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Gastric Tumors and Lung Lesions in the Rat following the Intragastric or Intraperitoneal Administration of *N*-(β-Chloroethyl)-*N*-nitrosourethan

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SUMMARY

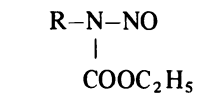
N-(β-Chloroethyl)-*N*-nitrosourethan given intragastrically to rats in one or two doses not exceeding 15 mg/kg body weight induced tumors among 12 rats that survived longer than 1 year, one of the esophagus, one of the forestomach, and three of the glandular stomach.

When a single dose of *N*-(β-chloroethyl)-*N*-nitrosourethan was given i.p. (about 5 mg/kg body weight), there were many early deaths from exudative and proliferative alveolar changes in the lungs, and none of the rats survived longer than 6 months.

The possible reasons for the high efficacy of *N*-(β-chloroethyl)-*N*-nitrosourethan as a carcinogen are discussed.

INTRODUCTION

Among the carcinogenic alkylnitroso compounds (5), MNU[1] (I) and its ethyl homolog, ENU (II), were effective carcinogens for the gastrointestinal tract. MNU was the more active; when given to young rats by stomach tube, it induced squamous carcinoma of the forestomach and the esophagus and/or adenocarcinoma of the glandular part of the stomach even with a single dose, 50 to 100 mg/kg body weight (6, 9, 11). When injected i.p., a single dose of MNU, 50 mg/kg body weight, induced adenoma and adenocarcinoma of the intestines (10). It was of interest to test in a comparable way for carcinogenic action the closely related CENU (III). The results are reported herewith.



in which R is CH_3 for MNU (I), CH_3CH_2 for ENU (II), and $ClCH_2CH_2$ for CENU (III).

MATERIALS AND METHODS

White young rats of the Porton strain bred in the Carshalton laboratories were used. They were housed in groups of 2 to 6 in metal cages and were given the normal diet (MRC Diet 41B) and water *ad libitum* throughout their lives. The animals were weighed at the beginning of the experiments and at monthly intervals (or more often) until they died or were killed (by coal gas) when obviously ill.

CENU (a yellowish oil) was a gift from Dr. Bebbington, Porton, Wiltshire, England. Solutions of it in arachis oil (1%) were freshly prepared before use.

In 1 experiment, 24 male weanling rats (45 to 60 g body weight) were given single doses of CENU by stomach tube (2 to 50 mg/kg body weight). Nine of these rats were given a 2nd intragastric dose (2 to 10 mg/kg body weight) 3 months later.

In a 2nd experiment, 4 male and 15 female rats (75 to 120 g body weight) were given a single dose of CENU in arachis oil by i.p. injection, 0.5 mg/rat or 4.2 to 6.5 mg/kg body weight.

RESULTS

Intragastric Administration of CENU

In those rats that received a dose higher than 10 mg/kg body weight, death occurred within 2 to 14 days. In these cases, the main autopsy findings were limited to the stomach (and esophagus), with a mucosa that showed superficial ulceration and necrosis together with submucosal edema. One rat showed acute duodenal ulceration at 14 days.

In the early cases, the lungs showed little significant pathological change apart from some increased cellularity in the alveolar walls.

Twelve rats survived for 12 to 25.5 months. In 6 out of the 12 rats, there was an unusually high incidence of lung infections (bronchitis and lung abscesses), including 1 case of adenomatosis. Three of these rats also had brain abscesses. The treatment and survival times of rats that had significant lesions are summarized in Table 1.

Tumors of the Stomach and of the Esophagus. Five out of these 12 rats showed gastric tumors at autopsy, 1 in the esophagus, 1 in the forestomach, and 3 in the glandular stomach. The tumor classification conformed to that of Stewart *et al.* (13).

Two squamous tumors occurred in rats that died at 17 and 25 months. The 1st tumor, of the esophagus, a poorly differentiated squamous cell carcinoma, penetrated through all the muscle coats to the serosa. There was no evidence of

[1] The abbreviations used are: MNU, *N*-methyl-*N*-nitrosourethan; ENU, *N*-ethyl-*N*-nitrosourethan; CENU, *N*-(β-chloroethyl)-*N*-nitrosourethan.

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Table 1
The treatment, survival times, and main lesions found in rats given CENU intragastrically

Animal Nos.	Dosage (mg/kg body weight)	Survival	Main lesions
1	50 + 50	5 days, K[a]	Stomach: mucosal necrosis and squamous atrophy
2	30	14 days, K	Acute duodenal ulcer
3	6 + 2	3 mo., D	Stomach: mucosal atrophy and submucosal edema. Loss of keratin
4	6 + 3	12 mo., K	Liver granulomata; brain chronic abscess
5	4 + 3	12.5 mo., K	Thickening of stomach wall and inflammatory cells
6	10	17 mo., K	Squamous cell carcinoma of esophagus
7	10 + 3	24.5 mo., K	Large s.c. fibrosarcoma
8	10 + 3	24.5 mo., K	Stomach: adenocarcinoma with ectopic bone
9	10	25 mo., K	Stomach: squamous cell carcinoma
10	6	25.5 mo., K	Stomach: "adenocarcinoma." 2 cerebral abscesses
11	6 + 3	25.5 mo., K	Stomach: "adenocarcinoma" with ectopic bone

[a] K, killed; D, died.

metastasis. The glandular part of the stomach showed mucosal atypia, with irregularly shaped and dilated glands and hyperchromatism of the cells, but there was no malignant change.

The 2nd tumor was a well-differentiated keratinizing squamous cell carcinoma of the forestomach penetrating through to the serosa (Figs. 1 and 2). The glandular part of the stomach again showed mucosal atypia, with a marked eosinophilic cellular infiltration in the wall and arterial thickening.

Tumors of the Glandular Stomach. The tumors of the *glandular* stomach were found in rats that died at 24, 25, and 25 months. The 1st rat had a large ulcerating and deeply penetrating tumor at the pyloric end of the stomach (Fig. 3). Histologically, it proved to be a fairly well-differentiated adenocarcinoma penetrating through to the serosa (Fig. 4). Ectopic bone was present in the tumor. In the forestomach, there was no marked mucosal change, but the submucosa showed edematous thickening.

The next rat had a well-differentiated tumor with cystic glandular elements. It penetrated the muscularis mucosae but no further. Ectopic bone was again present. As such, it should be regarded as a "precancer" (13).

The 3rd rat had a well-differentiated glandular tumor, which had provoked a marked host response of chronic inflammatory cells and fibrous tissue. Although the muscle coat was breached and filled in with scar tissue, it was difficult to identify tumor elements presenting at the serosa. The forestomach mucosa showed no significant change.

In those rats that did not develop gastric tumors after 12 months, the main changes observed in the stomach were mucosal atypia with glandular dilation and distortion, focal collections of inflammatory cells in the submucosa, and in 1 case a hyperplastic, warty appearance of the forestomach.

Administration of CENU i.p.

Administration of CENU by i.p. injection of a single dose not exceeding 6.5 mg/kg body weight resulted in a considerable number of early deaths, 8 dying and 1 being killed within 2 to 15 days, 5 dying and 4 being killed within 1 to 2.5 months, and only 1 rat surviving 6 months after the dose.

In the rats that died early, there was edema at the site of injection, pleural effusions, and congestion of the lungs.

The main lesions were in the lungs, which on histological examination showed generalized edema in the alveoli together with numerous alveolar macrophages and giant cells. The alveolar walls were very congested and cellular. Perivascular edema and adventitial cellular infiltrations of arterioles were also evident, together with increased connective tissue.

By 1 month after injection, the lung changes had become much more pronounced, with apparent "honeycombing" of the lung and thickening of the alveolar walls, together with numerous macrophages and giant cells. Some of the rats that survived longer than 1 month developed strikingly distended small bowel and cecum. Histologically, there was marked edema of the mucosa and dilation of the lymphatic vessels. In the solitary rat that survived 6 months, the lungs showed no residual fibrosis. Its forestomach had a warty hyperkeratotic appearance, but there was no invasion of the wall. No significant chronic changes were present in the livers of the rats regardless of whether they were given CENU intragastrically or by i.p. injections.

DISCUSSION

The results of this investigation show that CENU is a gastric carcinogen able to induce both squamous carcinomas of the forestomach and the esophagus and adenocarcinomas of the glandular part of the stomach with 1 or 2 doses. In this respect, it resembles MNU but appears to be more active, the effective carcinogenic dose being only about 10% of that of MNU. The localization of gastric tumors after CENU [as well as after MNU (9)] in both the forestomach and the pylorus could be due either to the possibility that the intragastric needle could have reached a different part of the stomach or that the amount of food could have been variable, as the rats were not starved before dosing.

The experiment in which a single dose of CENU not exceeding 6.5 mg/kg body weight was injected i.p. resulted in the death of 18 out of 19 rats in less than 10 weeks and of the last rat after 6 months.

The survival time of these rats was too short for tumors to develop. MNU injected i.p. induced intestinal tumors (10); the striking distention of the intestines seen among the rats that survived 5 to 10 weeks after i.p. injection of CENU indicates that CENU might also induce intestinal tumors if the dosage were appropriately adjusted to allow longer survival of the animals. Such experiments are in progress.

An additional point of interest from these experiments is in the marked lung changes that were present in the rat in the 1st 10 weeks after i.p. administration of relatively small quanties of the compound.

The marked exudative and proliferative changes observed in the lungs resemble in many ways those following the treatment of rats with MNU (10) and with pyrrolizidine alkaloids, especially those from the *Crotalaria* species plants such as fulvine (1) and monocrotaline (4), insofar as there was alveolar edema with thickening of the alveolar walls and apparent increase in connective tissue.

However, the presence of giant cells, together with the above proliferative changes, resembles also the features seen in rats after paraquat poisoning (2, 3).

The early deaths of the rats given CENU by the i.p. route very probably resulted from these lung changes, and the high incidence of lung infections following the intragastric administration of CENU might well have resulted from chronic lung damage in those rats that did not have gastric tumors.

Can the structural features that distinguish CENU from MNU explain the higher carcinogenic efficacy of the former? MNU has been shown to interact with free thiols with evolution of nitrogen as gas, the released methylating moiety substituting thiol or other nucleophilic groups *in vitro* (12) and *in vivo* (7, 8). The ability to interact with free thiols is a general property of alkynitrosourethans, including CENU (R. Schoental, unpublished results). However, in the case of CENU the released alkyl moiety contains chlorine, which being an additional functional group could react with another nucleophilic group of cell constituents and form an ethylene bridge inter- or intramolecularly. Such a bridge might persist locally and be more effective than 1-point substitutions by an alkyl group in causing the chronic effects.

In the present experiments, CENU was found to induce gastric tumors with a dose that is equivalent to only about 10% that of MNU. This indicates that the predominant part of the administered dose of MNU is probably involved in metabolic processes unrelated to the initiation of cancer.

CENU appears, therefore, to be a suitable compound for the investigation of the products and the mode of its action with cell constituents.

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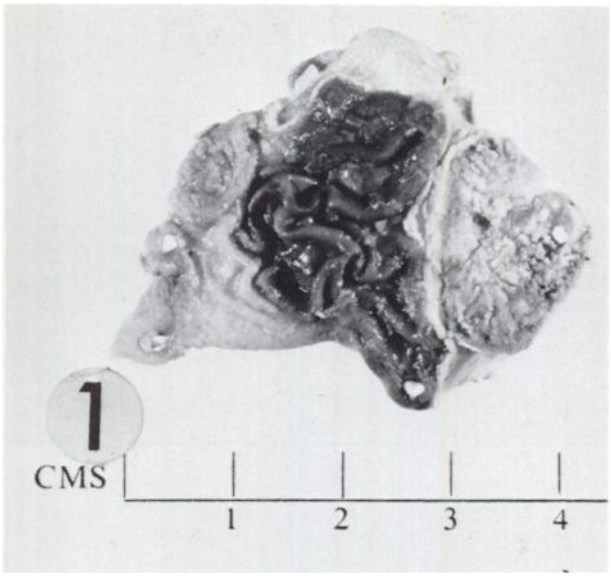

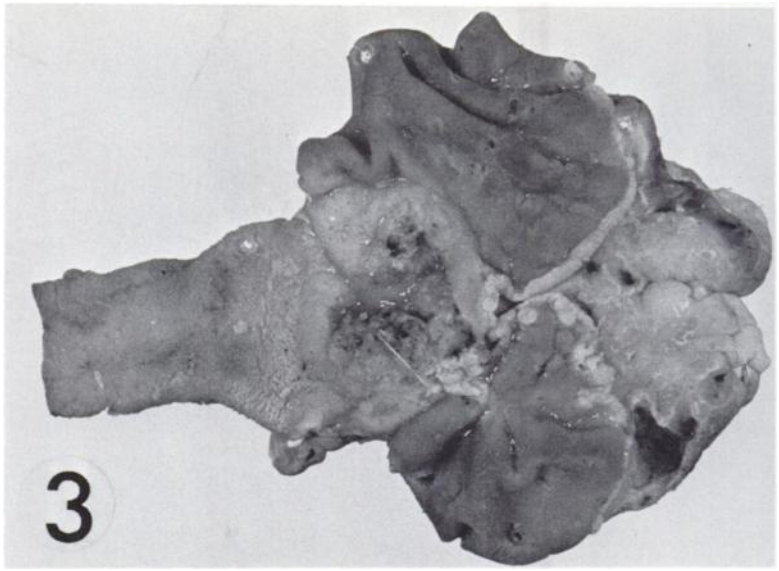

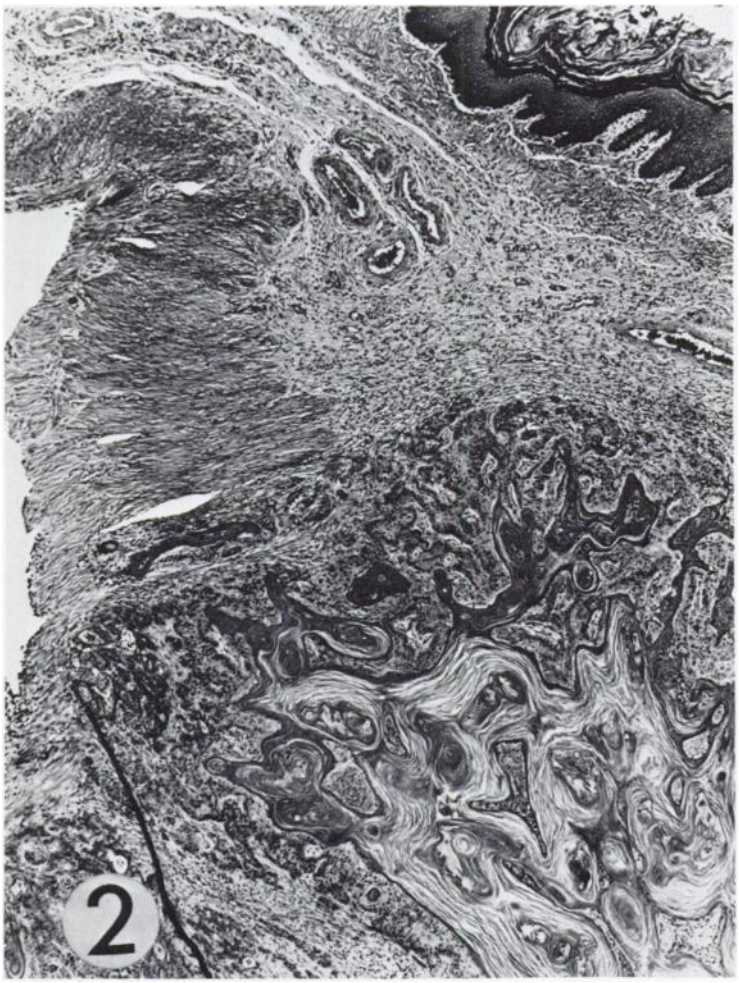

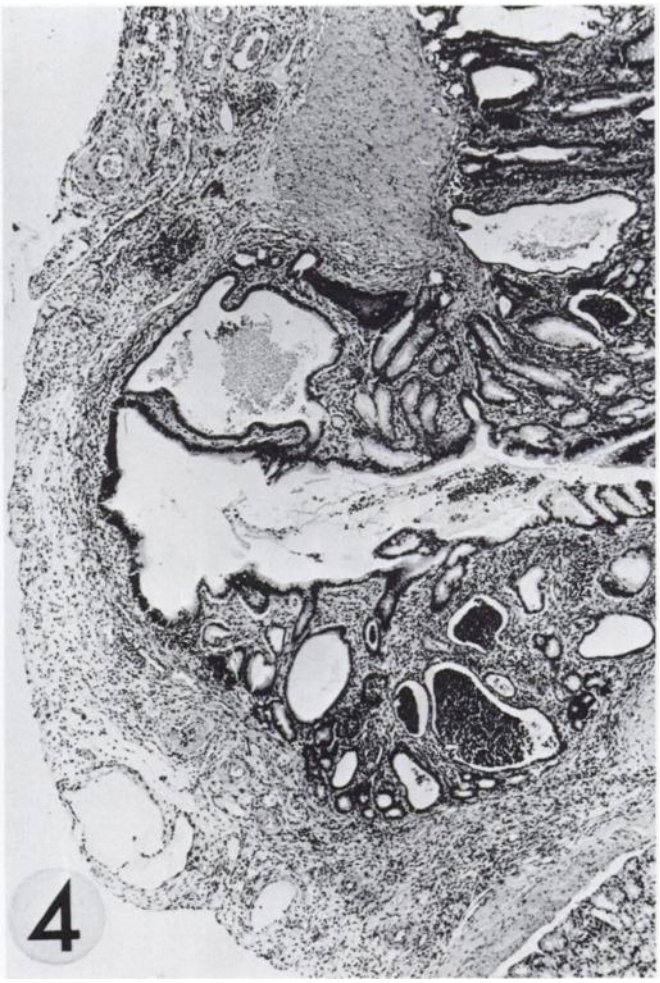

Fig. 1. Diffuse tumor of the forestomach of a rat killed 25 months after a single intragastric dose of CENU.

Fig. 2. Microscopic appearance of the keratinizing squamous cell carcinoma shown in Fig. 1. The tumor has penetrated through to the serosa. The pyloric end of the glandular stomach showed much glandular atypia. H & E, × 50.

Fig. 3. Ulcerating tumor at the pyloric end of the glandular stomach of a rat killed 24.5 months after the 1st of 2 intragastric doses of CENU. × 1.8.

Fig. 4. Microscopic appearance of the tumor shown in Fig. 3. Well-differentiated adenocarcinoma, which has penetrated through to the serosa. H & E, × 50.