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Induction of Experimental Atrophic Gastritis by N-Methyl-N'-Nitro-N-Nitrosoguanidine or Taurocholic Acid in Donryu Rats

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ABSTRACT

The morphology of the rat (Donryu) gastric mucosa was examined by light microscopy after administration of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) or taurocholic acid (TCA), a component of bile acids. MNNG was given to rats ad libitum from light-sealed bottles for 5 months and deionized water was given freely for 6 months thereafter. TCA was administered to rats freely for 11 months. Deionized water was given to rats as control (non-treated rats). Rats treated with MNNG or TCA and control rats were killed at 11 months after the beginning of the experiment. Using 3 micron tissue samples taken from the area of the gastric mucosa designated before the experiment, hematoxylin and eosin and azan stain were made for histopathological evaluation and fibrosis. Marked atrophic changes, such as reduction in the number of parietal cells, shortened mucosal length, inflammaotry cell infiltration, and proliferation of fibrosis, were present in the gastric mucosa of rats treated with MNNG as well as TCA. These findings were typical for atrophic gastritis. Such atrophic changes were slight in the gastric mucosa of the control rats. The frequency of tumourous lesions was very low in MNNG-treated rats. We have concluded on the basis of the present data that MNNG as well as TCA can induce atrophic gastritis in Donryu rats.

It has been generally accepted that peptic ulcer and/or gastric cancer coexist in atrophic gastritis of the gastric mucosa. Atrophic gastritis has also been regarded to be a predisposing factor of gastric cancer[16,25], particularly the intestinal type of gastric cancer[31]. It is therefore considered extremely important to develop atrophic gastritis experimentally in rats. However, this has been considered to be difficult with the exception of a couple of successful works based on immunological mechanism[11,18].

Such factors as mechanical, thermal, chemical, and radiation injury contribute to the development of atrophic gastritis in man and animals[10,13,15,20,32] and therefore these may be causative factors. These factors, however, lack direct evidences of developing atrophic gastritis in animals experimentally and also in man clinically. Duodenal fluid, particularly bile contained therein, is considered to be one of possible factors for atrophic gastritis because bile reflux can cause gastric mucosal changes. This has been demonstrated in gastric remnant following surgery for benign and malignant diseases[1,8,21,29,37].

A nitroso compound, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) known as a strong carcinogen[33,35], has also strongly damaging effects on the gastric mucosa of rats[2,19,30].

This importance of atrophic gastritis as mentioned earlier thus stimulated the authors to conduct the present study in which attempts those

made to produce atrophic gastritis experimentally in rats by administration of a component of bile acids, taurocholic acid (TCA), or MNNG for a long experimental period and to examine the gastric mucosa histopathologically as well as histochemically.

MATERIALS and METHODS

1. Animals

Male Donryu rats, weighing 100 g at the beginning of the experiment, were used in this study.

2. Production of atrophic gastritis

(1) MNNG

Solution containing 50 mg of MNNG (Sigma) dissolved in one liter of 0.04% Tween 60 was administered to rats ad libitum for light-sealed bottles for 5 months and deionized water was given freely for 6 months thereafter.

(2) TCA

Solution containing 5 mM sodium salt of TCA (Difco) was given to rats freely for 11 months.

3. Experimental groups

The experimental animals were divided the following three groups: Group A given MNNG alone (n=10), Group B given TCA alone (n=15), and Group C not treated but given deionized water (n=20) as used in Groups A and B.

4. Preparation of tissue samples

The rats of each experimental group were sacrificed 11 months after the beginning of experiment. The abdomen was opened and the stomach was resected and opened along the greater curvature. After macroscopical examination for possible tumourous lesions in the stomach and small intestine of Group A, Swiss-rolled tissue specimens were each taken from the five sites of the stomach of rats in these experimental groups (Fig. 1). These were immediately fixed in 10% neutral formalin for 24 hr at room temperature. After fixation, the rolled tissue specimens were embedded in paraffin wax and sectioned 3 microns in thickness.

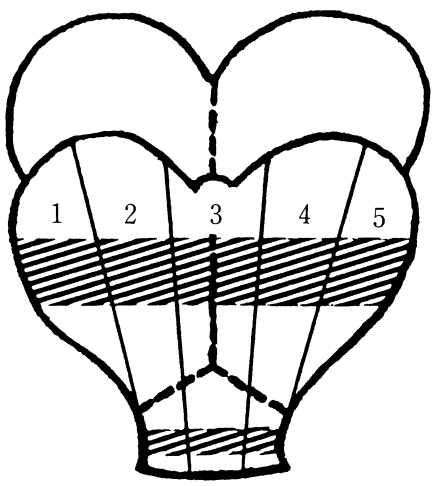

Fig. 1. Scheme of the stomach. The figure indicates Swiss rolled tissue samples taken.

5. Conventional histopathology and histochemistry

Sections were stained with hematoxylin and eosin for histopathological evaluation. Azan stain was made for evaluation of the fibrotic changes in the mucosa. Using ocular eye grid equipped in the eyepiece of the light microscope (Leitz), the number of parietal cells was counted per 0.25 cm square (unit area : UA) under × 400 magnification and the length of the mucosa of the area (shaded area in Fig. 1) arranged before the study were counted (mm) under × 100 magnification (Fig. 1). The degree of inflammatory cell infiltration and fibrotic changes was evaluated slight, moderate and severe according to the number of inflammatory cells in the lamina propria and the extent of staining with azan, respectively.

6. pH in the gastric juice

pH in the gastric juice was checked by dipping of pH test paper.

7. Definition of atrophic gastritis

In the present study atrophic gastritis was defined as (1) reduction of glands in both the fundobody and antral mucosa, especially reduction in the number of parietal cells of the fundobody mucosa, (2) shortening of the mucosal length of the stomach (shaded area), (3) development of interstitial fibrosis, and (4) inflammatory cell infiltration in the lamina propria mucosae. These were refered to in the evaluation of atrophic gastritis.

8. Statistical analysis

Data was expressed as mean ± standard deviation of the mean. Student-t tests were used as the statistical test. The difference with p value of less than 0.05 were considered statistically significant.

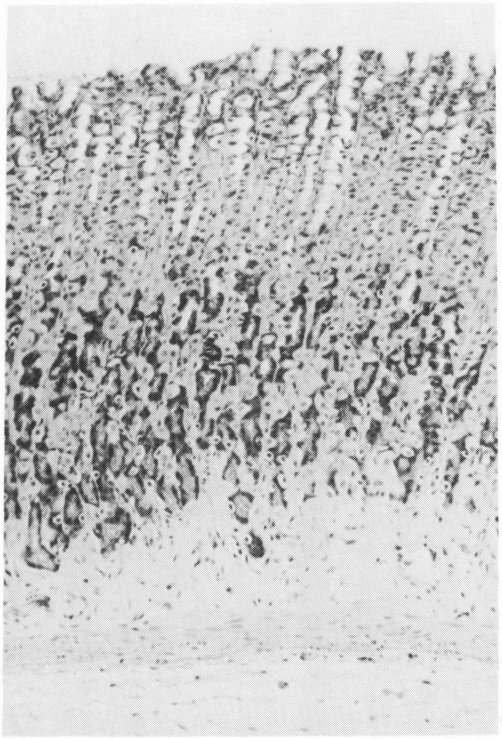

Fig. 2. Atrophic gastritis after MNNG shows marked reduction of parietal cells and fibrotic proliferation in Group A (HE stain, × 100).

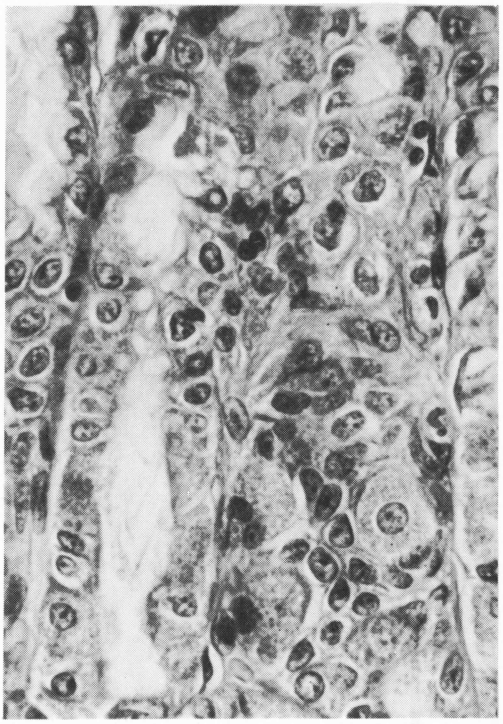

Fig. 3. Inflammatory cell infiltration in the lamina propria of the gastric mucosa in Group A (HE stain, × 100).

RESULTS

1. MNNG

The gastric mucosa of rats of Group A given MNNG alone showed such atrophic changes as shortened mucosal length, reduction of glands in both the fundobody and antral mucosa, irregularly shaped as well as disorganized mucosal structure, marked fibrous changes and inflammatory cell infiltration in the connective tissue (Fig. 2 and 3). Furthermore, surface mucosal lesions (erosions) were diffusely present in this group when compared to the control Group C (Fig. 4).

(1) Parietal cell population (Fig. 5)

The number of pariental cells per UA was 57.5 ± 11.3 cells in Group A, while it was 109.8 ± 16.1 in Group C. The difference in number was statistically significant ($p < 0.001$).

(2) Mucosal length (Fig. 6)

The length of the fundobody mucosa was 0.41 ± 0.06 mm and that of the antral mucosa was 0.21 ± 0.03 mm in Group A, while the length of the fundobody and that of the antral mucosa were 0.65 ± 0.05 mm and 0.27 ± 0.04 mm, respectively, in control Group C. The length of the fundobody and antral mucosa was each significantly different between Group A and C ($p < 0.001$).

(3) Fibrotic changes (Fig. 7)

Marked fibrotic changes were observed in the antrum of Group A when compared to those in the antrum of Group C. Fibrotic changes were moderate in the fundobody mucosa of Group A, while the changes were very slight in the fundobody of Group C.

(4) Inflammatory changes (Fig. 8)

Inflammatory cell infiltration was moderate but was always present in the lamina propria mucosae and muscularis mucosae of both the fundobody and antrum in Group A, while cell infiltration was slight in both the fundobody and antrum of Group C.

(5) pH in the gastric juice

pH in the gastric juice was 4 or 5 in Group A, while it was 2 in Group C.

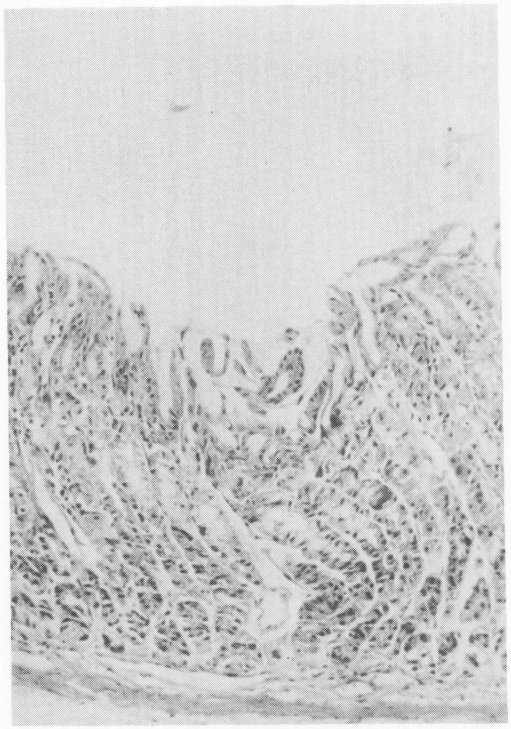

Fig. 4. A surface mucosal lesion (erosion), irregular structure, and sparse parietal cells in the stomach of Group A (HE stain, × 100).

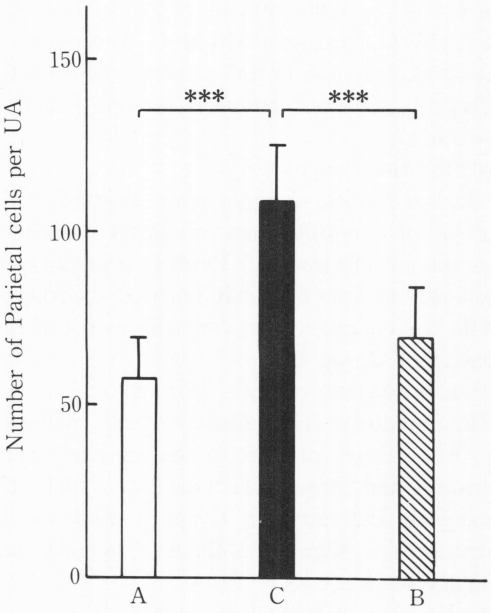

Fig. 5. Parietal cell population per unit area. Note significant reduction in the number of parietal cells per unit area in the gastric mucosa of Group A and B when compared to that of Group C.

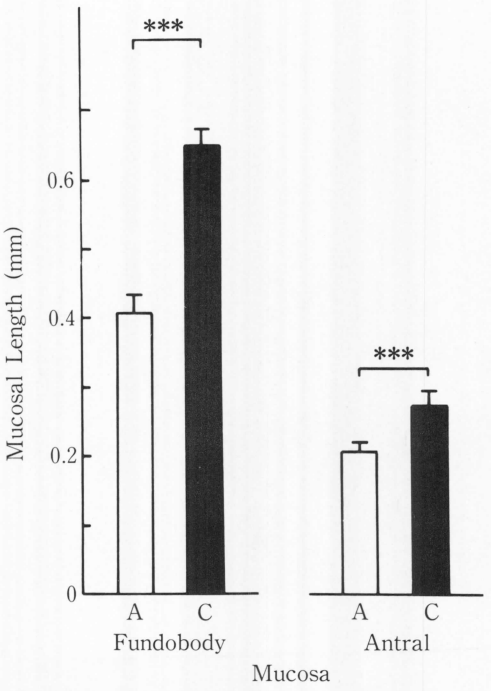

Fig. 6. Shortened length of both the fundobody and antral mucosa in rats of Group A when compared to that in Group C.

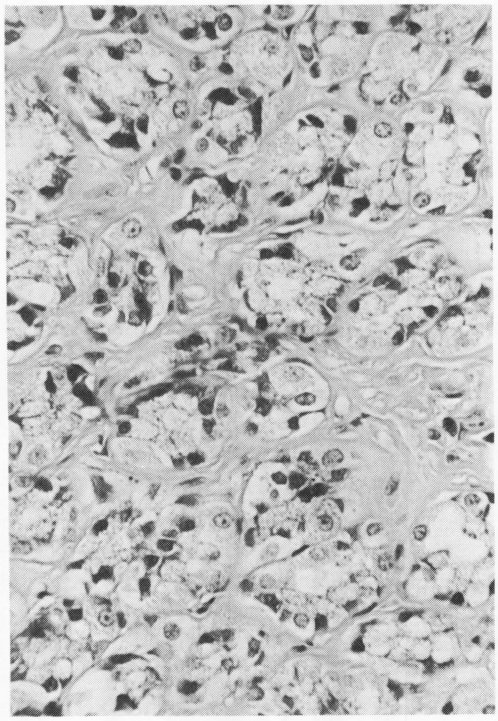

Fig. 7. Marked fibrotic changes in the fundobody mucosa of Group A (HE stain, × 100).

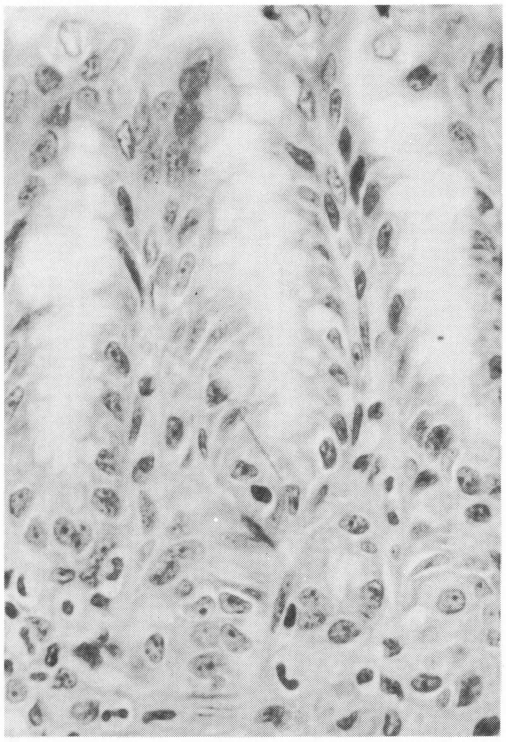

Fig. 8. Inflammatory cell infiltration in the antrum in Group A (HE stain, × 100).

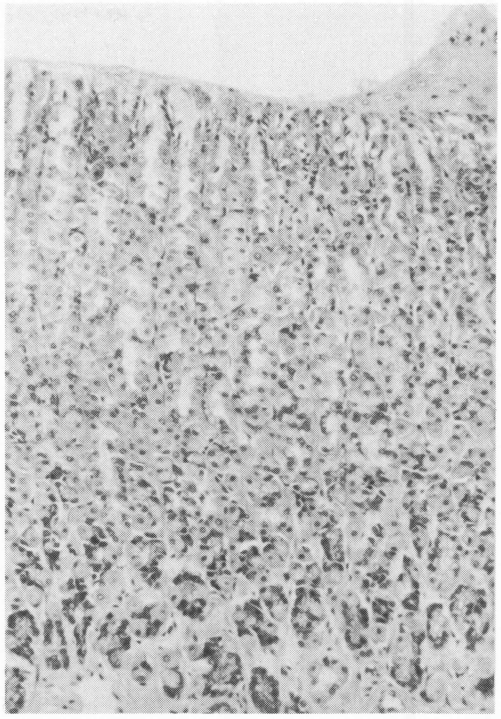

Fig. 9. Reduction of parietal cells and shortened mucosal length of the gastric fundobody after TCA in Group B (HE stain, × 100).

2. TCA

Atrophic changes were also present in the gastric mucosa in Group B given TCA alone. The atrophic changes were reduction of gastric glands as well as parietal cells and shortened mucosal length of both the fundobody and antrum (Fig. 9). The mucosal structure itself remained relatively regular in the fundobody mucosa, while the structure of the antral mucosa was somewhat irregular (Fig. 10).

Infiltration of inflammatory cells was marked in the stomach of Group B, but it was not marked in the stomach of Group C.

(1) Parietal cell population (Fig. 5)

The number of parietal cells per UA was 68.8 ± 15.6 cells in Group B and 109.8 ± 16.1 cells in Group C. The difference between the two was statistically significant ($p < 0.001$).

(2) Mucosal length (Fig. 11)

The length was 0.51 ± 0.06 mm in the fundobody mucosa and 0.21 ± 0.03 mm in the antral mucosa of Group B. The length was significantly reduced when compared to that of 0.65 ± 0.05 mm in the fundobody and that of 0.27 ± 0.04 mm ($p < 0.001$).

(3) Fibrotic changes (Fig. 12)

Fibrotic changes were severe in both the fundobody and antral mucosa of Group B, while the changes were slight in the fundobody as well as antral mucosa of Group C.

(4) Inflammatory changes (Fig. 13)

Round cell infiltration was severe in the lamina propria mucosae of both the fundobody and antral mucosa but more severe in the antrum than the fundobody of Group B, while it was slight in both the fundobody and antral mucosa of Group C.

(5) pH in the gastric juice

pH in the gastric juice was 3 or 4 in Group B and 2 in Group C.

DISCUSSION

It has been defined that atrophic gastritis is associated with reduction of gastric glands per se of the parietal cells in the gastric mucosa in man[24]. Atrophic gastritis was, therefore, also defined in the present study as reduction of

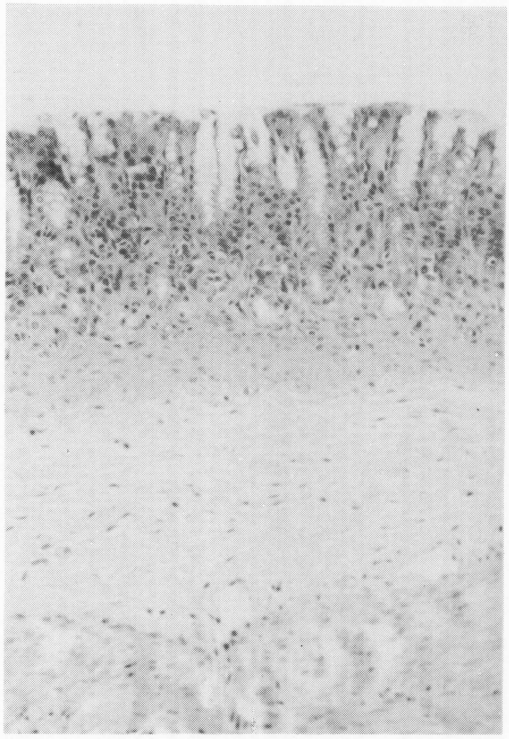

Fig. 10. The irregular structure of the mucosa and marked fibrosis were present in the antrum of Group B (HE stain, × 100).

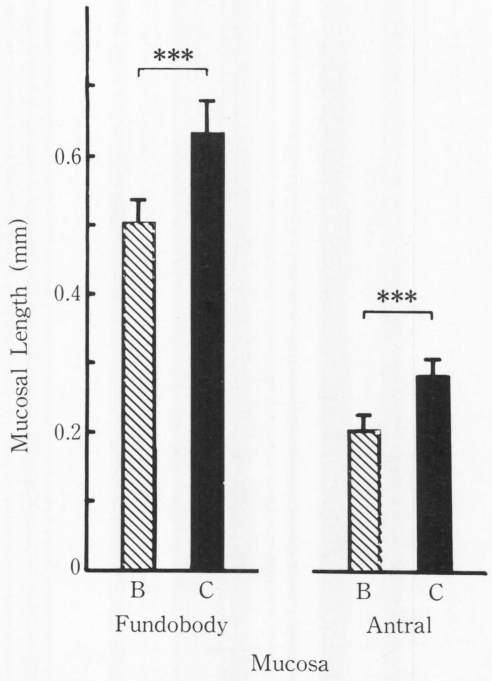

Fig. 11. A significant decrease in the length of both the fundobody and antral mucosa in Group B (shaded column) when compared to that in Group C (dark column).

parietal cells and shortened length of the mucosa together with cellular infiltration and development of fibrosis of the lamina propria mucosae. The present study showed that MNNG or TCA administration to rats resulted in development of atrophic gastritis which satisfied the foregoing definition.

It is generally known that MNNG is a strong carcinigenic agent in alkaline environment in animals, particularly in rats[23]. This can also develop atrophic changes in the mucosa, depending upon the dosage of this agent, being associated with various degrees of reduction of gastric glands per se, parietal cells and chief cells, intestinal metaplasia and hyperplasia of premature cells in the generalized zone of the glands in rats[36].

Present in Donryu rats of this study were marked reduction in the number of parietal cells in the fundobody mucosa, fibrous proliferation, and shortened mucosa in both the fundobody and antrum. These mucosal changes were typical for atrophic gastritis. Moreover, no tumourous lesions were observed in the stomach with the exception of one rat which had tumourous lesion, this rat was excluded from this study, although the dose of MNNG used here sufficient in developing tumour for eleven months. This is supported by the finding that development of tumours by MNNG is somewhat difficult in Donryu rats[26]. MNNG was administered to rats for 5 months and no treatment was given for more than 6 months thereafter. This is well accepted manner of developing experimental tumour in the stomach of Wistar rats. It may be considered that marked atrophic changes in the gastric mucosa of Donryu rats developed during the administration of the chemical for 5 months because it had been already observed at 3 months after administration[37]. Marked atrophic changes was still present at 11 months although no treatment was made to the rats after withdrawal of MNNG, suggesting that atrophic gastritis was irreversible. Such atrophic changes were more remarkable in the antral mucosa than in the fundobody mucosa. This suggests that the antrum

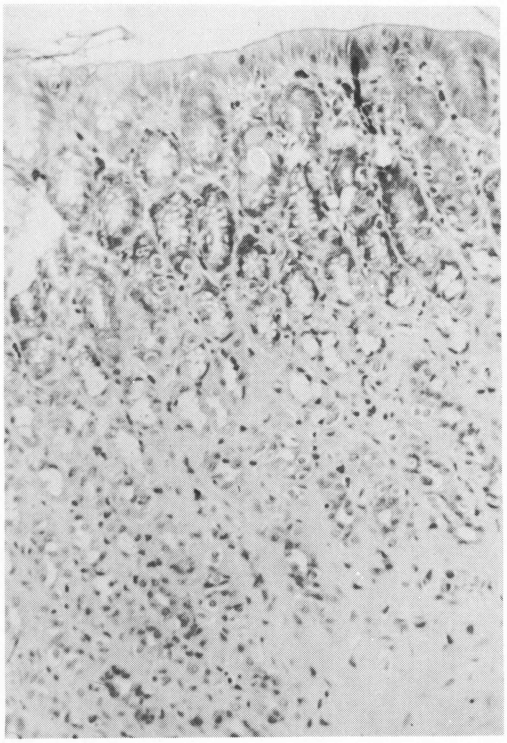

Fig. 12. Marked proliferation of fibrosis was present in the antrum of Group B (HE stain, × 100).

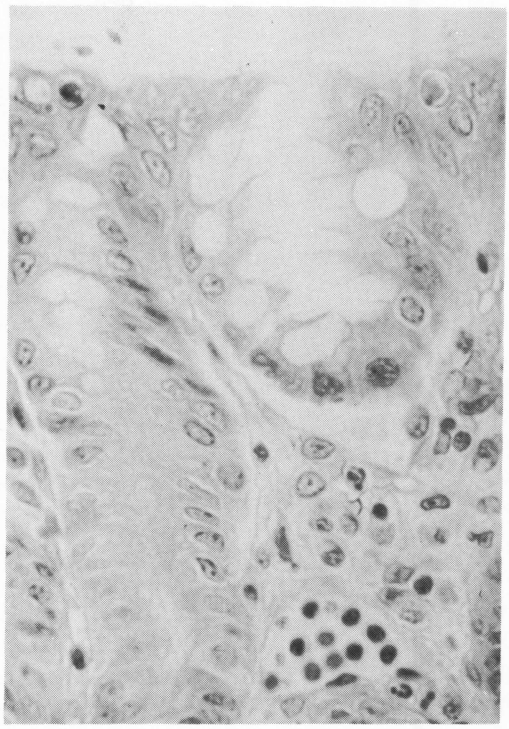

Fig. 13. Inflammatory cell infiltration was observed in the antrum of Group B (HE stain, × 100).

was more exposed to the chemical than the fundobody mucosa.

The mechanism of developing atrophic gastritis by MNNG could not be fully understand in the present study. MNNG, an acylalkyl nitrosoamine, is an active carcinogen which can directly react with multiple sites in the DNA molecule. This may affect the normal growth of gastric gland cells as well as epithelial cells and may alter cell renewal in the generalized zone. The mucosal surface of the glandular stomach actually developed an irregular cell arrangement and generative cell zone was injured in the initial phase after MNNG. This may cause mucosal atrophy. The amount of mucus in epithelial cells was also reduced. The damaged and diminished generative zone caused shortened length of the glandular mucosa. The mucosal changes including diffuse and multiple erosions continued during the administration of MNNG. The repeated cytotoxic effects of MNNG caused reduction of parietal cells and chief cells and changed the structure of glandular cells through gastric mucosal barrier[6,7] damaged by MNNG, which induced morphologically cellular irregularity of the luminal surface.

These may be the possible mechanism of the development of atrophic gastritis in Donryu rats by MNNG.

It has been pointed out that reflux of duodenal content, particularly bile, is likely important in the development of atrophic gastritis in the postoperative remnant stomach in man[1,8,21,29,39]. The action of bile on the gastric mucosa has also been demonstrated in animals by the authors[17] and other[34]. Lawson[20] showed that atrophic gastritis occurred in postgastrectomized remnant stomach in dogs by experimental reflux of the duodenal content into the stomach. The authors have observed that atrophic gastritis developed by oral administration of TCA, a compound of bile acids, suggesting that bile can be one of the causative factors of development of atrophic gastritis.

The gastric mucosal surface is covered with and protected by mucosal barrier[6,7]. It is

known that bile acids damage the gastric mucosal barrier and resulting gastric mucosa itself[5,9,27,34]. TCA, a compound of bile acids, may damage the gastric mucosal barrier as well as gastric mucosa, because gastric mucosal surface lesions were diffusely present in the stomach of rats with TCA in the present study. Cohen et al[4] also suggested that TCA may alter the normal gastric mucosal barrier. Ivey et al[14] and Ritchie and Shearburn[28] proposed a possible mechanism in which bile acids can break the mucosal barrier to ionic movement of sodium and hydrogen ions. This may result in back diffusion of hydrogen ions from the lumen with resultant mucosal damage[14,28,34]. The injury of TCA has been repeated to the gastric mucosa during administration of TCA for 11 months. This may cause cytolytic effect on parietal cells and resulting reduction of cells which leads to atrophic gastritis. Ritchie and Shearburn[28] and Black et al[3] have shown actual evidences that the concentration, pH, and type of bile acids are quantitatively related to these changes occurring in the gastric mucosa. Furthermore, it has been reported that unconjugated bile salts have greater inhibiting and cytolytic effects on parietal cells than conjugated bile salts[12] as a possible mechanism of development of gastritis. In the present study TCA administered for a long period can cause atrophic gastritis in Donryu rats by the foregoing mechanism.

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