

## Early Gastric Cancer Induced by N-Ethyl-N'-nitro-N-nitrosoguanidine in a Cynomolgus Monkey Six Years after Initial Diagnosis of the Lesion

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**A signet ring cell carcinoma in the gastric antrum of a Cynomolgus monkey induced by N-ethyl-N'-nitro-N-nitrosoguanidine was sequentially studied by endoscopy, biopsy, and autopsy. The carcinoma was first detected on the angulus of the stomach at the 38th month as a slightly elevated lesion. Sixty-one months later this tumor was found to be still in the "early" (intramucosal) stage. Another, independent, initial gastric cancer was also discovered. This is the first example of an induced gastric carcinoma remaining in the "early" stage during a six-year follow-up period after the initial histologic diagnosis.**

**Key words:** Early gastric cancer — Monkey carcinogenesis — Follow-up — N-Ethyl-N'-nitro-N-nitrosoguanidine

The term "early gastric cancer" (EGC) does not have any chronological meaning. This term was defined after investigation of the curable phase of human gastric carcinomas by Japanese researchers. It encompasses only the depth of the cancer infiltration (mucosa or mucosa and submucosa), regardless of the presence of lymph node metastasis or the extent of the lesion.<sup>1)</sup> Treatment of EGC is based on the presumption that, if left untreated, it will progress to advanced cancer. It is difficult to determine the time course of EGC, because after the initial diagnosis, there is no ethical basis to deny the patient treatment. However, in a limited number of retrospective<sup>2)</sup> or prospective studies<sup>3)</sup> in which the surgical operation was delayed or not carried out for some reason (for example, refusal of permission for operation by a patient or the family), the duration of EGC was estimated. These results permitted the conclusion that a certain number of EGC seem to have a relatively slow growth rate and remain "early" for several years.<sup>4)</sup>

By contrast, among many experiments investigating the sequential morphologic, radiologic, and endoscopic changes of chemically induced gastric carcinomas and their precarcinomatous lesions,<sup>5-11)</sup> there have been no data about EGC existing for several years. In this case report we present an example of an N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG)-induced EGC that remained in the "early" stage during a six-year follow-up period after the initial histologic diagnosis by endoscopic biopsy.

Other aspects of the experiment were previously published as the first report on the induction of gastric carcinoma by a chemical carcinogen in nonhuman primates.<sup>12)</sup> Case 5 in that report corresponds to the monkey whose pathology is presented here.

### MATERIALS AND METHODS

The young adult Cynomolgus monkey (*Macaca irus*) was purchased from CLEA Japan, Inc., Tokyo, housed in a metal cage and fed AB chow for monkeys (Oriental Yeast Co., Ltd., Tokyo) with fruit or vegetables daily. ENNG (CAS: 63885-23-4, Aldrich Chemical Co., Inc., Milwaukee, WI) was dissolved in distilled water at a concentration of 1 mg/ml, diluted with tap water to 200 µg/ml three times a week and given *ad libitum* for 26 months. The stomach was examined by endoscopy and biopsy in months 25, 32, 38, 50, 61, and 108. The monkey was killed in the 109th experimental month and autopsied carefully. The entire stomach and esophagus were cut into 5 mm-wide step sections, embedded in paraffin, and stained with hematoxylin and eosin (H-E). Selected parts of the stomach were sectioned serially.

### RESULTS

The estimated total ENNG intake was about 23 g in 26 months. Examination by endoscopy and biopsy in months 25, 32, 50, 61, and 108 did not reveal any lesion in the stomach. However, upon endoscopy in month 38, a slight elevation in the angulus and an increase in redness of the antral mucosa were detected. In the biopsy specimen taken from this lesion, intramucosal signet ring cell carcinoma was found (Fig. 1), as reported previ-

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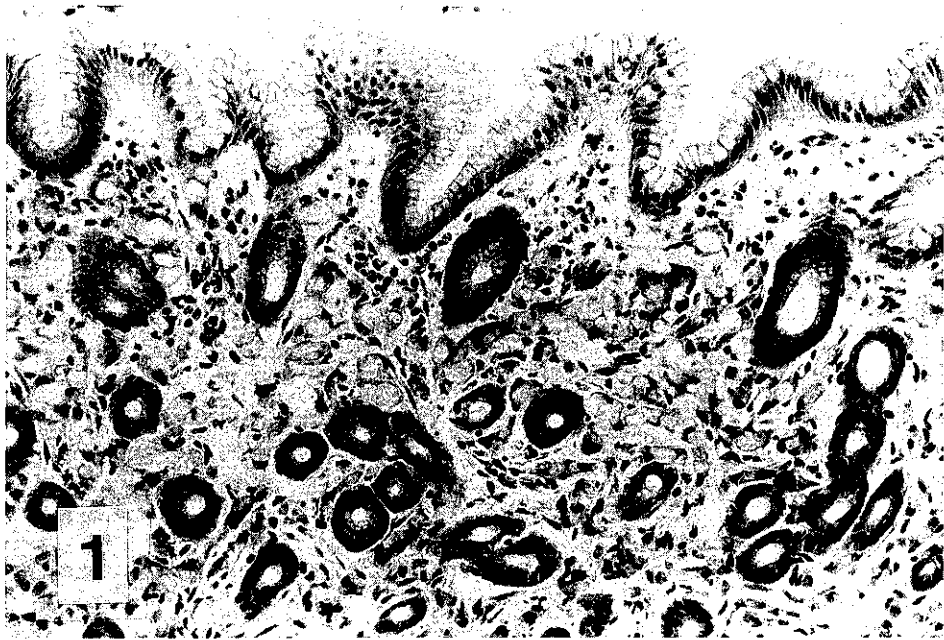


Fig. 1. Histologic appearance of signet ring cell carcinoma induced by ENNG in a biopsy specimen taken from the prepyloric mucosa along the lesser curvature, at the 38th experimental month. H-E.  $\times 200$ .

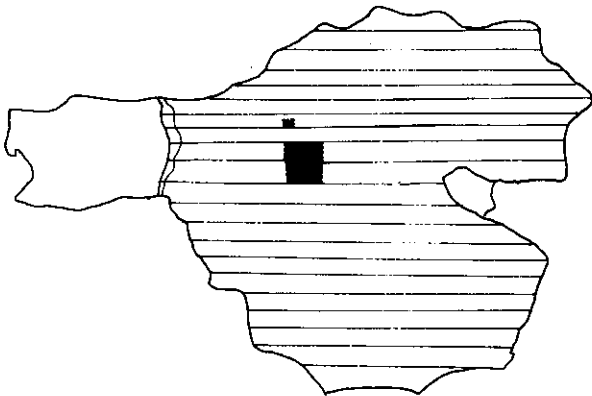


Fig. 2. Localization of the carcinomas in the autopsied stomach of the Cynomolgus monkey. Shaded areas represent carcinoma.

were found (Fig. 2). One was a microscopic poorly differentiated carcinoma located in the glandular neck region of the posterior wall of the prepyloric mucosa. The second was another tumor, 10 mm in diameter, consisting mainly of signet ring cells (Fig. 3A) and, in the central eroded part, of poorly differentiated cells with a trabecular pattern (Fig. 3B). The histologic structure of the carcinoma in the biopsy material is identical to the peripheral part of this tumor. In serial sections, no submucosal infiltration was found. Metastases could not be detected either.

#### DISCUSSION

As has already been reported, the histologic appearance of chemically induced gastric carcinomas in monkeys is very similar to those in human and to those induced by ENNG in dogs. Furthermore, it has been found that the period necessary for development of the induced tumor is longer in monkey than in rodent or dog, probably due to the longer life-span of monkeys.<sup>12, 13)</sup>

In the present case, a signet ring cell carcinoma was detected in a biopsy specimen obtained by endoscopy in the 38th month of the experimental period. However, we could not detect this tumor in three subsequent biopsy specimens, because the original, slightly elevated lesion became flat and therefore invisible to endoscopy, so the

ously.<sup>12)</sup> It was known that the tumor was not dissected out totally at the biopsy, because the margins of removed mucosal tissue were also infiltrated by carcinoma cells.

At autopsy, the antral mucosa did not show any visible lesion, but after fixation in 10% neutralized formalin, a small depression was detected in the prepyloric region along the lesser curvature. Two independent cancer foci

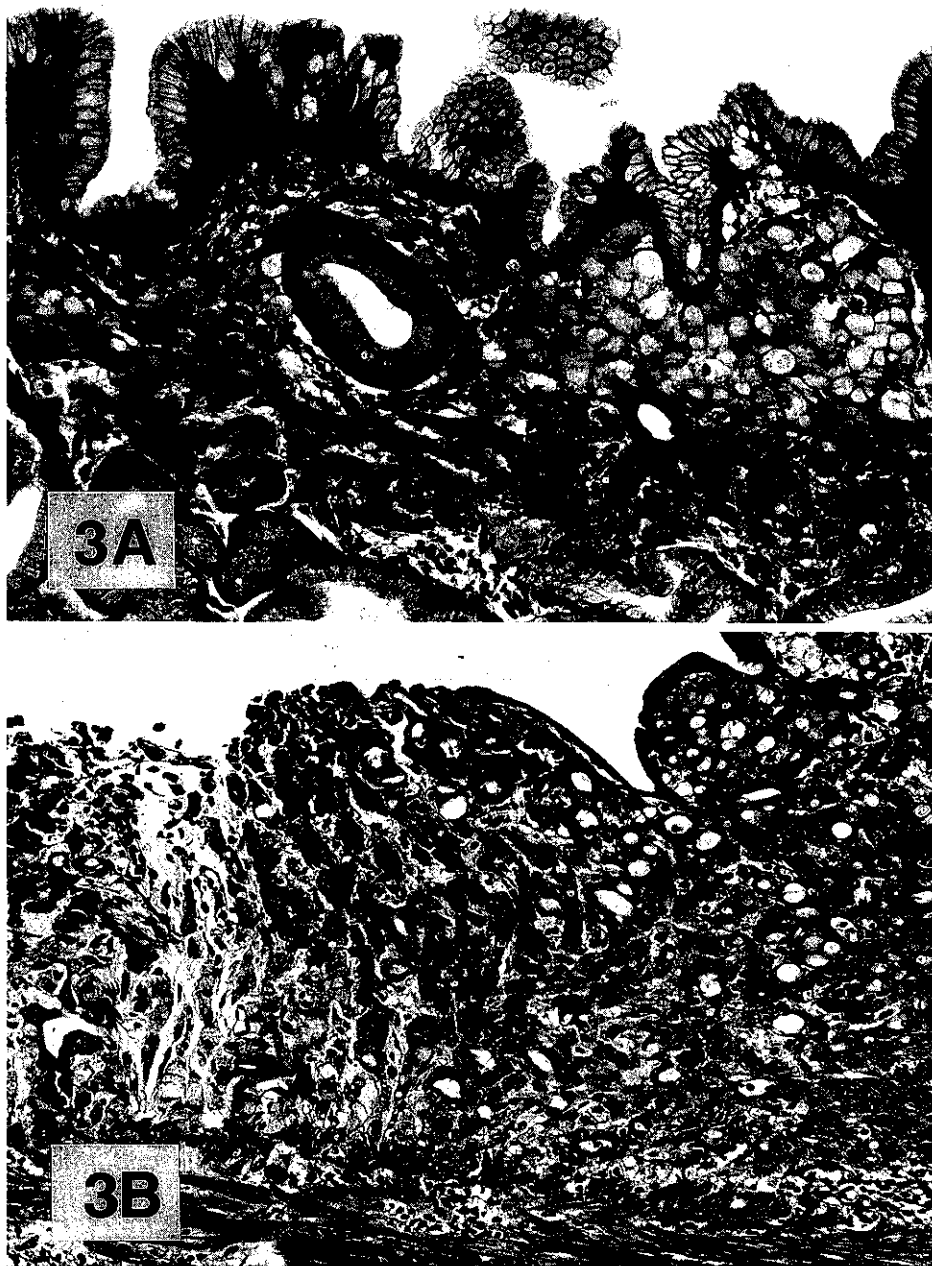


Fig. 3. A. Peripheral part of the larger gastric cancer consisting of signet ring cells in the upper 2/3 portion of the stomach mucosa. Autopsy specimen. H-E.  $\times 200$ . B. Central part of the tumor in Fig. 3A. Poorly differentiated tumor cells and signet ring cells arranged in trabecular fashion in the entire mucosa, with a shallow erosion but without submucosal infiltration. H-E.  $\times 200$ .

biopsy specimens were consequently taken from the wrong places (intermediate zone and fundus). A similar tendency of the macroscopic type of EGC to change in this way in humans has been reported.<sup>14)</sup> Sixty-one months later at the autopsy, nevertheless, the signet ring cell carcinoma was detected again in the same region of

the stomach as before, still in the "early" (intramucosal) stage. The histologic pattern of the second, microscopic carcinoma found only at the autopsy was different.

Sequential studies by radiology, endoscopy, and biopsy for investigation of the development of MNNG-induced gastric carcinomas have been carried out on dogs.<sup>5,6)</sup>

Progression was detected without the tumor first remaining in the "early" stage for a period of years. This phenomenon clearly shows that the EGC is a real carcinoma. Since there are no precarcinomatous conditions of the gastric mucosa comparable to any variants of the signet ring cell carcinomas that might lead to a misdiagnosis,<sup>10)</sup> it is easy to exclude the possibility of diagnostic error.

Although the spontaneous regression of an induced, well differentiated adenocarcinoma in the stomach of a dog has been described,<sup>15)</sup> in the present case there were no signs of tumor regression. Moreover, the induced gastric carcinomas of all the monkeys in this experiment showed a wide variety of histologic types,<sup>12)</sup> so it is highly

unlikely that the first carcinoma recognized six years ago underwent a complete regression and a new carcinoma appeared with the same histologic pattern.

A possible explanation for intramucosal gastric carcinoma persisting for a long period is based on the observation that experimentally induced signet ring carcinoma cells in the early stage have a tendency to spread in upward and lateral directions, rather than to the submucosa. Indeed, in the superficial layer the tumor exhibits a significant rate of cell loss with an estimated cellular turnover rate of 4.4 days.<sup>16)</sup> This might have the result that some gastric cancers remain at the "early" stage for years, possibly in both monkeys and humans.

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