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OBSERVATION OF CELL REGENERATION AS THE BACKGROUND OF GASTRIC CARCINOGENESIS

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INTRODUCTION

In recent years, it has become possible for neoplasm to be produced by chemical carcinogens in most organs. Several effective chemical carcinogens have been discovered and experimental studies on the gastrocarcinogenesis have been undertaken.

For producing carcinomas at a higher incidence, it was suggested that some conditions such as chronic ulceration by chemical agents[16,43] or surgical methods,[27,28] and a foreign body or plastic bead inserted into gastric lumen,[9] gastric vagotomy[8] and gastrectomy[32] might play a certain role.

Either acceleration of regenerative activity in the gastric mucosa or prolongation of carcinogenic stimulation in the stomach is considered to be one of the promoting factors for carcinogenesis.

For producing the acceleration of regenerative activity in the gastric mucosa, it is thought that the method, introduced by Matsuyama et al.[22] who employed a subcutaneously implanted homograft of the gastric mucosa, is worth undertaking. In this study, numerous undifferentiated or immature cells were observed in the gastric cysts subsequently formed from the implanted gastric mucosa. Furthermore, prolongation of carcinogenic stimulation could possibly be obtained in the gastric cysts because carcinogens are prevented from deactivation by HCI and their absorption by gastric mucosa is delayed. Utilizing experimental gastric cysts implanted as homografts and treated with a potent carcinogen, gastrocarcinogenesis may be analyzed.

The purpose of this study is to investigate, at the light-and electron-microscopic levels, the early morphological changes of gastrocarcinogenesis and cystogenesis in implanted gastric cysts of Donryu rats, with or without the effects of N-ethyl-N'- nitro

- N- nitrosoguanidine (ENNG).[40]

Materials and Methods

I. Production of gastric cysts

Sixty-two adults Donryu rats of both sexes, weighing 200-250 grams, were used. They were fasting for 24 hours before the operation.

Laparotomy was carried out under penthrane anesthesia. The stomach was opened and small segments of the mucosa from the fundic region containing a variety of cell types, surface mucous cells, mucous neck cells, parietal cells, chief cells, and a few endocrine cells, were removed quickly and immediately immersed in 0.9% NaCl solution. The segments of the gastric fundic mucosa were further trimmed into 2-3mm^3 cubes and homografted into the subcutaneous region of abdominal wall of Donryu rats.

Of 161 pieces of gastric mucosa implanted, 32 (19.9%) successfully formed cysts. Postoperatively, at 1, 2, 3, 4, 5, 6, 7, 8 and 10 days, at 2 and 3 weeks, and at 1, 2, 3, 6, 10 and 12 months, implanted gastric cysts were taken out from the animals (Table 1).

The tissue was immediately immersed in 2.5% glutaraldehyde solution for 2 hours, buffered at pH 7.4 by 0.2 M sodium cacodylate, and postfixed for the next 2 hours in s -collidine buffered 1% osmium tetroxide. After dehydration in a graded series of ethanol baths, the specimens were embedded in epoxy resin (Epon 812).

The blocks were sectioned on a Porter-Blum ultramicrotone. Semithin sections was stained with Azur A or toluidine blue and observed for orientation by light microscopy. Ultrathin sections were doubly stained with lead citrate and uranyl acetate.

In addition, the part of the specimens were stained with hematoxylin eosin and silver impregnation (Grimelius' stain).

II. Experiment for carcinogenesis in the implanted gastric cysts

Twenty-six Donryu rats of both sexes with 36 gastric cysts were given 0.5 ml of ENNG solution by subcutaneous injection (Table 1). ENNG (Aldrich Chemical Co., Milwaukee, U. S. A.) was dissolved in distilled water to a concentration of 1 mg/ml and stored in a dark bottle in a cold place.

ENNG in use was further dissolved in 0.9% NaCl at the concentration of 300 μg/ml and injected weekly for eight weeks. Cysts growing to a grossly identifiable size at two weeks to one month after implantation were used for carcinogen experimentation because of their known development of abundant immature to premature cells in this stage. After administration of ENNG, at 2, 2.5 and 5 months, animals were sacrificed and gastric cysts were quickly taken out from the subcutaneous portion. Specimens were processed for light-and electron-microscopic observation of mainly the earliest stage of experimental carcinogenesis in the gastric mucosa.

In order to compare morphologic changes of early carcinogenesis in gastric cysts, the following experiment was additionally undertaken. Thirty adult Donryu-rats weighing 200 to 300 grams received ENNG at the concentration of 100 μg/ml in their drinking

Table 1 Results of the Experiment

	No. of Rats	No. of Successes
1) Gastric cyst	62 (161)	28 (32)
2) Gastric cyst with ENNG	46 (72)	26 (36)
3) Total	108 (233)	54 (68)
Experimental gastric carcinoma	33	18

( ) No. of implantations

water for the first 40 weeks and then normal water ad libitum for the following one year. After administration of ENNG, animals were sacrificed at chronological stages from 1 month to 1 year. The stomach was cut into longitudinal strips along the lesser curvature. The tissue was stained with hematoxylin and eosin, and Grimelius' silver impregnation for the light-microscopic study. Morphological changes were observed light -and electron-microscopically.

RESULTS

The overall data of the present experiment are shown in Table 1.

I. Gastric cytogenesis

i) Gross and histologic study

The implanted gastric mucosa occasionally began to be grossly necrotic because of poor blood supply 24 hours after its implant, though was its configuration mostly maintained. Surviving gastric mucosa was surrounded by fibrous tissue in the subcutaneous region associated with proliferative capillaries, and gradually grew to a microcyst. Cystic lumen thus formed was filled with secretory fluid. In the period between 6 months to 1 year after implantation, the size of the implanted gastric cysts reached 20 mm in the largest dimension, most remaining with dimensions of 3 mm-10 mm.

Histologically, at 24 hours after implantation, the homografts showed irregular arrangement with occasional depletion of the gastric glands and flattening of the gastric pits. Gastric crypts disappeared and the surface-epithelium was replaced by a single layer of columnar cells (Fig. 1).

Progressive atrophy and degeneration of the cells in the fundic glands were evident. At 4 to 5 days, loose connective tissue started increasing among the gastric fundic glands. In some areas, the fundic glands were replaced by fibrous tissue, leaving only the surface -epithelium. The submucosal tissue was thickend with fibrosis, edema and many proliferating capillaries. At 7 days to 4 weeks, microcyst-formation composed of columnar cells arranged in one layer was produced (Fig. 2).

At 2 months, gastric glands with a close resemblance to the normal structure were found in the cystic wall along with occasional mural microcysts. These glands sequentially transformed to one-layered epithelial cells lining the inner surface of the cystic wall

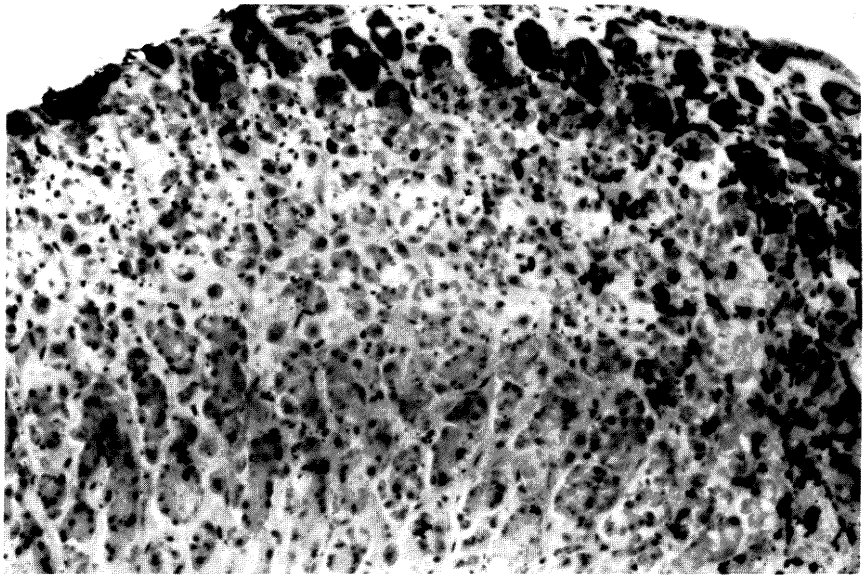


Fig. 1. In the top of the gastric pits, the surface-epithelium is replaced by a single layer of columnar cells. One day after implant. (Azur: ×100).

(Fig. 3).

At 6 months to 1 year, the gastric glands in the cysts showed occasional hyperplastic proliferation of the cells. In such hyperplastic areas there was slight structural distortion with no definite cellular atypism (Fig. 4). Gastric cysts revealed pleomorphic structures and multicystic formation could occasionally be seen (Fig. 5).

ii) Electron-microscopic study

At 24 hours after implantation, most epithelial cells of the gastric glands showed atrophy, degeneration and partial destruction of the cell membrane. Pyknosis of nuclear components was evident. Intracytoplasmic organells such as mitochondria and Golgi apparatus were destroyed. Microbleeding was seen in the broad intercellular space (Fig. 6). However, the pits of the gastric glands were covered with single-layered mucus-forming cells identical to immature surface-epithelial cells. These changes were progressive day after day. At 4 to 5 days after implantation, although mature glandular cells were atrophied, degenerated and in many areas, entirely depleted, one-layered immature epithelial cells remained to proliferate and line the inner surface of the gastric cyst wall (Fig. 7).

In occasional areas, stratified undifferentiated cells were found (Fig. 8). At 6 days to 2 weeks, there were many undifferentiated cells with dark cytoplasm containing numerous free ribosomes and a few mucous granules, with an increased nucleocytoplasmic ratio, tight interdigitation and well-developed desmosomes. Golgi apparatus and mitochondria were abundant, but lysosomal granules were scanty and microvilli were not developed. Undifferentiated cells with mucous granules and numerous coated-vasicles

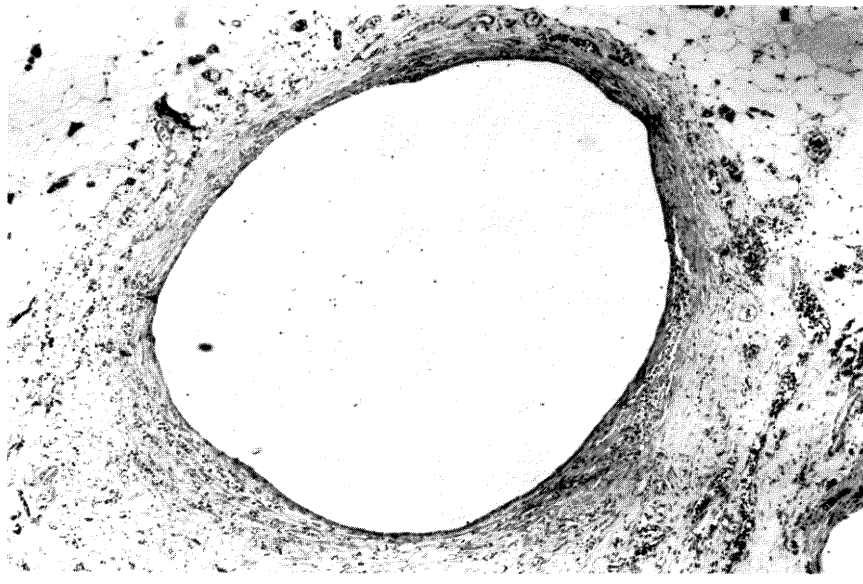


Fig. 2. Microcyst formation. One month after implant. (H. E.: ×40).

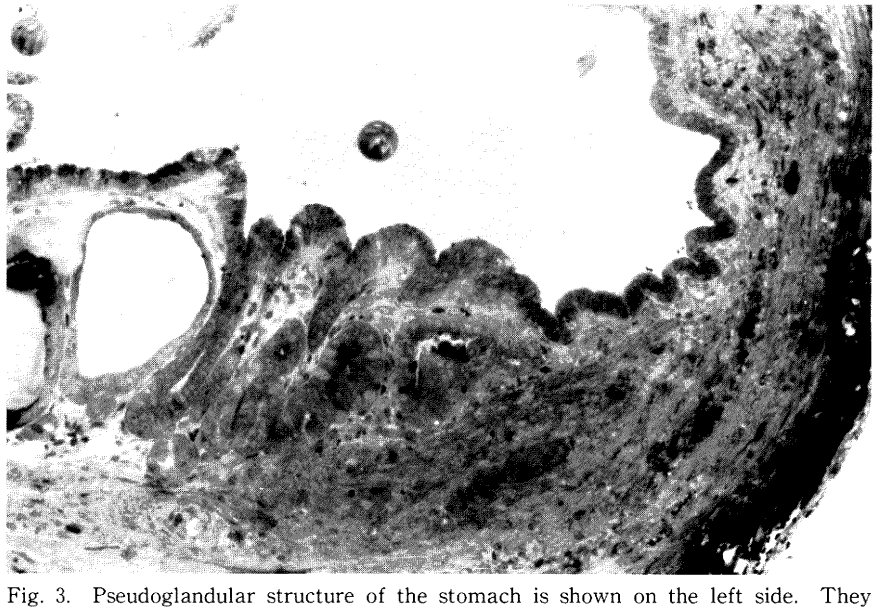


Fig. 3. Pseudoglandular structure of the stomach is shown on the left side. They transform to one layered epithelial cells toward the right side. Two months after implant. (Azur: ×100).

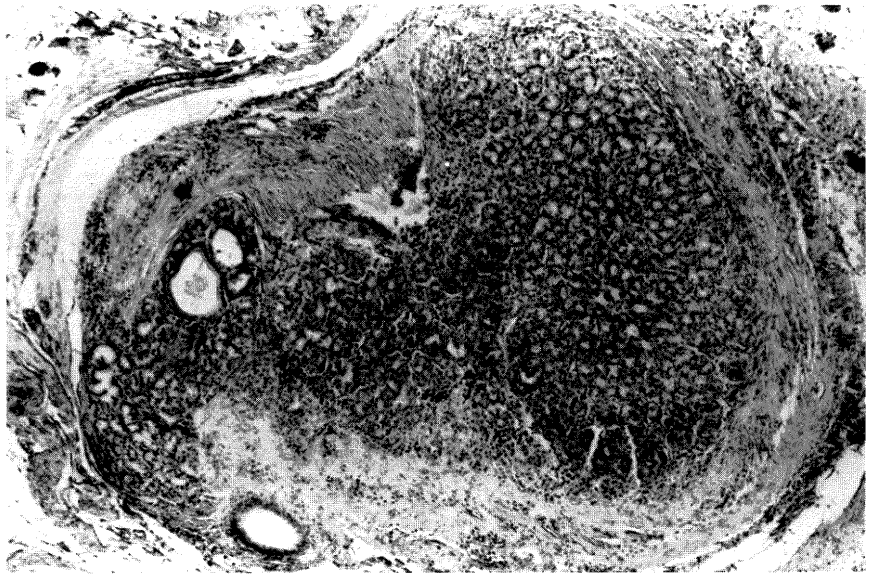


Fig. 4. Regenerated epithelium proliferating and obliterating the cystic lumen. One year after implant. (H. E.: ×40).

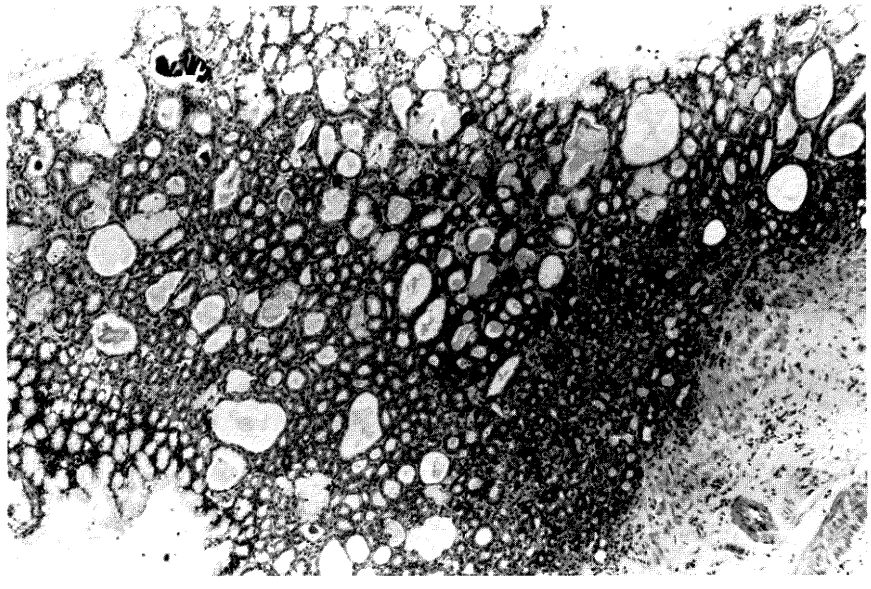


Fig. 5. Hyperplastic stage in cystic production with gastric gland protruding into the cystic lumen. Multicystic formation is evident. One year after implant. (H. E.: × 40).

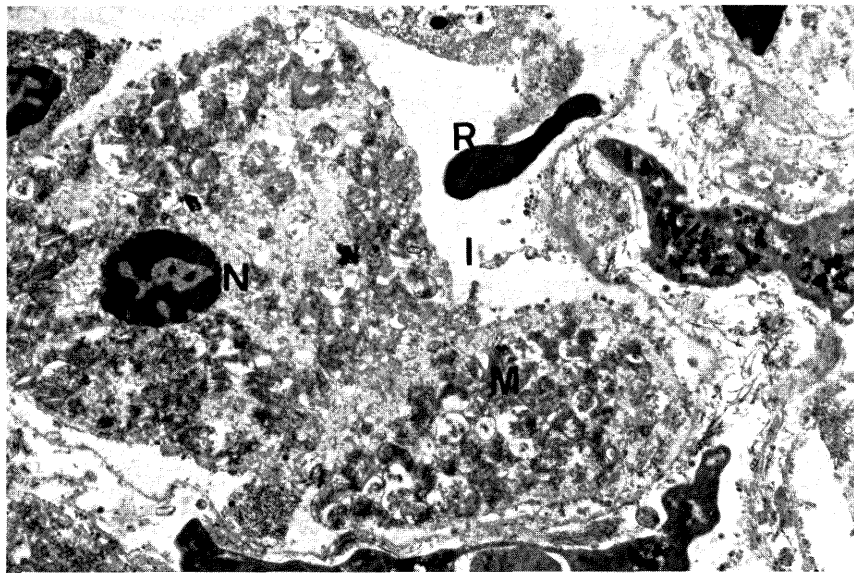


Fig. 6. Severe morphologic changes at 24 hours after implant. Glandular cells have almost degenerated, atrophied and are partially destroyed. N: piknotic nucleus. R: red blood cell. I : extended intercellular space. M: mitochondria. ×6,000 (orig. magnif. ×3,000).

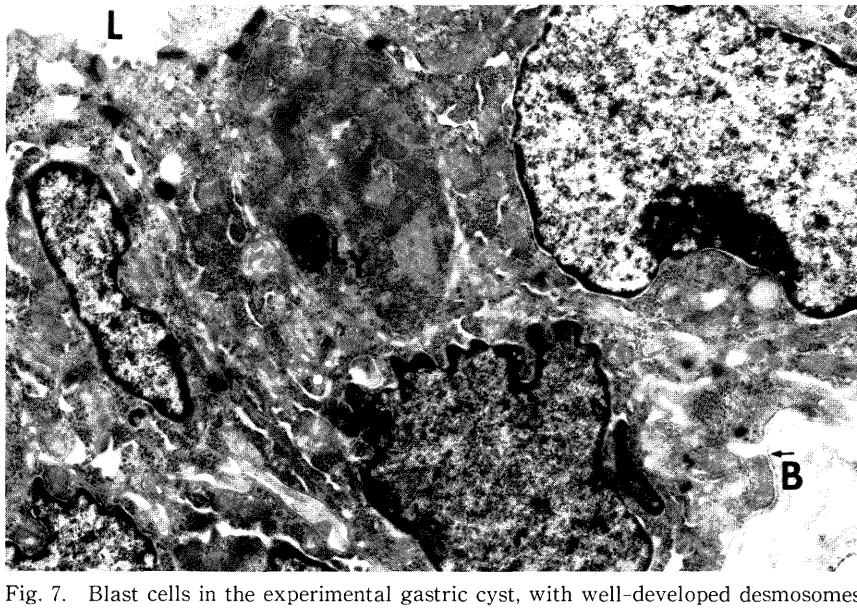


Fig. 7. Blast cells in the experimental gastric cyst, with well-developed desmosomes, abundant free ribosomes and polysomes, and an irregular nucleus Ly: lysomal granule. L: cystic lumen. B: basement membrane. Five days after implant. ×6,000 (orig. magnif. ×3,000).

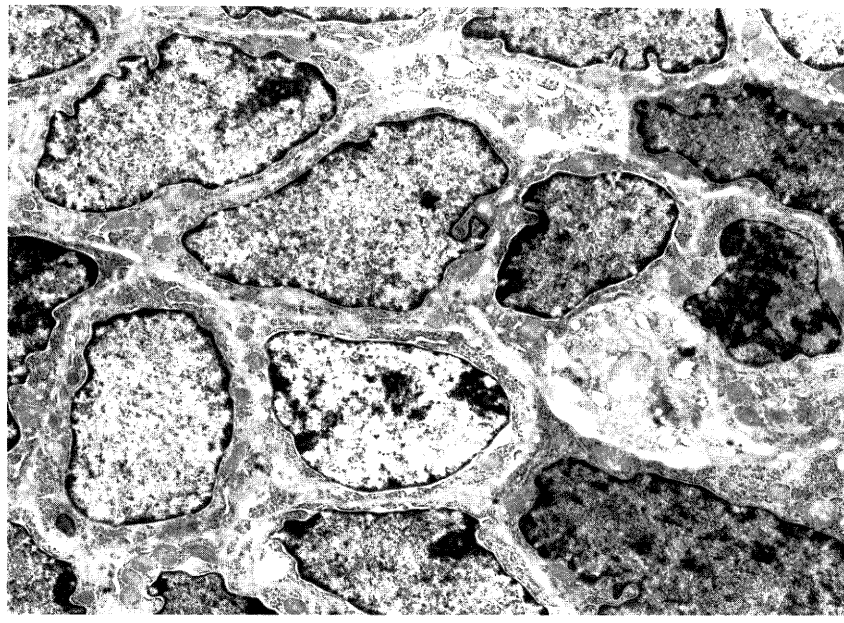


Fig. 8. A nodular conglomerate of stratified undifferentiated (blast) cells with dark cytoplasm containing numerous free ribosomes, few secretory granules, an increased nucleocytoplasmic ratio and tight interdigiration. Five days after implant. ×8,500 (orig. magnif. ×3,000).

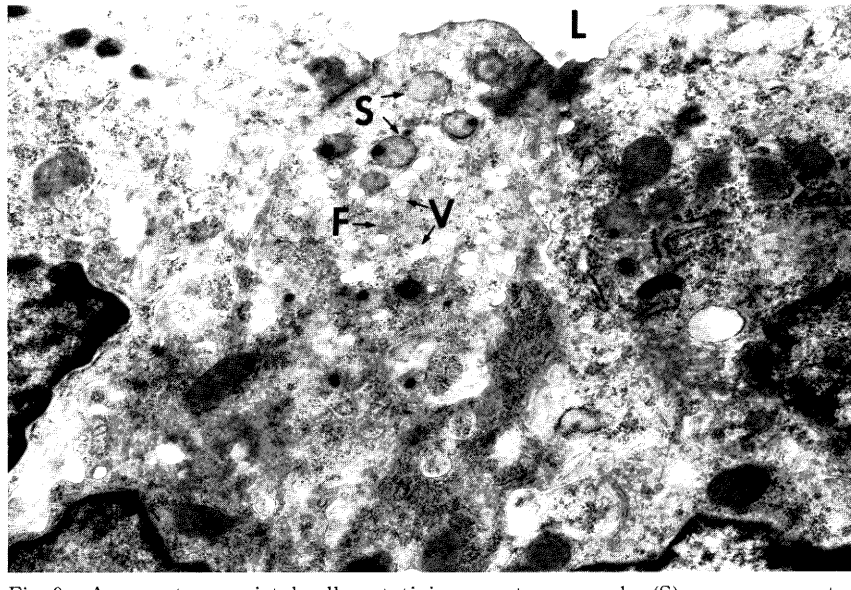


Fig. 9. A premature parietal cell contatining secretory granules (S), numerous coated-vesicles (V) in the apical cytoplasm, fibrillar bundles (F), and cystic lumen (L). Seven days after implant. ×10,000 (orig. magnif. ×5,000).

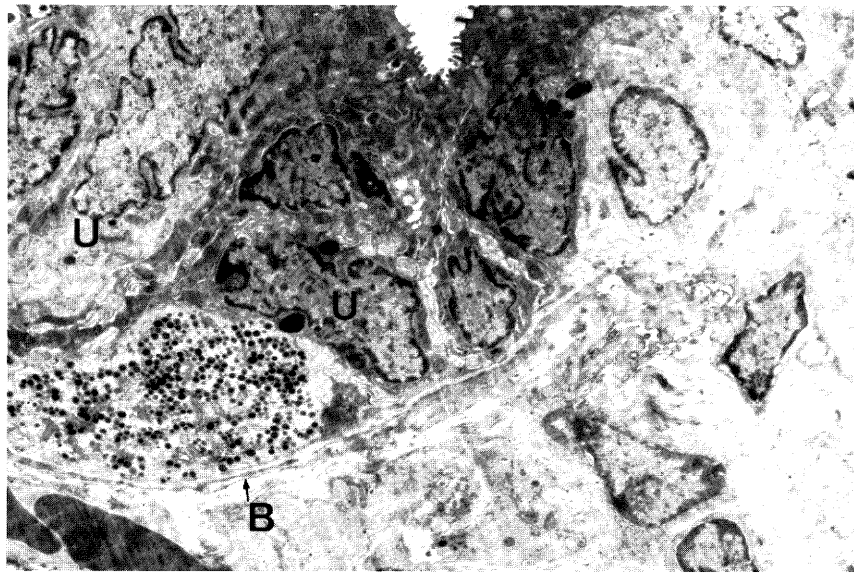


Fig. 10. A young endocrine cell among undifferentiated lining cells (U) in an experimental gastric cyst situtated on the basement membrane. Twenty-two days after implant. B: basement membane. ×4,000 (orig. magnif. ×2.000).

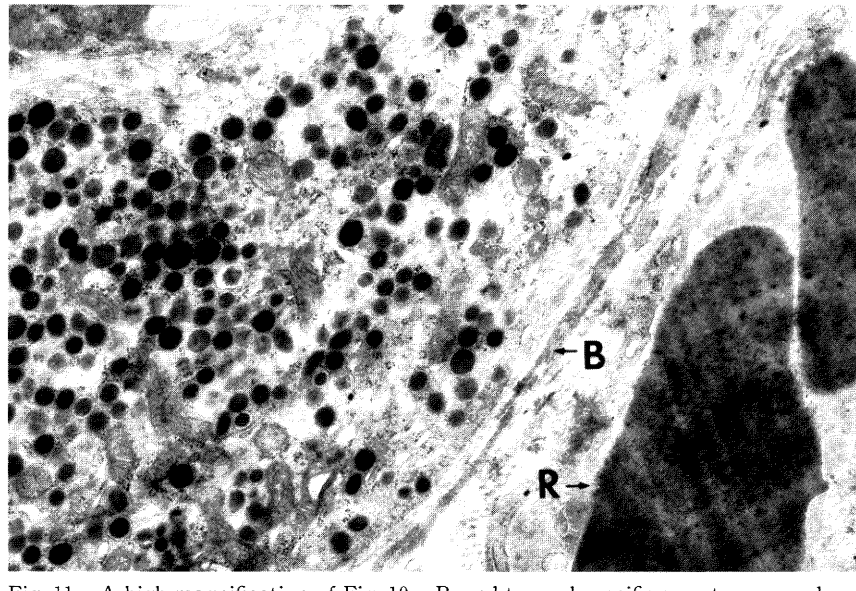


Fig. 11. A high magnification of Fig. 10. Round to oval specific secretory granules of endocrine type with a homogeneous high electron-dense central core measuring 130-230 mμ in diameter and surrounded by a limiting membrane. B: basement membrene. R: red blood cell. ×16,000 (orig. magnif. ×8,000).

in the apical cytoplasm were seen at 7 days. Some of them were suggestive of premature parietal cells (Fig. 9).

At 22 days after implantation, there were found endocrine cells containing round or oval secretory granules with a homogeneous, highly electron-dense central core measuring 130-230 mμ in diameter and surrounded by a limiting membrane; broad-haloed granules were also seen (Figs. 10 & 11). As to the other intracytoplasmic organells, mitochondria were small in size and scanty in number in comparison with those in surrounding undifferentiated cells, and Golgi apparatus was difficult to identify. These cells were considered to be premature endocrine cells of a closed type[18] situated on the basement membrane with accompanying undifferentiated cells. Most of these cells showed a certain resemblance to G-cells in the pyloric area of the normal Donryu rats. Mature parietal cells and chief cells were not identified at this stage. Two months after implantation, structures identifiable as gastric glands were found in to the cystic wall. The component cells of these glands were of abortive differentiation, in spite of their close similarity, under light-microscopy, to the normal fundic gland of the stomach represented by the presence of undifferentiated cells containing mucous granules along with mature mucous cells, premature parietal cells,[3] premature endocrine cells, and a few zymogen-granulated cells (Fig. 12). Occasionally, fibrovesicular cells as described by Hammond[10] and other authors[15,31] were observed. In their apical surface, short and broad (stubby) microvilli composed of parallel fibrillar bundles protruding into the cystic lumen were recognized (Fig. 13). Fibrillar bundles constituting cores of microvilli extended into the apical cytoplasm. Between these fibrillar bundles a large number of small vesicles and several lysosomal granules were present. Free ribosomes were moderate in number and mitochondria were small in size. Tonofibrils were much more abundant than other undifferentiated cells and tight junctions were well-developed. In addition, few mucous granules were seen in place (Fig. 14).

In the period between 6 months and 12 months after gastric mucosa implantation, many mucous-granulated cells, mature parietal cells and mature chief cells were encountered and hyperplastic changes were occasionally seen in the cystic glandular structure. Small mitochondria and immature endocrine cells,[5] triangular in shape, without specific secretory granules characterized by clear cytoplasm, and of small cell -size compared to the neighboring cells, were seen. (Fig. 15). In other places, premature endocrine cells of another type, with pleomorphic, (oval, roud, tear-drop shaped) endocrine secretory granules measuring about 250 mμ in diameter with a homogeneous central core surrounded by a limiting membrane were also evident (Fig. 16). Mitochondria were smaller than those of the neighboring parietal cells. These cells were identical to normal premature EC cells in the duodenum of the Donryu rats. A few endocrine cells with round to oval granules, some identical to the G cell, were also present (Fig. 17).

In this study, at least three types of undifferentiated cells were present. The first of them were blast cells seen at 7 deys after implantation; the second, so called fibrovesi-

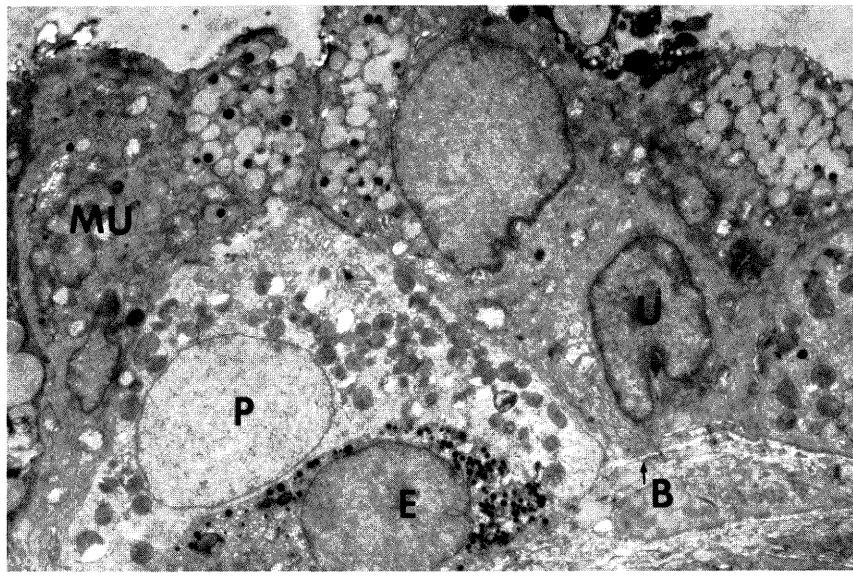


Fig. 12. Pseudogastric gland at 2 months after implant, composed of a young parietal cell (P), young endocrine cell (E), young mucous cell (Mu) and an undifferentiated cell (U). B: basement membrane. ×4,000 (orig. magnif. ×2,000).

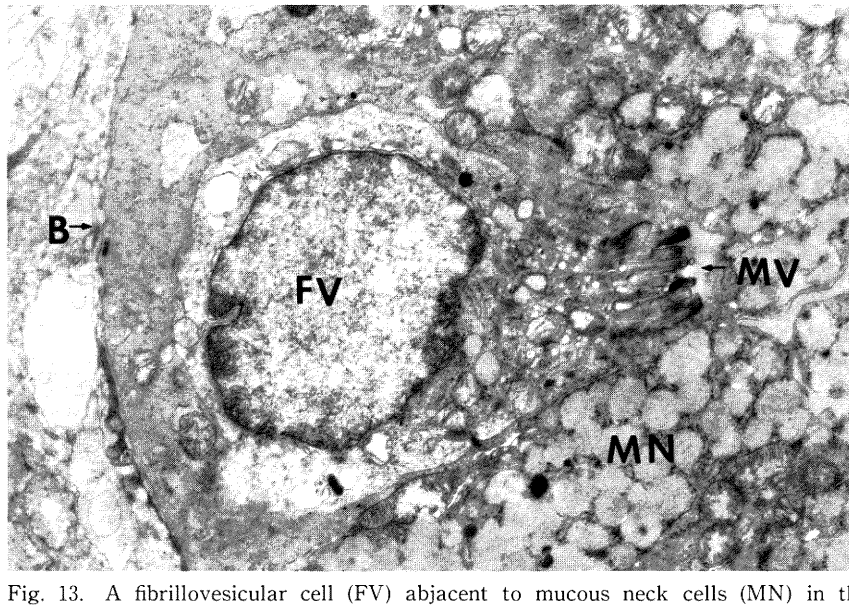


Fig. 13. A fibrillovesicular cell (FV) abjacent to mucous neck cells (MN) in the proliferated glandular structure. B: basement membrane. MV: microvilli. Two months after implant. ×8,000 (orig. magnif. ×4,000).

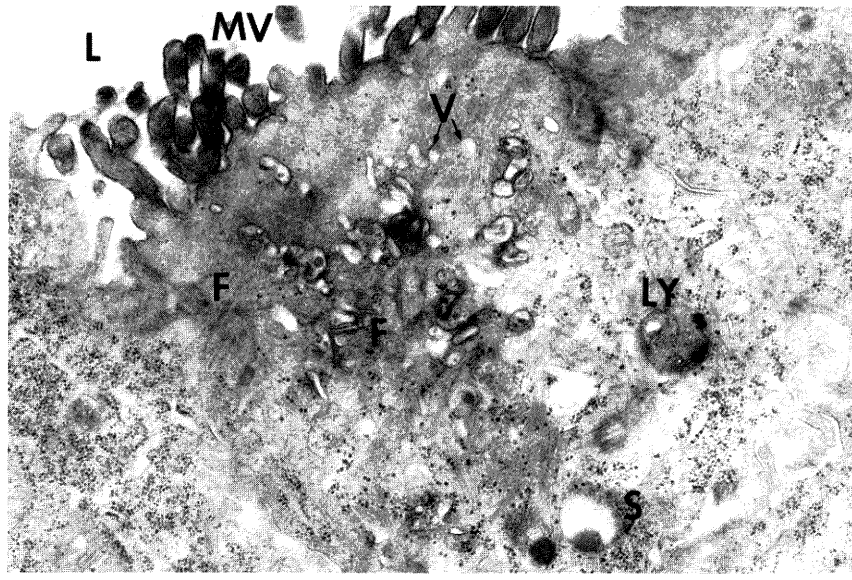


Fig. 14. A fibrillovesicular cell in the cystic wall at 25 days after implant with short broad microvilli (MV) protruding into the cystic lumen (L). In the apical cytoplasm, abundant fibrillar bundles (F) and small vesicles (V), few lyosomal granules (Ly) and secretory granules can be seen. ×20,000 (orig. magnif. ×10,000).

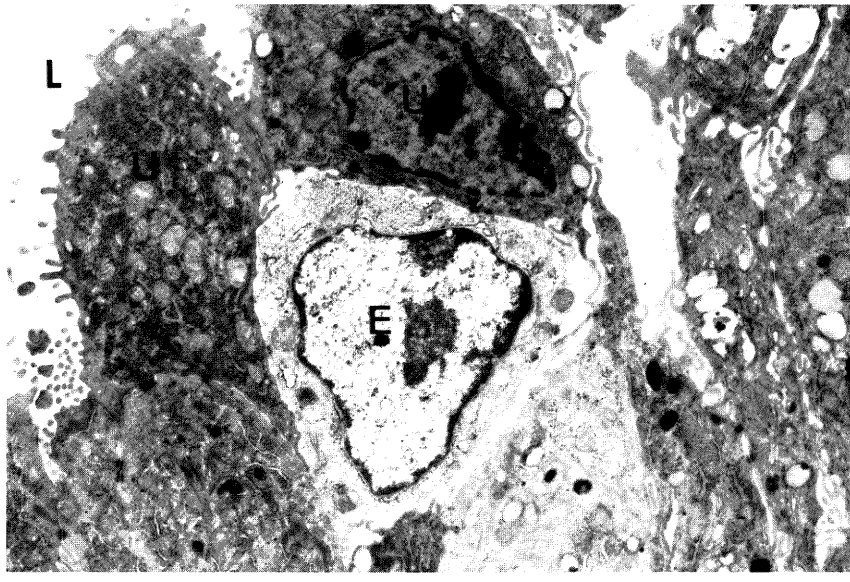


Fig. 15. A triangular immature endocrine cell (E) in the glandular stucture of the gastric cyst at 12 months after implant, characterized by clear cytoplasm, small and slender mitochondria and the absence of specific secretory granulas. L: cystic lumen. U: undifferentiated cell. ×6,000 (orig. magnif. ×3,000).

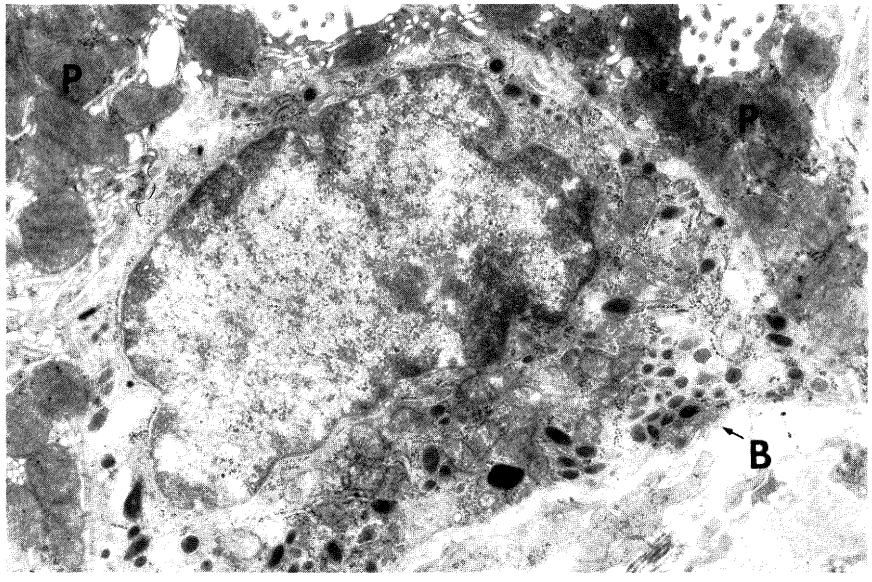


Fig. 16. A mature EC cell containing endocrine secretory granules measuring 250 mμ in diameter, the granules being pleomorphic with oval, round and tear-drop shaps. This cell is surrounded by mature parital cells (P). B: basement membrane. Ten months after implant. ×12,000 (orig. magnif. ×6,000).

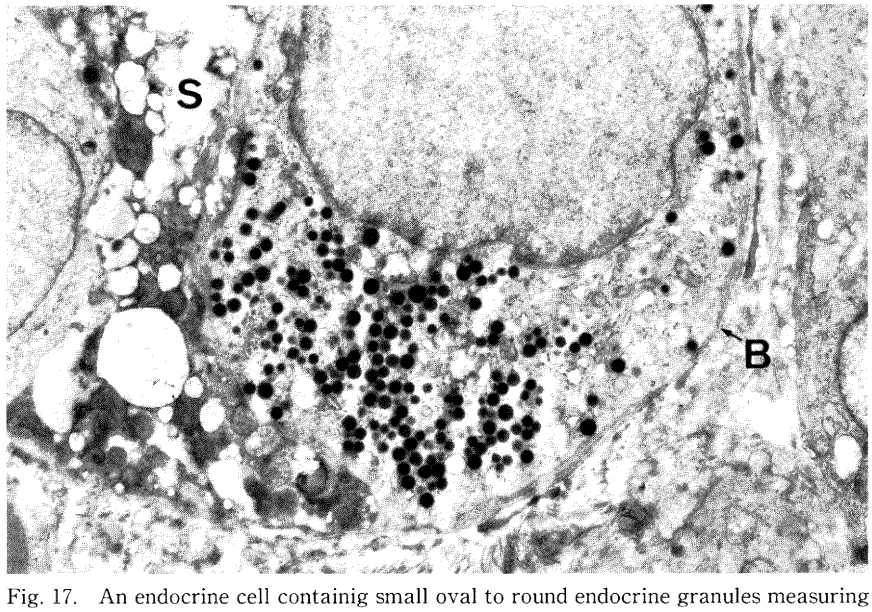


Fig. 17. An endocrine cell containig small oval to round endocrine granules measuring about 150 mμ in the proliferated pseudoglandular structure. S: secretory granulas in the neighboring cell. B: basmement membrane. Three months after implant. ×6,000 (orig. magnif. ×3,000).

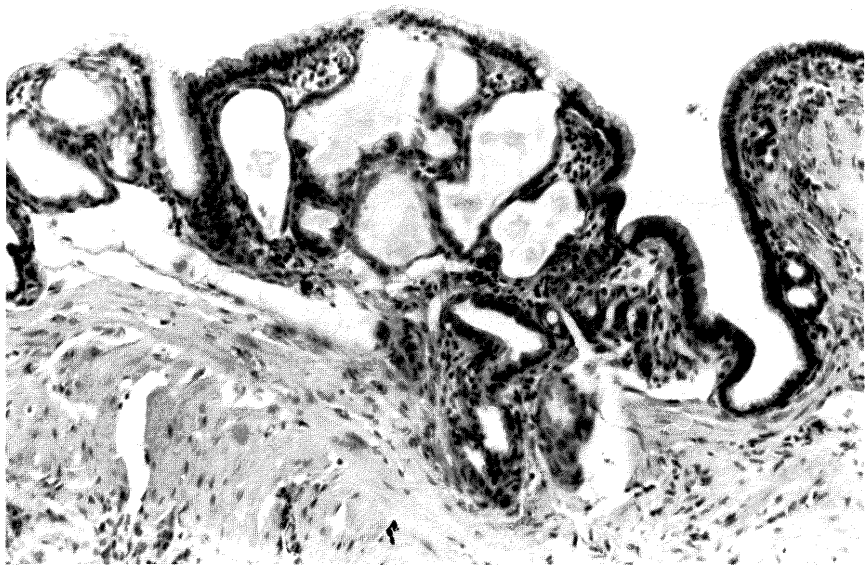


Fig. 18. Hyperplastic gastric mucosa with multiple microcysts, pseudoglandular structure and slight structural atypism. Two month after ENNG administration in an implanted gastric cyst. (H. E.: ×100).

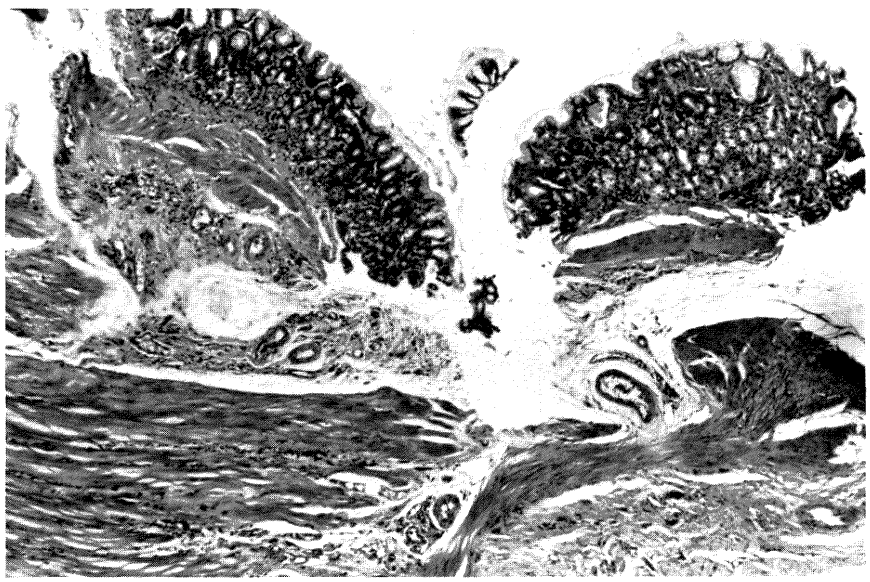


Fig. 19. Slight hyperplasia of the gastric glands. Two months after ENNG administration in an implanted gastric cyst. (H. E.: ×40).

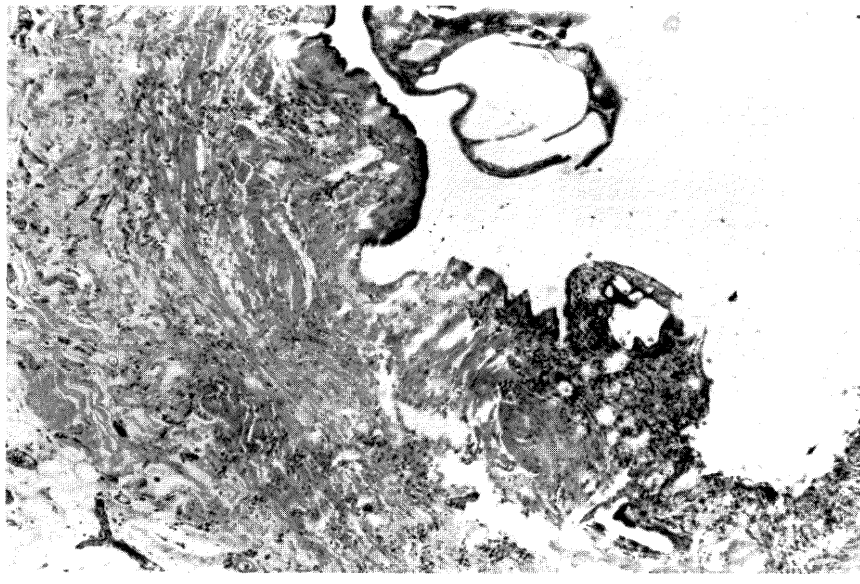


Fig. 20. Structural irregularity in the glandular structure of the cystic wall. One -layered epithelial cells are depleted in place. Two months after ENNG administration in an implanted gastric cyst. (H. E.: ×40).

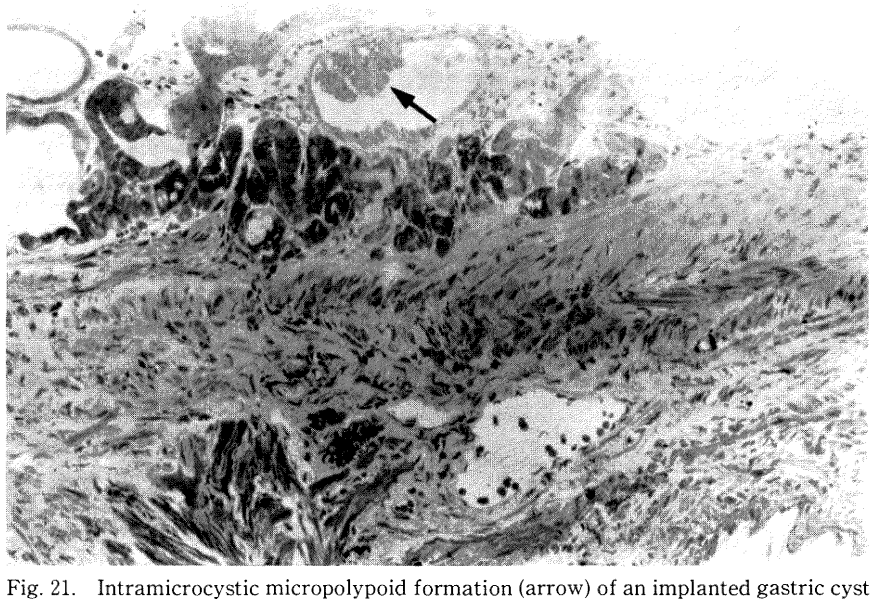


Fig. 21. Intramicrocystic micropolypoid formation (arrow) of an implanted gastric cyst 2 months after ENNG injection. (Azur: ×100).

cualr cells at 1 to 2 months; and the last were triangular cells characterized by clear cytoplasm and small mitochondria.

II. Carcinogenesis in the stomach of Donryu rats

Grossly and light-microscopically, slight hyperplastic changes of the component cells were observed in the implanted gastric cysts at 2 months after ENNG administration. Simultaneously microcyst formation in pseudoglandular structures and slight structural atypism were observed (Figs. 18, 19 & 20). Intramicrocystic polypoid lesions were also characteristic features (Fig. 21).

During the 6 months after carcinogen administration, no carcinoma was observed on a gross basis. Certain characteristic features at the electron-microscopic level were, however, seen at 2 months (Fig. 22). Undifferentiated cells lining the inner surface of the cystic wall showed certain morphologic changes including the swelling of rough ERs, nuclear irregularity, well-preserved lamellar structure of the Golgi apparatus, and an increased number of intracytoplasmic ribosomes and polysomes. Mitochondria were swollen in a round shape. Intramitochondrial cristae were decreased in number and shortened in length. Secretory granules and lysosomal granules were scattered throughout the cytoplasm.

At high magnification these lesions showed a well-preserved Golgi apparatus, a moderate mumber of secretory granules and enlarged rough ERs. Basement membrane was relatively well-preserved (Fig. 23).

Some of the other lining cells of the gastric cyst showed intracytoplasmic myelin-like figures, well-preserved Golgi apparatus, a small amount of long and slender rough ERs, and sparsely protruded microvilli on the apical cytoplasm. Mitochondria were moderately abundant and intramitochondrial granules were clearly identified (Fig. 24). The nucleocytoplasmic ratio was increased. Certain cells with long tonofibrils continuous to a desmosome were observed (Fig. 25). The other mature cells, such as parietal cells, mucous neck cells, and chief cells, however, which were demonstrated in pseudoglandular structures of the implanted gastric cyst, remained under normal condition (Fig. 26).

On the other hand, gross changes in the early stage of carcinogenesis after ENNG administration consisted of the formation of erosion, ulceration and micropolyps. Gastric carcinoma was produced at 36 weeks and in most cases localized in the antral region and in a few cases in the fundic region of the stomach. One of the experimental gastric carcinomas showed a tendency to be a well-differentiated adenocarcinoma (Fig. 27) with occasional admixture of signet ring cell variety. In silver impregnation with Grimelius' stain, argyrophil cells were not observed within the nest of carcinoma cells, but rather, were increased in number in the neighboring glands (Fig. 28 & 29). Electron-microscopically, carcinoma cells were characterized by a large irregular-shaped nucleus and well-developed microvilli. Cells with mitosis were occasionally observed. The component cells contained well-developed intracytoplasmic organells such as Golgi apparatus, abundant polysomes and enlarged mitochondria. In the apical cytoplasm a

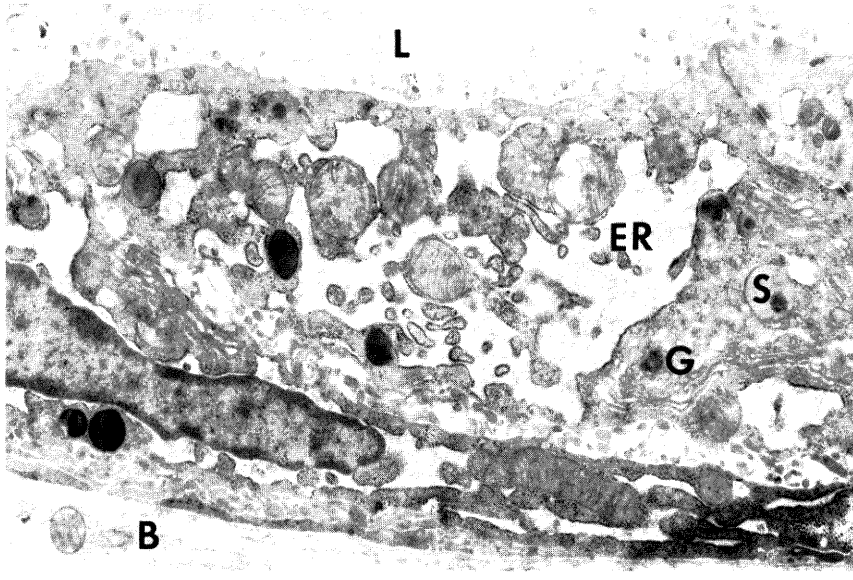


Fig. 22. The lining cell of the gastric cyst 2 months after ENNG administration with severe changes characterized by swelling of rough ER (ER), round-shaped mitochondria, few microvilli, secretory granules (S) and lysosomal granules. Golgi apparatus (G) is well preserved. L: cystic lumen. B: basement membrane. ×12,000 (orig. magnif. × 6,000).

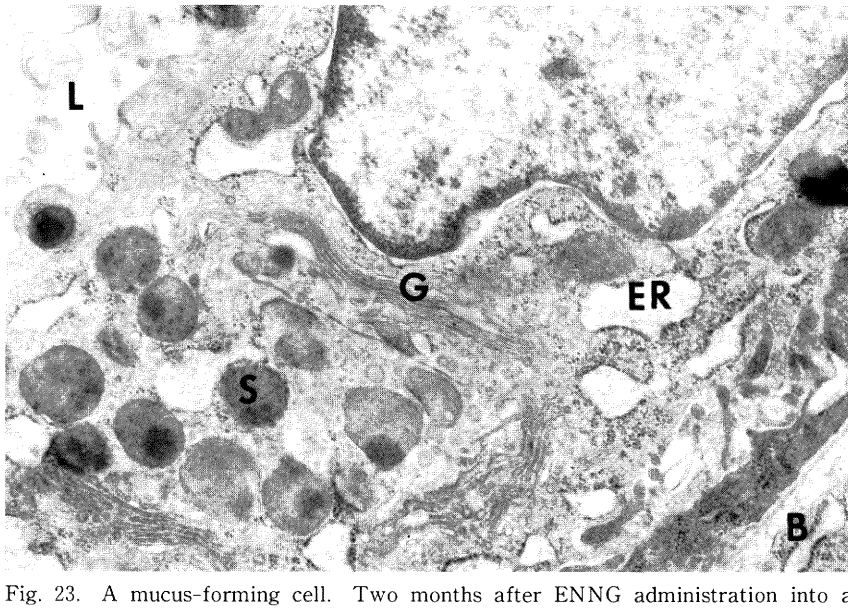


Fig. 23. A mucus-forming cell. Two months after ENNG administration into an implanted gastric cyst. L: cystic lumen. S: secretory granules. G: Golgi apparatus. ER: rough ER. B: basement membrane. ×20,000 (orig. magnif. ×10,000).

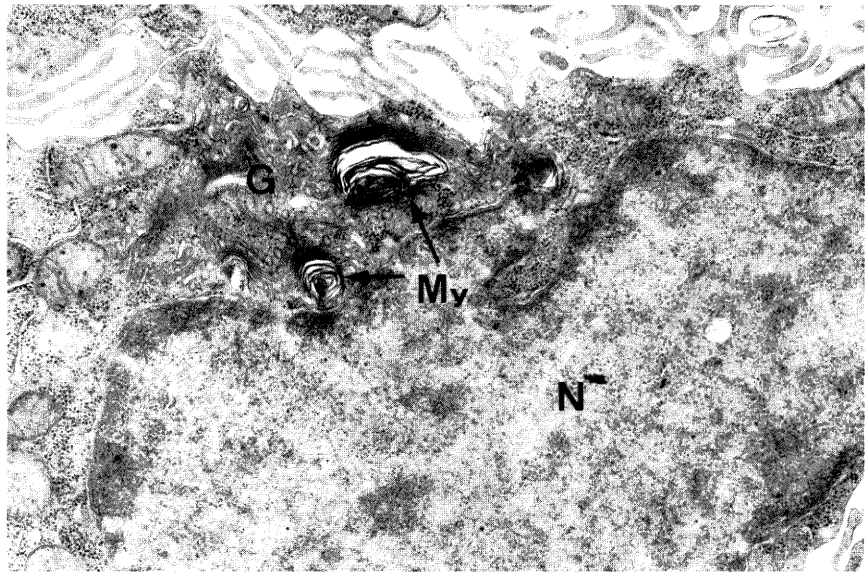


Fig. 24. A lining cell of an implanted gastric cyst 2 months after ENNG administration contains myelin-like figures (My), a large nucleus, abundant mitochondria with few intramitochondrial granules, Golgi apparatus and abundant free ribosomes. ×20,000 (orig. magnif. ×10,000).

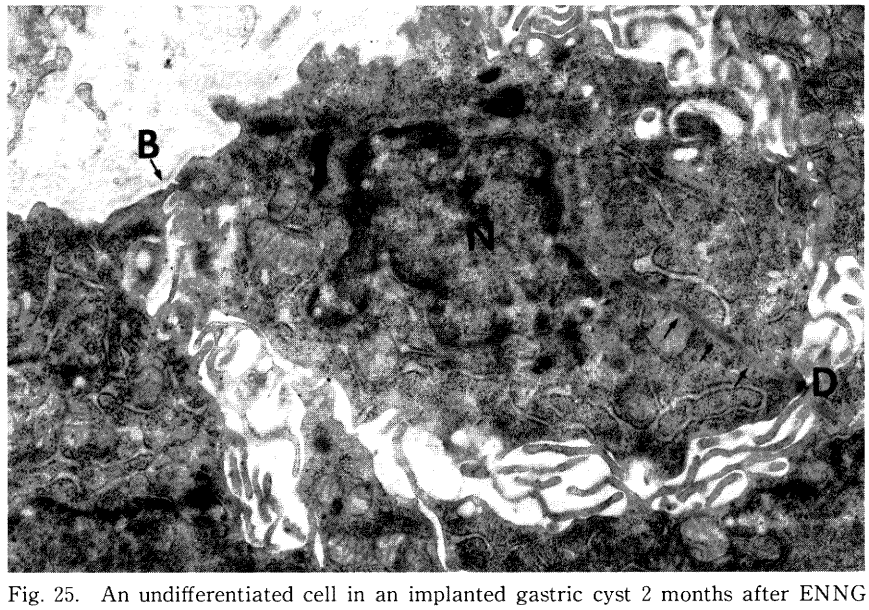


Fig. 25. An undifferentiated cell in an implanted gastric cyst 2 months after ENNG injection; shows a bundle of long and slender tonofibrils continous to a desmosome (D) (arrows), irregular nucleus (N) and abundant free ribosomes. B: basement membrane. ×12,000 (orig. magnif. ×6,000).

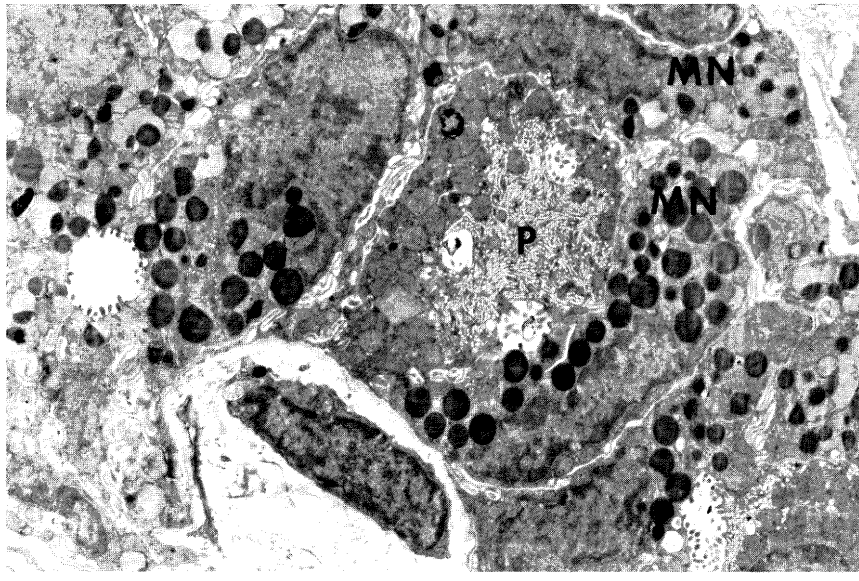


Fig. 26. An experimental gastric cyst showing mature epithelial cells such as parietal cell (P) and mucous neck cells (MN) remain in normal condition. Two months after ENNG administration. ×6,000 (orig. magnif. ×3,000).

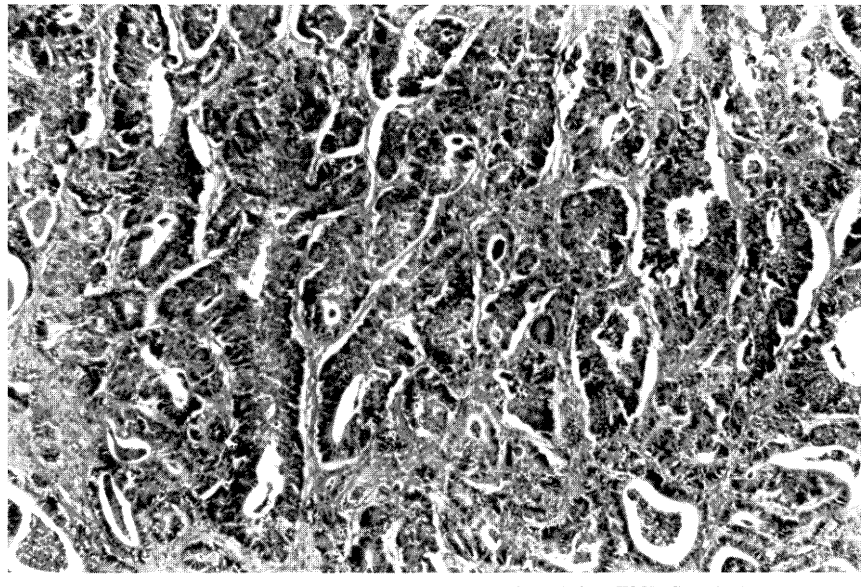


Fig. 27. Well-differentiated adenocarcionoma induced by ENNG administration in drinking water (H. E. ×100).

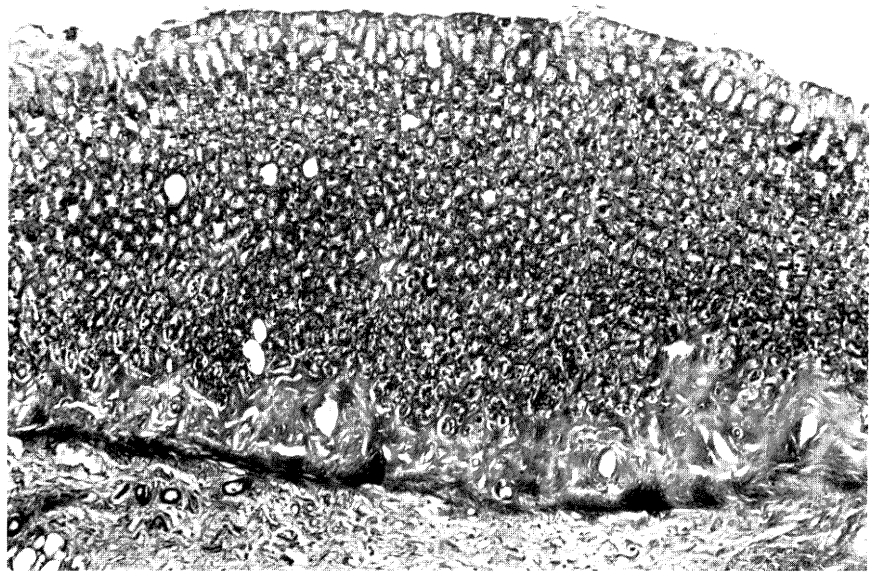


Fig. 28. Argyrophil cells are increased in number in the neighboring glands of nests of carcinoma cells. (Grimelius' stain: ×40).

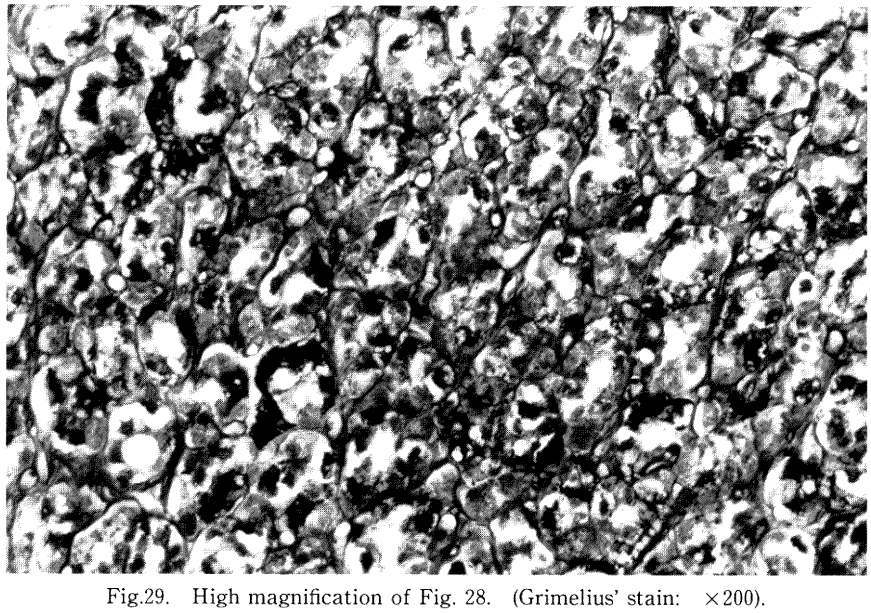


Fig.29. High magnification of Fig. 28. (Grimelius' stain: ×200).

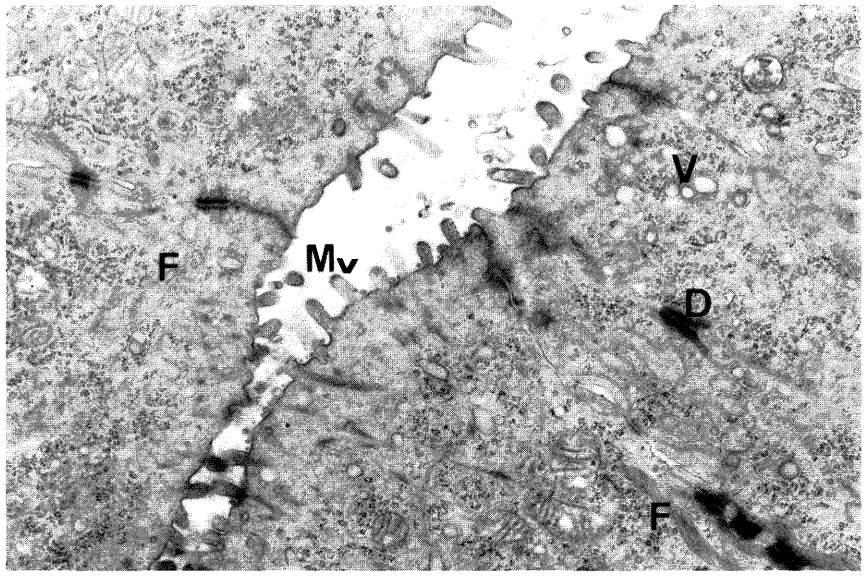


Fig. 30 Well-differentiated adenocarcinoma induced by ENNG administration and characterized by well-developed microvilli (Mv), tight junctional complex, well-developed desmosomes (D) and abundant fibrillar component (F). Several small vesicles (V) are evident. ×12,000 (orig. magnif. ×6,000).

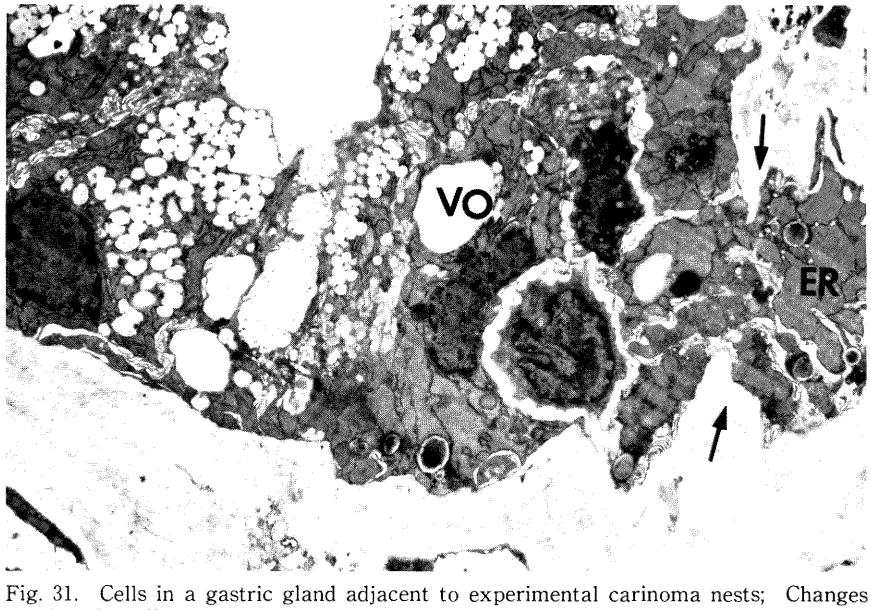


Fig. 31. Cells in a gastric gland adjacent to experimental carinoma nests; Changes consist of swollen rough ER (ER), an increased mumber of vacuoles (VO), conspicuous disappearance of basment membrane and focal microinvasion (arrows). ×4,000 (orig. magnif. ×2,000).

relatively large number of small vesicles were observed (Fig. 30). Secretory granules and lysosomal granules were not observed while microinvasive phenomenon was observed in places. Rough ERs were long and slender in shape. Although parietal cells and chief cells were of an almost normal morphology at the light-microscopic level, severe changes were revealed electron-microscpically in cells of the glands adjacent to carcinoma nests. These changes consisted of swollen ERs filled with amorphous proteinaceous fluid, atrophied Golgi apparatus, an increased number of vacuoles, conspicuous disappearance or destruction of the basement membrane, and a decreased number of the other intracytoplasmic organells. These cells appeared to be degenerated and subsequently replaced by carcinoma cells (Fig. 31). Endocrine cells, however were well-preserved.

DISCUSSION

I. Histogenesis of gastric epithelial cells

The changes demonstrated at 24 hours after implantation might well be a result of the operative procedure at least with respect to both physical violence and the resultant ischemia. Although most of implanted mature gastric epithelial cells were atrophied, degenerated, and gradually depleted, a few remained to proliferate and resulted in the production of a gastric cyst. Many investigators[6,7,13,20,46] have reported on the life span of gastric epithelial cells in mammalia, including man. Messier[23] stated that transit time in the rat stomach and colon was 3 days. Creamer et al.[4] claimed a migration time of 1 day in the mouse stomach. It is suggested that gastric epithelial cells regenerate from the generative area within 7 days. In the present study mature gastric epithelial cells are considered to disappear during the 7 days following the operative implantation. In general, a parietal cell was recognized to originate from immature cells in the neck area of the fundic gland.[3,21,41,45] Immature parietal cells, containing few mucous granules,[36] were also observed in this study.

Regarding the histogenesis of chief cells, there have been two main different hypotheses. The first of them is a theory explaining that this cell type is derived from undifferentiated cells or mucous neck cells.[3,12,41] The explanation is that mucous cells are an immature form of chief cells and they develop into chief cells as they migrate toward the base of the glands.[11] The other theory, by Stoffels et al.[39], avers that chief cells do not originate from mucous cells, but probably proliferate with a mitotic activity in the normal human stomach. In the present study, a chief cell has been seen to reappear in the cystic wall 2 months after implantation, and in the experimental study on the carcinogenic effect on gastric cysts, parietal cells containing zymogen granules were found (Fig. 32). It is considered, therefore, that chief cells might be differentiated from the same origin as parietal cells. It is suggested that chief cells orginate from some of the undifferentiated cells.

Most endocrine cells found in normal fundic glands of the Donryu rat were scattered

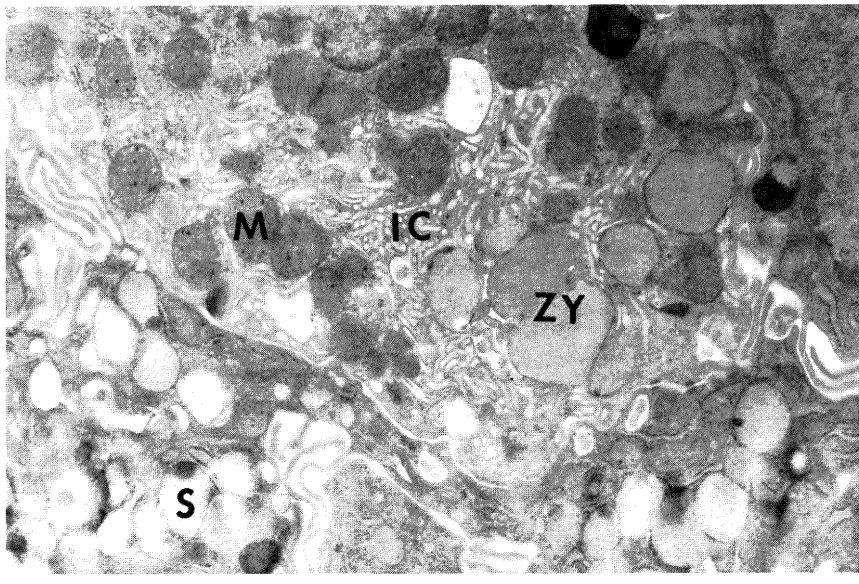


Fig. 32. A parietal cell containing zymogen granules (ZY), 5 months after ENNG administration. IC: intracellular secretory canaliculi. S: secretory granules. M: mitochondria. ×12,000 (orig. magnif. ×6,000).

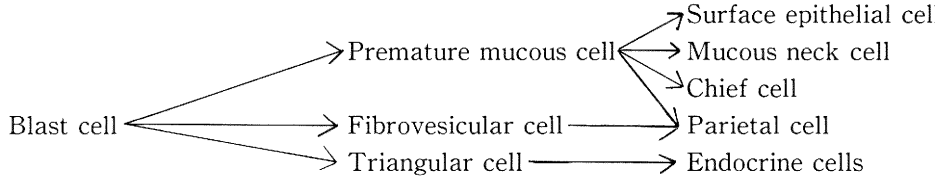
among glandular cells and contained relatively small, round-to-oval secretory granules measuring 150-250 mμ in diameter. They had a close similarity to cells seen in the normal antral glands. As to endocrine cell origin, many authors have reported the results of their investigations. Pearse et al.[29] reported that endocrine cells originated from the neural crest. Yeomans et al.[47] expressed their opinion that endocrine cells, like zymogen cells, were not replaced mainly by differentiation of stem cells, but constituted self-renewing populations in developing gastric mucosa. On the other hand, Soga[36] and Tahara[41,42] found endocrine cells containing mucous granules or zymogen granules at electron-microscopic level. They concluded that endocrine cells originated from undifferentiated (mucus-forming) cells of the gastric mucosa. Fujimoto et al.[6,7] reported that the longest life time of an endocrine cell might be 50 days.

In this study, when premature endocrine cells were observed 3 weeks after implantation, immature endocrine cells, mature G-like cells and mature ECL cells were present, suggesting their origin in immature or undifferentiated cells in gastric epithelium. It is possible that endocrine cells also originate in certain undifferentiated cells in the implanted gastric epithelium.

Three types of undifferentiated cells, a blast cell, a fibrovesicular cell and a triangular cell, were closely related to histogenesis of gastric epithelial cells. The blast cells containing few mucous granules and seen at 7 days after implantation were probably the stem cells of the gastric epithelium and would later transform into a premature mucous

cell, a fibrovescular cell and a triangular cell. It was thought that the premature mucous cell would develop into a mature mucous neck cell, a surface-epithelial cell, a parietal cell and a chief cell. It is still not cetain whether the fibrovesicular cell is a new cell type or the precursor of a parietal cell and a mucous neck cell. In this study, a cell having mucous granules in the cytoplasm was observed. Therefore, this cell also was thought to be the precursor of a mucous cell. The triangular cell was not characterized by its cell type because it had neither mucous granules nor specific secretory granules. But several types of endocrine cells could be observed in the experimental gastric cyst at 1 year after implantation. The triangular cell was probably derived from the blast cell and would later develop to a mature G cell, an ECL cell or another cell type.

Hypothetic histogenesis of cell development in gastric glandular cells is shown as follows:



II. Cystogenesis

The developmental process of the implanted gastric cysts in Donryu rats was divided into the following four stages (Fig. 33).

i) Degenerative stage (1-4 postoperative days)

Implanted gastric mucosal cells seemed to degenerate quickly due mainly to ischemic changes. Almost all mature gastric epithelial cells disappeared and few immature cells survived. Gastric cysts were lined with a single layer of few mucous-containing cells seemingly related to the surface-epithelial cells.

ii) Proliferative stage (5 days to 1 month)

Among degenerating cells, clumps of undifferentiated cells remained in some places. They began proliferatng to form a stratified structure consisting of many blast cells containing few secretory granules of mucus nature. Continuous proliferation resulted in the conglomeration of these clumps. Microcysts consisting of many undifferentiated cells were thus completed. In this stage, no mature gastric mucosal cells such as parietal cells, chief cells, and endocrine cells were identified.

iii) Cystic stage (2 to 6 months)

Microcysts were increased in size at 2 months and the cystic cavity was filled with secretory fluid. In this stage, a pseudoglandular structure of the stomach appeared in the cystic wall. This structure consisted of mature parietal cells, premature chief cells, mature endocrine cells, premature parietal cells, and premature mucous cells.

In some cystic structures lined with a single layer of cells, no mature cells were found.

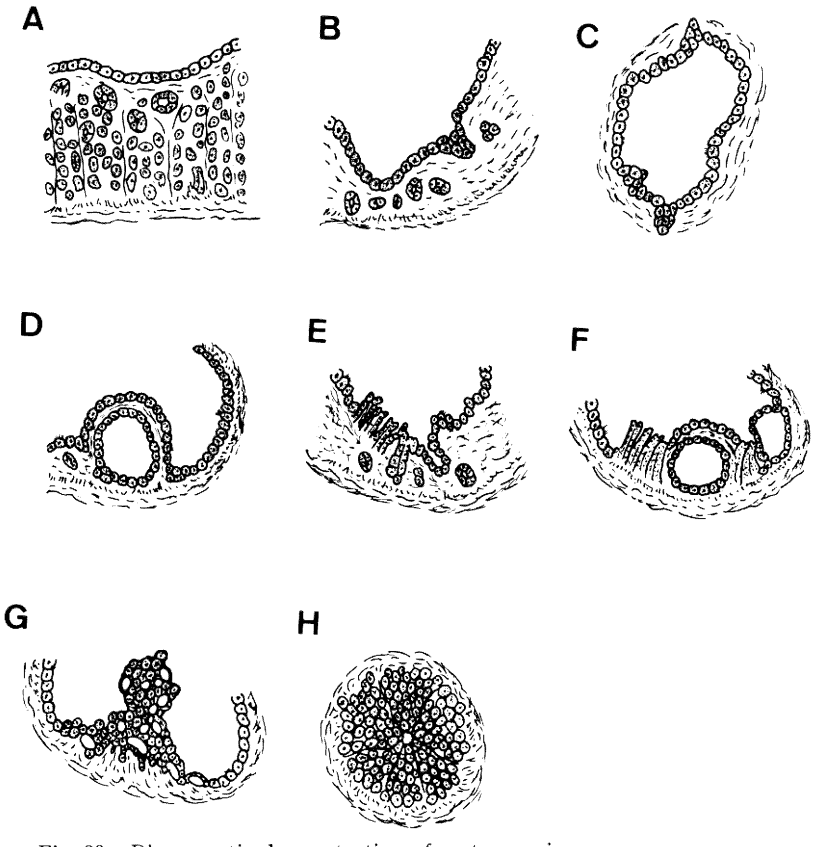


Fig. 33. Diagramatic demonstration of cystogenesis.

A: 1 day after implant —— Degenerative stage
B: 7 day after implant —— Proliferative stage
C: 1 month after implant
D: 3 months after implant —— Cystic stage
E: 6 months after implant
F: 10 months after implant
G: > 1 year after implant —— Hyperplastic stage
H:

iv) Hyperplastic stage (6 months to 1 year)

In this stage, gastric cysts were more increased in size, and glandular structures were further developed with occasional hyperplasia and focal structural distortion without cellular atypism. It is worthwhile to note that in the earliest stage of the experimental carcinogenesis in the present study, intramucosal microcysts similar to those observed under the condition of atypia in man and experimental carcinogenesis in dog[34] were produced. Some authors reported similar observations in clinical cases.[2,30] Cholangiocellular or hepatocellular cysts have been known to arise in the course of chemically induced liver carcinomas.[17] It has been concluded that liver cysts have arisen from the

retention of the billiary substance caused by excessive and irregular proliferation of hepatoparenchymal neoplastic cells.[17]

In the implanted gastric cysts, it is understood that normal secretory stream is lost because of the structural disotrtion of the glands in the implants which result in the retention of the secretory substance.

Experimental cystogenesis in the gastric mucosal implants thus seems to be accounted for by resulting retention of secretory substance from irregularly proliferating undifferentiated cells that have been previously described.

III. Carcinogenesis

As for morphologic changes in the early stage of gastric carcinogenesis,[24] Saito et al.[33,34] have reported in detail that atrophy and erosion of the mucosa occur first, followed by regenerative glandular hyperplasia and that, in the next stage, adenomatous hyperplasia with slight celluar atypism and eventual production of an invasive adenocarcinoma may occur. Tanaka[44] has reported proliferation of cells in the neck region of gastric glands in the course of gastric carcinogenesis. It is supposed that regenerative hyperplasia may occur as the result of acceleration of regenerative activity following the direct carcinogenic damage done by atrophy or erosion. In the experimental series of the present study, ENNG has injected into implanted gastric cytst at 2 months. Although mature cells such as parietal cells and chief cells remain with normal morphological figures, undifferentiated cells are observed to have moderate morphological changes characterized by degenerated Golgi apparatus, excess of secretory fluid in the rough ERs resulting in enlargement of intracisternal spaces, increased tonofibrillar bundles, and abundant free ribosomes. On rare occasion, a parietal cell including zymogen-like granules was observed. It could be suggested that undifferentiated cells are more influenced by the effect of ENNG than the other mature cells and are able to transform into various mature gastric epithelial cells and sometimes into other heterotopic is metaplastic epithelium as seen in intestinal metalasia. This transformation potential is thought to be maintained for a long time under abnormal conditions. These morphologic changes may be deviated or distorted, some toward malignant transformations through the influence of chronic carcinogenic stimuli.

On the other hand, the effect of ENNG on mature cells is supposed to result in atrophy, degeneration and the final disappearance of cells. In fact, on observation of the neighboring glands of the experimental gastric carcinoma of a well-differentiated type with occasional admixture of signet ring cell variety, severe changes have been electron-microscopically revealed in cells such as swollen rough ERs filled with secretory fluid, an increased number of vacuoles, and decreased intracytoplasmic organelles other than mitochondria. Parietal cells and chief cells often maintained an almost normal morphology at the light-microscopic level. In silver impregnation (Grimelius' stain), argyrophil cells are not observed within the nests of carcinoma cells but are rather increased in number in the neighboring glands. It is thought that the stimulation of carcinogens

results in certain deviations in the mechanism of argyrophil cell proliferation.[26] Iwanaga[14] has referred to heterotopic multiple cysts of the stomach in the precancerous stage. It is considered thet undifferentiated cells are increased in number at the precancerous stage by chronic carcinogenic stimuli. Such a phenomenon may be identical to structures of both experimental gastric cysts after 6 months and of experimental gastric carcinoma.

As to the question of when undifferentiated cells are affected by the carcinogen in the course of neoplatic transformation, the M•U theory by Soga[37,38] may be employed to explain the mechanism of carcinogenesis. If carcinogenesis happens on the anlage cells in the M-phase of blastic stage, differentiation of carcinoma may yield a multidirectional pattern with a veriety of histologic types. If it happens on the anlage cells in the U-phase, it will develop an unidirectional pattern with a uniform histologic type. For example, Nakamura et al.[25] have reported, from the histogenetic view-point, that gastric carcinoma is divided into two types, gastric and intestinal; carcinoma cells of the gastric type arise from the gastric mucosa and those of the intestinal type dfferentiate from the intestinal metaplastic epithelium.

A reported case of so-called parietal cell carcinoma[19,35] may be explained by the U-phase carcinogesesis.

On the other hand, Azzopardi et al.[1] reported that gastric carcinomas may originate in tissue with the potential of differentiating a large variety of cell types. In clinical cases, we rarely encounter simultaneous multiple gastric carcinomas of different pathologic characteristics. Such neoplasms are considered to have transformed in the M-phase.

In this series, it is proved that lower-graded undifferentiated cells are more affected by carcinogenic agents than the others.

Erosion, ulceration and glandular atrophy are explained to be caused by chemical stimuli of carcinogenic agents. Following this stage, regenerative activity is accelerted, resulting in regenerative hyperplasia of cells and the production of immature cells. These immature cells seem to correspond to the anlage cells or preprimordial and primordial calls in the M-phase and U-phase. Carcinogenic stimuli may act on these cells at this stage and the malignant transformation of the cell will occur with different characteristics depending on the stage of the M- or U-phase.

In the present study, immature or undifferentiated cells were demonstrated in the implanted gastric cysts and the possibility of these cells to undergo malignant transformation is supposed, but the final definitive conclusion is still undetermined and remains for the furhter investigation.

Summary and Conclusion

Experimental homograft implantation of the gastric mucosa was undertaken for the purpose of studying the histogenesis of the gastric epithelial cells and cystogenesis as the

background of carcinogenesis, using 62 adult Donryu rats. In the second phase of the experiment, a chemical gastrocarcinogen (ENNG) was used in order to investigate whether or not the early changes of possible malignant transformation could be demonstrated in undifferentiated cells produced in the implanted gastric cysts. The resuls of this experimentation are summarized as follows:

1) The gastric fundic mucosa of Donryu rats was homografted into the subcutaneous region of the abdominal wall. Rate of successful implantation was 29.4% (68/233). The implanted gastric mucosa was observed to subsequently form cystic structures.

2) Although mature epithelial cells disappeared at 7 days after implantation, in the subsequent stages all varieties of gastric epithelial cells were observed to regenerate from the blast cells in the implanted gastric cysts. It is conjectured that gastric epithelial cells originate from undifferentiated cells which, at the proliferative stage, constitute the lining of the gastric cysts.

3) Regarding histogenesis and cystogenesis in the implanted gastric mucosa, four stages were classified as follows:

i) Degenerative stage (1-4 days)

ii) Proliferative stage (5 days to one month)

iii) Cystic stage (one month to 6 months)

iv) Hyperplastic stage (6 months to 1 year)

4) Histogenesis of cyst formation is thought to be the result of the retention of secretion due to structural distortion of the glands and inflammation of the interstitium.

5) Histologic examination was performed on 39 implanted gastric cysts in 28 Donryu rats after weekly subcutaneous administration of 0.5 ml of ENNG at a concentration of 300 μg/ml. On the 6 month observation slight hyperplastic changes were demonstrated in the implanted gastric mucosa.

6) Electron-microscopic examination likewise disclosed certain morphologic changes toward malignant transformation from the undifferentiated cells. While mature cells were not affected by the carcinogen (ENNG) and remained in normal condition, the undifferentiated cells showed definite ultrastructual changes possibly due to ENNG, and suggestively toward malignant transformation.

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REFERENCES

1) Azzopardi, J. G. and Pollock, D. J.: Argentaffin and argyrophil cells in gastric carcinoma. *J. Path. Bact.* **86**: 443-451, 1963.
2) Chakravorty, R. C. and Schatzki, P. F.: Gastric cystic polyposis. *Digest. Dis.* **20**: 981-989, 1975.
3) Corpron, R. E.: The ultrastructure of the gastric mucosa in normal and hypophysectomized rats. *Amer. J. Anat.* **118**: 53-63, 1966.
4) Creamer, B., Shorter, R. and Bamforth, J.: The turnover and shedding of epithelial cells. Part I. The turnover in the gastrointestinal tract. *Gut* **2**: 110-116, 1961.
5) DeLemos, C.: The ultrastructure of endocrine cells in the corpus of the stomach of human fetuses. *Amer. J. Anat.* **148**: 359-384, 1976.
6) Fujimoto, S. et al.: Tritiated thymidine autoradiographic study on the origin and renewal of gastrin cells in the pyloric gland of hamsters. *Igaku-no-Ayumi* **108**: 549-551, 1979.*
7) Fujimoto, S. et al.: Tritiated thymidine autoradiographic study on the origin and renewal of gastrin cells in the pyloric gland of hamsters: II Turnover time and cell migration. *Igaku-no-Ayumi* **110**: 87-89, 1979.*
8) Fujita. M. et al.: Enhancement of gastric carcinogenesis in dogs given N-methyl-N'-nitro-N-nitrosoguanidine following vagotomy. *Cancer Res.* **39**: 811-816, 1979.
9) Fukushima, S.: Effect of plastic bead on gastric carcinogenesis in rats treated with N-methyl-N'-nitoro-N-nitrosoguanidine. *GANN* **67**: 197-206, 1976.
10) Hammond, J. B. and Ladeur, L.: Fibrillovesicular cells in the fundic glands of the canine stomach: Evidence for a new cell type. *Anat. Rec.* **161**: 393-412, 1968.
11) Hattori, T.: On cell proliferation and differentiation of the fundic mucosa of the golden hamster. *Cell Tiss. Res.* **148**: 213-225, 1974.
12) Helander, H. F.: Ultrastructure and function of gastric mucoid and zymogen cells in the rat during development. *Gastroenterol.* **56**: 53-70, 1969.
13) Hunt, T. E. and Hunt, E. A.: Radioautographic study of proliferation in the stomach of the rat using thymidine-H^3 and compound 48/80. *Anat. Rec.* **142**: 505-517, 1962,
14) Iwanaga, T.: Diffuse heterotopic mutiple cysts and carcinoma of the stomach. Jap. J. Cancer Clin. **19**: 971-979, 1973.*
15) Jonson, F. R. and Young, B. A.: Undifferentiated cells in gastric mucosa. *J. Anat.* **102**: 541-551, 1968.
16) Kanahara, H.: An experimental study on atypical epithelial proliferations associated with chronic ulceration of the stomach with special reference to early carcinogenesis. *Acta Med. Biol.* **21**: 133-168, 1974.
17) Karaki, Y.: Light-and electron-microscopic analysis of hepatic tumors in mastomys: Their histopathogenesis. *Acta Med. Biol.* **26**: 163-203, 1979.
18) Kobayashi, S., Fujita, T. and Sasagawa, T.: Electron microscope studies on the endocrine cells of the human gastric fundus. *Arch Histol. Jap.* **32**: 429-444, 1971.
19) Kondo, K., Tamura, H. and Taniguchi, H.: Intracellular microcyst in gastric cancer cells. *J. Electronmicroscopy* **19**: 41-49, 1970.
20) MacDonald, W. C., Trier, J. S. and Everett, N. B.: Cell proliferation and migration in the stomach, duodenum and rectum of man: radioautographic studies. *Surgery* **46**: 405-417, 1964.
21) Matsuyama, M.: Differentiation of immature mucous cells into parietal, argyrophil, chief cells in stomach graft. *Science* **169**: 385-387, 1970.
22) Matsuyama, M., Suzuki, H. and Nakamura, W.: Induction of subcutaneous gastric cyst in mice. *Nagoya Med. J.* **14**: 97-99, 1968.
23) Messier, B.: Radioautographic evidence for the renewal of the mucous cells in the gastric mucosa of the rat. *Anat. Rec.* **136**: 242-253, 1960.
24) Murakami, T. and Habu, H.: Pathological studies on early gastric cancer—its histogenesis and development. *Surgical Therapy* **34**: 39-48, 1976.*

25) Nakamura, K., Sugano, H. and Takagi, K.: Histogenesis of gastric cancer: Similarity of cancer cells to the normal epithelial cells of the stomach. *J. Clin. Electron Microscopy* 8: 302-303, 1975.
26) Ogata, T.: A study on the endocrine cells in the atypical epithelial lesion of the stomach. GEP endocrine system. *Igaku-no-Ayumi* 84: 625-630, 1973.*
27) Oohara, T.: Production of experimental chronic gastric ulcer in the rat. *Jap. J. Cancer Clin.* 17: 457-464, 1971.*
28) Oohara, T.: Experimental ulcer-cancer in the rat-Effect of oral administration of N-methyl-N'-nitro-N-nitorosoguanidine preceded by experimental chronic gastric ulcer in the rat. *Jap. J. Cancer Clin.* 17: 543-550, 1971.*
29) Pearse, A. G. E. and Polak, J. M.: Neural crest origin of the endocrine polypeptide (APUD) cells of the gastrointestinal tract and pancreas. *Gut* 12: 783-788, 1971.
30) Rosen, Y., Vaillant, J. G. and Yermakov, V.: Submucosal mucous cysts at a colostomy site: Relationship to colitis cystica profunda and report of a case. *Dis. Col. & Rect.* 19: 453-457, 1976.
31) Rubin, W. et al.: The normal human gastric epithelia—a fine structural study— Lab. *Invest.* 19: 598-626, 1968.
32) Rumpf, P. et al.: Die chemische Erzeugung von Magen-carcinomem bei der Ratte nach Vagotomie und Magenresektion nach Billroth II. *Chir. Forum. Exp. Klin. Forsch.* : 58-62, 1977.
33) Saito, T. et al.: Sequential morphological changes in N-methyl-N'-nitro-N-nitrosoguanidine carcinogenesis in the glaudular stomach of rat. *Cencer Inst.* 44: 769-783, 1970.
34) Saito, T. et al.: Follow-up studies of morphological changes in N-Methyl-N'-nitro-N-nitrosoguanidine carcinogenesis in stomach of dogs. *Jap. J. Cancer Clin.* 21: 881-891, 1975.*
35) Seki, M.: Parietal cell carcinoma. *J. Electronmicroscopy* 11: 40-46, 1962.
36) Soga, J.: Fine structure of the gastric mucosa of the cancer producing African rodent, Rattus (mastomys) natalensis. I Normal general structure. *Acta Med. Biol.* 14: 1-21, 1966.
37) Soga, J.: Histogenesis of carcinoid tumor. *Stomach and Intestine* 10: 625-633, 1975*.
38) Soga, J.: Histogenesis of carcinoid in relation to ordinary carcinomas. *Acta Med. Biol.* 30: 17-33, 1982.
39) Stoffels, G. L., Preumont A. M. and DeReuck, M.: Cell differentiation in human gastric gland as revealed by nuclear binding of tritiated actinomycin. *Gut* 20: 693-697, 1979.
40) Sugimura, T. and Fujimura, S.: Tumor production in glandular stomach of rat by N- methyl-N'nitro-N-nitorosoguanidine. *Nature* 216: 943-944, 1967.
41) Tahara, E.: Regeneration of the gastric epithelia in mice: an electron microscopic study. *Hiroshima J. Med. Sci.* 20: 65-99, 1971.
42) Tahara, E.: Fine structure of several types of endocrine cells in mouse gastric mucosa with special reference to the histogenesis. *Hiroshima J. Med. Sci.* 20: 255-268, 1971.
43) Takahashi, M. et al.: Effect of fundic ulcers induced by iodoacetamide on develoment of gastric tumors in rat treated with N-metyl-N'-nitro-N-nitrosoguanidine. *GANN* 67: 47-54, 1976.
44) Tanaka, S.: Kinetics of proliferation on the gastric epithelium in the early stage of carcinogenesis in rat. *Igaku-no-Ayumi* 88: 581-586, 1974.*
45) Willams, G. and Lahy, T.: Radioautographic and quantitative studies on parietal and peptic cell kinetics in the mouse -A selective effect of gastrin on parietal cell proliferation- *Gastroenterol.* 69: 416-426, 1975.
46) Yeomans, N. D. et al.: Maturation and differentiation of cultured fetal stomach. *Gantroenterol.* 71: 770-777, 1976.
47) Yeomans, N. D. and Trier, J. S.: Epithelial cell proliferation and migration in developing rat gastric mucosa. *Developmental Biol.* 53: 206-216, 1976.

*: Written in Japanese