LEIOMYOSARCOMAS INDUCED BY ORAL ADMINISTRATION
OF N-METHYL-N'-NITRO-N-NITROSOGUANIDINE IN
GASTRIC CYSTS GRAFTED IN SUBCUTANEOUS
TISSUE OF MICE

(Plates LXXXIV~LXXXV)

Mutsushi MATSUYAMA,*1 Harumi SUZUKI,*1 and Takaaki NAKAMURA*2
(Aichi Cancer Center Research Institute*1)

Synopsis
Mice neonatally grafted with glandular stomach were continuously given N-methyl-N'-nitro-N-nitrosoguanidine (NG) dissolved in drinking water (50 mg/L) for 10 months. This dose of NG did not induce gastric carcinoma, but gave rise to leiomyosarcomas in the wall of the gastric cysts in the subcutaneous tissue, in 5 out of 26 mice. Atypical hyperplasia of epithelial cells in the cysts was also found in 2 mice. A strengthened and remote action of this carcinogen in gastric cysts was suggested.

INTRODUCTION
Sugimura and Fujimura9) succeeded in inducing tumors in the stomach of rats by the administration of N-methyl-N'-nitro-N-nitrosoguanidine (NG) dissolved in drinking water. The tumors were confined to the glandular stomach and upper part of the intestine in their experiment. A technique for the induction of the cyst of glandular stomach, which contains acid solution, has been established in the subcutaneous tissue of mice.5) These findings prompted us to test whether NG had a remote action of carcinogenesis in the gastric cyst in the subcutaneous tissue when it is applied orally.

MATERIALS AND METHODS
Newborn mice of the dd/I strain, of both sexes, were grafted with sheets of the glandular stomach of the litter mates, as previously described.5) When the animals became 2 months old, at which the gastric cysts were established in the subcutaneous tissue in the left axillary region, they were divided into two groups (Table I). One group of mice was continuously administered with NG (K and K Laboratories, Inc., N.Y.), dissolved in drinking water at a concentration of 50 mg/L, for 10 months (Group 1). Thereafter, they were given plain water without NG. Another group of mice was given plain drinking water throughout the experiment, as a control (Group 2). Mice grafted neonatally with fragments of the heart (Group 3) and nonoperated mice (Group 4) were also given NG, same as the mice in Group 1 (Table I). The water bottles on cages were shielded by aluminum foil to protect NG from degradation by light and refilled

*1 Kanokoden, Tashiro-cho, Chikusa-ku, Nagoya 464 (松山隆司, 鈴木泰重).
*2 Present address: Department of Pathology, Nagoya City University Medical School. Kawa-
sumi-cho, Mizuho-ku, Nagoya 466 (中村隆昭).
Table I. Tumors Induced by Oral Administration of NG in dd/I Mice Bearing Subcutaneous Gastric Cysts

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Neonatal grafting</th>
<th>Treatment</th>
<th>No. mice at termination of treatment</th>
<th>No. of mice with</th>
<th>other tumors</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>graft</td>
<td>sarcoma of cyst</td>
</tr>
<tr>
<td>1</td>
<td>Stomach</td>
<td>NG*</td>
<td>33</td>
<td>26</td>
<td>5</td>
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<td>2</td>
<td>Stomach</td>
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<td>3</td>
<td>Heart</td>
<td>NG*</td>
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<tr>
<td>4</td>
<td>None</td>
<td>NG*</td>
<td>18</td>
<td>(−)</td>
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* NG, dissolved in drinking water (50 mg/L), was continuously given for 10 months, beginning when the mice were 2 months old.
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with a freshly prepared NG solution 3 times a week. All the mice were fed freely with CMF diet (Oriental Yeast Co., Tokyo). The animals were autopsied when they died or were killed at the termination of the experiment, 18 months after birth. The stomach, duodenum, stomach grafts, and other organs affected were fixed in 10% Formalin, followed by routine procedures of dehydration and embedding, and the sections were stained with Hematoxylin and Eosin. Other sections of these organs were stained with Prussian Blue, periodic acid-Schiff, Alcian Blue, Elastica-van Gieson's, and silver impregnation, when needed.

RESULTS

Incidence of the grafts and tumors is recorded in Table I. Since there was no sex difference in the incidence, except ovarian tumors, the results from both sexes were totaled in each group. In agreement with previous studies,3-5) the stomach grafts usually formed macroscopic cysts at the site of grafting, except in a few cases (Table I). All of the cysts of the mice given NG (Group 1) contained chocolate-colored, condensed solution (Photo 1), whereas content of the cysts of the mice given water alone (Group 2) was clear and serous. Histological examinations revealed severe bleeding in the muscle and submucosal layers of the cysts, forming a thick band of accumulation of macrophages full of hemosiderin granules, in all the mice of Group 1 (Photo 2). Tumors in the grafts developed in 5 out of 26 mice in Group 1 after 311-415 (average 361) days from the beginning of the treatment (Table I and Photo 3). The tumors consisted mainly of spindle-shaped cells with scattered large polygonal cells and some mitotic figures, showing irregularly interwoven pattern (Photo 4). These were diagnosed as leiomyosarcomas. Four of these were thumb-tip sized and contained large area of necrosis. At the time of autopsy no gastric cysts, which had been palpated before, was recognized. One other was found as a small sarcoma nodule in the cyst wall (Photo 5). Atypical hyperplasia of epithelial cells was also found in the cyst wall in 2 mice of Group 1 (Table I, and Photos 6 and 7). These hyperplasia, consisting of cells which showed no definite reaction to periodic acid-Schiff reagent, were multi-focal, but never found in the mice of Group 2. The mice grafted with heart fragments (Group 3) had no macroscopic remnants or tumors in the grafted region (Table I). Microscopic examination was not made with these grafts. All the cysts of the mice in Group 2, given water alone, retained their normal structure.

Majority of the host stomach in the mice of Groups 1, 3, and 4 treated with NG showed moderate to severe atrophy in the mucosa, especially severe in the pyloric region, but no tumor was found in the stomach, except one hemangioma. Duodenal tumors develope in 7 and 5 mice in Groups 1 and 4, respectively (Table I). These tumors were found 340−548 (average 461) days after the beginning of the treatment. In the mice of Group 2 there was found no tumor in the cysts or in the gastrointestinal tract. Tumors of the other organs were found in a few mice of each group (Table I).

DISCUSSION

NG has been reported as "local" carcinogen, producing tumors at the site of application.1,7,9,10) Schoental and Bensted8) have also revealed that NG induces in rats mainly tumors of the forestomach, when given intragastrically, and tumors of the intestines
when injected intraperitoneally. The results of the present study revealed, however, that NG had carcinogenic potency to gastric cysts in the subcutaneous tissue, even when it was given orally. The mechanism of the carcinogenic action of NG in the subcutaneous cysts is obscure, but it is apparent that NG or its derivative(s) can reach the cysts via blood stream and induces severe hemorrhage and tumors. Saito, Iguchi, Takayama, and Sugimura§ found that NG labeled with $^{14}$C at guanidino-carbon was incorporated in a higher concentration into the stomach and small intestine in rats. This may also be the case for gastric cysts in the subcutaneous tissue of mice. The possibility of retention of NG in the lumen of the cysts can be excluded, since the carcinogen is degraded rapidly into noncarcinogenic derivatives in an acid condition.

In parallel experiments, in which multipotent carcinogens (N,N′-2,7-fluorenylenebisacetamide and urethan) were administered to mice bearing grafts of the stomach or the pancreatic islets, tumor of the grafts was scarcely produced, whereas many tumors were found in various other organs (Ito, Matsuyama, and Nagayo, unpublished data). These results may be related to the fact that the grafted organs, even ectopically implanted, are fairly resistant to multipotent carcinogens applied by a remote route.

Direct injection of 7,12-dimethylbenz[a]anthracene into the cysts gave rise to leiomyosarcomas originating from the muscle layers of the cysts, but produced no definite adenocarcinoma. In the present experiment, orally administered NG induced the same tumors, instead of epithelial tumors, in the cyst wall. Moreover, these sarcomas in the gastric cysts developed earlier than the tumors of the duodenum. It was also revealed that the stomach of the mouse was insensitive to NG, even though a larger dose (200 mg/L, dissolved in drinking water) was used. These results may lead to the conclusion that epithelial cells are less susceptible to carcinogens than muscle cells in the mouse stomach.

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REFERENCES

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EXPLANATION OF PLATES LXXXIV~LXXXV

Photo 1. Female dd/I mouse administered with NG for 10 months. Gastric cyst of dark color, due to hemorrhage, in the left chest wall. Hemorrhage in the gastrointestinal tract may be noted through the abdominal wall. ×1.0.

Photo 2. Cyst wall with accumulated macrophages, full of hemosiderin granules. Inside of the wall was covered by one-cell layered surface mucous cells (arrows), but erosions are sometimes found. In the lumen (left) bled cells and debris are seen. Prussian Blue stain. ×57.

Photo 3. Male dd/I mouse administered NG. Large tumor in the grafted area of the left axilla. ×0.9.

Photo 4. Leiomyosarcoma mass consisting of spindle-shaped cells with some mitotic figures. Hematoxylin and Eosin stain. ×166.

Photo 5. Branched cyst containing thick and thin mucosa, and a small sarcoma nodule (arrow) in the muscle layers. Hematoxylin and Eosin stain. ×5.9.

Photo 6. Branched cyst containing erosive thin mucosa, well-developed thick mucosa, and an area of atypical hyperplasia (arrow). Hematoxylin and Eosin stain. ×4.8.

Photo 7. Atypical hyperplasia of the epithelium of the cyst wall, invading the muscle layers (left). Hematoxylin and Eosin stain. ×166.