

## Effect of Milk Diet on Gastroduodenal Malignancy Induced by N-methyl-N'-nitro-N-nitrosoguanidine in Rats

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**= Abstract =** An experimental study on the effect of a milk diet on carcinogenesis induced by N-methyl-N-nitro-N-nitrosoguanidine (MNNG) was designed in rats to elucidate its mechanism. A total of 136 Sprague-Dawley rats were divided into 6 groups according to the milk dosages in each diet. The entire group of 136 rats was fed the MNNG (100  $\mu$ g/ml) and milk for the initial 28 weeks. Thereafter for the next 12 weeks the group was fed a normal diet only. After this 40 week experiment 109 rats survived. These rats were then dissected with the results being summarized as follows:

Suppression of gastroduodenal malignancy was evidenced by the increase of milk concentration in the diet except for the group given MNNG and the lowest concentration of milk (6% milk).

Significant differences in the rate of cancer association were present between the regenerative hyperplasia (22.2%) and adenomatous hyperplasia (57.9%).

The incidence of benign lesions increased proportionally with the concentration of milk in the diet, especially in regenerative hyperplasia.

In the group which had been given the lowest concentration of milk there was a significant increase of the serum gastrin level in the rats with gastric cancer or precancerous benign lesions like regenerative hyperplasia or adenomatous hyperplasia.

**Key Words:** *MNNG, Milk in the diet, Serum gastrin, Carcinogen*

### INTRODUCTION

In the pursuit of pathogenesis of human gastric cancer Farber (1981) and Mirvish (1983) considered the initiating and promoting factors. Carcinogen and procarcinogen are studied in animals and humans as initiating factors. Hirayama (1971), Kubo *et al.* (1981) and Mirvish (1983) regarded the environment as an important element of the initiating factor because there is a difference in the incidence of stomach cancer between western and oriental countries. In addition, this incidence is now decreasing when compared with that of the past, and it also reveals a peculiar picture with regard to the study of immigrant. Higginson (1966) pointed out the importance of extrinsic factors as a causative factor for cancer: soil, air pollution, occupation, social environment, smoking and alcohol consump-

tion are all considered as an important factors (Miller 1982). Sugiura (1981), Wynder *et al.* (1963), Hirayama (1971), McGuigan *et al.* (1973), Farber (1981) and Miller (1982) emphasized that diet is the most important causative factor of gastrointestinal cancer. The mechanisms of the dietary effect for carcinogenesis can be explained by the facts that it contains the carcinogen, acts as an activator for carcinogen or acts as a micronutrient in the process of intrinsic cancer development (Farber 1981). On the other hand Hirayama (1967 & 1971) noticed the difference of the intake of milk between the diets of cancer patients and noncancer patients. After an epidemiological study he concluded that as a solitary food, milk had the most protective effect on gastric cancer. Mirvish (1983) also reported the protective effect of milk in gastric carcinogenesis. Sugimura (1951) and Miller (1941)

performed animal experiments to find out the protective effect of milk toward cancer.

Intrinsic factors such as sex, age, blood type, heredity, pernicious anemia and gastric benign lesions are considered as having some causal connection with gastric cancers. At the same time gastric acidity and gastrin are regarded as factors affecting gastric cancer (Wynder *et al.* 1963). Effect of gastrin has been discussed controversially as an inhibitor as well as a promotor of gastric cancer (Tannenbaum *et al.* 1974; Deveney *et al.* 1970; Tatsuta *et al.* 1982).

In the present study, to find out the effect of milk in gastroduodenal cancer we performed an animal experiment feeding rats with MNNG and milk in their diet. At the same time to discern the mechanism of milk in the process of cancer inhibition we analyzed the relationship of our pathologic findings of gastroduodenum with serum gastrin levels.

## MATERIALS & METHODS

### 1. Animal and grouping

136 male Sprague-Dawley rats weighing about 150g each were divided into 6 groups. Group 1 consisted of 20 rats as a control group. Group 2 consisted of 20 rats given a 6%-milk diet. Group 3 consisted of 24 rats given the MNNG *ad libitum* as drinking water for 28 weeks and then maintained on tap water without MNNG for an additional 12 weeks. Group 4 consisted of 24 rats with MNNG and 6%-milk diet for 28 weeks and fed like group 3. Group 5 consisted of 24 rats given MNNG and 13%-milk diet and fed like group 3. Group 6 consisted of 24 rats given MNNG and 26%-milk diet and fed like group 3.

Rats were housed 5 or 6 in a plastic cage under natural lighting and room temperature.

### 2. Chemicals

MNNG was purchased from Aldrich Chemical Co., Inc., Milwaukee, Wis. A stock solution of MNNG (1 mg/ml), kept in a dark, cold place was appropriately diluted with tap water to obtain the 100  $\mu$ g/ml in concentration. The water bottles were covered with aluminum foil to protect the MNNG from the degradation by light and were refilled with a solution of MNNG every 2 days.

### 3. Milk

Milk was purchased from Seoul Milk Co. as a whole milk powder. According to the each group designed, above milk was mixed with the diet and fed as a concentration of 6, 13 and 26% in dry weight.

### 4. Histologic examination

Complete autopsies were performed on all rats when they died or became moribund during the experiment or they were sacrificed at week 40. The stomach was opened along the greater curvature, pinned flat on a cork board, and fixed with 15% neutralized formalin. 4-mm wide step sections were made along the lesser curvature, and sections were stained with hematoxylin and eosin, PAS-alcian blue and Masson trichrome if necessary.

### 5. Serum gastrin analysis

Concentration of gastrin in fasting rat sera was measured at the end of this experiment by radioimmunoassay.  $^{125}$ I-Gastrin was purchased from the Radioassay Systems Laboratories, Inc., Carson, CA. The rabbit antibody to human gastrin I & II and second antibody of goat anti-rabbit gamma globulin were used.

The results were statistically analyzed by  $\chi^2$  test and *t* distribution.

## RESULTS

Body weight decreased in rats administered with MNNG only, but the growth rate returned to the range in other groups after the withdrawal of MNNG. There was no difference in the growth and survival rates regardless of the association of gastrointestinal malignancy.

The incidence of gastric cancer was 25% in group 3, 36.8% in group 4, 27.8% in group 5, and 14.3% in group 6, reflecting the suppression of carcinogenesis by the increase of milk concentration in the diet, but the statistical significance could not be verified in each group ( $p > 0.05$ ) (Fig. 1).

There were statistically significant differences in the rate of cancer association with regenerative hyperplasia (22.2%) and adenomatous hyperplasia (57.9%) ( $p < 0.05$ ). The incidence of benign lesions increased proportionally with the concentration of milk in the diet, especially in regenerative hyperplasia with low frequency of cancer association.

The incidence of small bowel cancer was 5% in group 3, 21.1% in group 4, 22.2% in group 5, and 9.5% in group 6.

But there were no statistical differences among each group.

Serum gastrin levels showed  $128.6 \pm 30.6$  pg/ml in group 1,  $85.5 \pm 3.1$  pg/ml in group 2,  $116.2 \pm 69.0$  in group 3,  $155.3 \pm 57.4$  in group 4,  $108.4 \pm 39.7$  in group 5, and  $99.2 \pm 24.5$  pg/ml in group 6. Therefore group 4 revealed the significant increase

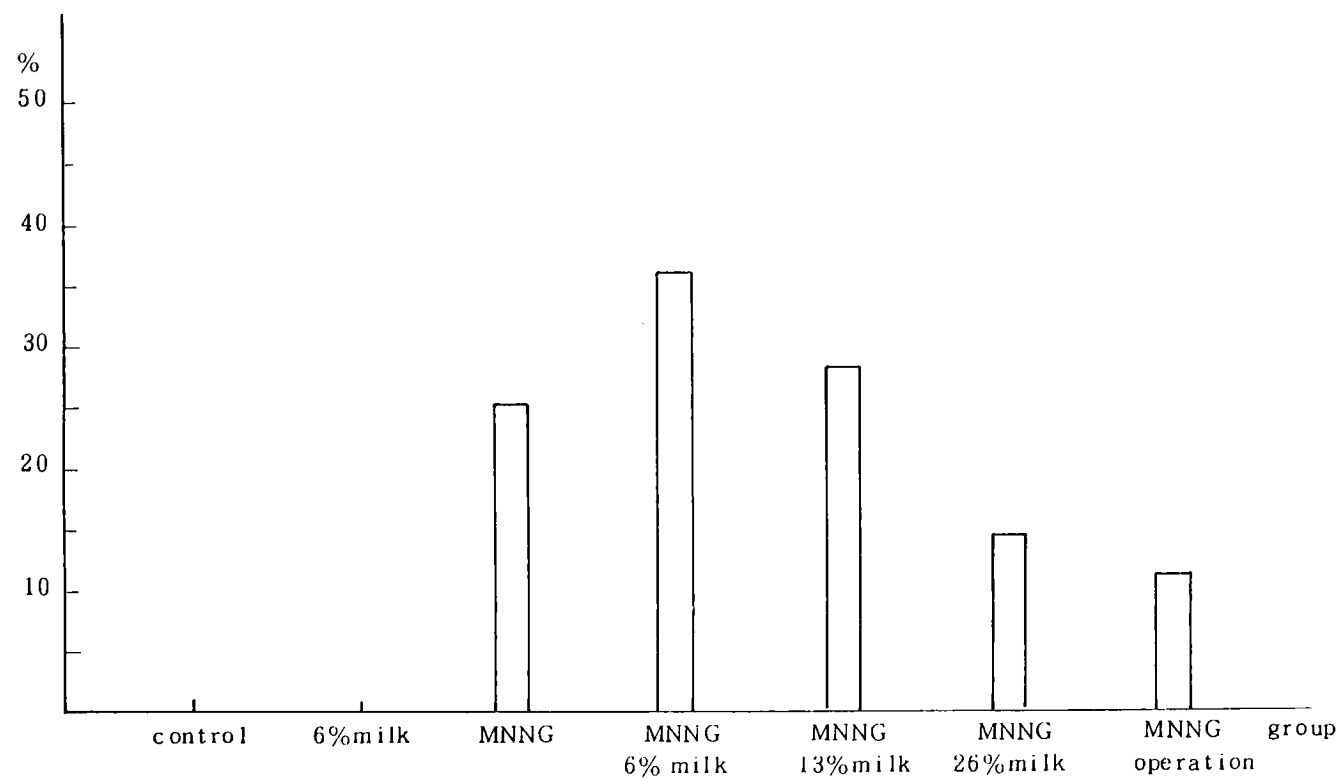


Fig. 1. Incidence of MNNG-induced gastric carcinomas.

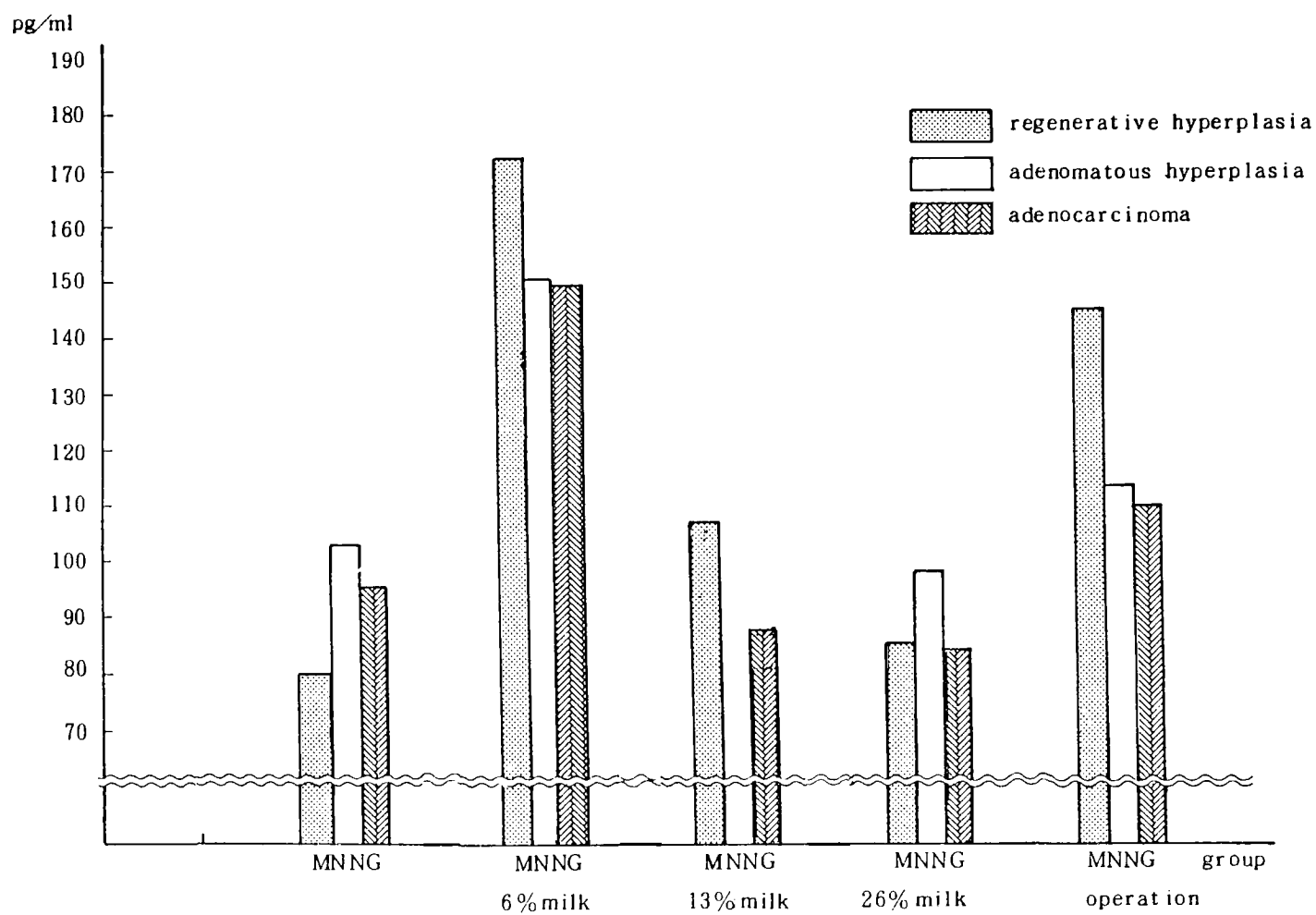


Fig. 2. Serum gastrin level according to gastric histologic type.

of serum gastrin level compared with other groups ( $p < 0.01$ ).

Such a finding was also evident in group 4 with all the precancerous and malignant lesions ( $p < 0.05$ ) and its difference was more pronounced in rats with regenerative hyperplasia which had the lowest frequency of cancer association ( $p < 0.05$ ) (Fig. 2).

The above results may lead to the assumption that a high milk diet inhibits the development of gastric cancer in rats by delaying the carcinogenesis from benign precancerous conditions, such as regenerative hyperplasia and adenomatous hyperplasia, and the increase of serum gastrin plays a significant role in gastric carcinogenesis.

### DISCUSSION

As one of the environmental factors, the diet contributes much to the difference of cancer incidence according to the epidemiological study in regional levels of social status and immigrants (Haenszel 1972 & 1975; Mirvish 1983). In the gastrointestinal tract, the stomach is primarily affected by dietary carcinogen. This can be understood because the stomach is the first place for the contact of food in the gastrointestinal tract (Kinosita 1969; Hirayama 1971; Miller 1982). The mechanism by which food is associated with the development of cancer can be explained by the fact that food acts as a media of a carcinogen like aflatoxin, contains carcinogen during food processing, acts as a modulator in the activation of carcinogen, and acts as a micronutrient during the intrinsic formation of carcinogen (Hirayama 1971; Farber 1981). The foods containing carcinogen include dimethylamine in dried fish, benzpyrene in smoked fish and meat, hydroperoxide in fried food, and precursor of nitrosamine in salted fish (Dungal 1961; Kinosita 1969; Mirvish 1983). At the same time fried food and hard grain cause erosions in gastric mucosa (Correa *et al.* 1975). Salted food destroys the gastric mucosal barrier and highly concentrated starch food and hard grain cause the disturbance of gastric acid secretion (Mirvish 1983). To the contrary vitamin C & E inhibit the formation of carcinogen by blocking the transformation of nitrosamine from nitrite (Farber 1981). In addition vitamin B complex, A, choline and cystine have protective effects upon hepatic cancer (Hirayama 1971). According to the epidemiological study, the intake of fruits and dairy products have a correlation with the incidence of gastric cancer. In Japanese research on

the epidemiological study of stomach cancer (Hirayama 1967 & 1971; Haenszel 1972) milk was considered as an important factor causing the difference in the incidence of gastric cancer. Furthermore the amount of milk intake showed some relation with the inhibition of cancer. In an experimental study using butter yellow, milk inhibited the development of hepatic cancer (Sugiura 1951) and fresh milk showed a more protective effect upon cancer than dried forms (Cornelia 1946).

In our experiment to research the milk effect upon gastric cancer we discovered that with the greater increase of milk in the diet, there was a decline in the amount of cancer that developed. However the premalignant gastric lesions such as regenerative hyperplasia and adenomatous hyperplasia showed a reverse correlation. From the above results we concluded that milk in the diet inhibited the development of cancer from the precancerous lesion even though premalignant conditions were not suppressed.

Milk contains vitamins, minerals such as calcium, phosphorus, and iron as well as major energy resources such as carbohydrate, fat and protein.

The mechanisms of milk in the inhibition of cancer were explained from many aspects: as a nutritional effect (Hirayama 1967 & 1971), protein of milk, physiochemical effect, change of gastric acid secretion, effect on gastric mucosa, and the prostaglandin effect it contains (Reide *et al.* 1980; Materia *et al.* 1982). Protein as a nutritional component of milk makes mucus, improves the protective and reparative process, and has a buffering effect on gastric acid (Sugiura 1951; Johnson 1976; Miller 1982; Mirvish 1983). The component of protein, an amino group, combines with methyleurea in competition with nitrite. Phospholipid in milk is a surfactant functioning as a barrier of gastric mucosa (Dial *et al.* 1984). In an animal experiment Kinosita (1969) showed that an irritant which induced gastric erosion was decreased by milk in the diet. Milk has a buffering effect on gastric acid initially, but after one or two hours protein and calcium increase the gastric acid secretion. Reid *et al.* (1980) and Materia *et al.* (1982) reported that prostaglandin  $E_2$  &  $F_2\alpha$  are contained in human milk. Hill *et al.* (1983) and Lichtenberger *et al.* (1983) described how the cytoprotective effect of prostaglandin is mediated by phospholipid. Prostaglandin has an effect on gastric acid secretion, gastric mucus production, smooth muscle contraction, control of local blood flow and shifting of glu-

cose and electrolyte, enzyme excretion at brush border, and absorption of zinc. To define the mechanism of milk in carcinogenesis pathologic analysis of gastric premalignant lesion is to say that it is indispensable. Nagayo (1981) considered gastric dysplasia as a premalignant condition. Morgenstern *et al.* (1973) suspected that chronic gastritis-induced regenerative polyp, inflammatory polyp, or adenomatous polyps might be precancerous lesions. In an animal study, Fujimura *et al.* (1970) and Saito *et al.* (1970) observed the gastric erosion or regenerative hyperplasia after 5 weeks ingestion of MNNG by rats, adenomatous hyperplasia after 20 weeks, and adenocarcinoma after 30–40 weeks. These precancerous lesions might have had either a reversible course to normal tissue or an irreversible course to malignancy. During the course of cancer formation intrinsic or extrinsic co-carcinogens could have acted as promoting factors. Ippoliti *et al.* (1976) reported that serum gastrin is increased by milk intake. Johnson (1976) described that gastrin acts as a trophic hormone on gastrointestinal mucosa. There are two controversial explanations for the role of gastrin in cancer development. Tatsuta *et al.* (1977 & 1982) and Deveney *et al.* (1980) observed that gastrin inhibited the carcinogenesis via the trophic effect on gastric mucosa, increased blood flow, and increased gastric acid secretion. On the contrary Bralow *et al.* (1970) and Tahara (1982) considered that gastrin promoted the growth of gastric cancer by the trophic effect on gastric malignant lesions as well as gastric mucosa itself. In an experimental animal study Kishimoto *et al.* (1983) observed the increase of gastrin in animals with cancer.

This experiment showed that in the group which had been given the MNNG and 6% milk diet, the incidence of gastric cancer was the highest even though its statistical significance is not verified ( $p > 0.05$ ). At the same time there was a significant increase of the serum gastrin level in the rats with gastric cancer, as well as precancerous benign lesions such as regenerative hyperplasia or adenomatous hyperplasia in that group.

We can suspect that increased serum gastrin might play a significant role in gastric cancer development, however, an explanation why gastrin is increased specifically in group-4 remains for further indepth experimentation and study.

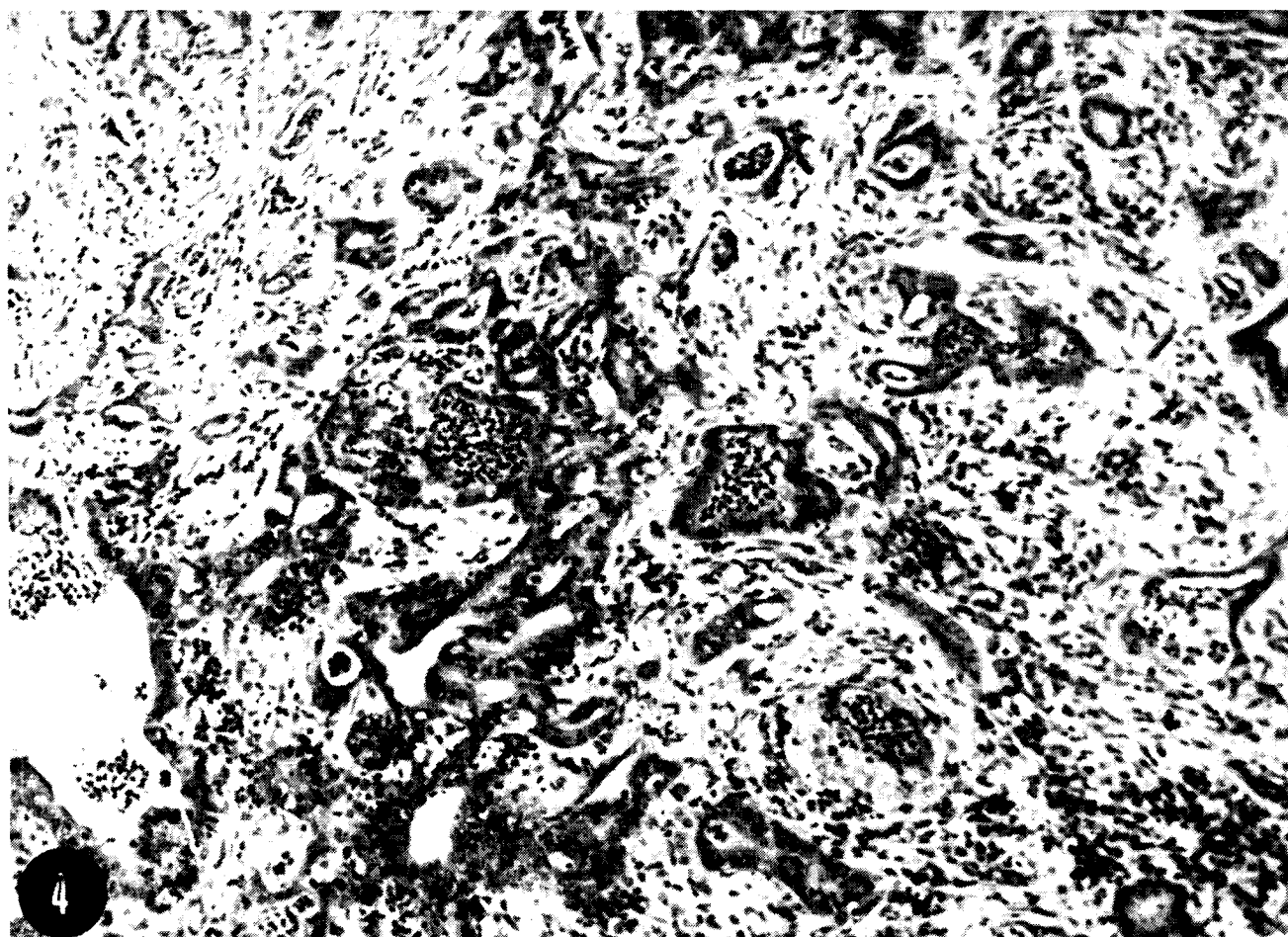
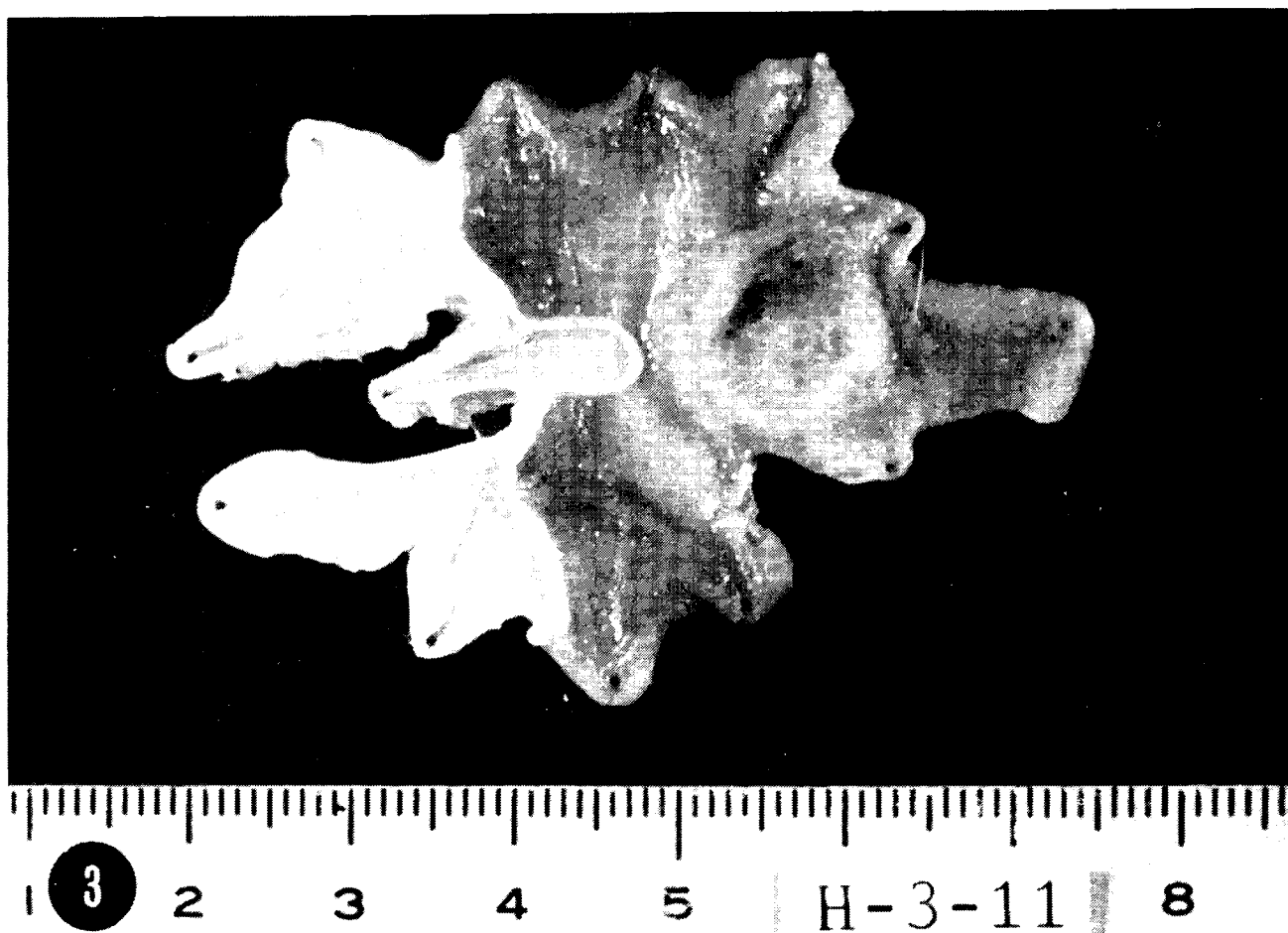
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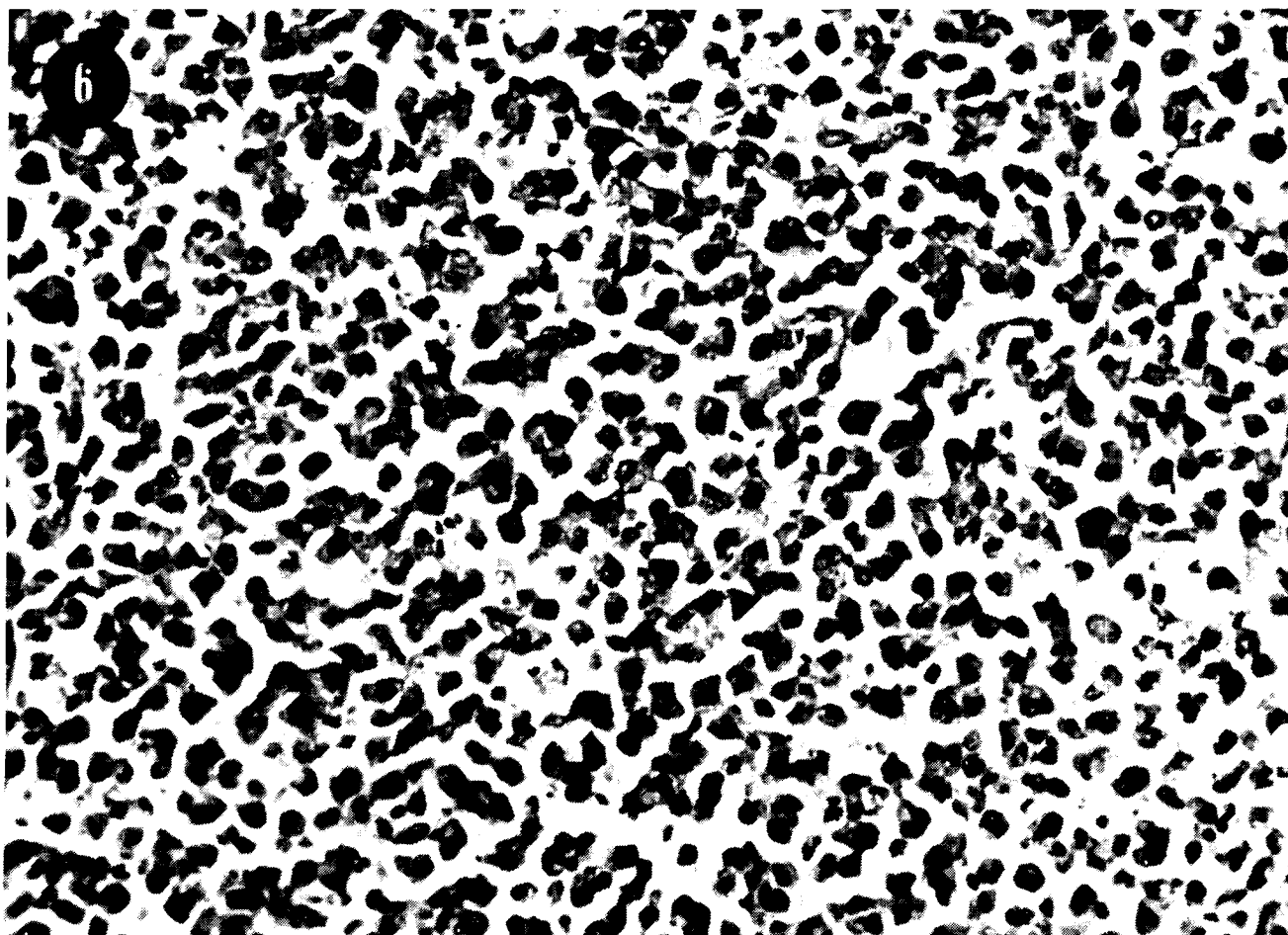
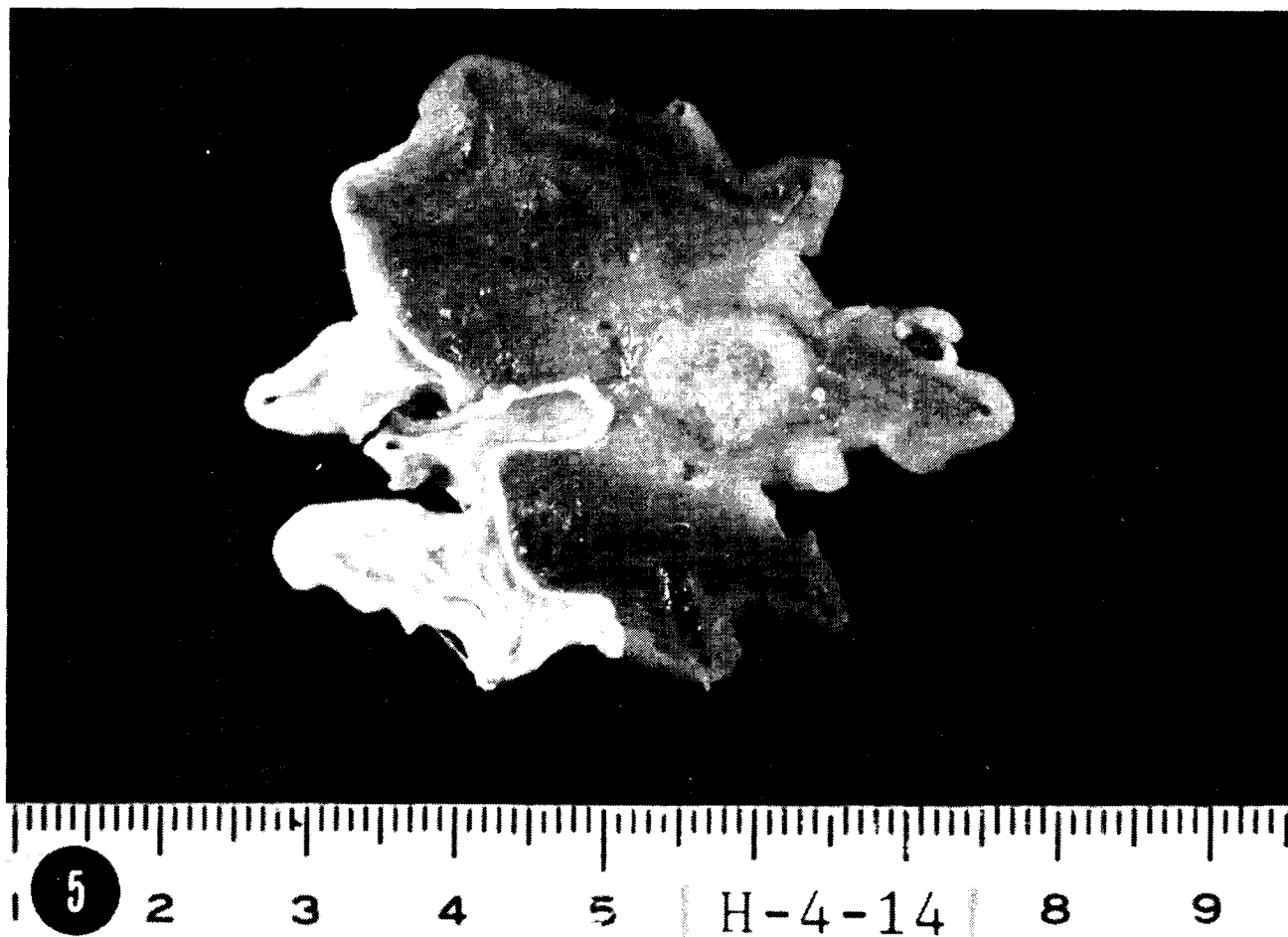
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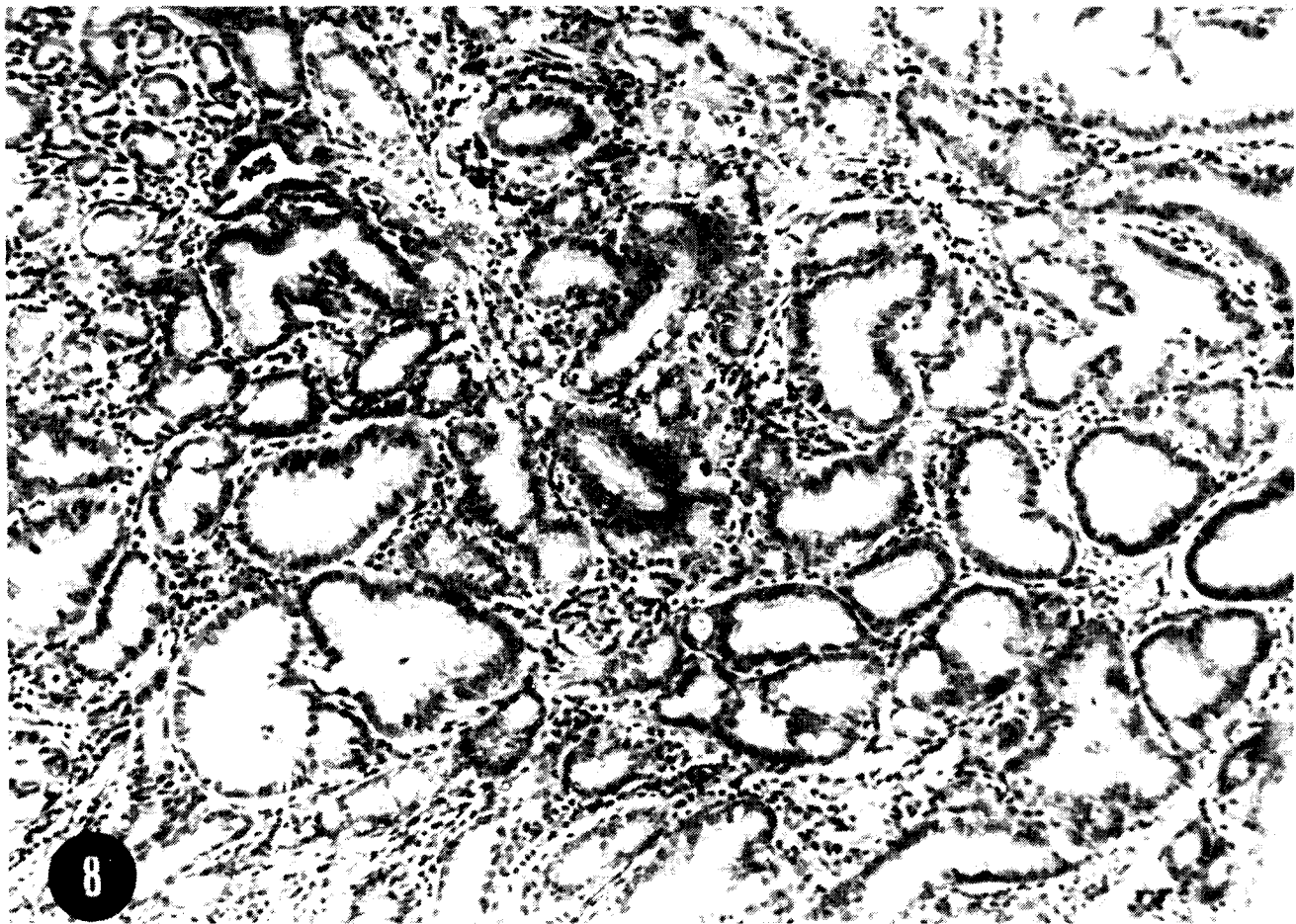
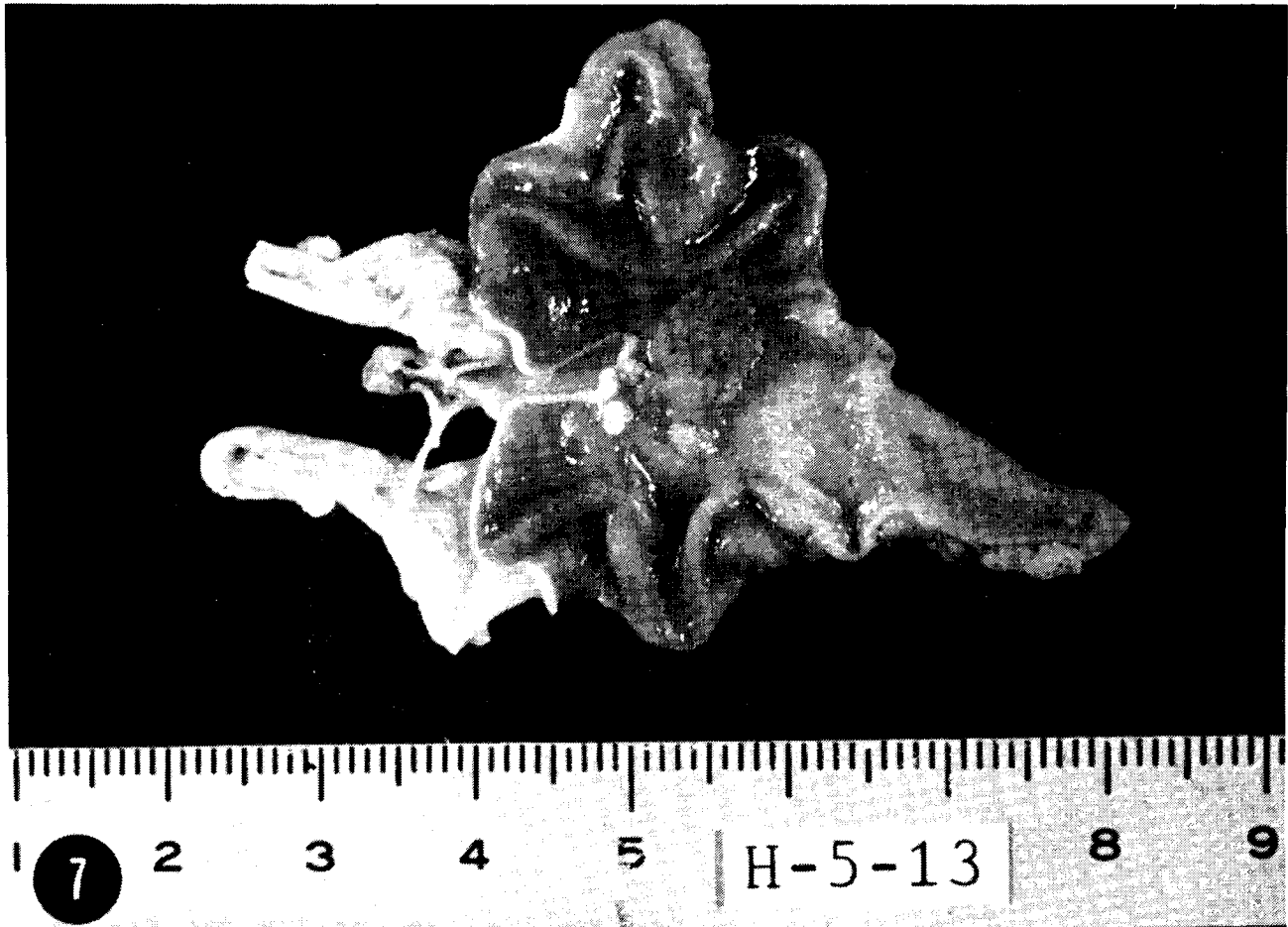
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## LEGENDS FOR FIGURES

- Fig. 3. (H-3-11) Gross photograph of gastric carcinoma in the glandular stomach. A large ulcerofungating tumor mass with thick rim of peripheral heaping and central ulceration involves almost whole length of the lesser curvature.
- Fig. 4. (H-3-11) Microphotograph of Fig. 10. The actively branching glands infiltrate into the underlying tissue with desmoplastic stroma accompanied by cellular exudates. HE, X200.
- Fig. 5. (H-4-14) Gross photograph of gastric lymphoma in the glandular stomach. The lesion is a diffusely elevated round mass with central depression.
- Fig. 6. (H-4-14) Microphotograph of Fig. 16. The tumor cells are relatively monotonous but their nuclei are hyperchromatic and varying in size. HE, X200.
- Fig. 7. (H-5-13) A flat fungating mass with slight papillary surface is seen at the proximal portion of glandular stomach. The tumor is basically adenoma, but its upper margin contains carcinomatous foci.
- Fig. 8. (H-5-13) Microphotograph of gastric adenoma with slight atypicality. Lining cells are focally dysplastic, but glandular configuration is still maintained. H & E, X100.







= 국문초록 =

## 우유투여가 N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) 유발 위십이지장 암 발생에 미치는 영향에 관한 실험적 연구

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MNNG 투여에 의한 백서 위십이지장암 발생에 있어서 우유의 영향을 조사하고 그 원인을 분석키 위해 조직학적 및 혈청학적 분석을 시도하였다. 실험군은 생후 8주 전후의 Sprague-Dawley 백서 136마리로서 일반사료만 준 대조군 20마리(제1군)와 6% 우유사료만 준 군 20마리(제2군), MNNG(100  $\mu\text{g}/\text{ml}$ ) 투여군 24마리(제3군), MNNG 및 6% 우유사료군 24마리(제4군), MNNG 및 13% 우유사료군 24마리(제5군), MNNG 및 26% 우유사료군 24마리(제6군)로 분류한 후 군별에 따라 28주간 발암제 및 우유사료를 투여하고 실험시작 40주째 생존한 109마리에 대해 다음과 같은 결과를 얻었다.

1. MNNG 단독 투여로 실험시작 12주 이후의 성장에 영향을 주었으나 ( $P < 0.01$ ) 가역적이었고 생존에 미치는 영향은 없었다.

2. 위암발생은 대조군, 우유사료군에서 없었고 제3군 25%, 제4군 36.8%, 제5군 27.8%, 제6군 14.3%로 우유사료군에서 우유농도 증가에 따라 위암발생의 감소가 관찰되었고 제6군에서는 제3군보다 암발생이 억제되었다. 그러나 제4군에서는 제3군보다 상회하는 발암율을 보였다.

3. 위의 양성병변은 재생성 과증식, 선종성 과증식, 섬유 증식증 등이었고 MNNG 투여 각 우유사료군간의 분포는 우유농도 증가에 따라 위 양성병변이 증가하였으며 특히 암 수반율이 작은 재생성 과증식 발생군에서 뚜렷하였다. 재생성 과증식군에서의 암 수반율은 22.2%, 선종성 과증식군에서는 57.9%로 유의한 차이를 보였다 ( $P < 0.05$ ).

4. 소장암 발생은 위암에서와 같이 십이지장의 선암이 주이었고 종양 발생 빈도는 3군에서 5%, 4군에서 21.1%, 5군에서 22.2%, 6군에서 9.5%이었으나 발생예수가 작아 각 군간의 통계적 유의성은 없었다.

5. 혈중가스트린치는 암발생이 많았던 제4군에서 증가되었고 ( $P < 0.01$ ), 양성 위 병변과 관련된 혈중 가스트린치도 제4군에서 증가하였으며, 특히 재생성 과증식 수반동물군에서의 가스트린 증가는 유의하였으며 ( $P < 0.05$ ), 위암발생군에서도 가스트린이 유의한 증가를 보였다 ( $P < 0.05$ ).

이상의 성적을 바탕으로 위암발생은 우유농도 증가에 따라 감소되며 고농도의 우유사료군에서 발생이 억제되고 있음은 전암성 병변으로부터의 암 발생을 억제하려는 우유의 암 발생 지연효과인 것 같다. 저농도 우유사료군에서의 암발생율의 증가는 혈중 가스트린치의 증가가 그 한 요인으로 해석되며 암발생군에서의 가스트린치의 증가와 더불어 ( $P < 0.05$ ) 전암성 병변인 재생성 과증식군에서의 가스트린 증가가 ( $P < 0.05$ ) 이를 뒷받침하고 있다.