RADIOLOGICAL STUDY OF CANINE STOMACH CANCER INDUCED BY N-METHYL-N’-NITRO-N-NITROSOGUANIDINE*1

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Aqueous solution of N-methyl-N’-nitro-N-nitrosoguanidine (MNNG) was given orally for 15 months in 4 dogs and follow-up examination of their stomach was made successively by radiography, endoscopy, and biopsy. Double-contrast photography revealed fine gastric mucosal damages. Dogs were sacrificed when they became moribund during the follow-up period. Eight cancers and one leiomyosarcoma were found histologically in the cardiac and pyloric portions of the stomach. Seven of these 9 lesions were visualized clearly in radiographs. Diagnosis of the lesions was 6 cancers or suspected cancers, and one polyp. The progress of these lesions was followed by radiographic examination.

X-Ray diagnosis of human gastric cancer has made a great advance in recent years. The double-contrast photography has played an especially important rôle in detecting many cases of early gastric cancer with a high degree of reliability.2,5,7) The work of Sugimura et al. has made it possible to produce stomach cancer in experimental animals including rats,8,9) hamsters,1) and dogs6) by the administration of N-methyl-N’-nitro-N-nitrosoguanidine (MNNG) solution in place of drinking water. In experiment on rats administered with MNNG, Kurihara et al.3,4) conducted X-ray examination on the animals before sacrifice. They succeeded in demonstrating the presence of a cancer while the rats were still alive, thus verifying the applicability of radiographic technique even with such small animals. This paper reports radiographic follow-up studies on the stomach of dogs after the discontinuation of MNNG administration.

MATERIALS AND METHODS

Animals  Four 3-month-old mongrel dogs (1 male and 3 females), weighing an average of 4.7 kg, were given a solution of 167 µg/ml of MNNG for the first month of the experiment and of 83 µg/ml for the following 14 months in place of drinking water. Pellet diet (No. 4, CLEA Japan Inc.) was given throughout the experiment. After 463 days, the administration of MNNG solution was discontinued and follow-up studies by radiography and endoscopy were started (Table I). As a control, a 3-month-old male dog weighing 5 kg was given water without the carcinogen.

Methods  Food was withdrawn the night before examinations and examinations were usually made on the evening of the following day. To anesthetize the animals, 0.5 ml/kg of Nembutal (Abbott Laboratories, U.S.A.) was injected intravenously. Then 0.5 ml of Buscopan (Boehringer Sohn, Ingelheim) was injected intramuscularly to block the parasympathetic nerves. Dogs were then placed on the fluoroscopic table in a prone position and 100% barium meal with a few drops of Gascon Drop (anti-foaming solution, Kissei Pharmaceutical Co., Ltd., Mastumoto) was introduced into the stomach through a gastric tube using a syringe. Radiographs were taken with a high-voltage fluoroscope, Toshiba KX-15 (Toshiba Nucleonics Co., Ltd., Tokyo) at 75 kV, 150 mA, with 0.08 sec exposure using Kodak RP X-ray films (Eastman Kodak Co., Rochester).

*1 This work was supported by grants from the Ministry of Education and from the Ministry of Health and Welfare. This paper forms Part IX of a series entitled “Production of Stomach Cancer in Experimental Animals by N-Methyl-N’-nitro-N-nitrosoguanidine.” Preliminary results have been presented at the 29th Meeting of the Nippon Societas Radiologica and at the 13th Annual Meeting of the Japanese Society of Gastroenterology.

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First, filling films were taken with 150 to 200 ml of barium meal in the prone position. Then double-contrast radiographs were taken in the prone, supine, right anterior oblique, and left anterior oblique positions. Endoscopy was also performed under conditions similar to that for radiography. Before endoscopy, mucous materials were removed by repeated washing with water containing Gascon Drops using a gastric tube and a syringe. A fiberscope, Olympus Model GTF-A (Olympus Optical Co., Tokyo) or fiber gastroscope Type BL (Machida Endoscope Co., Tokyo) was used.

### RESULTS

**Control Dog** The form and mucosal relief of the stomach, revealed by radiography, resembled those of humans, as shown in Photos 1 and 2. However, stomach of the dog was shorter than that of the human and axis of the stomach was at right angles to the esophagus at the esophago-cardiac junction. These differences are because the dog stands on four legs. No duodenal bulb or Kerckring's folds were observed.

**Case 1 (Dog No. 2):** Radiography was performed on day 466, 3 days after the discontinuation of MNNG administration. A slight angular deformity was demonstrated on the double-contrast radiograph in the supine position. An irregular mucosal relief with small filling defects and barium flecks were also observed on the posterior wall of the antrum, as shown in Photo 3. The lesions in the antrum were suspected to be malignant and to be shallowly ulcerated. A filling defect was also present in the small intestine, as shown in Photo 4.

The dog was sacrificed on day 518. Macroscopically, the antral mucosa was atrophic and slightly nodular, as suspected from the radiographic findings. A polyp was found in the small intestine.

Histological examination showed microscopic foci of intramucosal adenocarcinoma in the antrum and on the border of a healed ulcer in the angular portion near the lesser curvature. In the cardiac portion near the greater curvature, an adenocarcinoma was also observed, although the lesion was not detected in the radiographs. The polypoid lesion of the small intestine, which corresponded to the filling defect seen on radiograph, was a leiomyosarcoma, measuring $3 \times 3 \times 4.5$ cm.

**Case 2 (Dog No. 4):** Radiographic examination was first made on day 473, ten days after discontinuation of MNNG administration. A slight angular deformity was noticed. Mucosal ir-

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**Table I. Follow-up Study after Discontinuation of MNNG**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>MNNG administration</th>
<th>Follow-up study</th>
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<tbody>
<tr>
<td></td>
<td>30</td>
<td>500</td>
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<td></td>
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<td>900</td>
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<td></td>
<td></td>
<td>1100</td>
</tr>
</tbody>
</table>

- R = Radiology
- E = Endoscopy
- B = Biopsy
- * = Sacrificed

MNNG concentration in drinking water $\square 167 \mu g/ml \square 83 \mu g/ml$
regularity with shallow barium flecks was visualized in the antro-angular portion near the lesser curvature.

About 4 months later, on day 591, radiograph was again taken. A shortening of the lesser curvature and folds converging towards the gastric angle were visualized. One of the folds abruptly terminated at the margin of the depressed lesion and resembled a human gastric cancer. In the small intestine a large tumor with a crater was observed.

This dog was sacrificed on day 594. Macroscopically, the antral mucosa was atrophic and pale with a few shallow ulcers of up to 9 × 5 mm in size at the gastric angle along the lesser curvature. One fold converging towards the ulcerative lesion was interrupted near the demarcated margin, as seen in the radiographs. Microscopically, adenocarcinomas were present near the border of the healed ulcers and also on the top of the interrupted fold. In the cardiac portion near the greater curvature, another adenocarcinoma invading the submucosal layer was found. A large leiomyosarcoma (9 × 8 × 6 cm) was found in the small intestine.

Case 3 (Dog No. 1): The first radiography was performed on day 477, two weeks after the discontinuation of MNNG administration. As in Cases 1 and 2, an angular deformity and a slight shortening of the lesser curvature were observed. Poor antral distension was noted on the filling films. Irregular gastric areas were seen on the posterior wall of the antro-angular portion near the lesser curvature on the double-contrast films. These were suspected to represent a malignant lesion.

In the second radiograph taken on day 663, about seven months later, the angular lesion was not distinct. The posterior wall of the cardiac portion was not clearly visualized in radiographs on the first examination, but on the second examination, a large crater was seen with several small filling defects surrounding it (Photo 5). The lesion resembled a human gastric cancer of Borrmann's type III.

The dog was sacrificed on day 698, about one month after the last examination. Macroscopically, a tumor with two ulcers (1.3 × 0.9 and 1.0 × 0.6 cm) was present in the posterior wall of the fundic portion near the greater curvature. The borders of the ulcers were irregularly elevated, while the antral mucosa was widely atrophic and irregularly depressed. Slightly elevated granular lesions were also observed in this lesion.

Microscopic examination showed that these two lesions were adenocarcinomas, one infiltrating the serosal layer, and the other infiltrating the mucosal layer. As in Cases 1 and 2, a leiomyosarcoma was observed in the small intestine.

Case 4 (Dog No. 3): After discontinuation of MNNG, 5 radiographic examinations, two endoscopic examinations, and one biopsy were performed over a period of 19 months.

The first radiography was performed on day 463, just before discontinuation of MNNG administration. As in Cases 1, 2, and 3, indications of ulcerative changes, such as shortening of lesser curvature, converging folds, and poor antral distension, were observed, although these changes were more distinct. The lesion of the converging folds was diagnosed as an ulcerative type of cancer (Photos 6 and 7).

On day 728, about 9 months after the first radiography, several niches developed within the demarcated lesion near the gastric angle. On day 960, about eight months later, another large niche (shown by a wide arrow in Photo 8) appeared in the lesser curvature of antrum. Diffuse mucosal irregularity was evident near the gastric angle.

Endoscopy, performed on day 960, revealed a gray zonal mucosal area at the gastric angle with white coated patches and petechias (Photo 9). From histological examination of the biopsy specimens, this lesion was diagnosed as a mucocellular adenocarcinoma (Photo 10).
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In the cardiac portion, a sessile polypoid lesion about 9 mm in diameter was detected by radiography on day 878 (Photo 12a). Three months later, it seemed to have grown larger on the radiograph (Photo 12b). The lesion was also detected by endoscopy (Photo 13), but the biopsy specimens from the lesion did not show any cancerous growth.

The dog was sacrificed on day 1045, about 3 months after the final examination. Macroscopically, a depressed lesion, 2.8 × 2.7 cm in diameter, with a deeper ulceration within the lesion was observed in the angular portion (Photo 11). Some of the folds converging towards the lesion were interrupted midway. In the cardiac portion, near the greater curvature, there was a sessile polypoid lesion (1.3 × 1.2 cm) with a slight central depression. The surrounding mucosa was pale and rough, as suspected from radiographic findings. Histological examination showed multiple adenocarcinomas on the antro-angular portion near the lesser curvature and carcinoma cells invading the muscular layer beneath the deep ulceration. The polypoid lesion on the cardiac portion was a leiomyosarcoma. Another adenocarcinoma was present in the cardiac portion (as shown by double arrows in Photo 12b) and in extirpated specimen (Photo 11). An intestinal tumor was also found as in other dogs.

DISCUSSION

In this work we carried out radiological examination on dogs during development of stomach cancer after MNNG administration. We know of no previous reports on radiological examination of cancerous or precancerous lesions in the stomach of dogs. This work was performed in order to see whether it was possible to follow the development of cancers in dog stomach, since if this were possible, information from this kind of experiment should be very useful in relation to the development of human gastric cancer.

On postmortem examination, 9 main lesions were demonstrated macroscopically and histologically in the 4 dogs. They were 4 adenocarcinomas near the angular portion at the lesser curvature, and 4 adenocarcinomas and one leiomyosarcoma in the cardiac portion near the greater curvature.

Our final radiological interpretation of the lesions before sacrifice was 4 cancers (3 in the angular and one in the cardiac portion), 2 suspected cancers (one in the angular and one in the cardiac portion), and one polypoid lesion in the cardiac portion, as summarized in Table II.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Radiological diagnosis</th>
<th>Histological diagnosis&lt;sup&gt;a)&lt;/sup&gt;</th>
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<tbody>
<tr>
<td></td>
<td>Angular portion</td>
<td>Upper portion</td>
</tr>
<tr>
<td>1</td>
<td>Carcinoma</td>
<td>Not visualized</td>
</tr>
<tr>
<td>2</td>
<td>Carcinoma</td>
<td>Not clearly visualized</td>
</tr>
<tr>
<td>3</td>
<td>Suspected carcinoma</td>
<td>Carcinoma</td>
</tr>
<tr>
<td>4</td>
<td>Carcinoma</td>
<td>Suspected carcinoma, Polyp</td>
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</tbody>
</table>

<sup>a)</sup> Depth of invasion indicated in parentheses

Among these 7 lesions, which were diagnosed positively, 3 angular and 2 cardiac lesions could be followed in the radiographs. The 3 angular lesions were interpreted as two suspected cancers and one cancer from the first radiograph. After the follow-up examination of these three lesions, we concluded that one of the two suspected cancerous lesions progressed to resemble a human...
gastric cancer. This kind of changes seen from the second radiograph was less evident in the case of another suspected cancerous lesion. The lesion in the angular portion, which could be interpreted as cancer even in the first radiograph, became gradually demarcated during the following 19 months after the first radiography, indicating a more malignant type. Several ulcerations also appeared and subsided within and besides the margin of this cancerous lesion. Thus, 2 of 3 angular lesions developed a more malignant appearance in successive radiographs while one did not change much.

Among 5 cardiac lesions, 3 were seen in radiographs during the follow-up period while 2 were not seen. One of the 3 cardiac lesions was a distinctly ulcerative cancer resembling an advanced human cancer of Borrmann’s type III in the second radiograph, although it was not observed in the first. The other lesion was suspected to be an early cancer from the first radiograph but it subsequently remained flat and irregular without distinct ulceration for 7 months. The third lesion was a polypoid lesion, about 9 mm in diameter, which was first seen on day 878 and seemed to have grown larger 3 months later.

The malignant lesions, diagnosed radiologically, were classified into three types; flat, ulcerative, and polypoid. The most common sites of these lesions were the antro-angular portion near the lesser curvature and the cardiac portion near the greater curvature. These flat or ulcerative lesions seen in the radiograph were found to be adenocarcinoma on histological examination. In the two lesions with a crater, cancer cells infiltrated into the muscular or serosal layer, while in the flat or slightly depressed lesions, cancer cells were localized within the mucosal layer. Namely, deeply ulcerative lesions were advanced gastric cancer while flat or slightly ulcerative lesions were early cancer. These results support the validity of radiological diagnosis in this experiment and the usefulness of this method.

Some cancerous lesions in dogs resembled early human gastric cancer and some resembled advanced cancer. However, the demarcation at the cancerous lesions in radiographies was not so sharp in dogs as in humans.

Apart from the cancerous lesions mentioned above, other lesions were observed similar to those of human erosive gastritis and atrophic gastritis. On day 878, about 14 months after the discontinuation of MNNG administration, dog No. 3 showed several small filling defects accompanied with central depressions in the cardiac portion. Three months later, however, these lesions could not be observed in the radiograph, and another lesion resembling erosive gastritis appeared in the angular portion, as shown in Photos 8 and 12a. Another radiological finding supporting gastritis was the presence of decrease in the mucosal relief, especially in the angular portion near the lesser curvature and in the cardiac portion near the greater curvature. Histological examination revealed glandular atrophy in these two portions in all the dogs. In cardiac portion of dog No. 3, the thickening of mucosal muscle was observed corresponding probably to the places where erosions were found by radiology. Further studies are required to see whether these two kinds of lesions are related to carcinogenesis.

The occurrence of leiomyosarcoma in the small intestine was easily diagnosed by radiographic examination. The development of leiomyosarcomas in the small intestine interfered with follow-up studies on the process of development of stomach cancer, because stenosis, bleeding from sarcomas, and infection of necrotized tumor tissue frequently resulted in weakness or even death of experimental animals. Now we remove intestinal sarcomas by operation when they are diagnosed radiologically so that we can observe the process of development of stomach cancer for a sufficiently long period.

Further follow-up studies by radiography and endoscopy are in progress using Beagle dogs.
The authors express their sincere thanks to Dr. K. Tsukamoto, President of the National Cancer Center, and to Prof. M. Komiya and Dr. T. Katayama, First Department of Internal Medicine, Tokyo Medical and Dental University, for their encouragement and advice.

(Received July 19, 1973)

REFERENCES


EXPLANATION OF PLATES

Photo 1. Control dog. Double-contrast radiograph in the supine position.
Photo 2. Control dog. Filling film in the prone position.
Photo 3. Case 1. Double-contrast radiograph in the supine position after administration of MNNG for 15 months. Irregular barium flecks and small filling defects are seen in the antrum (arrows).
Photo 4. Case 1. Filling film in the prone position. Angular deformity and a large filling defect (arrow) can be observed in the small intestine.
Photo 5. Case 3. Double-contrast radiograph in the supine position, 8 months after the discontinuation of MNNG administration.
Photo 6. Case 4. Filling film in the prone position just before the discontinuation of MNNG administration. Antral distension is poor and the gastric angle is deformed.
Photo 7. Case 4. Double-contrast radiograph in the supine position. Folds are converging towards the gastric angle.
Photo 8. Case 4. Another profile of the niche (wide arrow) near the margin of the demarcated lesion, taken 14 months after Photo 7. Several round filling defects with central depressions are observed near the angular portion (small arrow).
Photo 9. Case 4. Endoscopic appearance of gastric angle, 8 months after Photo 8. Cancerous erosion with a white coating is observed near the angle of the lesser curvature.
Photo 11. Case 4. Gross appearance of the stomach of Case 4 (dog No. 3). A depressed lesion (2.8 × 2.7 cm) with a deep ulceration near the angle and a sessile polyp (1.3 × 1.2 cm) surrounded by pale and rough mucosa are recognized on the cardiac portion.
Photo 12a. Case 4. Double-contrast radiograph in the left anterior oblique position taken 13 months after discontinuation of MNNG. A sessile polyp (wide arrow) was found on the posterior wall of the cardiac portion. Polypoid lesions resembling those in human erosive gastritis (small arrow) were found on the posterior wall of the body.
Photo 12b. Case 4. Three months after Photo 12a. The polyp (wide arrow) seemed to have grown in diameter. The possible erosive gastritis could not be observed. Mucosal irregularity is observed in the posterior wall of the cardiac portion (arrows).
Photo 13. Endoscopy at the same time as Photo 12b. A sessile polyp is observed on the cardiac portion.
X-RAY STUDY OF CANINE GASTRIC CANCER

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