Table I—Temperature and Time Effects on I Recovery from Plasma

Hours	Recovery[a] 25°	37°
0.0	90.5 ± 3.0	80.4 ± 3.0
0.5	82.5 ± 4.8	76.6 ± 3.7[b]
4.0	91.7 ± 2.6	55.6 ± 1.2[b]

[a] Percent recovery and percent relative standard deviation of quadruplicate analyses. [b] Percent relative standard deviation of a minimum of five samples.

of Compound A indicated that there were two replaceable hydrogens. The highest mass observed was 323 (odd mass), indicating that *m/e* 323 was either a fragment produced from a higher even mass or a molecular ion containing an odd number of nitrogens. Furthermore, there was no indication of chlorine in *m/e* 323. When compared to the chlorambucil molecule, it is most likely that *m/e* 323 was the molecular ion and the chlorambucil nitrogen was preserved. The carbon isotope ratio ($^{12}C/^{13}C$) indicated that 16 carbons was a reasonable assignment. Two major fragments, *m/e* 191 and 179, most likely were due to a highly stable styrene-like fragment and the *p*-aminobenzyl ion.

All three compounds showed the loss of 131 amu [$(CH_3)_3SiOCOCH_2$], indicating that the carboxyl group of I was intact. Based on these observations, Compound A (Fig. 3A) was suggested to be *p*-aminophenylbutyric acid. From similar observations, Compounds B and C (Figs. 3B and 3C) were suggested to be 4-pyrrolylphenylbutyric acid and 4-[3-(hydroxy)pyrrolyl]phenylbutyric acid. No *p*-aminobenzyl-like ion was observed in Compound B or C, probably due to the stabilization by the pyrrole group. The dark-brown color observed in the nonaqueous solution as well as in powdered preparations such as ^{14}C-I most likely was due to these pyrrole derivatives.

The α,β-unsaturated compounds 2-[4-*N*,*N*-bis(2-chloroethyl)]-aminophenyl-2-butenoic acid and 2-[4-bis(2-chloroethyl)]aminophenylacetic acid were found in rats given 8 mg of I/kg ip (13). Both compounds are products of the β-oxidation of I. The phenylacetic acid derivative in human urine and plasma was found (Figs. 1 and 5), and attempts to find the α,β-unsaturated compound were unsuccessful. More extensive studies of I metabolism are in progress.

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Antiradiation Compounds XVII: Binding Ability of Radiation-Protective *N*-Heterocyclic Aminoethyl Disulfides and Thiosulfates to DNA

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Abstract □ Binding parameters for a series of *N*-heterocyclic aminoethyl disulfides and thiosulfates to DNA were determined at different ionic strengths and pH values. None of the thiosulfates showed any binding ability, but the disulfides revealed DNA binding abilities that were suppressed both by increased ionic strength and hydrogen-ion concentration. No correlation between DNA binding ability and radiation protective activity in mice was evident.

Keyphrases □ Antiradiation compounds—binding ability of radioprotective *N*-heterocyclic aminoethyl disulfides and thiosulfates to DNA □ Radioprotective compounds—*N*-heterocyclic aminoethyl disulfides and thiosulfates, DNA binding ability □ DNA—binding by radioprotective *N*-heterocyclic aminoethyl disulfides and thiosulfates □ *N*-Heterocyclic aminoethyl disulfides and thiosulfates—DNA binding ability, radiation protection evaluated

The ability of the radioprotective aminothiols cystamine and bis(2-guanidinoethyl) disulfide to bind reversibly to DNA, RNA, and other nucleoproteins has been demonstrated (1), but the significance of this ability in radiation protection of mammals is not clear. Evidence has accumulated that the aminothiol protecting agents cause a temporary inhibition of nucleoprotein synthesis and facilitate repair of radiation damage (2–5), but the necessity for complexation in this process has not been shown.

To demonstrate whether or not DNA binding is important to radiation protection or repair, a series of *N*-heterocyclic substituted aminoethyl disulfides and thiosulfates showing varying degrees of radiation protection to mice was observed for the ability to bind to DNA. A correlation with protecting ability could show DNA-binding ability to be essential to the protective or repair process.

Most studies of the mechanism of radiation protection in mammals utilized the *N*-unsubstituted aminothiols and simple *S*-derivatives, such as 2-mercaptoethylamine, its phosphorothioate, thiosulfate, or disulfide (cystamine), or 2-mercaptoethylguanidine. Since *N*-substituted de-

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Table I—Preparation of ^{35}S-Thiosulfates [a]

$R_2{}^+NHCH_2CH_2S{}^*SO_3{}^-$

$R_2{}^+NHC$-H_2CH_2X R_2N	X	Weight, g[a]	Yield, g	Melting Point	Literature Melting Point
NH_2	Br	0.1640	0.040	178–180°	189° (18)
O⟨⟩N	Cl	0.1488	0.055	180–182°	181–183° (9)
⟨⟩N	Cl	0.1472	0.150	179–181°	180–182° (9)
⟨⟩N	Cl	0.1360	0.077	159–161°	165–167° (9)

[a] All compounds were prepared using 1.1 ml of sodium ^{35}S-thiosulfate solution; these quantities represent equal moles (0.8 mmole).

rivatives of 2-mercaptoethylamine have shown greater protection in mammalian tests and provided compounds having activities ranging from good to nonprotective, such a series should be suitable for observing a possible correlation between DNA binding ability and radiation protective ability. Accordingly, a series of *N*-heterocyclic substituted 2-mercaptoethylamine derivatives (6) having the $HetNHCH_2CH_2SX$ structure (I) was utilized (Het = aromatic or alicyclic heterocycle and X = SO_3H, SCH_2CH_2NHHet). The possible mechanisms by which the aminothiols act as radiation-protective agents were reviewed (7).

EXPERIMENTAL[1]

Synthesis of Radiolabeled Thiosulfates and Disulfides—Synthesized compounds were identified[2] by comparison of IR spectra with those of authentic samples (6). Purity was checked by TLC on silica gel plates with detection of spots by exposure to iodine vapor. Both radiolabeled and nonradiolabeled compounds were prepared on a small scale.

Preparation of Sodium ^{35}S-Thiosulfate Solution—An ampul of sodium ^{35}S-thiosulfate[3] (22.5 mCi/mmole) was broken, and ~0.0074 g was weighed and spiked with cold sodium thiosulfate to give a weight of 0.5745 g (3.6 mmoles). The sodium thiosulfate was transferred to a 5-ml volumetric flask, and distilled water was added to the mark. Each milliliter of this solution contained 0.114 g (0.72 mmole) of sodium thiosulfate. The remaining sodium thiosulfate was stored *in vacuo* over phosphorus pentoxide.

The synthetic procedures for the organic thiosulfates were essentially those already described (6). Weights of starting materials, yields, and melting points are listed in Table I. The disulfides were prepared as follows.

2-Aminoethyl ^{35}S-Disulfide Dihydrochloride—A solution of iodine (0.2487 g, 0.98 mmole) in 5 ml of alcohol was added dropwise to a solution of ^{35}S-2-aminoethanethiosulfuric acid in 1 ml of water. The solution was refluxed for 90 min, and ethanol was removed by distillation. The aqueous residue was extracted with chloroform after the pH was adjusted to 9.0 with 10% NaOH.

The chloroform extract was dried over magnesium sulfate, and the chloroform was evaporated under reduced pressure (40°). The resulting oil was treated with hydrochloric acid in ethanol, and the hydrochloride was twice recrystallized from absolute ethanol, giving 0.042 g (20% yield), mp 219–221° dec. [lit. (8) mp 211°].

2-(N-Morpholinyl)ethyl ^{35}S-Disulfide Dihydrochloride—A solution of ^{35}S-2-(*N*-morpholinyl)ethanethiosulfuric acid (0.449 g, 1.96 mmoles) and potassium iodide (0.065 g, 0.392 mmole) in 1 ml of water was heated, and a solution of iodine (0.2487 g, 0.98 mmole) in 5 ml of alcohol was added dropwise. The solution was refluxed for 90 min, and the ethanol was removed under vacuum at 40° in a rotary evaporator. A little water was added, the solution was filtered, and the filtrate was extracted with ether after the pH was adjusted to 9.0 with 10% NaOH.

The ether layer was dried over magnesium sulfate and evaporated under reduced pressure to an oil, which was treated with hydrochloric acid in ethanol. The solution was evaporated to dryness, and the residue was twice recrystallized from absolute ethanol, giving 0.1056 g (30% yield) of white crystals, mp 225–226° dec. [lit. (9) mp 229–230°]; R_f 0.53 (methanol–water, 6:4).

2-(N-Piperidinyl)ethyl ^{35}S-Disulfide Dihydrochloride—The preceding procedure was followed with ^{35}S-2-(*N*-piperidinyl)ethanethiosulfuric acid (0.441 g, 1.96 mmoles), giving 0.11 g (31% yield) of white crystals, mp 267–269° dec. [lit. (9) mp 270–271°]; R_f 0.28 (*n*-butanol–water–acetic acid, 49.5:49.5:1).

Anal.—Calc. for $C_{14}H_{28}N_2S_2{\cdot}2HCl$: C, 46.52; H, 8.37; N, 7.75; S, 17.74. Found: C, 46.6; H, 8.5; N, 7.7; S, 17.4.

2-(N-Pyrrolidinyl)ethyl ^{35}S-Disulfide Dihydrochloride—The preceding procedure was used with ^{35}S-2-(*N*-pyrrolidinyl)ethanethiosulfuric acid (0.4135 g, 1.96 mmoles), giving 0.067 g (20% yield), mp 231–233° dec. [lit. (9) mp 227–228°]; R_f 0.25 (*n*-butanol–water–acetic acid, 49.5:49.5:1).

Preparation of Stock DNA Solutions—Stock solutions of DNA[4] were prepared in phosphate buffers of the following ionic strengths and pH:

Ionic Strength 0.14, pH 7.2—potassium phosphate (monobasic), 0.0158 *M*; sodium phosphate (dibasic), 0.0404 *M*; sodium diethylenediaminetetraacetate, 0.003 *M*; and carbon dioxide-free distilled water, *qs*, 1000 ml.

Ionic Strength 0.021, pH 7.2—phosphate buffer of ionic strength 0.14 and pH 7.2, 75 ml; and carbon dioxide-free distilled water, *qs*, 1000 ml.

Ionic Strength 0.02, pH 6.0—potassium phosphate (monobasic), 0.0132 *M*; sodium phosphate (dibasic), 0.0013 *M*; sodium diethylenediaminetetraacetate, 0.003 *M*; and carbon dioxide-free distilled water, *qs*, 1000 ml.

The DNA concentration was measured[5] at 259 nm using the molar absorptivity $E(P)$ = 6650 (10). Appropriate dilutions were made, and a Beer–Lambert plot was constructed using the regressed absorption values. Spectral scans of DNA in the different stock solutions were identical. The DNA concentration in micromoles of DNA phosphorus per milliliter was determined:

$$\text{concentration of DNA phosphorus } (\mu\text{moles/ml}) = \frac{1000}{6650} \times 12.5 \times \text{dilution factor} \times \text{absorbance} \quad \text{(Eq. 1)}$$

The concentration values ranged from 1.7 to 2.1 μmoles of DNA phosphorus/ml for the different stock solutions.

Preparation of Stock Solutions of Radiolabeled Thiosulfates and Disulfides—Stock solutions containing 3 μmoles/ml of radiolabeled thiosulfates and disulfides were prepared in the appropriate phosphate buffer and stored at 4°. For the equilibrium dialysis experiments, concentrations of 0.5, 1.0, 1.5, 2.0, and 2.5 μmoles/ml were prepared at the time of the experiments.

Equilibrium Dialysis—The equilibrium dialysis experiments were performed as described by Wagner and Arav (11), using 5-ml glass or acrylic dialysis cells (12). A cellular dialysis membrane (mol. wt. cutoff 12,000) was pretreated by five consecutive 30-min boilings in distilled water and storage at 4° in phosphate buffer for 3–4 hr. The filled dialysis cells were immersed in a thermostated shaker bath at 37 ± 0.5° and shaken until equilibrium was reached (generally 24 hr).

The concentrations of compound in each chamber were determined by radioactivity measurements, or by absorbance measurements in the case of the quinoxalinyl derivative. For radioactivity determinations, 2-ml aliquots from each chamber were transferred to liquid scintillation vials (20 ml). To each was added 15 ml of a liquid scintillation cocktail[6], and the solution was shaken vigorously and counted[7]. An average of five readings was taken, and experiments were carried out in triplicate.

Concentrations of free (C_F) and bound (C_B) species were calculated:

$$C_F = \frac{CT_F}{CT_T} \times \text{total concentration of radiolabeled compound} \quad \text{(Eq. 2)}$$

$$C_B = \frac{CT_T - CT_F}{CT_T} \times \text{total concentration of radiolabeled compound} \quad \text{(Eq. 3)}$$

[1] Elemental analyses were carried out by Dr. F. B. Strauss, Oxford, England. Melting points were determined on a Mel-Temp capillary melting-point block.
[2] Perkin-Elmer 457 S grating spectrometer.
[3] Amersham-Searle Corp.
[4] Type I, sodium salt, Sigma Chemical Co., St. Louis, Mo.
[5] Beckman DB spectrophotometer.
[6] Phase Combining System, Amersham-Searle Corp.
[7] Packard Tri-Carb liquid scintillation spectrometer.

Table II—Binding Parameters of Disulfides with DNA [a]

pH	Ionic Strength	Compound	K_a, mM^{-1}	$1/n$	n
7.2	0.14	2-Aminoethyl disulfide	0.51	3.57	0.28
		2-(*N*-Morpholinyl)ethyl disulfide	0.0	0.0	—
		2-(*N*-Piperidinyl)ethyl disulfide	0.40	3.70	0.27
		2-(*N*-Pyrrolidinyl)ethyl disulfide	0.49	5.05	0.20
7.2	0.021	2-Aminoethyl disulfide	10.84	2.48	0.41
		2-(*N*-Morpholinyl)ethyl disulfide	0.0	0.0	—
		2-(*N*-Piperidinyl)ethyl disulfide	5.22	1.81	0.55
6.0	0.020	2-Aminoethyl disulfide	6.93	1.65	0.61

[a] The values were determined from reciprocal (Klotz) plots.

The mathematical treatment of the dialysis experiment data to give association constants, if all binding sites of DNA exhibit the same affinity toward a small molecule, can be expressed as:

$$K_a = \frac{r}{C_F(n - r)} \quad \text{(Eq. 4)}$$

where K_a is the intrinsic association constant, r is the number of adsorbates bound per reference unit (DNA base pair), n is the number of available binding sites per reference unit (DNA base pair), and C_F is the equilibrium concentration of free unbound species.

Plots of both r/C_F *versus* r according to Scatchard (13):

$$r/C_F = nK_a - rK_a \quad \text{(Eq. 5)}$$

and $1/r$ *versus* $1/C_F$ according to Klotz (14):

$$1/r = 1/n + 1/K_a(n)(C_F) \quad \text{(Eq. 6)}$$

were used to determine K_a and n. The binding data were plotted according to both equations. Both gave linear plots, but the correlation coefficient for the Klotz equation was better (>0.91). Values of K_a and n are listed in Table II as determined by the Klotz equation.

DISCUSSION

Binding studies of the heterocyclic aminoethyl disulfides and thiosulfates were carried out by equilibrium dialysis (11), and the concentrations of free and bound species at equilibrium were determined by radioactivity measurements. Radiolabeled thiosulfates were synthesized using sodium ^{35}S-thiosulfate and the heterocyclic aminoethyl chlorides where the heterocycles were morpholine, piperidine, and pyrrolidine; 2-bromoethylamine was also converted to the thiosulfate. The thiosulfates were converted to the radiolabeled disulfides by oxidation with iodine (15) since the thiosulfate was labeled on the outer sulfur atom. Yields from both reactions were generally in the 30–40% range, but piperidinyl thiosulfate was obtained in an 83% yield.

2-(2-Quinoxalinyl)aminoethanethiosulfuric acid (6) also was studied but was not labeled with sulfur 35. It exhibited strong UV absorption at 249 and 359 nm, which did not interfere with the absorption peak of DNA (256 nm at pH 7.2) and could be used as such. The concentration of bound compound was found by breaking the complex with dimethyl sulfoxide (16).

The results of the equilibrium dialysis experiments for the *N*-heterocyclic aminoethyl thiosulfates indicate that no binding took place with DNA. In mice (Table III), the quinoxalinyl derivative and aminoethanethiosulfuric acid had high radiation protective activity (>50% survival at 30 days), the piperidinyl derivative had moderate radiation protective activity, and the morpholinyl and pyrrolidinyl derivatives were inactive. Apparently, DNA binding is not a requirement for protective activity by the aminoalkyl thiosulfates, at least of the intact molecule. The negatively charged thiosulfate probably is repelled by the phosphate groups of DNA and the positively charged amino groups probably remain associated with the thiosulfate groups. *In vivo*, it is possible that the thiosulfates are metabolized to the disulfides, a reaction that occurs readily under hydrolytic conditions (17).

The binding parameters for the heterocyclic aminoethyl disulfides are listed in Table II. Comparison of the radioprotective activities (Table III) with the DNA binding data indicates that the heterocyclic compound with good activity (morpholinyl) did not exhibit binding ability whereas

Table III—Intraperitoneal Radiation Protective Activities of Thiosulfates and Disulfides in Mice

Compound	Drug Dose, mg/kg	Radiation Dose, rads[a]	Survival at 30 Days, %
2-Aminoethanethiosulfuric acid[b]	150	800R[c]	73
2-(*N*-Morpholinyl)ethanethiosulfuric acid	100	849	0
2-(*N*-Piperidinyl)ethanethiosulfuric acid	60	849	20
2-(*N*-Pyrrolidinyl)ethanethiosulfuric acid	70	849	0
2-(*N*-Quinoxalinyl)ethanethiosulfuric acid	400	849	70
2-Aminoethyl disulfide[d]	146	650R[c]	60
2-(*N*-Morpholinyl)ethyl disulfide	20	849	50
2-(*N*-Piperidinyl)ethyl disulfide	30	849	10
2-(*N*-Pyrrolidinyl)ethyl disulfide	20	849	10

[a] Radiation dosage was from ^{137}Cs γ-irradiation given at a rate of 141.5 rads/min except where indicated otherwise. [b] Data from D. L. Klayman, M. M. Grenan, and D. P. Jacobus, *J. Med. Chem.*, **12**, 510 (1969). [c] Dose is expressed in roentgens of X-radiation. [d] Data from M. L. Beaumariage, *C. R. Soc. Biol.*, **151**, 1788 (1957), using rats.

the poor protective agents (piperidinyl and pyrrolidinyl) showed some binding at ionic strength 0.14 and good binding at ionic strength 0.021. However, 2-aminoethyl disulfide showed both good binding ability and radioprotective ability, although the binding ability was depressed at the higher ionic strength which would resemble physiological conditions more closely. Obviously, no correlation can be drawn between DNA binding ability and radiation protective ability for the disulfides.

Some useful information may be deduced from the binding parameters. Straight-line plots were obtained with both Scatchard and Klotz equation, but the Klotz equation, after linear regression analysis, gave the best correlation coefficient (>0.91). The linear binding isotherms for the disulfides indicate that only one type of binding site on the DNA molecule interacted with these compounds. Comparison of the association constants at two different ionic strengths, 0.14 and 0.021, shows that binding at the higher ionic strength was suppressed, probably through competition with other ions. Comparison of the n values also indicates that the number of available binding sites was reduced. In addition, the lower binding constants at pH 6.0, in comparison to those at pH 7.2, indicate competition with hydrogen ions. At the lower pH, the greater degree of protonation of the amino groups might be expected to lead to greater association with phosphate groups of DNA.

The inability of the morpholine derivative to bind to DNA might be assumed to result from the polar nature of the hetero oxygen atom. However, the fact that the piperidinyl derivative gave lower association constants than the unsubstituted 2-aminoethyl disulfide at both ionic strengths suggests that steric hindrance prevented the association of the charged nitrogen with DNA phosphate groups. Apparently, only ionic interactions occurred. Also, since the association constants were suppressed at the higher ionic strength, it is questionable that the disulfides, including cystamine, would bind to DNA appreciably under physiological conditions. From the effects of ionic strength and pH on the binding constants and the lack of correlation with radiation protective activity, it may be concluded that binding to DNA is not a requisite for radiation protection or nucleic acid repair.

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Biodistribution of ^{14}C-Lomustine in an Animal Tumor Model

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Received May 14, 1979, from the *Department of Radiology, Massachusetts General Hospital, Boston, MA 02114.* Accepted for publication August 13, 1979.

Abstract □ A formulation of ^{14}C-lomustine in propylene glycol–ethanol (4:1) was administered intravenously to rats infiltrated with glioma tumors of the astrocytic series (RT6). The organ and tumor distribution of this agent was followed at 1, 4, 12, and 24 hr. Rapid blood disappearance (0–1 hr) of the label concomitant with an increase in all organs except the lung, muscle, and brain was observed. Only the blood, liver, and muscle contained >1% of the dose after 24 hr. The bladder, liver, small bowel, and kidneys concentrated the highest percentages throughout the study. The distribution of ^{14}C-lomustine in the tumor relative to the brain, muscle, and blood showed a maximum 4–12 hr after administration.

Keyphrases □ Lomustine, ^{14}C-labeled—biodistribution, rat tumor model □ Antineoplastic agents—^{14}C-lomustine, biodistribution, rat tumor model □ Biodistribution—^{14}C-lomustine, rat tumor model

Early detection of malignant tumors serves as a basis for improving cancer control. One experimental approach is to label minute quantities of antineoplastic drugs with appropriate radionuclides and to follow their biodistribution in a suitable animal tumor model. One example involved the *N*-nitrosoureas, a class of chemotherapeutic drugs for various malignant tumors (1). The structures of several *N*-substituted nitrosoureas are shown in Table I, where the chlorine- or fluorine-substituted haloethyl derivatives are the most active, with the methyl-substituted nitrosourea possessing the least chemotherapeutic activity (2).

The agent investigated in this study, lomustine [1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea[1], CCNU, I], is an asymmetrical *N*-substituted nitrosourea used for the palliative treatment of primary and metastatic brain tumors and for Hodgkin's disease (3). This investigation followed the radiopharmacodynamics of ^{14}C-I in tumor-bearing rats for 24 hr after intravenous injection.

EXPERIMENTAL

Materials and Methods—The *N*-nitrosoureas are lipophilic agents whose solubility characteristics require formulations with hydroalcoholic vehicles (4). Propylene glycol–ethanol (4:1) was the vehicle chosen for intravenous administration of ^{14}C-I[1] (5).

The ^{14}C-I [1-(2-chloroethyl-U-^{14}C)-3-cyclohexyl-1-nitrosourea] had a specific activity of 12.156 mCi/mmole (52.017 μCi/mg; 1.0195 mCi in 19.6 mg) and a radiochemical purity of 99.7%. The ^{14}C-I was labeled at the chloroethyl moiety (Table I). Prior to the animal studies, the ^{14}C-I was added to 10 ml of the propylene glycol–ethanol mixture.

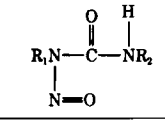
Tissue Distribution—The rat tumor model (RT6) was a brain malignancy induced by repeated intravenous injections of *N*-nitrosomethylurea (6). The induced tumors were gliomas of the astrocytic series, the histology of which did not vary significantly with serial passage through tissue culture, subcutaneous implantation, or freezing (6). This model was used previously for the determination of the tumor affinity of various ^{99m}Tc-labeled compounds (7). Early detection of this type of tumor in patients with glioblastoma multiforme would be advantageous since the current survival estimate for this pathology 2 years after surgery is ~10% (8).

To determine the pharmacodynamics of ^{14}C-I, 0.2 ml was injected intravenously *via* the tail vein in adult male rats. Three rats were sacrificed at 1, 4, 12, and 24 hr postadministration. Each 0.2 ml of solution had 0.385 mg (1.65 μmoles) of ^{14}C-I (20 μCi of ^{14}C).

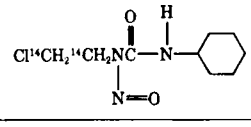
At the time of sacrifice, the blood (7% of the body weight), brain, liver, spleen, pancreas, lungs, small bowel, kidneys, heart, bladder, bone marrow, muscle, and tumor fluid, capsule, and necrotic center were isolated from each animal, and their radioactivity content was measured. The dose percentage per gram of tissue, the dose percentage per organ, and the mean ± standard deviation (n = 3) were calculated.

Activity Measurement—Approximately 100 mg of each tissue was placed in a glass scintillation vial containing 2 ml of tissue solubilizer (9).

Table I—Structures of Several *N*-Substituted Nitrosoureas



R_1	R_2	Name
Methyl	H	1-Methyl-1-nitrosourea
Fluoroethyl	Fluoroethyl	1,3-Bis(2-fluoroethyl)-1-nitrosourea
Chloroethyl	Chloroethyl	1,3-Bis(2-chloroethyl)-1-nitrosourea
Chloroethyl	Cyclohexyl	1-(2-Chloroethyl-U-^{14}C)-3-cyclohexyl-1-nitrosourea



[1] Synthesized by the Monsanto Chemical Co. under contract with the National Institutes of Health and provided by Dr. R. Engle of the National Cancer Institute (sample NSC-79037,350-4H).

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