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Effects of Gastrin and Histamine on Gastric Carcinogenesis Induced in Rats by *N*-Methyl-*N'*-Nitro-*N*-Nitrosoguanidine

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Abstract—*The effects of gastrin and histamine on the incidence and histology of gastric cancer induced by* N-*methyl*-N'-*nitro*-N-*nitrosoguanidine were investigated in Wistar strain rats.*

It was found that prolonged administration of gastrin or histamine after treatment with N-*methyl*-N'-*nitro*-N-*nitrosoguanidine resulted in a significant reduction in the incidence of adenocarcinomas of the glandular stomach. Histological examination showed that unlike the highly differentiated adenocarcinomas with a typical glandular structure in the control group and the histamine-treated group, the adenocarcinomas that did develop in rats after prolonged administration of gastrin had little or no typical glandular structure.*

These results showed that both gastrin and histamine were related to reduction in the incidence of adenocarcinomas in the glandular stomach, whereas the histological type of adenocarcinoma was affected only by gastrin.

INTRODUCTION

THE FREQUENCY of gastric carcinoma is much greater in achlorhydric individuals than in acid-secreting individuals [1–3]. Furthermore, it is well known that gastric cancer is rarely found in patients with duodenal ulcers accompanied by hypersecretion of acid [4, 5]. Moreover, a close relationship was found between the gross and histological types of gastric carcinomas and acid-secreting function [6]: carcinomas were ulcerated and histologically undifferentiated when the acid-secreting area was large, whereas they were polypoid and histologically differentiated when there was little or no acid-secreting area. Thus it seems likely that gastric acid secretion influences the development and histological type of gastric cancer, although the mechanisms responsible for these effects are not known.

In the present study, the effects of gastric acid secretagogues on the incidence and histological types of gastric carcinomas were examined by injecting gastrin or histamine in depot form into rats that had been treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) [7].

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MATERIALS AND METHODS

A total of 100 young male Wistar-strain rats of about the same age, initially weighing 100–150 g, were given drinking water containing 50 μg MNNG (Wako Pure Chemical Industries, Ltd., Osaka, Japan) per ml for 25 weeks. The MNNG solution was changed every other day and given to the rats from bottles covered with aluminum foil or painted a dark color. After the period of MNNG treatment, 10 rats were killed, and their stomachs were examined grossly and histologically. From Week 26 the rats were given normal tap water and divided randomly into 4 groups. The groups were treated as follows: Group 1 (30 rats) received no treatment; Group 2 (15 rats) was given the vehicle, olive oil, only; Group 3 (21 rats) was given 4 mg of histamine dihydrochloride (Katayama Chemical Industries, Ltd., Osaka, Japan) per day by depot injection; and Group 4 (24 rats) was given 300 μg of tetragastrin (C-terminal tetrapeptide of gastrin, Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) per day by depot injection. Tetragstrin and histamine were given as suspensions in olive oil. From the beginning of Week 26 to the end of Week 29 the rats in Groups 3 and 4 were given one

injection every day, and then from Week 30 to the end of the experiment they were given one injection every other day. All injections were given subcutaneously in a volume of 0.5 ml between 2 and 3 p.m. each day, different sites of injection being chosen. Group 2 was treated in the same way as Groups 3 and 4, but given 0.5 ml of olive oil only.

The experimental groups were kept in different cages in the same room under otherwise identical conditions throughout the experimental period, and tap water and a diet of rat chow pellets were provided *ad lib.*

Animals that survived for more than 35 experimental weeks were included in the effective numbers. Rats were killed when they became moribund, and animals that died or were killed during the experimental period were autopsied. The stomach and other organs were examined carefully. The stomach was opened along the region of greater curvature, pinned flat on a cork mat, and fixed with 10% neutralized formalin solution for histological examination. The fixed stomach was cut into 3 mm wide, longitudinal strips to the region of lesser curvature. Tissues were embedded in paraffin, cut into thin sections, and stained with hematoxylin and eosin. Selected tissues were stained with alcian blue and periodic-acid–Schiff. Slides were examined without knowledge of their origin.

The results were analyzed by Fisher's exact probability test [8]. The word "significant" indicates a calculated P value of less than 0.05.

RESULTS

Incidence of gastric cancers

The incidence and histology of gastric cancers in each group are summarized in Table 1 and Fig. 1.

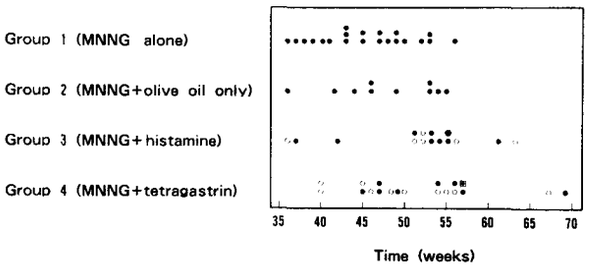

Fig. 1. Incidence and histology of gastric tumors in the glandular stomach of MNNG-treated rats after 35 experimental weeks. For explanation of Groups, see Table 1. ○, no tumor in the glandular stomach; ●, adenocarcinoma; ◉, leiomyosarcoma; ▣, mixed tumor.

Table 1. Incidence and histology of gastric tumors in the glandular stomach of MNNG-treated rats

Group No.*	No. of effective rats	No. of rats with gastric tumors	Histology	
			Adenocarcinoma	Miscellaneous
1. MNNG alone	21	21 (100)†	21	0
2. MNNG + olive oil only	10	10 (100)	10	0
3. MNNG + histamine	15	9 (60.0)‡	8	1‖
4. MNNG + tetragastrin	19	9 (47.3)§	8	1¶
Total	55	39 (70.9)	37	2

*MNNG alone: 50 μg/ml MNNG was given in the drinking water for 25 weeks.
MNNG + olive oil only: 0.5 ml of olive oil was administered after exposure to 50 μg/ml MNNG for 25 weeks.
MNNG + histamine: 4 mg of histamine dihydrochloride in depot form was administered after exposure to 50 μg/ml MNNG for 25 weeks.
MNNG + tetragastrin: 300 μg of tetragastrin in depot form was administered after exposure to 50 μg/ml MNNG for 25 weeks.
†Numbers in parentheses are percentage incidences.
‡Difference from value of Group 2 statistically significant ($P<0.05$).
§Difference from value of Group 2 statistically significant ($P<0.005$).
‖Leiomyosarcoma.
¶Mixed tumor.

Grossly the gastric tumors appeared as sessible tumors, ulcerations, plaque-like lesions, or large polypoid masses, sometimes obstructing the lumen. No rats had more than one cancerous lesion. The lesions were almost always confined to the antrum, but two tumors were located in the oxyntic mucosa (i.e., a leiomyosarcoma in Group 3 and a mixed tumor in Group 4).

In Group 1, treated with MNNG alone and

in Group 2, treated with MNNG and then olive oil only, gastric tumors were found in all rats examined. The incidences of tumors in Group 3, treated with MNNG and then histamine, and in Group 4, treated with MNNG and then tetragastrin, were significantly less than those in control Group 2.

No gastric cancer was found in rats treated with MNNG and killed in Week 25.

Histological types of adenocarcinomas

The adenocarcinomas of the glandular stomach were histologically classified into 3 main types on the basis of their differentiation; highly differentiated, well differentiated and poorly differentiated adenocarcinomas. Well differentiated and poorly differentiated adenocarcinomas were also subdivided into 2 subtypes on the basis of their mucin-producing activity.

1. *Highly differentiated adenocarcinoma.* Tumors of this type featured a glandular structure which was fairly comparable in the arrangement of cells to that enclosing normal gastrointestinal crypts. These glands infiltrated extensively into the underlying submucosa and muscle layer. The individual lining cells, which were usually in a single layer, perhaps were taller and wider than those of normal glands. Nuclei were large and were not basally oriented (Fig. 2).

2. *Well differentiated adenocarcinoma.* a. Common type: In this type of adenocarcinoma, gland formation was discernible but occurred with less regularity than highly differentiated adenocarcinomas. Neoplastic glands were lined by low columnar or cuboidal atypical cells of various sizes and shapes. Nuclei were large and hyperchromatic, giving the individual cells an over all dark appearance. A small amount of mucous substance was observed in the cytoplasm of the apical side of the cells, but mucinous nodules were not seen in this type of adenocarcinoma (Fig. 3).

b. Mucinous carcinoma type: Mucin-secretion was active, resulting in mucinous nodules containing large amounts of extracellular mucin, with only a few isolated groups of tumor cells. The tumor cells were regularly arranged and, often, showed glandular structure (Fig. 4).

3. *Poorly differentiated adenocarcinoma.* a. Anaplastic type: These carcinomas consisted of highly anaplastic cells scattered individually without accompanying fibrous stroma. The tumor cells showed no typical glandular or tubular differentiation. This type of adenocarcinoma was not seen in the present experiment.

b. Signet-ring cell carcinoma: These carcinomas consisted of cells with intracellular mucin, giving the cell a signet-ring appearance (Fig. 5).

Table 2 shows the distribution of these histological types in 21 control, 10 olive oil-treated, 8 histamine-treated and 8 tetragastrin-treated rats. Highly differentiated adenocarcinomas were found in all rats in the control Groups 1 and 2, and in 87.5% of those in Group 3 treated with MNNG and then histamine. The difference between the incidence in Groups 2 and 3 was not significant. In Group 4, treated with MNNG and then tetragastrin, the incidence of highly differentiated adenocarcinomas was only 25.0%, the other 75% of the tumors being adenocarcinomas with little or no glandular structure. The incidence of common type, mucinous carcinoma type and signet-ring cell carcinomas were 37.5%, 25.0% and 12.5%, respectively. The difference between the incidence of highly differentiated adenocarcinomas in Groups 2 and 4 was statistically significant.

DISCUSSION

The present results show that prolonged administration of tetragastrin or histamine in depot form after MNNG-treatment for 25 weeks resulted in a reduced incidence of gastric cancer in the glandular stomach of rats. The results on the effect of tetragastrin confirm our previous report [9]. The mechanism of this effect of tetragastrin or histamine is unknown, although 3 possible explanations may be considered.

The 1st explanation is the effect of gastrin or histamine on alternations in cell proliferation kinetics. It is well known that gastrin regulates the metabolism and growth of various tissues of digestive tract [10, 11], such as oxyntic gland mucosa, duodenal mucosa, and colonic mucosa. However, pentagastrin has not been observed to stimulate DNA synthesis in the antral mucosa of rats nor does gastrin increase the synthesis of proteins in this tissue [10, 11]. On the contrary, in dogs, intravenous infusion of gastrin is followed by only a slight decrease in DNA synthesis and mitotic indices in antral glands [12]. In rats, pentagastrin was shown to inhibit normal cell proliferation

Table 2. Histological types of adenocarcinomas in the glandular stomach of MNNG-treated rats

Group No.*	No. of rats with gastric cancer	Highly-differentiated	Well differentiated		Poorly differentiated
			Common type	Mucinous type	Signet-ring Cell Carcinoma
1. MNNG alone	21	21 (100)†	0	0	0
2. MNNG + olive oil only	10	10 (100)	0	0	0
3. MNNG + histamine	8	7 (87.5)	1 (12.5)	0	0
4. MNNG + tetragastrin	8	2 (25.0)‡	3 (37.5)	2 (25.0)	1 (12.5)

*For explanation of groups, see Table 1.
†Numbers in parentheses are percentage incidences.
‡The difference between the incidences of highly-differentiated adenocarcinomas in Groups 2 and 4 was statistically significant ($P<0.002$).

in the antral mucosa [13]. Therefore, influences of gastrin on epithelial cell proliferation in the glandular stomach after exposure to the carcinogen may be related to reduction of cancer formation. It is also reported that histamine influenced epithelial cell proliferation in the jejunum of rats [14]. However, the effect of histamine on antral stem cell proliferation in rats is unknown. The mechanism of the effect of histamine on the reduction of cancer formation needs further investigation.

The second possibility is the digestion of cancerous lesions as a result of increasing gastric secretion. Mallory [15] and Palmer and Humphreys [16] reported that cancerous lesions are easily ulcerated by gastric acid. In the present study, no cancers were found during treatment with MNNG for 25 weeks. Therefore, they must have developed after MNNG treatment for 25 weeks, and it seems likely that in Groups 3 and 4 they may be digested as a result of increased acid secretion. We reported previously that prolonged administration of tetragastrin after treatment with MNNG resulted in a significant increase in gastric acid secretion [9]. But it was not demonstrated in this work that hypersecretion of acid had actually occurred during the adminstration of depot histamine. Therefore, at this point, we cannot conclude that acid secretion alone decreased cancer formation.

Modifications in mucosal blood flow is another interesting explanation. In general, secretagogues, such as gastrin, histamine, insulin and food, also stimulate mucosal blood flow [17, 18]. For the same rate of acid secretion, histamine increased mucosal blood flow more than gastrin did. It was found that histamine [17] and gastrin [19] also increased mucosal blood flow to areas of the stomach which do not secrete acid. But in order to clear up this effect of mucosal blood flow changes on tumor formation, further studies are needed.

Unlike human cancers, the gastric tumors in rats induced by MNNG consisted of highly differentiated epithelial cells [7, 20–23]. These cells sometimes appeared similar to the cells in normal gastrointestinal epithelium. Therefore, in the present paper, we classified the experimentally induced carcinomas into 3 main types on the basis of their differentiation: Highly differentiated adenocarcinomas, well differentiated adenocarcinomas, and poorly differentiated adenocarcinomas. No uniform standards are available for classification of experimental gastric cancer, making it difficult to compare the distribution of different histological types, but undifferentiated adenocarcinomas seem to be rare [7, 20–23], although so-called undifferentiated adenocarcinomas have been observed in very low incidence in rats treated with MNNG in combination with oral administration of NaCl solution [24] or surfactant [23].

In our experiment, the incidence of highly differentiated adenocarcinomas was significantly less in Group 4, treated with MNNG and then tetragstrin, than in Group 2, treated with MNNG and then olive oil alone, or in Group 3, treated with MNNG and then histamine. On the contrary, adenocarcinomas characterized by little or no typical glandular structure were common; signet-ring cell carcinoma was found in Group 4. In our previous work [9], gastric cancers in rats were classified into 2 types on the basis of human gastric cancer; well differentiated and poorly differentiated adenocarcinomas. According to the above new classification of experimentally induced cancers, however, few gastric cancers that did develop after prolonged administration of tetragastrin in our previous work [9] were of the common type of well differen-

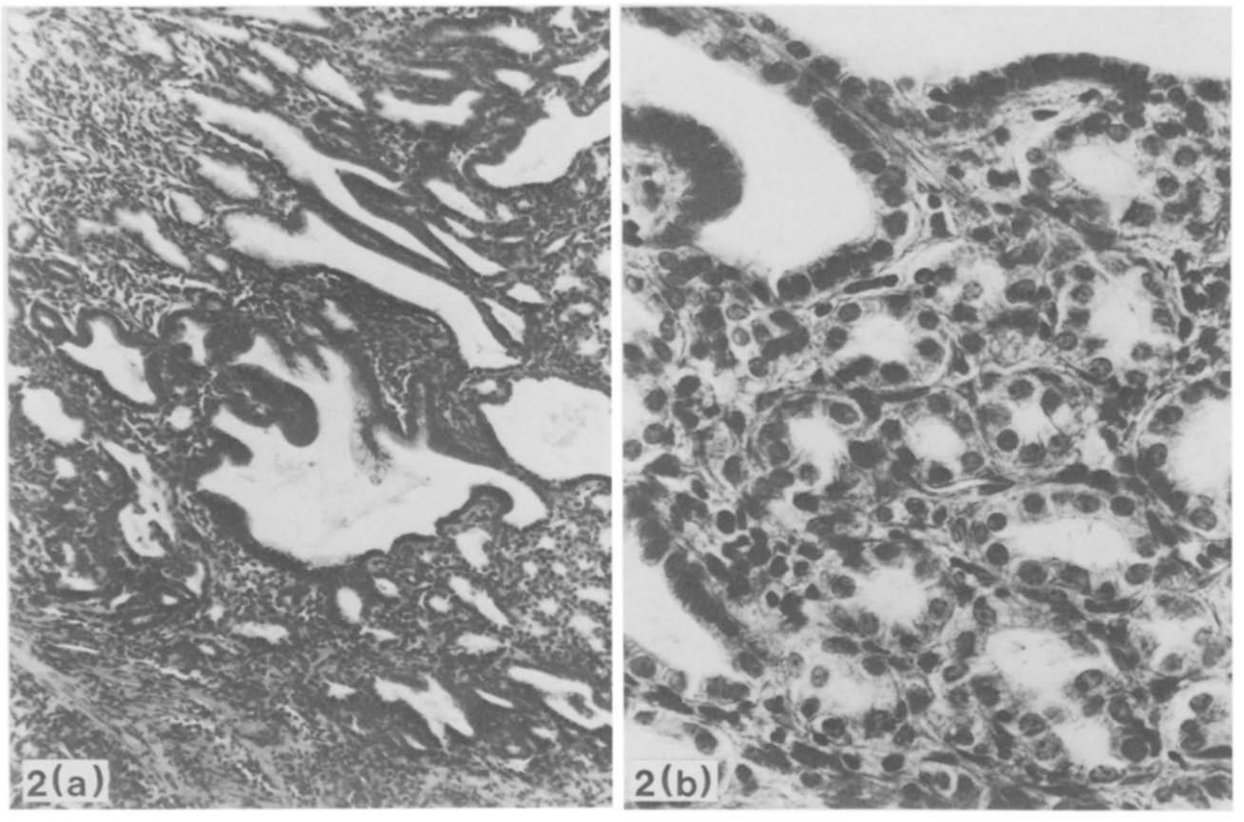

Fig. 2. Highly differentiated adenocarcinoma in control Group 1. H & E, ×100 (a); ×200 (b).

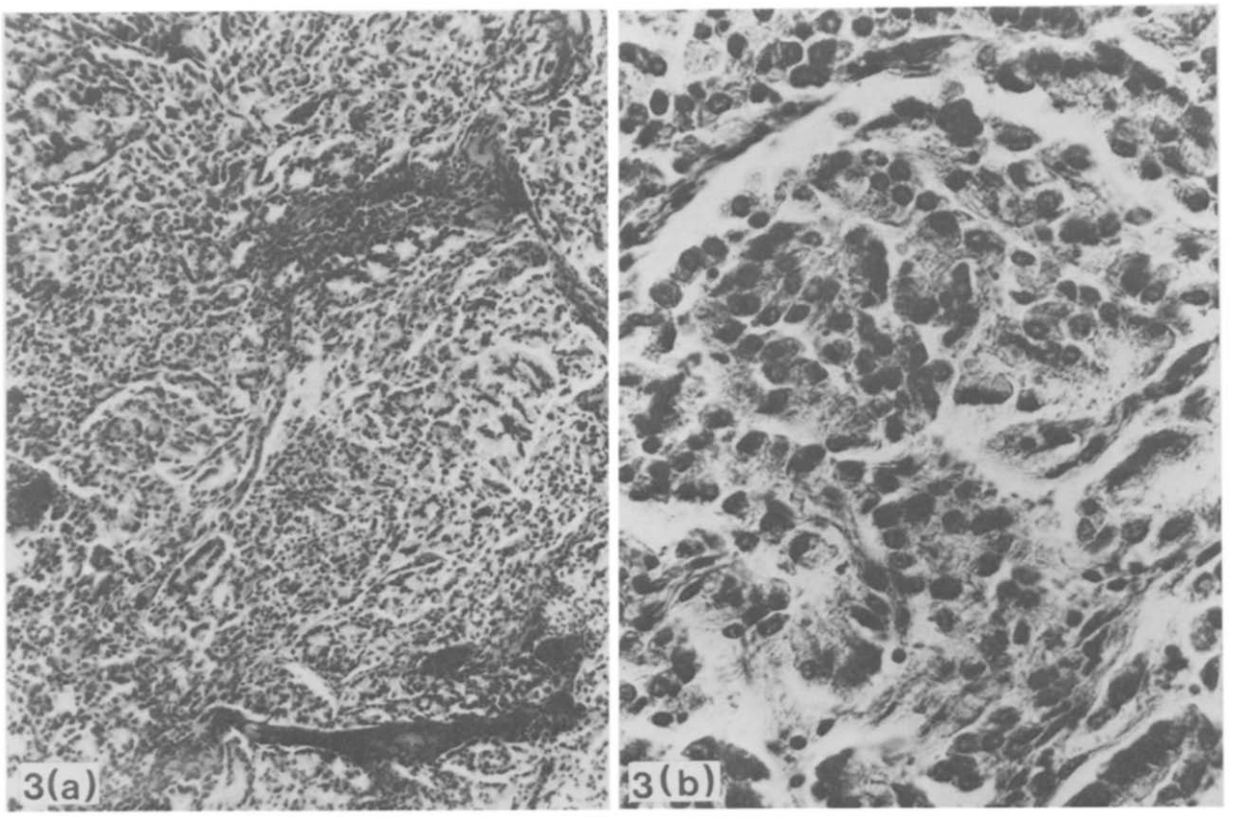

Fig. 3. Common type of well differentiated adenocarcinoma in Group 4 treated with MNNG and then tetragastrin. H & E, ×100 (a); ×200 (b).

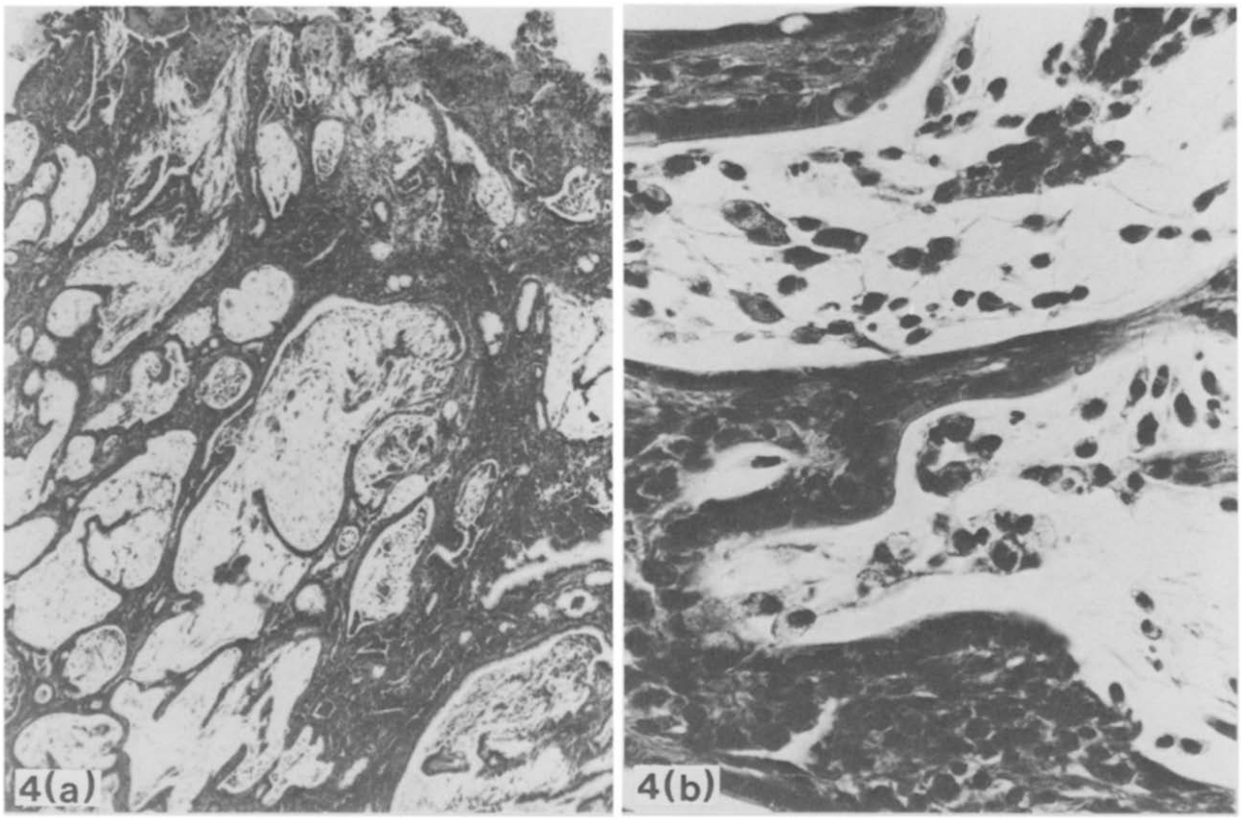

Fig. 4. *Mucinous adenocarcinoma of well differentiated adenocarcinoma in Group* 4 *treated with M.NNG and then tetragastrin. H & E,* ×100 (*a*); ×200 (*b*).

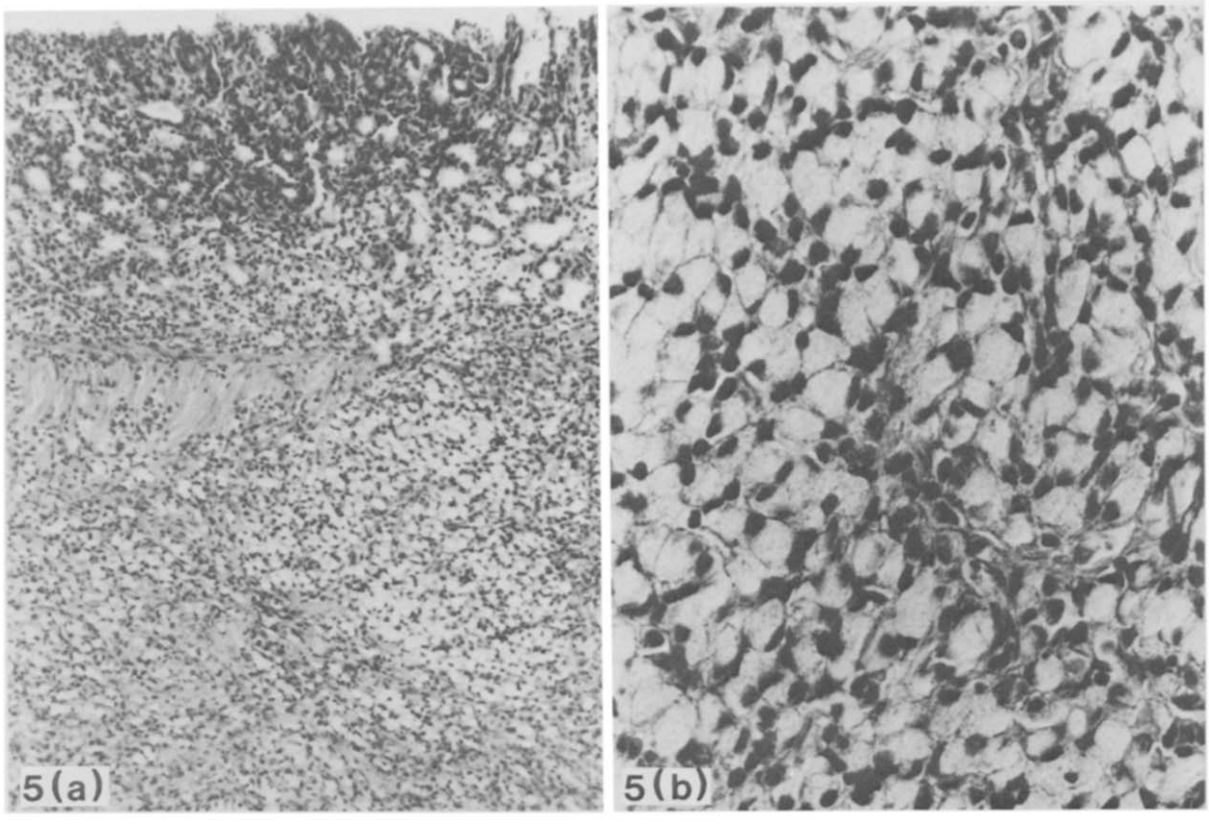

Fig. 5. *Signet-ring cell carcinoma in Group* 4 *treated with M.NNG and then tetragastrin. H & E,* ×100 (*a*); ×200 (*b*).

tiated adenocarcinomas, whereas gastric cancers in the control group were all of highly differentiated adenocarcinomas. Similar findings have been reported by other investigators: Tahara *et al.* [25] and Furukawa *et al.* [26] reported that administration of a small dose of gastrin during MNNG treatment resulted in the development of so-called undifferentiated adenocarcinomas in the glandular stomach of rats.

Our results show that both gastrin and histamine are related to reduction of gastric tumor formation, but that only gastrin, and not histamine, affects the histological type of the adenocarcinomas that did develop. However, the mechanism of this effect of tetragastrin is unknown.

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