in the intensity of the ninhydrin-positively stained spots. The peptides occurring in both types of sera have a different aminoacid composition. The peptides designated X_1 are characterized by the presence of basic amino acids, and in the neoplastic sera by an additional content of arginine and valine (Figure 2). The X_2 peptides from the control sera contained leu, val, met, phen amino acids. The X_2 peptides from neoplastic sera contained aspartic acid in addition to the other amino acids.

The investigations showed that in the total arginine content of the sera from persons with neoplasms is comprised of free L-arginine in an amount corresponding to that of the sera from healthy persons and a peptide-bound arginine which was not found in the control sera. In neoplastic diseases, therefore, peptides characterized by the presence of the amino acid arginine appear in the blood serum. SKARZYNSKI and SARNECKA-KELLER[5] were the first to suggest that in certain pathological conditions, for example malignancy, and in tissue damaged by radiation, compounds not found in healthy person's sera may be found in the sera of such patients. HAMMERSTEN and SANDELL[6] in investigations on sera from leukaemia patients, isolated a peptide with an amino acid content similar to that found in our investigations, which always contained arginine and besides serine and methionine.

It is interesting to note that, in all the sera from the patients with neoplasms, no matter what the localization of the tumor, and identical amino acid composition was found in the X_1N peptides. It is suggested that these peptides may have originated from disintegrated tumor basic proteins. Such proteins in human tissue tumors[7] and in an experimental by induced Guerin tumor in rats[8] has been found recently.

It is thought that, under the influence of specific intracellular proteases and endopeptidases, the proteins are degraded into peptides. As a result of disturbances in the permeability of the cell membranes, the protein degradation products may escape into the extracellular spaces and thus into the blood stream.

Zusammenfassung. Nachweis von argininreichen Peptiden in menschlichen Seren mit neoplasmatischen Krankheiten.

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Inhibitory Effects of Nitrososarcosine on Mouse Liver Mixed Function Oxidase Activity

Potential human hazards from environmental exposure to dialkylnitrosamines have become a matter of major concern[1,2]. That humans are sensitive to the toxic effects of nitrosamines is shown by observations that human liver contains enzymes which can activate dimethylnitrosamine[3]. Nitrosamines are rather inert, chemically, and derive biological action from enzymatic activation to carbonium ions[4]. The organ distribution of enzymes which activate nitrosamines generally determines the organotropic action of the various nitrosamines[5].

Nitrososarcosine induces cancer of esophagus in rats[6] and squamous cell carcinoma of the nose in mice[7]. The 7-day oral LD_{50} in mice is 3.15 g/kg with little noticeable histological changes. Therefore, since there is no acute liver pathology, it seems unlikely that enzymes necessary to activate nitrososarcosine would be present in mouse liver.

It is the purpose of the present communication to report biochemical effects of nitrososarcosine on mouse liver-namely, inhibition of microsomal enzyme activity. The significance of this phenomenon is that it is one of the only biological effects of a diakylnitrosamine unassociated with apparent enzymatic activation.

Male Swiss albino mice (ICR/dub) were used in all studies. Animals were housed in shoebox type cages with constant access to Purina Chow and water. Mice were killed by cervical dislocation and mouse liver microsomal enzyme function was assessed by quantitating aminopyrine demethylase and aniline hydroxylase activity. Microsomal suspensions were prepared as described previously[8].

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Table I. Dose response of mouse liver aminopyrine demethylase and aniline hydroxylase activity to nitrososarcosine

Treatment	No. of animals	Aminopyrine demethylase activity[a]	Aniline hydroxylase activity[a]
		(Mean ± S.E.)	(Mean ± S.E.) × 10
Control	10	3.75 ± 0.39	7.81 ± 0.09
Nitrososarcosine (250 mg/kg)	10	2.74 ± 0.32[b]	6.42 ± 0.03
Nitrososarcosine (500 mg/kg)	10	1.89 ± 0.24[c]	5.30 ± 0.06[c]
Nitrososarcosine (1000 mg/kg)	10	1.52 ± 0.10[c]	2.98 ± 0.03[c]

[a] Expressed as mMoles of product per g liver per 10 min. [b] Statistically different from controls at $p < 0.05$. [c] Statistically different from controls at $p < 0.01$. Groups of mice were treated with nitrososarcosine, p.o., and sacrificed 3 h later.

Table II. Time course of nitrososarcosine induced inhibition of mouse liver aminopyrine demethylase and aniline hydroxylase activities

Time (h)	No. of animals	Aminopyrine demethylase activity[a]	Aniline hydroxylase activity[a]
		(Mean ± S.E.)	(Mean ± S.E.) × 10
0	5	2.36 ± 0.34	5.66 ± 1.19
0.25	5	1.94 ± 0.17	4.58 ± 0.43
1	5	1.29 ± 0.21[b]	2.71 ± 0.37
3	5	1.27 ± 0.08[b]	3.17 ± 0.36
24	5	1.79 ± 0.09	5.63 ± 0.41

[a] Expressed as mMoles product per g liver per 10 min. [b] Statistically different from controls at $p < 0.01$. Groups of mice were treated with 1,000 mg/kg p.o. at 0 time and killed at 0.25, 1, 3, or 24 h.

All incubation mixtures were buffered at pH 7.4 and contained 75 μmoles nicotinamide, 4.5 μmoles NADP, 15 μmoles $MgCl_2$, 30 μmoles $MnCl_2$, 15 μmoles DL-isocitrate, and 75 μg isocitrate dehydrogenase in a final volume of 3 ml[8]. In assaying for aminopyrine demethylase, microsomes from 75 mg of liver were used, and 45 μmoles of neutralized semicarbazide were added to the medium. After incubation at 37 °C for 30 min, the incubation was deproteinized with 10% TCA and formaldehyde was assayed as the reaction product[8]. The concentrations of aminopyrine in the kinetic studies were 1.7, 2.5, 5, and 10 mM; for other aminopyrine demethylase assays, a concentration of 10 mM aminopyrine was used. Assays for aniline hydroxylase activity were based on microsomes from 125 mg of liver and 0.5 mM aniline was used as substrate. After incubation at 37°C for 30 min., solutions were deproteinized following submersion in boiling water. Measurements of formation of p-aminophenol were performed by coupling p-aminophenol with phenol to form indophenol[8].

Dose response of microsomal enzymes to nitrososarcosine was determined 45 min subsequent to treatment.

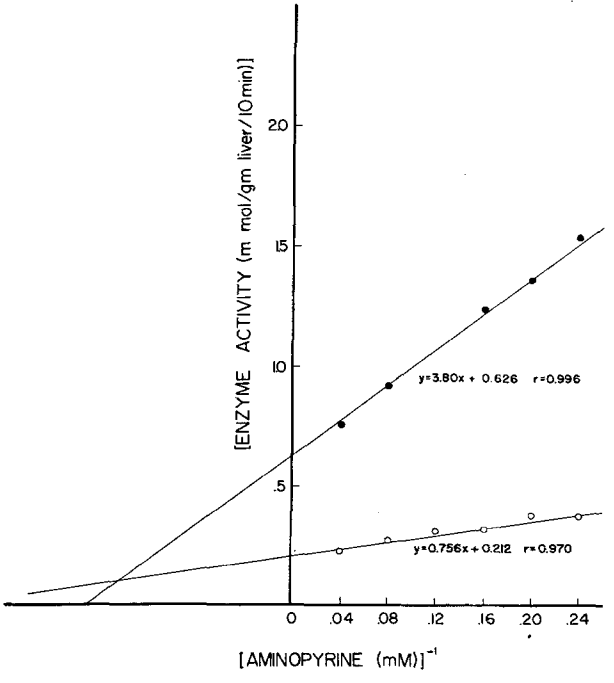

Fig. 1. Effect of nitrososarcosine on kinetics of mouse liver aminopyrine demethylase activity.

Groups of 5 mice were treated with 1000, 500, 250, or 0 mg/kg nitrososarcosine per os. Animals were then killed and livers removed for aminopyrine demethylase and aniline hydroxylase determinations.

The time course of the response was measured at various times after oral administration of 1000 mg/kg nitrososarcosine. Animals were sacrified 0, 0.25, 1, 3, and 24 h after treatment and samples were taken for aminopyrine demethylase and aniline hydroxylase activity.

In experiments to quantitate the effects of nitrososarcosine on kinetics of aminopyrine demethylase, groups of 6 mice were injected with 1,000 mg/kg and killed 3 h later. Livers from 6 mice were pooled and kinetic constants of aminopyrine demethylase determined[8].

Effects of dibutylnitrosamine and dimethylnitrosamine were determined 45 min and 3 h to subsequent to oral administration each compound. Dibutylnitrosamine was dissolved in dimethylsulfoxide and administered at doses of 1000, 500, or 250 mg/kg. Relevant solvent controls were always performed. In the case of dimethylnitrosamine, mice received 500 mg/kg, dissolved in water.

The inhibitory effects of nitrososarcosine on microsomal aminopyrine demethylase and aniline hydroxylase activities are shown in Table I. At a dose of 1000 mg/kg nitrososarcosine produced 59% and 62% inhibitions of aminopyrine demethylase and aniline hydroxylase, respectively. However, at low doses (250 mg/kg) inhibition of aminopyrine demethylase and aniline hydroxylase activities was only 27% and 18% of which only the first was marginally statistically significant ($p < 0.05$).

As can be seen on Table II, maximum inhibition of microsomal enzyme activity occurs about 1 to 3 h subsequent to treatment. In the case of aminopyrine demethylase, enzyme activity was suppressed by 45% and

Table III. Effect of nitrososarcosine on aminopyrine demethylase kinetics

Treatment	Km[a]	Vm[b]
	(Mean ± S.E.)	(Mean ± S.E.)
Control	3.72 ± 0.55	4.34 ± 0.38
Nitrososarcosine	6.14 ± 0.81	1.31 ± 0.21[c]

[a] Expressed as mM aminopyrine. [b] Expressed as μmoles CHO formed per g liver per 10 min. [c] Statistically different from control at $p < 0.01$. Groups of 6 mice were treated with 1000 mg/kg nitrososarcosine and killed 3 h later.

Table IV. Effect of dimethylnitrosamine on mouse liver aminopyrine demethylase activity

Treatment	No. of animals	Time	Aminopyrine demethylase activity[a]
			(Mean ± S.E.)
Control	7	—	1.81 ± 0.15
Dimethylnitrosamine	7	45 min	1.56 ± 0.16
Dimethylnitrosamine	7	180 min	1.44 ± 0.14

[a] Expressed as mMoles product per g liver per 10 min. Groups of mice were treated with 500 mg/kg i.m. and killed at times indicated. Neither treated groups was different from control.

46% at 1 and 3 h respectively while only 18% and 24% at 0.25 h and 24 h. Similarly aniline hydroxylase activity was inhibited by 52% and 44% at 1 and 3 hours and by only 19% and 1% at 0.25 and 24 h. In the case of each enzyme, differences were statistically significant at 1 and 3 h but not at 0.25 or 24 h.

Data presented in Table III indicate that nitrososarcosine non-competively inhibits aminopyrine demethylase activity. Although nitrosarcosine induced a 1.65 fold increase in *Km*, this difference is not statistically significant. In contrast, there is a 70% suppression of Vm in nitrososarcosine-treated mice, a difference which is highly significant ($p < 0.01$). To more clearly illustrate these differences, a Lineweaver-Burke plot from a typical experiment is shown in Figure 1.

In order to determine whether these effects were unique to nitrososarcosine, 2 other nitrosamines were tested under similar conditions. As can be seen from Table IV, dimethylnitrosamine at 20 times the LD_{50} induced no inhibition of aminopyrine demethylase activity within 3 h. Similarly dibutylnitrosamine, a bladder carcinogen in the mouse, induced little inhibition of microsomal enzyme activity (Figure 2). At 45 min after treatment, there was a 22% inhibition of microsomal enzyme activity which was statistically different from DMSO controls ($p < 0.01$). However, neither point was different from the naive controls. Similar results were obtained 3 h subsequent to treatment. Aminopyrine demethylase activity in mice receiving 1000 mg/kg dibutylnitrosamine was marginally different from that of DMSO controls ($p < 0.05$). Enzyme activity in mice receiving 500 mg/kg, although similar to the 1000 mg/kg group, was not different from DMSO controls.

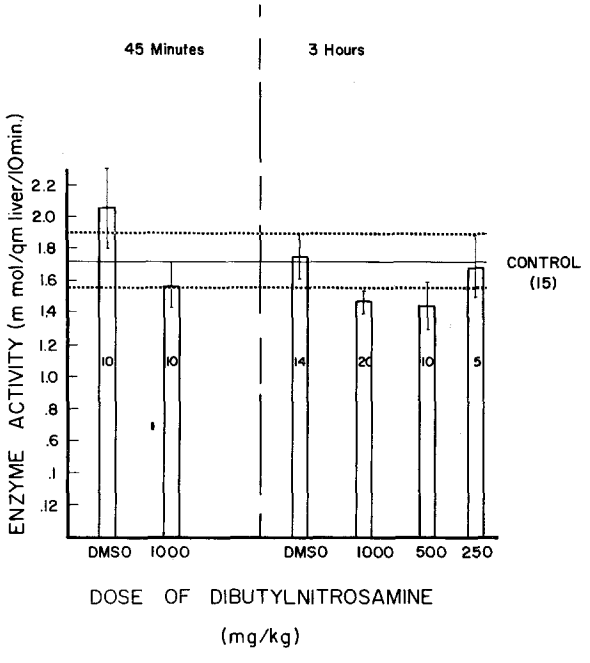

Fig. 2. Effect of dibutylnitrosamine on mouse liver aminopyrine pemethylase activity.

Data presented here indicate that nitrososarcosine induces a marked transient inhibition of mouse liver microsomal enzyme activity. The magnitude this inhibition appears maximal at 1 h after exposure and is totally reversed at 24 h. Doses of nitrososarcosine necessary to induce this effect are 10-fold lower than the toxic doses for nitrososarcosine. At LD_{50} levels of nitrososarcosine, there is no hepatocellular necrosis[7]. That these inhibitory effects are observed at doses lower than acute lethality levels and in an organ where there is no acute histopathology observed, suggests that this is a selective inhibitory response.

The relevance of observations that nitrososarcosine inhibits microsomal mixed function oxidase activity does not lie primarily in the loss of this important function to the animals. Certainly the observation that this compound induces cancer indicates that inhibition of microsomal function is of secondary importance. However, it is unique that nitrosarcosine may exert effects in an organ on which it has no toxicity and thereby appears to lack the enzymes to activate it. The complexity of responses to this nitrosamine may therefore be much greater than with other nitrosamines.

Zusammenfassung. Nachweis, dass Nitrosarcosin im Gegensatz zu Dimethyl- und Dibutylnitrosamin die nichtfunktionelle Oxidase in der Mäuseleber hemmt.

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