Metabolism of N-Methyl-N'-nitro-N-nitrosoguanidine in Rats

N-Methyl-N'-nitro-N-nitrosoguanidine (MNNG) is well known as a potent mutagen[1,2] and locally acting carcinogen. Since SCHOENTAL[3], DRUCKREY et al.[4] and SUGIMURA et al.[5] reported the carcinogenesis of MNNG, a number of papers have been presented on its biochemical properties[6–14]. MNNG seems to be one of the most suitable chemicals for production of stomach cancer in the rat[15]. This paper deals with the fate, distribution, metabolism and metabolic pathways of MNNG in rats using tracer technique.

Material and methods. Methyl- and guanidino-labelled MNNG were used in the experiment and the specific activity of each radiomer was 0.074 μCi/mg and 2.59 μCi/mg, respectively. Labelled MNG[16] was also prepared by denitrosation of the corresponding labelled MNNG. The purity of each labelled compound was checked chemically and radiochemically. Labelled MNNG was given orally to male Wistar albino rats (body weight: 200–330 g) by stomach tube at a dose of 100 mg/kg in DMSO (20 mg/1.4 ml). The rats were kept in metabolic cages for collection of urine and faeces and given a standard diet and water ad libitum. The expired CO_2 was collected in NaOH solution for 48 h at different intervals and an aliquot of the diluted alkali sample was taken for radioactive assay with a scintillation counter.

A portion of the collected urine, and portions of dried faeces and organs were oxidized to CO_2 for measuring ^{14}C in a phosphor according to the method of JEFFAY and ALVAREZ[17,18].

Results and discussion. Results on the fate of MNNG and MNG are summarized in Table I.

MNNG was rapidly eliminated, mainly via kidneys, but a small amount of radioactivity was found in the faeces. Methyl-labelled MNNG was oxidized to CO_2 in the range of 20 to 30% of the dose. On the other hand, the conversion of guanidino-labelled MNNG into CO_2 amounted at most to 1.5% of the total dose. The production of CO_2 from both methyl- and guanidino-labelled MNG was negligible. 3 urinary metabolites of guanidino-labelled MNNG were easily separated by either paper- or thin-layer chromatography. The major radioactive metabolite (I) and the second metabolite (II) coincided with MNG and NG, respectively.

The reversed isotopic dilution analysis for further identification of these compounds was undertaken. Their identity with authentic samples was established on the basis of the fact that the specific activity of each compound

Table I. Fate of MNNG and MNG shown as percentages of the dose given

	MNNG(4)[b]	MNNG(3)[a]	MNG(2)[b]	MNG(1)[a]
Urine (24 h)	63.1	40.4	89.8	77.3
(48 h)	65.2	43.8	97.1	79.6
Faeces (48 h)	2.8	1.4	0.8	0.6
^{14}C exhaled as CO_2 (48 h)	0.7	24.1	–	>0.1

The results for MNNG[b], MNNG[a] and MNG[b] are each the mean of measurements on 4, 3 and 2 rats, respectively, except those for MNG[a] on 1 rat. [a] Labelled at methyl-carbon. [b] Labelled at guanidino-carbon.

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[16] Abbreviation: MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; MNG, N-methyl-N'-nitroguanidine; NG, nitroguanidine; DMSO, dimethylsulfoxide.
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Table II. RSA of ^{14}C-radioactivity at 48 h after dosing of labelled MNNG

	MNNG[a]	MNNG[b]		MNNG[a]	MNNG[b]		MNNG[a]	MNNG[b]
Forestomach	0.32	0.69	Spleen	0.20	0.24	Brain	0.05	0.003
Stomach contents	0.35	0.16	Glandular stomach	0.17	0.21	Testicle	0.07	0.02
Liver	0.39	0.24	Esophagus	0.19	–	Adrenal glands	0.15	0.03
Intestine	0.30	0.54	Heart	0.17	0.09	Prostate	–	0.07
Kidneys	0.30	0.18	Lungs	0.17	0.02	Muscles	–	0.007

$$\text{RSA} = \frac{\dfrac{\text{activity in the organ (cpm)}}{\text{organ weight (g)}}}{\dfrac{\text{total dose (cpm)}}{\text{body weight (g)}}}$$

Figures are each the mean of measurements on 2 rats. [a] Labelled at methyl-carbon. [b] Labelled at guanidino-carbon.

remained constant during 3 recrystallizations. In another experiment, MNG was isolated as pure crystals from the cummulative urine of rats receiving non-radioactive MNNG in DMSO at a dose of 100 mg/kg at 4-days intervals for one month (total dosage: 1100 mg) and identified by admixture with authentic sample. The third metabolite (III) has not yet been identified. It does not appear to be the general glucuronide because there was no change in chromatographic behavior of the radioactive spot on the chromatogram, even after the action of β-glucuronidase at 37 °C for 24 h in acetate buffer (pH 5.0).

In determining the metabolic sequence of MNNG, it is plausible to presume at least 2 possible pathways for the formation of NG in rats.

The first would be the N-demethylation of MNG and the second alternative is the nucleophilic attack of amino group on guanidine carbon, probably after elimination of hypothetic compound CH_3–N = N–OH.

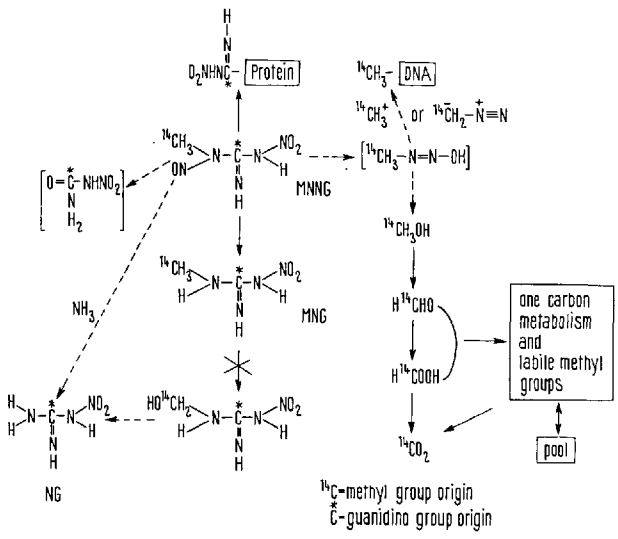
The first possibility was rejected due to the following in vivo and in vitro data. After an oral dosage of MNG (4.6 μCi/kg), methyl-labelled MNG was excreted unchanged in the rat and no $^{14}CO_2$ was detected in the expired air from rats, as shown in Table I. When methyl-labelled MNG was incubated in vitro with the rat liver homogenate fortified with co-factors at 37 °C for 30 min in air, and with constant shaking to determine the N-demethylation of MNG by the method of NASH[19], a measurable amount of formaldehyde labelled with ^{14}C was not obtained from the incubation mixture of MNG. Thus this fact is in favor of the hypothesis that MNG is very stable both in vivo and in vitro, and NG is not likely to be formed as a result of N-demethylation of MNG. Therefore, the alternate pathway in which MNNG reacts with amino group to give NG in the body would seem to predominate and can account for the formation of NG. Furthermore, our suggested metabolic route was supported by the fact that MNNG reacted with ammonia to give NG in vitro[20].

The authors wish to propose that the metabolic pathways of MNNG in rats are as shown in the Figure.

The distribution of ^{14}C in the organ at 48 h after a single oral dose of MNNG is shown in Table II. The organ-affinity was compared for level of ^{14}C in terms of relative specific activity (RSA). Although significant difference of the affinity among these organs was not observed, the values of RSA can be divided into 3 groups. The first group of relatively high RSA was found in forestomach, stomach contents, liver, intestines and kidneys. The second group indicating medium RSA-values included spleen, glandular stomach, oesophagus, heart and lungs with RSA value in the spleen close to the first group. Third group showed low RSA values and included brain, testicle, adrenal glands, prostate and muscles.

From the above data, one cannot say distinctly that there is stomach-specific affinity, since the comparison of the RSA values in our experiments did not clearly indicate sharply high values in the stomach. However, stomach-affinity must be examined at different time intervals before an exact conclusion could be attained.

The present results differ somewhat from those of KAWACHI et al.[14] in the experimental conditions, the oxidative ratio of guanidino- and methyl-carbons of MNNG and one of the metabolites, although the isolation of MNG is a common finding to us and the other group[14,21].



Presumed metabolic pathways of MNNG in rats.

Zusammenfassung. Versuche mit ^{14}C-markiertem Carcinogen MNNG ergaben an Ratten nach einmaliger Verabreichung der Substanz eine vorwiegend über die Niere erfolgende Elimination. Die Metabolite von MNNG in Verknüpfung mit verschiedenen Stoffwechselstufen und ihre Verteilung im Gewebe wurden näher verfolgt.

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Occurrence of Dihydromurexine (Imidazolepropionylcholine) in the Hypobranchial Gland of *Thais (purpura) haemastoma*[1]

The hypobranchial gland of the Mediterranean snail *Murex trunculus* contains large amounts of urocanylcholine or murexine[2]. The same choline ester occurs also in *Murex brandaris, Tritonalia erinacea, Murex fulvescens, Thais lapillus, Urosalpinx cinerea, Concholepas concholepas*[3–5] and a number of other molluscs belonging to the families of Muricidae and Thaisidae[6]. The hypobranchial gland of *Thais floridana, Thais chocholata* and other related species contains, in its turn, senecioylcholine or β,β-dimethylacryloylcholine[7,8], and that of