

ratio is worse than that by  $(F^1/F^2)_{K-T}$ . Finally, the urinary recovery ratio is the worst approximation of all, which is not unexpected since renal excretion is only a minor elimination route ( $f < 0.50$ ).

## DISCUSSION

An approximate solution for relative bioavailability estimation between nonintravascular doses was suggested previously (1). Since then, numerous inquiries have been received concerning the nature and source of its inexactitude, particularly under the assumption of constant nonrenal clearance between treatments. Simulation studies were not revealing; reasonably accurate estimates usually were obtained except when unrealistically large perturbations were considered. On the other hand, the present theoretical analysis appears to offer new insights.

Whereas the ratio of areas under the plasma curve and of urinary excretions are exact relative bioavailability determinants when their respective assumptions prevail, Eqs. 8 and 13 are only approximations when nonrenal clearance remains constant between treatments. In addition, Eqs. 8 and 13 give different answers for a given data set. However, criteria were established so that the more accurate of the two estimates always can be identified. The choice of reference for calculations should be made for each comparison within a study to ensure the best possible estimates.

Despite their approximate nature, Eqs. 8 and 13 always are preferable to area ratios and often are superior to urinary excretion ratios when nonrenal clearance is constant. The only exception is when the drug is

eliminated predominantly by renal excretion. Predominance now has been defined as when  $\dot{V}_{cl,r}/\dot{V}_{cl,p}$  is greater than either  $1/2(1 + U^*/D^*)$  or:

$$\frac{1}{2} + \left\{ \frac{1}{2} + \frac{(\dot{V}_{cl,p}^*]_{ex} - \dot{V}_{cl,r}^*) - (\dot{V}_{cl,p}^*]_{ex} - \dot{V}_{cl,r}^*)}{[\dot{V}_{cl,p}^*]_{ex} - \dot{V}_{cl,r}^* + \dot{V}_{cl,r}^*} \right\} \frac{U^*}{D^*}$$

depending on whether Eq. 8 or 13 is to be used, respectively. Thus, given some idea of the usual fraction excreted unchanged following an intravenous dose, a decision can be made whether the urinary excretion ratio, Eq. 8, or Eq. 13 should be chosen.

The constant nonrenal clearance assumption ordinarily is favored for another reason. Given that observed renal clearances differ between treatments, the likelihood that compensatory nonrenal clearance changes will occur to maintain a constant plasma clearance or a constant ratio of renal to plasma clearance seems remote. On the other hand, adjustments in plasma clearance based only on observed changes in renal clearance simply corroborate experimental evidence.

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# Mass Spectrometry of Chlorambucil, Its Degradation Products, and Its Metabolite in Biological Samples

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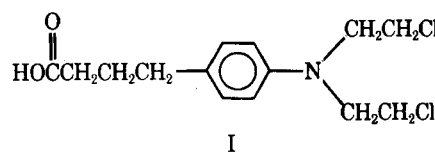
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**Abstract** □ A sensitive and specific method for the determination of chlorambucil and its metabolite in biological fluids is reported. The method is based on selected-ion monitoring detection following simple extraction of the parent compound, its metabolite, and an internal standard (chlorambucil- $d_8$ ) from plasma and urine samples. The precision (reproducibility) of the method was  $94.3 \pm 1.3\%$  with 200 ng of chlorambucil added to 1 ml of plasma. Chlorambucil degradation or alkylation of plasma proteins was minimal with plasma incubated at 24° for 4 hr. However, chlorambucil recovery decreased to 56% after plasma incubation at 37° for 4 hr. Three chlorambucil degradation products in ethyl acetate solution were found, and their structures were studied by mass spectrometry.

**Keyphrases** □ Chlorambucil—analysis, mass spectrometry, degradation products and metabolite, human plasma and urine □ Antineoplastic agents—chlorambucil, degradation products and metabolite, mass spectrometry, human plasma and urine □ Mass spectrometry, selected-ion monitoring—chlorambucil, degradation products and metabolite, human plasma and urine

The anticancer drug chlorambucil (I) is useful in the treatment of chronic lymphocytic leukemia, ovarian carcinoma, nodular lymphocytic lymphoma, and myelocytic leukemia (1, 2). Various analytical methods were reported for the quantitation of I, including the colorimetric determination of 4-(*p*-nitrobenzyl)pyridine derivatives (3, 4) and UV spectrophotometric (5) and chlorine titrimetric (6) methods. None of these methods provides the sensitivity and accuracy needed to study I pharmacokinetics and metabolism in humans. The mass spectrometric determination of I in plasma was reported recently (7). This



method requires several extraction steps, including a back-extraction of I from the aqueous solution at alkaline pH.

The quantitative method presented here is based on a one-step extraction followed by a determination of I and a metabolite in plasma and urine by mass spectrometry using a deuterated I internal standard. This method was used to study the *in vitro* I stability in plasma as well as to characterize the *in vitro* degradation products. The method was applied to the quantitation of I and a metabolite in human plasma and urine samples.

## EXPERIMENTAL

**Chlorambucil- $d_8$  Synthesis**—The synthesis of chlorambucil- $d_8$  (labeled at *N*-chloroethyl) was adapted from a literature method (8). 4-(*p*-Nitrophenyl)butyric acid<sup>1</sup>, 500 mg, was reacted with ethereal diazomethane<sup>1</sup>. The resulting methylnitrophenylbutyrate was dissolved in ethyl acetate-methanol (9:1 v/v) through which hydrogen gas was bubbled continuously for 8 hr in the presence of palladium<sup>2</sup> with the solvent

<sup>1</sup> Aldrich Chemical Co., Milwaukee, Wis.  
<sup>2</sup> Ventron Corp., Danver, Mass.

at constant volume. The ethyl acetate then was evaporated using nitrogen gas, yielding the reddish-brown aminophenylbutyric acid methyl ester, which was dissolved immediately in 20 ml of 50% acetic acid ( $\text{CH}_3\text{COOD}^3$  in  $\text{D}_2\text{O}^3$ ). In a cold room ( $4^\circ$ ), 2 ml of ethylene oxide- $d_4^3$  (99% isotopic purity) was added.

The reaction mixture was allowed to stand at room temperature for 24 hr. The reaction was terminated by adding ~10 ml of water and sodium bicarbonate until no significant carbon dioxide evolved. The yellowish solution was extracted with  $5 \times 8$  ml of ethyl acetate, and the ethyl acetate was dried with anhydrous sodium sulfate and then evaporated using nitrogen gas. All traces of water were removed by azeotroping with methylene chloride.

The gummy, dark-brown product was refluxed with 4 ml of freshly distilled phosphorus oxychloride for 45 min. Excess reagent was removed by partitioning several times with water-methylene chloride. The methylene chloride layer was evaporated using a nitrogen stream and refluxed with concentrated hydrochloric acid for 6 hr at  $100^\circ$ . The product was extracted with ethyl acetate-hexane (1:1) and partitioned with 0.01 M phosphate buffer (pH 9.0)-0.01 M NaCl. The aqueous phase was removed and washed twice with methylene chloride, and the pH was adjusted to 3 using 2 N HCl.

Chlorambucil- $d_8$  was extracted from the acidic aqueous solution with methylene chloride. The final product in methylene chloride was examined by high-performance liquid chromatography<sup>4</sup> and mass spectrometry<sup>5</sup>. The product had >95% chemical purity and >99% isotope purity as revealed by mass spectrometry for the molecular and base ions of deuterated and undeuterated chlorambucil. The yield was ~30%.

**Chlorambucil- $d_8$  Standardization**—Synthetic chlorambucil- $d_8$  was used as an internal standard without further purification. Its chlorambucil- $d_8$  concentration was determined against a known amount of I<sup>6</sup> by monitoring  $m/e$  326 (M -  $\text{CH}_2\text{Cl}$ ) and 328 for I and  $m/e$  332 (M -  $\text{CD}_2\text{Cl}$ ) and 334 for chlorambucil- $d_8$ . Also,  $m/e$  326 and 328 of I were monitored against  $m/e$  383 and 385 of chlorambucil- $d_8$ . Interferences due to other ions were not observed.

**Extraction of I**—Deuterated I (five to 10 times the maximum I amount expected) and 0.5 ml of 4% perchloric acid were added to 0.5 ml of plasma or urine. Ethyl acetate-hexane (1:1 v/v), 0.75 ml, was added to the precipitated sample, and I was extracted into the organic layer. The sample was centrifuged for 5 min at 3000 rpm<sup>7</sup>, and the organic layer was transferred into a 1-ml microvial with a polytetrafluoroethylene-lined cap<sup>8</sup>. The solvent was evaporated with purified nitrogen gas at room temperature, and several drops of dry methylene chloride were added to the dried residue. Any water present in the dried residue was removed azeotropically with methylene chloride.

**Derivatization of I**—Dried samples were converted to their trimethylsilyl (TMS) derivatives for GLC-mass spectrometric analysis. *N,O*-Bis(trimethylsilyl)trifluoroacetamide<sup>9</sup>-acetonitrile-methylene chloride (20:10:1 v/v/v) solution, 100  $\mu\text{l}$ , was added to the samples and allowed to react at  $100^\circ$  for 10 min. The trimethylsilyl derivatives were analyzed by GLC-mass spectrometry.

**GLC-Mass Spectrometry**—A mass spectrometer<sup>5</sup> was interfaced with a gas-liquid chromatograph<sup>9</sup>. All analyses were performed at 70 eV. The derivatized samples were injected directly onto a GLC column (0.5 m  $\times$  2 mm, 3% OV-17 on 100-120-mesh Gas Chrom Q) at  $190^\circ$ . After the solvent was vented for 45 sec at  $190^\circ$ , the column temperature was raised directly to  $310^\circ$ . A I trimethylsilyl derivative eluted at  $230^\circ$ .

Trimethylsilyl I was monitored at  $m/e$  326 (M -  $\text{CH}_2\text{Cl}$ ) and 328, and the internal standard (trimethylsilyl chlorambucil- $d_8$ ) was monitored at  $m/e$  383 (M<sup>+</sup>) and 385. Deuterated I was monitored at its molecular and chlorine isotope ions ( $m/e$  383 and 385) instead of at its most intense fragment ions [ $m/e$  332 (M -  $\text{CD}_2\text{Cl}$ ) and 334] due to an interfering ion at  $m/e$  332. Because  $m/e$  383 was ~10 times less abundant than  $m/e$  332, 10 times more of the internal standard relative to the expected I concentration was added to give an approximately equal height compared to that of  $m/e$  326.

**Animal and Human Studies**—Rats were given 5.6 mg of I/kg by intravenous bolus injections over 1 min into a jugular venous catheter (9). Patients were given 0.6 mg of oral I<sup>10</sup>/kg. Animal and human plasma and

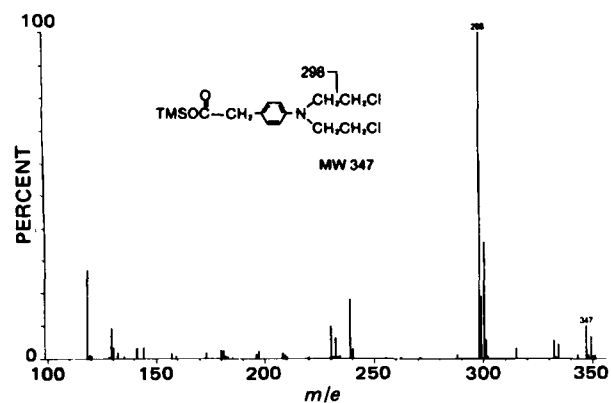


Figure 1—Mass spectrum of metabolite, *p*-[4-*N,N*-bis(2-chloroethyl)aminophenyl]acetic acid; isolated from human plasma and urine.

urine samples were kept on ice during collection and stored at  $-70^\circ$  until extracted for I.

**Recovery Studies**—Quantitative extraction was studied with  $^{14}\text{C}$ -labeled I.  $^{14}\text{C}$ -I<sup>11</sup> (505 ng,  $3.5 \times 10^6$  cpm) was added to 1 ml of plasma, the solution was extracted three times, and the  $^{14}\text{C}$ -recovery in the organic layer was examined in each extraction. The precision (reproducibility) of the recovery was studied by mass spectrometry. At least three samples of 5 and 1  $\mu\text{g}$  and 200, 100, and 50 ng of I and appropriate amounts of the internal standard were added to 1-ml plasma samples. A single extraction was then carried out. The expected ratio of I to the internal standard was used to calculate recovery precision.

**Stability of I**—The I stability (10  $\mu\text{g}/\text{ml}$ ) in plasma at  $37^\circ$  was studied. At designated times, a sample was taken, the internal standard was added, and the extraction, derivatization, and analyses were performed. The disappearance rate constant,  $\kappa$ , of I was calculated according to  $\kappa = 2.3 \log ([C]_0/[C]_t)/t$ , where  $[C]_0$  and  $[C]_t$  are the concentrations of I in the medium at times zero and  $t$ .

Drug stability in a nonaqueous medium also was studied. A solution of I in ethyl acetate was left at room temperature under ordinary laboratory conditions (with fluorescent light) for 2 weeks. The intensity of the brown color of the ethyl acetate solution increased with prolonged exposure to light. This brownish I solution was examined by GLC-mass spectrometry as the trimethylsilyl derivatives.

**Calculations**—The I concentrations in plasma and urine were calculated as follows:

$$\text{nanograms of I/aliquot} = \frac{\text{response of I sample}}{\text{response of internal standard sample}} \times \frac{1}{\text{relative equal weight response}} \times \text{nanograms of internal standard} \quad (\text{Eq. 1})$$

where:

$$\text{relative equal weight response} = \frac{\text{response of I}}{\text{response of internal standard}} \quad (\text{Eq. 2})$$

for standards of equal weight.

**Identification and Determination of Phenylacetic Acid Mustard (*p*-[4-*N,N*-bis(2-chloroethyl)aminophenyl]acetic Acid)**—Plasma and urine samples were extracted and derivatized as described for chlorambucil. Trimethylsilyl derivatives of samples were introduced directly into the GLC system, and the mass spectra of the resolved peaks were examined. Phenylacetic acid mustard was synthesized and highly purified.

The spectrum of the trimethylsilyl derivative of synthetic phenylacetic acid was compared directly to the GLC spectra of plasma and urine. The trimethylsilyl derivative of phenylacetic acid mustard eluted earlier than that of chlorambucil. For routine analysis of phenylacetic acid mustard in biological samples,  $m/e$  298 (M -  $\text{CH}_2\text{Cl}$ ) was monitored simultaneously with chlorambucil. The quantitation was performed as with

<sup>11</sup>  $^{14}\text{C}$ -Chlorambucil (labeled at the chloroethyl moiety) was supplied by Dr. R. Engles, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md.

<sup>3</sup> Merck and Co., St. Louis, Mo.

<sup>4</sup> Waters Associates, St. Louis, Mo.

<sup>5</sup> Model 3300, Finnigan, Sunnyvale, Calif.

<sup>6</sup> Supplied by Burroughs-Wellcome Co., Research Triangle Park, N.C.

<sup>7</sup> GLC-2B, DuPont Instrument Co., Wilmington, Del.

<sup>8</sup> Regis Chemical Co., Morton Grove, Ill.

<sup>9</sup> Model 950 GC, Finnigan, Sunnyvale, Calif.

<sup>10</sup> Leukeran, supplied by Burroughs-Wellcome Co., Research Triangle Park, N.C.

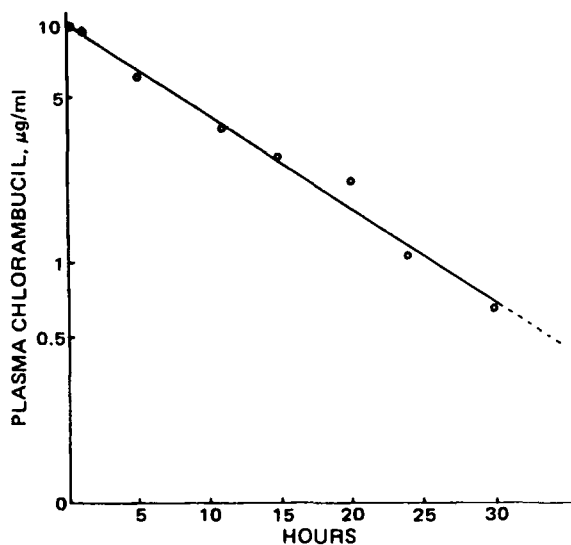


Figure 2—Disappearance of I from plasma at 37°. Ten micrograms of I was added to 1.0 ml of plasma and incubated at 37°.

chlorambucil. The initial calibration was carried out using the synthetic compound against chlorambucil- $d_8$ .

**Linearity**—Linearity of the I determination was studied at 1 µg and 100, 50, 10, 5, 2, 1, and 0.5 ng. Each sample was derivatized independently

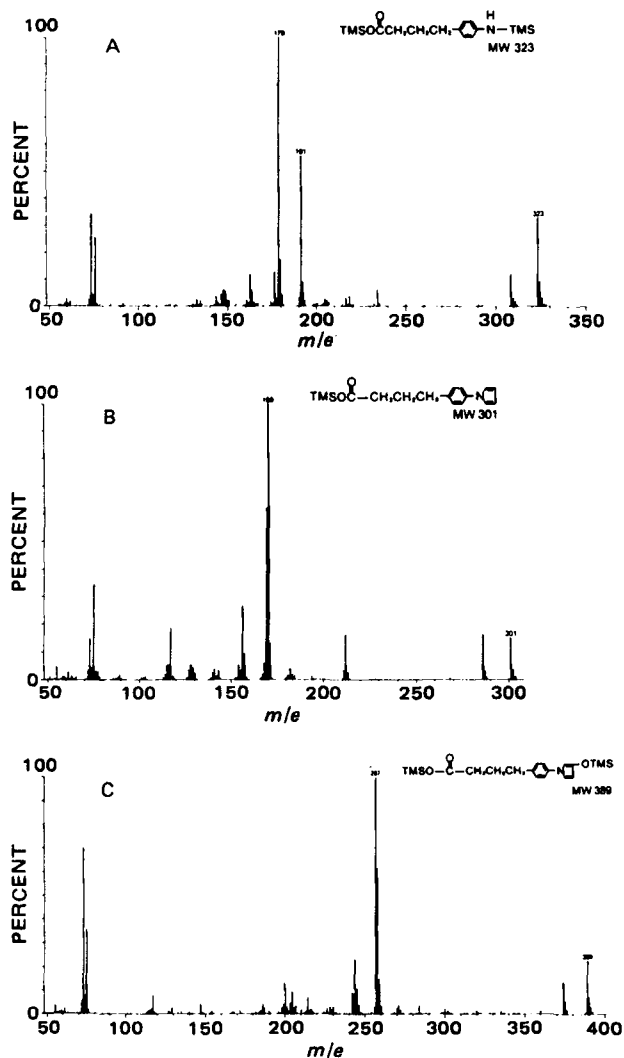


Figure 3—Mass spectra of the degradation products of I in ethyl acetate.

along with the appropriate amount of internal standard and analyzed. The linearity was evaluated against  $y = x$ .

Since an excess of the internal standard was used, the dynamic range of the relative equal weight response also was examined. Both a 100-fold excess of the internal standard to I and a fivefold excess of I to the internal standard were studied. The concentration range used was 5 µg–0.5 ng for I and 10 µg–50 ng for the internal standard.

## RESULTS

The spectrum of *p*-[4-*N,N*-bis(2-chloroethyl)]aminophenylacetic acid, a metabolite detected in human plasma and urine (Fig. 1), was identical to that of the synthesized compound.

Studies with  $^{14}\text{C}$ -labeled I indicated that more than 70% of added I was recovered from plasma at 25°. Eighty-four percent of the I was recovered when the extraction was repeated three times. The extraction of I at concentrations of 5 and 1 µg and 200, 100, and 50 ng added to 1 ml of plasma showed percent recoveries and standard deviations of  $100.4 \pm 2.0$ ,  $90.5 \pm 3.0$ ,  $94.3 \pm 1.3$ ,  $88.2 \pm 4.2$ , and  $91.3 \pm 12.8$ , respectively. At least three samples of each concentration were used for statistical calculations.

The linearity of the I determination between 1 µg and 500 pg showed a correlation coefficient,  $r$ , of 0.999. There was no statistical difference from  $y = x$  ( $p > 0.05$ ). The relative equal weight response calculated from either a 100-fold excess of the internal standard to I or a fivefold excess of I to the internal standard was consistent.

The disappearance of I incubated in plasma (10 µg/ml) is shown in Fig. 2. The disappearance rate constant of I was  $k = 0.09 \text{ hr}^{-1}$  for the plasma (Fig. 2). The correlation coefficient,  $r$ , for all data points to  $C = C_0 e^{-kt}$  was 0.993. Depending on the source of the plasma used, there was an ~10% variation in the disappearance rate constant.

In contrast to its degradation in an aqueous medium, I stored in a nonaqueous solution (ethyl acetate) at room temperature under fluorescent light resulted in three major products (Fig. 3). With deuterated *N,O*-bis(trimethylsilyl)trifluoroacetamide- $d_8$ , the number of trimethylsilyl groups per molecule was two for Compound A (Fig. 3A), one for Compound B (Fig. 3B), and two for Compound C (Fig. 3C).

Stability data for I in plasma at 25 and 37° are presented in Table I. About 90% of the I was recovered from the plasma after 4 hr of incubation at 25° whereas only 56% of the I was recovered at 37° after that time.

Figure 4 shows a plasma I disappearance curve following an intravenous bolus injection of 5.6 mg/kg in a rat. The plasma disappearance of 0.55 mg of I/kg given orally and of *p*-[4-*N,N*-bis(2-chloroethyl)]aminophenylacetic acid in a patient with chronic lymphocytic leukemia is shown in Fig. 5. The selected-ion chromatograms of plasma I and the background are shown in Fig. 6.

## DISCUSSION

The method presented is based on a one-step extraction from biological fluids, and the selected-ion monitoring method was used for the subsequent determination of I and a metabolite. The method can be used to study pharmacokinetics and metabolism in cancer patients. Compound I was monitored at  $m/e$  326 and 328 with deuterated I at  $m/e$  383 and 385. When 500 pg was derivatized and analyzed, there was sufficient sensitivity to detect quantities at the 100-pg level. However, to prevent I adsorption, the column had to be preconditioned with two to three repeated injections of I (~0.5 µg). The chlorine isotope ratio gave additional selectivity. The deuterated I synthesis can be carried out in most laboratories using basic organic chemistry techniques and starting materials readily available commercially.

A method for the determination of I in plasma was published recently (7) that depends on several extraction steps followed by mass spectrometric analysis. This method is not only lengthy, but it also requires back-extraction from the buffer at pH 9.0. The instability of the chloroethyl group at alkaline pH is well documented (6, 10, 11). At room temperature, there was ~13% hydrolysis of I in aqueous solution at pH 9.0 over 30 min (7). Alkaline exposure at higher temperatures could result in even greater hydrolysis. Although such hydrolysis is a spontaneous phenomenon observed in *in vitro* situations, the *in vivo* hydrolysis rate may be far greater. Quantitative determination of the formation of hydroxy products is necessary for an overall understanding of the pharmacokinetic behavior of I.

Table I shows the effect of the incubation time and temperature on the stability and recovery of plasma I. Compound I was stable in plasma at room temperature, but recovery decreased significantly at 37°. This low recovery probably resulted from both alkylation and hydrolysis. There

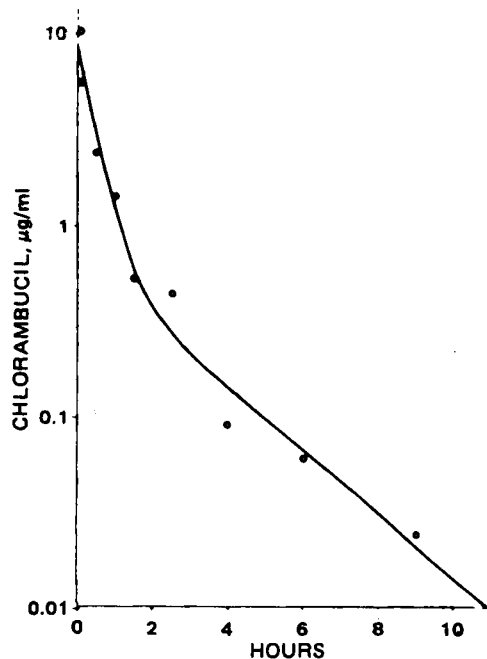


Figure 4—Disappearance of I in rat plasma.

may be a protective effect against hydrolysis by plasma proteins, as was shown previously for I and phenylalanine mustard (6, 12).

Three major compounds were observed in the nonaqueous system. The mass spectra with deuterated *N,O*-bis(trimethylsilyl)trifluoroacetamide

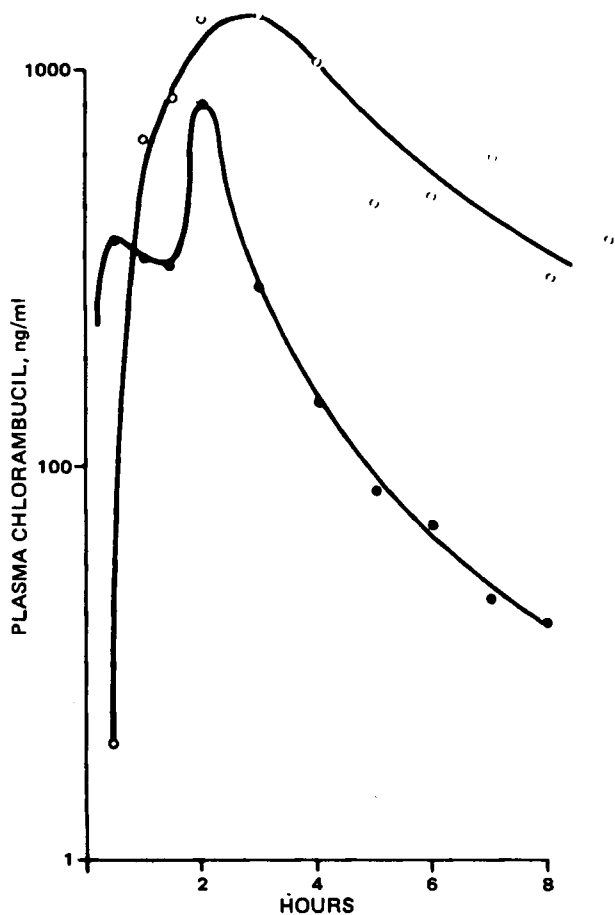


Figure 5—Plasma I disappearance in a patient with chronic lymphocytic leukemia. The patient was given 0.55 mg/kg orally, and samples were drawn at designated times and analyzed for p-[4-N,N-bis(2-chloroethyl)]aminophenylacetic acid (O) and I (●).

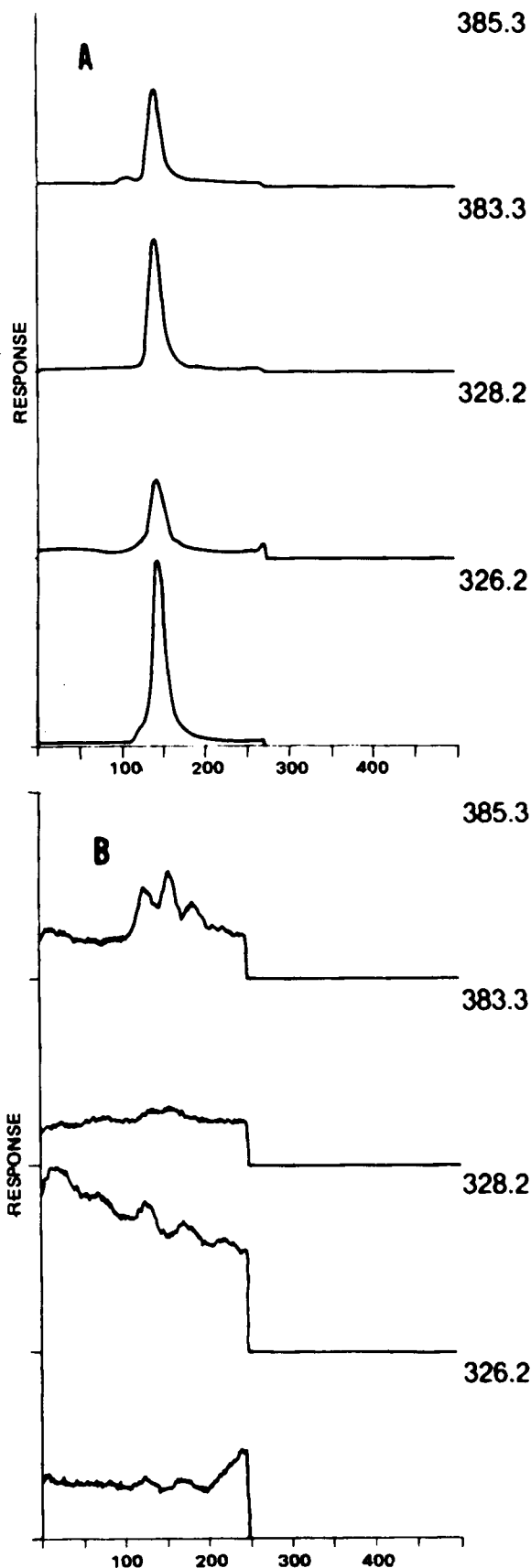


Figure 6—Ion chromatograms of plasma I and plasma background. Compound I, 200 ng, and 2.0 µg of the internal standard were added to 1.0 ml of plasma. After extraction and derivatization, 0.1 ml of equivalent plasma was analyzed (Fig. 6A). In Fig. 6B, 1.0 ml of plasma was extracted and derivatized, and 0.5 ml of equivalent plasma was analyzed.

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

Hours	Recovery <sup>a</sup>	
	25°	37°
0.0	90.5 ± 3.0	80.4 ± 3.0
0.5	82.5 ± 4.8	76.6 ± 3.7 <sup>b</sup>
4.0	91.7 ± 2.6	55.6 ± 1.2 <sup>b</sup>

<sup>a</sup> Percent recovery and percent relative standard deviation of quadruplicate analyses. <sup>b</sup> 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 (<sup>12</sup>C/<sup>13</sup>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<sub>3</sub>)<sub>3</sub>SiOCOCH<sub>2</sub>], 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 <sup>14</sup>C-I most likely was due to these pyrrole derivatives.

The  $\alpha,\beta$ -unsaturated compounds 2-[4-*N,N*-bis(2-chloroethyl)]-aminophenyl-2-butenic 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  $\beta$ -oxidation of I. The phenylacetic acid derivative in human urine and plasma was found (Figs. 1 and 5), and

attempts to find the  $\alpha,\beta$ -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-