Takagi, K.: Carcinoma of the stomach in incipient phase. Gann **59**, 251–258 (1968)

Onoe, T.: Electron microscopic studies of human carcinoma. J. Electronmicro. **11**, 70–84 (1962)

Planteydt, H.T., Willighagen, R.G.J.: Enzyme histochemistry of gastric carcinoma. J. Path. Bact. **90**, 393–398 (1965)

Saito, T., Inokuchi, K., Takayama, S., Sugimura, T.: Sequential morphological changes in N-methyl-N′-nitro-N-nitrosoguanidine carcinogenesis in the glandular stomach of the rats. J. nat. Cancer Inst. **44**, 769–783 (1970)

Sasano, N., Nakamura, K., Arai, M., Akazaki, K.: Ultrastructural cell patterns in human gastric carcinoma compared with non-neoplastic gastric mucosa. J. nat. Cancer Inst. **43**, 783–802 (1969)

Seki, M.: Parietal cell carcinoma. J. Electromicr. **11**, 40–46 (1962)

Stewart, H.L., Snell, K.C., Morris, H.P.: Histologic criteria for the diagnosis of experimentally induced carcinoma and precancerous lesions of the glandular stomach of inbred strains of rats and comparison with transplantability. Gann Monogr. No. **8**, 353–375 (1969)

Sugimura, T., Fujimura, S., Baba, T.: Tumor production in the glandular stomach and alimentary tract of the rat by N-methyl-N′-nitro-N-nitrosoguanidine. Cancer Res. **30**, 455–465 (1970)

Takayama, S., Saito, T., Fujimura, S., Sugimura, T.: Histological findings of gastric tumors induced by N-methyl-N′-nitro-N-nitrosoguanidine in rat. Gann Monogr. No. **8**, 197–208 (1969)

Toner, P.G., Carr, K.E., Wyburn, G.M.: The digestive system – An ultrastructural atlas and review. London: Butterworths 1971

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