ABSTRACT

The effects of mouse plasma, human plasma, and purified porcine liver carboxylesterase on nitrosourea, nitrosamide, and nitrosocarbamate chemical stability, mutagenicity, and DNA cross-linking activity were compared. These three classes of N-nitroso compounds are chemically similar but displayed different biological activities and were affected differently by plasma and carboxylesterase. Nitrosourea stability as well as mutagenicity and DNA cross-linking activity were affected negligibly by esterase or plasma. In contrast, nitrosamide and nitrosocarbamate stability, mutagenicity, and DNA cross-linking activity were rapidly decreased in the presence of plasma or carboxylesterase. For example, chemical half-lives were from 10- to 20-fold shorter for the nitrosamides and nitrosocarbamates in the presence of 5% mouse plasma. Similar decreases were seen for mutagenicity and DNA cross-linking activity. Preliminary studies indicated one active plasma component to be an enzyme, possibly an esterase. Additional factors such as sulfhydryls may also participate. Whereas some nitrosoureas are active antitumor agents, the lack of antitumor activity for analogous nitrosamides and nitrosocarbamates may reside predominantly in their rapid in vivo inactivation. These results may help to account for the high in vitro mutagenicity as compared with the low in vivo activities of nitrosamides and nitrosocarbamates.

INTRODUCTION

The nitrosoureas, nitrosamides, and nitrosocarbamates are a class of alkylating agents related by their ability to decompose under physiological conditions to diazotates that are active alkylating species (Chart 1) (6, 7). All 3 groups of compounds have been shown to be direct-acting base-pair substitution mutagens in microbial test systems (5, 14, 21). The carcinogenicity of N-nitroso compounds has been well documented; greater than 100 are now known to be carcinogenic, and carcinogenicity has been detected in 39 species among 17 orders and 5 classes of animals (3, 16, 19). These classes of compounds exist in the environment and can be formed in vivo by nitrosation of ingested ureas, amides, and carbamates in the digestive tract. Ureas and carbamates are especially prevalent in the environment; they are formed from food constituents and are used as drugs and insecticides (15, 18).

Some of the nitrosoureas possess antitumor activity. Several nitrosoureas such as BCNU6 and N-(2-chloroethyl)-N'-cyclohexyl-N-nitrosourea are currently used to treat a variety of human cancers. This effective antitumor activity is attributed to the alkylating ability of these compounds (20). However, in spite of their close chemical relationship to the nitrosoureas, the simple alkyl nitrosamides and the nitrosocarbamates possess negligible antitumor activity in vivo (5, 11). Understanding the divergent biological activities of these compounds may assist not only in the definition of relative carcinogenic potential but also in the search for more effective antitumor agents.

We have compared the effects of mouse and human plasmas and porcine liver carboxylesterase on the chemical stability, mutagenicity, and DNA cross-linking activity of several nitrosoureas, nitrosamides, and nitrosocarbamates. In all cases tested, the amides and carbamates were rapidly destroyed or inactivated by plasma and carboxylesterase while the nitrosoureas were negligibly affected. A preliminary report of some of this work has been published (2).

MATERIALS AND METHODS

Source of Drugs and Chemicals. BCNU was provided by the Drug Development Branch of the National Cancer Institute. CNA and MNA were prepared as described previously (5).

CNC was prepared as follows. Chloroethyl isocyanate (3 g, 28 mmol) was added to anhydrous ethanol (15 ml) and stirred for 2 hr at 25°. Removal of solvent under reduced pressure gave an oil (4.5 g). The unpurified carbamate (750 mg, 5 mmol) was dissolved in formic acid (10 ml, 88%) and was stirred at 0°. A solution of sodium nitrite (700 mg, 10 mmol) in water (3 ml) was added slowly. After stirring for 1 hr at 0°, methylene chloride and water were added. The organic phase was separated and dried with sodium sulfate, and the solvent was removed under reduced pressure. Preparative thin-layer chromatography (benzene on silica gel) gave a yellow oil (650 mg);

1H NMR (CDCl3) δ 1.4 (3H, t), 3.4 (2H, t), 4.1 (2H, t), 4.5 (2H, q).

MNC was prepared as follows. Ethyl chloroformate (2.2 g, 20 mmol) was added to a mixture of methylamine hydrochloride (2.6 g, 44 mmol), potassium hydroxide (2.5 g, 44 mmol), water (20 ml), and methylene chloride (20 ml) and was stirred at 0°. After 30 min, the organic phase was separated and dried with sodium sulfate, and the solvent was

The abbreviations used are: BCNU, N,N'-bis(2-chloroethyl)-N-nitrosourea; CNA, N-(2-chloroethyl)-N-nitrosoacetamide; MNA, N-methyl-N-nitrosoacetamide; CNC, N-(2-chloroethyl)-N-nitrosoethy carbamate; NMR, nuclear magnetic resonance; t, triplet; q, quartet; s, singlet; d, doublet; br, broad singlet; MNC, N-methyl-N-nitrosoethy carbamate; DMNU, N,N' -dimethyl nitrosourea; 1H, mutagenic half-life; 1/2, chemical half-life.

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removed under reduced pressure to give an oil (1.9 g). The unpurified carbamate was nitrosated as in the synthesis of CNC. Preparative thin-layer chromatography (benzene on silica gel) gave a yellow oil (500 mg): 'H NMR (CDCl3) δ 1.4 (3H, t), 3.1 (3H, s), 4.5 (2H, q).

DMNUs was synthesized as follows. Phosgene was bubbled into a stirred mixture of methylamine hydrochloride (3.3 g, 50 mmol), water (50 ml), and potassium hydroxide (5.6 g, 100 mmol, powdered) at 0° until the solution became acidic. After a stirring at 0° for 2 more hr, 1 N hydrochloric acid (5 ml) was added followed by the slow addition of a solution of sodium nitrite (3.6 g, 50 mmol) in water (10 ml). After a stirring for 2 hr, the solution was extracted with methylene chloride, the organic phase was dried, and the solvent was removed under reduced pressure. Chromatography (chloroform on silica gel) gave a pale yellow solid: m.p. 96-98° (literature m.p. 96°) (10). 'H NMR (CDCl3) δ 3.0 (3H, d), 3.1 (3H, s), 7.0 (1H, br s).

Carboxylesterase (EC 3.1.1.1) from porcine liver, bovine serum albumin, and L-cysteine-HCl were obtained from Sigma Chemical Company. Glutathione (reduced form) was obtained from Calbiochem Company. Preparation of Plasma. Mouse plasma was prepared by bleeding female C57BL/6 × DBA/2 F1 mice from the eye with a heparinized pipet. The blood was immediately centrifuged, diluted to 10% (v/v) with 0.1 M sodium phosphate buffer at pH 7.4, and stored frozen at -10°C. Human plasma was obtained from one healthy male donor and was prepared and stored in the same fashion as the mouse plasma.

Chemical Decomposition Studies. A 25 mM solution of nitrosourea in 100% ethanol was diluted 1:30 with 0.1 M sodium phosphate buffer, pH 7.4. This was then added to an equal volume of either buffer, 10% plasma, or porcine liver esterase (20 g/ml; 2.7 units/ml) in the same buffer and was incubated at 37°C. Aliquots were removed at intervals and assayed for N-nitrosourea content on a Waters Model 440 high-pressure liquid chromatography instrument equipped with a C18 reversed-phase column and with a Model 450 variable-wavelength detector set at 230 nm. Methanol/water was used as eluant, 50% methanol for the methyl nitrosourea, and 70% methanol for the chloroethyl compounds. The flow rate was 1 ml/min.

DNA Cross-Linking Studies. Mouse L1210 cells were grown in Roswell Park Memorial Institute Medium 1640 supplemented with 10% (v/v) fetal bovine serum. DNA was labeled by exposing the cells to [14C]thymidine (0.05 μCi/ml and 10^-6 M) for 24 hr at which time they were resuspended in isotope-free medium at 37°C for 21 hr before treatment with chemical agents. Exposure to the N-nitrosourea compounds was for 30 min at 37°C followed by 2 washes in fresh medium. The treated cells were incubated for an additional 5 hr at 37°C to allow maximum appearance of DNA cross-links. Cells were prepared for alkaline elution by suspension in 0.075 M NaCl:0.024 M EDTA, pH 7.4, at 0°C. Each cell suspension was split into 4 equal portions which received 100, 200, 300, or 400 rads γ-irradiation (137Cs source) at 0°C. The irradiated cells were kept at 0°C before being loaded onto 2-μm porosity filters (Acropor; Gelman Sciences, Inc.). The cells were lysed with 0.1 M NaCl:0.02 M EDTA, pH 10 (30 min); the filters were washed with 0.02 M EDTA, pH 10 (30 min); and the DNA was eluted with tetrapropylammonium hydroxide:0.02 M EDTA, pH 12.1 (7.5 hr), at a flow rate of 0.04 ml/min. The eluate was collected as one fraction. The radioactivity remaining on the filter and that in the eluate were determined by liquid scintillation counting and were used to calculate the fraction of DNA retained on the filter. The data are expressed by plotting the logarithm of the fraction of DNA retained versus the radiation dose in rads to produce a straight-line plot reminiscent of a process exhibiting apparent first-order kinetics.

Bacterial Strains. Mutagenicity tests were performed on Salmonella typhimurium strain hisG46. Mutant hisG46 is the parent strain for the Ames tester strains TA1535 and TA100; it detects base substitution mutations, by far the predominant mutagenic lesions produced by short-chain alkyl N-nitrosourea compounds (5, 8, 14). The uvrB mutation and the pKM101 plasmid factors which enhance mutagenicity in Salmonella of other classes of mutagens, had little effect on the mutagenicity of these direct-acting N-nitrosourea compounds (5). Also, the liquid preincubation assay was found to be more sensitive than the standard Salmonella plate incorporation assay (5). For these reasons, all of the mutagenicity tests described in this paper were liquid preincubation assays with the hisG46 strain and not the Ames tester strains TA1535 or TA100.

Mutagenicity Assay. The N-nitrosourea compound in 3.3% ethanol was diluted into an equal volume of either 0.1 M sodium phosphate buffer, pH 7.4, 10% plasma in 0.1 M sodium phosphate buffer, pH 7.4, or porcine liver esterase (17 μg/ml, 2 units/ml) in 0.1 M sodium phosphate buffer, pH 7.4. These mixture were preincubated at 37°C and aliquots were removed at intervals, diluted 1:20 into a suspension of bacteria that had been grown overnight in Difco nutrient broth, centrifuged, and then resuspended to the same volume in 0.1 M sodium phosphate buffer, pH 7.4. Following a 10-min incubation at 37°C, duplicate 0.1-ml samples were mixed with top agar and plated. Plating medium consisted of Vogel-Bonner E medium (29), 1.25% (v/v) liquid Difco nutrient broth, and 1.5% agar with a top agar containing 0.5% NaCl solution and 0.6% Bacto-agar. Since some N-nitrosourea compounds are photosensitive, all manipulations were performed in subdued light (17). Plates were hand counted after 48 hr incubation at 37°C in the dark. The average number of spontaneous revertants per plate for hisG46 was 2 (an average of >100 plates). Spontaneous revertants were subtracted before data were plotted. The concentrations of the N-nitrosourea compounds used were picked so as to give between 400 and 700 revertants/plate before the preincubation was begun. This range was chosen to give enough revertants to follow decreases in number with time of treatment of the N-nitrosourea compound but not enough to cause inaccuracies in counting. Because the compounds vary in mutagenic potency, they were not all tested at the same concentration as was done in the chemical decomposition studies.

RESULTS

Chemical Decomposition. The chemical decomposition of CNA, CNC, and BCNU in 0.1 M phosphate buffer (pH 7.4) at 37°C occurred with a half-life of 30, 100, and 40 min, respectively (Charts 2 and 3). In the presence of 5% mouse plasma or purified porcine liver esterase (10 μg/ml), the decomposition rate for CNA or CNC at pH 7.4 was significantly enhanced, the half-life being reduced to ≤5 min. In contrast, the half-life of...
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Chart 2. BCNU and CNA decomposition in 0.1 M phosphate buffer (pH 7.4), 5% mouse plasma, or porcine liver carboxylesterase was followed as described in “Materials and Methods.” Briefly, a 0.83 mM solution of test compound was mixed 1:1 with either 0.1 M phosphate buffer (pH 7.4), 10% mouse plasma, or esterase (2.7 units/ml) at 37°. Aliquots were removed at different times and assayed for N-nitroso compound content by high-pressure liquid chromatography with a UV detector set at 230 nm.

BCNU was not measurably decreased by the addition of either mouse plasma or esterase to the reaction mixture. In the presence of mouse plasma, the half-life of CNC was greater than that of CNA. In contrast, with the purified esterase the half-life of CNC was less than that of CNA. The methyl compounds (MNA, MNC, DMNU) gave results analogous to those of the chloroethyl compounds, although MNA and MNC were not as rapidly destroyed by mouse plasma or by esterase as were CNA and CNC (data not shown).

The ability of mouse plasma to destroy CNC was greatly reduced by pretreatment of the plasma with cyclohexyl isocyanate or by heating at 60° for 1 hr (Chart 3). Human plasma was not as active at destroying nitrosocarbamates as was mouse plasma (Chart 3). In the presence of 5% untreated human plasma, CNC had the same half-life as in 5% mouse plasma pretreated with cyclohexyl isocyanate (Chart 3). Simultaneous addition of equimolar BCNU (which would yield an alkyl isocyanate upon spontaneous decomposition) and CNC to mouse plasma did not change the initial rate of plasma-mediated CNC decomposition (Chart 4).

Mutagenicity. Both CNA and CNC were potent mutagens in the Salmonella mutagenicity assay, this study being the first to demonstrate mutagenicity of CNC. The nitrosocarbamates and nitrosamides were approximately 100-fold more mutagenic than were the corresponding nitrosoureas (Chart 5). The mutagenicity of aqueous solutions of CNA and CNC decreased slowly due to spontaneous decomposition, and these decreases could be characterized by $t_{1/2}$ as defined in the time it takes to reduce the mutagenicity of a solution by one-half. In dose-response assays for these compounds, where mutagenicity was evaluated as a function of dose, the resultant curves had slopes $\geq 1$ making the $t_{1/2}$ not necessarily equal to the $t_{1/2}$. For CNA and CNC in buffer at pH 7.4, $t_{1/2}$ values were 40 and 200 min, respectively (Charts 6 and 7). As can also be seen in Charts 6 and 7, in the presence of 5% mouse plasma, the $t_{1/2}$ of CNA and CNC were reduced 12- and 19-fold, respectively; whereas, in the presence of porcine esterase, the $t_{1/2}$ of CNA and CNC were reduced about 5- and 46-fold, respectively. As found for CNC, MNC mutagenicity also was greatly decreased by the addition of 5% mouse plasma or esterase. In contrast, the rates of loss of mutagenicity of DMNU with and without plasma were not statistically different (data not shown).

The rate at which CNA lost its mutagenicity in the presence of plasma was linearly dependent on the concentration of mouse plasma; higher plasma concentrations caused a faster rate of loss of mutagenicity (Chart 8). Pretreatment of plasma with heat or cyclohexyl isocyanate, as for the chemical decomposition studies described above, lessened the plasma effect on mutagenicity (data not shown).

Several specific plasma factors were tested for their effects on the mutagenicity of CNA. Reduced glutathione or cysteine alone at a 2.6 molar excess to the N-nitroso compound caused a 2- to 3-fold increase in the rate of loss of CNA mutagenicity (Chart 6). In contrast, bovine serum albumin in a 1:10 molar ratio with CNA had a negligible effect on $t_{1/2}$. Human plasma had less activity than did mouse plasma (Chart 6).

DNA Cross-Linking. The alkaline elution method used differed from that described by Kohn et al. (12). Rather than obtaining one detailed elution time course, we obtained multiple data points from a given experimental condition. This permitted statistical analysis of our data through linear regression. The point in time at which the elution was stopped (7.5 hr) was close to that used by Kohn et al. (12) and was not critical to...
the validity of either approach. To calculate the cross-link factor (23), the "control" slope was divided by the "treated" slope.

The results depicted in Chart 9 are typical of this method of alkaline elution data expression. The DNA of untreated control cells responded to increasing radiation-induced strand breaks by an increased elution (expressed as a decreased DNA retention on the filter) which yielded a value of log fraction DNA retained per rad of approximately $-1.9 \times 10^{-3}$. The DNA of cells exposed to bifunctional alkylating agents was more resistant to alkaline elution and yielded data which were expressed as less negative slopes. This decreased elution of cross-linked DNA is believed to result from structural changes which resist complete strand separation in alkali and increase the size of the DNA fragments (13).

Exposure of cells to either 0.5 μM CNA or CNC or to 50 μM BCNU produced large changes in DNA elution which can be expressed as cross-link factors of 3.8, 2.8, and 2.1, respec-
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Chart 7. Mutagenicity of CNC in S. typhimurium strain hisG46. See "Materials and Methods" for protocol. A 2.1 ml stock of CNC in 3.3% ethanol was mixed 1:1 with either 0.1 M phosphate buffer (pH 7.4), 10% mouse plasma, or esterase (2 units/ml). •, 0.1 M phosphate buffer; ●, mouse plasma; ▲, esterase.

Chart 8. Decay rate of the mutagenicity of CNA as a function of mouse plasma concentration. CNA final concentration in the mixture with mouse plasma was 375 μM as in Chart 5.

Chart 9. Influence of preincubation of CNA, CNC, and BCNU with mouse plasma on alkaline elution of DNA of L1210 cells exposed to these agents. The N-nitroso compounds (2.5 nmol CNA or CNC, or 250 nmol BCNU) were incubated for 10 min at 37° in 500 μl 0.1 M potassium phosphate buffer (pH 7.4) containing 5 μl of 100% mouse plasma (O, □, △). Plasma was omitted from control incubations (•, •, ▲). The solutions of N-nitroso compounds were diluted 10-fold with L1210 cells in culture medium, incubated for 30 min at 37°, and handled further as described in "Materials and Methods." •, ●, ▲, N-nitroso compounds in 0.1 M phosphate buffer; O, □, △, N-nitroso compounds plus plasma. x, buffer alone, no N-nitroso compound; ▲, △, CNA; □, O, CNC; ●, □, BCNU.

Chart 10. DNA cross-linking in L1210 cells exposed to various N-(2-chloroethyl)-N-nitroso compounds and nitrogen mustard. L1210 cells were exposed to the cross-linking agents for 30 min and assayed for DNA cross-links by the alkaline elution method either immediately (nitrogen mustard) or after 5-hr incubation in drug-free medium. •, nitrogen mustard; △, CNA; O, CNC; ●, N-(2-chloroethyl)-N-nitrosourea; □, BCNU.

DISCUSSION

The chemical and biological properties of several structurally related nitrosoureas and nitrosamides have been compared...
previously (5). Both classes of N-nitroso compounds were found to be direct-acting mutagens, the nitrosamides being more active than the nitrosoureas in the Salmonella test system. Whereas many of the nitrosoureas were active antitumor agents, the nitrosamides, in contrast, were not active against L1210 leukemia in vivo. The compounds containing 2-chloroethyl groups were active DNA cross-linking agents; CNA was 100-fold more potent than was CNU (Chart 10). The nitroso- carbamates are a third class of N-nitroso compound. This third class resembled the nitrosamides in being more potent mutagens and DNA cross-linking agents than were the nitrosoureas and in lacking appreciable in vivo antitumor activity. 

We have shown here that mouse plasma contains a factor that promoted rapid degradation of the nitrosamides and nitroso- carbamates but had a negligible effect on the nitrosoureas tested. $t_{1/2}$ and $T_{1/2}$ of the nitrosamines and nitrosocarbamates were shortened 10- to 20-fold upon addition of 5% mouse plasma, whereas nitrosourea half-lives remained essentially unaffected (Charts 2, 3, 6, and 7). Similarly, brief incubation with plasma removed the ability of CNA or CNC solutions to cross-link DNA but did not affect the cross-linking ability of BCNU (Chart 9). Using CNA as an example, extrapolation to a mouse plasma concentration of 100% would cause an approximately 200-fold increase in the rate of loss of mutagenicity. 

Several lines of indirect evidence led to the conclusion that the plasma factor (or one of the plasma factors, should there be several) was an enzyme. Heating plasma caused considerable loss of activity, and treatment with cyclohexyl isocyanate, a known inhibitor of esterases (4), also drastically decreased plasma activity (Chart 3). The majority of the plasma activity remained after dialysis (data not shown). Furthermore, activity of 5% mouse plasma, while strongly inhibited by a level of 50 $\mu$M cyclohexyl isocyanate, had the capacity to destroy >400 $\mu$M N-nitroso compound in the absence of inhibitor. In addition, we have found that commercial porcine liver esterase (or an unidentified component in the preparation) destroyed both the nitrosamines and the nitrosocarbamates but not the nitrosoureas tested. Our working hypothesis is that the plasma factor is an enzyme, perhaps an esterase, that is able to cleave nitrosamides and nitrosocarbamates.

It is likely that mouse plasma contains more than one factor active in accelerating the decomposition of nitrosamines and nitrosocarbamates. High concentrations of sulphydryl compounds, such as reduced glutathione and cysteine, accelerated the loss of CNA mutagenicity although not as much as did 5% mouse plasma (Chart 6). Schoental (27, 28) has shown that both reduced glutathione and cysteine reacted with N-alkyl-N-nitrosocarbamates. In contrast, sulphydryls did not accelerate decomposition of nitrosoureas such as methylhydrosourea and BCNU (31). Given even the highest total plasma glutathione concentration reported in the literature (which includes both reduced and oxidized glutathione) (1), the free sulphydryl content of plasma appeared >700-fold too low to account for the inactivation of the N-nitroso compounds that we report here. Human plasma had a much lower inactivating capacity for N-nitroso compounds than did mouse plasma. In fact, the activity of human plasma approximated that found with mouse plasma which had been treated with cyclohexyl isocyanate (Chart 3).

White et al. (32) have studied the hydrolysis by chymotryptsin of nitrosamides related to phenylalanine. With N-isobutyryl-N'-nitrosophenylalanine benzyl amides, both the $\delta$ and $\lambda$ isomers were substrates, but only the $\delta$ isomer was an inhibitor. The inhibition of activity was probably due to alkylation of the enzyme by the benzyl carbonium ions generated at the active site. In addition, Gold and Linder (9) have shown nitrosocarbamates to be inhibitors of porcine liver carboxylesterase. We have not noticed inhibition of activity of either mouse plasma or porcine esterase by the nitrosamides or nitrosocarbamates used in this study, but the nitrosoureas were inhibitors (Chart 4). Since one of the products of nitrosourea decomposition is an isocyanate, this inhibition could well be due to carbamylation of the enzyme by the isocyanate generated. The lack of activity of plasma toward the nitrosoureas did not result from a rapid inhibition of the enzyme, since simultaneous addition of equimolar BCNU and CNC to mouse plasma did not alter the initial rate of CNC decomposition. However, inhibition of CNC decomposition was evident after approximately 10 min, or one half-life (Chart 4).

Several investigators have shown that the decomposition of nitrosoureas is accelerated in the presence of serum (6, 22). These experiments were performed using human serum at concentrations close to 100%, and the $t_{1/2}$ of the nitrosoureas were reduced relatively slightly, approximately 2- to 3-fold. This acceleration of nitrosourea decomposition was attributed to nonspecific chemical interactions between the nitrosoureas and serum proteins. Thus, the reactions were noncatalytic, and 800 $\mu$M bovine serum albumin had the same effect as did 100% human plasma (30). Serum-accelerated decay of BCNU was not apparent in our studies with 5% plasma as inactivating agent. Thus, serum-accelerated decay of nitrosoureas appeared to be a phenomenon quite separate from the plasma-catalyzed decay that we have detected for the nitrosamides and nitrosocarbamates. Using 5% mouse plasma, we saw the $t_{1/2}$ and $T_{1/2}$ change severalfold more than the minor changes observed by other workers using 100% human plasma and nitrosoureas. Additionally, in our studies, the $t_{1/2}$ and $T_{1/2}$ were virtually unchanged by 30 $\mu$M bovine serum albumin, a concentration corresponding to the level of albumin present in 5% plasma (data not shown).

Further experiments will be necessary to characterize individually the active components present in the plasma and organs of different mammalian species. Such work is essential if experiments on one animal species are to be used in extrapolation to another species. If potent modes of inactivation of N-nitroso compounds operate in laboratory animals but not in microorganisms, then microbial tests would tend to overestimate potential risk. Similarly, tests in mice could underestimate risk to humans should human plasma prove to contain much less inactivating capacity than does rodent plasma. Rapid destruction of nitrosamides and nitrosocarbamates by mouse plasma may also account for the lack of in vivo antitumor activity of these compounds in mice. For the chloroethyl analogues used in this study, the nitrosamides and nitrosocarbamates were $>100$-fold more potent mutagens in bacteria than the nitrosoureas. In sharp contrast to this, Russell and Montgomery (24) and Russell et al. (25, 26) have found ethyl nitrosocarbamate to be much less potent, in terms of mutagenicity, embryotoxicity, and teratogenicity, than ethylhydrosourea is in both the specific-locus test and the spot test in mice.

In summation, there exists in mouse plasma at least one factor, enzymatic in nature and perhaps an esterase, that rapidly destroyed nitrosamides and nitrosocarbamates as mea-
sured by high-pressure liquid chromatography, DNA cross-linking activity, and mutagenic activity. Nitrosoureas were negligibly affected. Whereas this activity may be fortuitous, it is possible that the activity serves to protect mammals against particular classes of N-nitroso compounds.

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