The Absorption, Distribution, Excretion, and Biotransformation of the Carcinostatic 1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea in Animals\textsuperscript{1}

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SUMMARY

The physiological disposition of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, a highly active agent against intraperitoneal and intracranial mouse leukemia L1210, was studied in mice, rats, dogs, and monkeys with the \textsuperscript{14}C label in each of 3 positions of the molecule, the ethylene, carbonyl, and cyclohexyl moieties. 1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea is lipid soluble and, after parenteral dosage, rapidly degraded in mouse and dog plasma with 2 exponential phases. The half-life of the initial phase is about 5 min, while the 2nd phase extends over 1 hr. During the 6 hr after i.v. injection of ethylene-labeled 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea in dogs, radioactivity in the cerebrospinal fluid exceeded that of plasma 3-fold. With the label in the cyclohexyl moiety, plasma radioactivity was about 50\% of cerebrospinal fluid \textsuperscript{14}C levels. The drug following biotransformation is primarily excreted by the kidneys with excretion being essentially complete during the first 24 hr in rodents and monkeys, but more protracted in dogs. Biliary secretion and reabsorption from the gastrointestinal tract have been demonstrated. The cyclohexyl portion of the molecule was bound extensively (40 to 60\%), while the ethylene moiety was not bound to plasma proteins of dogs. In mice, 10 to 20\% of the carbonyl and 4 to 6\% of ethylene carbon atoms were recovered in expired CO\textsubscript{2} 1 day after parenteral or oral dosage. The detection of cyclohexylamine, \textit{N,N'}-dicyclohexylurea, among other predominant unidentified molecular species, supports the hypothesis of intermediate hydroxydiazoalkane and isocyanate formation during the degradation of nitrosoureas in \textit{vivo}. However, the identified catabolites and cyclohexyl isocyanate were found to be inactive against the mouse leukemia L1210.

INTRODUCTION

In the search for new and more effective antitumor agents, the combined efforts of several research institutions have recently produced a family of antitumor agents, the nitrosoureas, with great efficacy in rodent tumor screens (6, 8, 16, 18) and man (1, 13). Only one of these agents, BCNU\textsuperscript{2} (NSC 409962), has received intensive clinical trial and was found to be effective against a wide spectrum of advanced solid tumors, including Hodgkin's disease (1, 9, 11), acute lymphocytic leukemia, and its meningeal spread in children (7, 15).

Although intensive investigations have been made of the biochemistry (5, 20, 21) and pharmacological disposition of BCNU in animals and man (2, 10), the active moiety of this agent is still unknown. This is attributable to the pronounced instability of BCNU in biological systems and to the high reactivity of its daughter products (12).

Another related compound, CCNU (NSC 79037, Chart 1), is at least as or more active than BCNU against mouse leukemia L1210 and is more lipid soluble, which might enhance passage across the blood-brain barrier. Also, in contrast to BCNU, CCNU has only a single \textbeta-chloroethyl group and a cyclohexyl group which can serve as a "handle" in following the metabolic fate of the drug, since it might be expected to be less reactive than the chloroethyl moiety and not as susceptible to degradation. Therefore, we have studied the physiological disposition of CCNU in animals in hopes of better elucidating its own metabolic fate and mode of action and that of the class of nitrosoureas.

MATERIALS AND METHODS

Radioactive CCNU was obtained from the Cancer Chemotherapy National Service Center with the \textsuperscript{14}C label in 3 separate positions of the molecule: the carbon atoms of the 2-chloroethyl moiety (ethylene-\textsuperscript{14}C-labeled CCNU, 1.37 mCi/m mole), the carbonyl moiety (carbonyl-\textsuperscript{14}C-labeled CCNU, 1.14 mCi/m mole) and uniformly throughout the carbon atoms of the 3-cyclohexyl ring (cyclohexyl-\textsuperscript{14}C-labeled CCNU, 1.13 mCi/m mole). The radioactively labeled drugs were of greater than 95\% purity as determined by thin-layer chromatography on...
Distribution and Excretion of Radioactivity

**Mice.** CDF1 male mice were given 50 mg/kg (approximate LD_{50}, effective antitumor dose) of each of the labeled CCNU preparations p.o. or i.p. The animals were kept in glass metabolism cages, and urine and stool were collected separately at various time intervals by the anal cup technique (14).

When assays were to be made for parent compound and biotransformation products, the urine was collected in flasks containing 0.2 ml 0.1 N hydrochloric acid immersed in a Dry Ice bath. Organs and tissues were excised from sacrificed animals and homogenized in distilled water, and dried aliquots were combusted by the oxygen flask technique to determine radioactivity content as described earlier for BCNU (2). Expired \(^{14} \text{CO}_2\) was collected from each of 6 animals over a 24-hr period following drug administration, and the radioactivity was assayed by Steinberg’s (17) anthracene system. Urine and plasma samples were processed and chromatographed on thin-layer Silica Gel G plates as described for BCNU (2), except that chloroform:95% ethanol (98:2, v/v) was used as the developing solvent.

All labeled compounds were stored in the dark at 0° until use. For p.o. administration or i.p. injections in mice and rats, the dose was prepared immediately prior to administration by dissolving CCNU in 95% ethanol and diluting with propylene glycol until the final volume contained a glycol:alcohol ratio of 5:0.6 (v/v). For i.v. administration in dogs and monkeys, the compound was dissolved in either a mixture of 99% ethanol:0.9% NaCl solution: DMSO (7:5:5, v/v) or 99% ethanol: propylene glycol (7:3, v/v).

Radioactive determinations on all dose solutions and biological samples were carried out as described previously for BCNU (2).

Recovery and Identification of CCNU and Metabolites in Biological Fluids

The modified Greiss reaction (2) was used to identify intact CCNU in solution and on thin-layer chromatograms (Silica Gel G). The thin-layer plates and radioautograms of the plates were prepared as previously described in the BCNU studies (2). Urine samples were spotted on plates directly, with a standard reference solution of CCNU. For assay of intact CCNU, 1.0-ml samples of plasma and CSF were extracted twice with 2.0 ml ether in a stopped tube. The combined ether layers were evaporated under a gentle stream of nitrogen at 45° and 0.13 ml ice-cold ethanol (95%) was added to the tube which was then agitated on a Vortex mixer for several sec. Duplicate 50-\(\mu\)l samples were then spotted on a thin-layer chromatogram, one at the origin, and the other ahead of the solvent front which served as a control to indicate the amount of radioactivity at the origin. The plates were then developed in a system of chloroform:95% ethanol (98:2, v/v) and the radioactive spots were either located by using a Baird-Atomic model RSC-363 radiochromatogram scanner or by preparing a radio-
Oliverio, Vietzke, Williams, and Adamson

Table 1
Excretion of radioactivity in mice after a single parenteral or oral dose of 50 mg/kg CCNU-\textsuperscript{14}C

<table>
<thead>
<tr>
<th>Position of label</th>
<th>Cumulative % of dose</th>
<th>Ethylene-\textsuperscript{14}C</th>
<th>Carbonyl-\textsuperscript{14}C</th>
<th>Cyclohexyl-\textsuperscript{14}C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine 6 hr</td>
<td>46.4</td>
<td>16.0</td>
<td>28.8</td>
<td>32.6</td>
</tr>
<tr>
<td>24 hr</td>
<td>86.2</td>
<td>79.9</td>
<td>57.3</td>
<td>57.9</td>
</tr>
<tr>
<td>Co (24 hr)</td>
<td>3.5</td>
<td>5.8</td>
<td>24.4</td>
<td>10.6</td>
</tr>
<tr>
<td>Feces (24 hr)</td>
<td>≤0.1</td>
<td>1.2</td>
<td>1.3</td>
<td>1.4</td>
</tr>
<tr>
<td>Total</td>
<td>89.7</td>
<td>86.9</td>
<td>83.0</td>
<td>69.9</td>
</tr>
</tbody>
</table>

Table 2
Chromatographic properties, color reactions, and relative distribution of urinary biotransformation products from mice 24 hr after a single i.p. injection of 50 mg/kg cyclohexyl-\textsuperscript{14}C-labeled CCNU

<table>
<thead>
<tr>
<th>Compound</th>
<th>% of total dose</th>
<th>$R_v$ value in solvent*</th>
<th>Color reaction*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCNU</td>
<td>≤0.1</td>
<td>0.90 0.89 0.76</td>
<td>Purple*</td>
</tr>
<tr>
<td>$N,N'$-Dicyclohexylurea</td>
<td>3.5</td>
<td>0.13 0.71 0.73</td>
<td>Yellow*</td>
</tr>
<tr>
<td>Cyclohexylamine</td>
<td>14-18</td>
<td>0.07 0.52 0.56</td>
<td>Purple*</td>
</tr>
</tbody>
</table>

* The solvent systems were: A, chloroform:95% ethanol (98:2); B, n-butyl alcohol:acetic acid:water (4:1:2); and C, isopropyl alcohol:ammonium hydroxide:water (9:1:1).

* Color reaction given by: *Griess reagent (2); *4-dimethylaminobenzene (Ehrlich's reagent); and ninhydrin reagent.

Antitumor Studies in Mice

Male and female CDF\textsubscript{1} mice were inoculated i.p. with 0.1 ml 1:1000 dilution of L1210 ascitic fluid obtained from a donor mouse on Day 7. The inoculum contained about $8 \times 10^3$ cells. Control mice (nondrug-treated, but treated with appropriate solvent) consistently had median survivals of 8 to 9 days. Drugs used for antitumor testing were CCNU, $N,N'$-dicyclohexylurea (K and K Laboratories, Inc., Plainview, N. Y.), cyclohexyl isocyanate (Aldrich Chemical Company, Cedar Knolls, N. J.), and cyclohexylamine (J. T. Baker Chemical Company, Phillipsburg, N. J.). CCNU was suspended in 1% methyl cellulose, cyclohexyl isocyanate was suspended in 0.5% methyl cellulose, cyclohexylamine was diluted with water, and $N,N'$-dicyclohexylurea was dissolved in DMSO and then diluted to a final concentration of 10 or 20% DMSO. $N,N'$-Dicyclohexylurea was given to mice at doses up to 100 mg/kg; all other drugs were injected in

![Chart 2. Plasma disappearance of CCNU in mice during 1st hr following a single i.p. injection of 50 mg/kg cyclohexyl-\textsuperscript{14}C parent drug.](chart)

Each point represents the average value of pooled samples from 4 mice.

Table 3
Distribution of radioactivity in mice 24 hr after a single i.p. injection of 50 mg/kg CCNU-\textsuperscript{14}C

<table>
<thead>
<tr>
<th>Position of label</th>
<th>Ethylene-\textsuperscript{14}C</th>
<th>Carbonyl-\textsuperscript{14}C</th>
<th>Cyclohexyl-\textsuperscript{14}C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>0.7 63</td>
<td>0.3 33</td>
<td>0.3 12</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.3 68 n.d.</td>
<td>0.2</td>
<td>0.2 29</td>
</tr>
<tr>
<td>Lung</td>
<td>≤0.1 33 n.d.</td>
<td>≤0.1 14</td>
<td>≤0.1 11</td>
</tr>
<tr>
<td>Brain</td>
<td>≤0.1 13 n.d.</td>
<td>≤0.1 4</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>≤0.1 23 n.d.</td>
<td>≤0.1</td>
<td></td>
</tr>
<tr>
<td>Small intestine</td>
<td>2.8 2.0</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>Large intestine</td>
<td>0.3</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>0.1 16 0.8</td>
<td>0.1 6</td>
<td></td>
</tr>
<tr>
<td>Gonads</td>
<td>≤0.1 14 n.d.</td>
<td>≤0.1 5</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>≤0.1 20 n.d.</td>
<td>≤0.1 12</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>≤0.1 n.d.</td>
<td>n.d. 3</td>
<td></td>
</tr>
<tr>
<td>Carcass</td>
<td>2.4 1.0</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Feces</td>
<td>≤0.1 1.4</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>CO\textsubscript{2}</td>
<td>3.5 10.6</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>86.2 57.9</td>
<td>78.4</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>96.6 64.0</td>
<td>84.7</td>
<td></td>
</tr>
</tbody>
</table>

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doses that ranged from nontoxic to lethal. Mice were treated by i.p. injections on Day 1 only (24 hr after inoculation) or daily from Day 1 until death. Ten mice were used for each dose level of each drug and the same number was used for solvent or untreated controls. Results are expressed as percentage of increase in median survival over controls.

RESULTS

Mice. Despite the route of administration and the position of the label in the parent drug, excretion was rapid and predominantly by the urinary route (Table 1). More than 75% of the dose was recoverable in the urine 24 hr following administration of the ethylene-14C- or cyclohexyl-14C-labeled CCNU. Following dosage with the carbonyl-14C-labeled CCNU, more than 50% of the radioactivity appeared in the urine during the ensuing 24 hr. The latter reduction in urinary excretion of 14C is accountable by the greater proportion of the radioactivity which appeared in the expired 14CO2. The cyclohexyl moiety of the parent molecule apparently remains intact since no radioactive 14CO2 was expired by mice during the 24-hr period following administration of the cyclohexyl-labeled CCNU. With each of the labeled CCNU preparations, fecal excretion of radioactivity was minimal after parenteral or oral dosage.

In urine samples examined by thin-layer chromatography on Silica Gel G followed by spraying with Griess reagent, less than 0.1% of the radioactivity could be associated with intact CCNU used as a standard reference. Radioactivity from urine samples of mice treated with ethylene-labeled CCNU did not migrate from the origin of thin-layer plates in either polar or nonpolar solvent systems with the exception of intact drug (≤0.1%).

Conversely, urine samples of mice treated with cyclohexyl-labeled CCNU yielded a number of distinct radioactive spots which migrated from the origin to give various RF values in several polar solvent systems. Incubation of untreated mouse urine with cyclohexyl-labeled CCNU at 37° for periods up to 1 hr did not yield the biotransformation products obtained from in vivo experiments. In these control incubations, only unchanged CCNU was recovered. From urine samples of treated animals, in addition to a trace of unchanged CCNU, 2 of the radioactive spots were identified in 2 chromatographic systems as cyclohexylamine (14 to 18% of radioactive dose) and N,N'-dicyclohexylurea (3 to 5%). The RF values for both compounds in the various solvent systems using the known reference compounds are shown in Table 2. Cyclohexylamine spots were located on thin-layer plates sprayed with ninhydrin reagent and then heated for 10 min in an oven at 110°. N,N'-dicyclohexylurea spots were located on the chromatograms sprayed with 4-dimethylaminoazobenzene and exposed to concentrated hydrochloric acid vapors. Incubation of the urine samples of mice treated with the cyclohexyl-labeled CCNU with sulfatase or glucuronidase at 37° for various time intervals up to 4 hr did not yield radioactive components which might have been present in the urine as these conjugates. Thus, approximately 75% of the 14C label in the cyclohexyl moiety of CCNU represents other biotransformation products which have not been identified. Urine samples of mice treated with the carbonyl-labeled compounds were only examined for the presence of unchanged CCNU.

Chart 2 depicts the rapid decline of intact CCNU in the plasma of mice (pooled samples from 4 mice at each designated time) after a single i.p. injection. The half-life during the first 30 min of exponential fall appears to be in the order of 5 to 6 min. Thereafter, the drug disappears more slowly with an estimated half-life of approximately 100 min. Although the chemical half-life of CCNU in plasma appears to be of shorter duration than BCNU (2, 10), the plasma disappearance patterns of each drug are similar.

In mice given a single p.o. dose of 50 mg/kg cyclohexyl-labeled CCNU, radioactivity was detectable in plasma as early as 3 min and peaked at 10 min following drug administration. Despite this rapid absorption, less than 0.5% of the plasma radioactivity was associated with intact parent compound.

The 24-hr tissue distribution studies (Table 3), regardless of the position of the label in the administered CCNU, showed a high percentage of isotope in the intestines, liver, and carcass. When the results were calculated in terms of tissue specific activity, the differences...
Rats. Recovery of radioactivity in the urine (≈75%) and feces (≈4%) of 2 rats given i.p. doses of the ethylene- or cyclohexyl-labeled CCNU was similar to recoveries obtained in mice. Biliary and urinary excretion of the labeled CCNU compounds was studied in 12 male Sprague-Dawley rats. In the first 6 hr following intragastric or i.m. injection of 5 mg/kg CCNU-14C, 15 to 30% of the dose was excreted, and by 24 hr (Table 4) 25 to 47% of the dose was recovered in the bile. Minimal differences were found when the position of the label in the molecule varied. Urinary excretion of radioactivity in most of the animals was somewhat decreased, averaging about 23% (with the exception of 2 animals) of the 14C dose, but the total recovery via bile and urine approximated the recovery obtained with noncannulated rats.

Dogs. The results of urinary excretion of radioactivity in female mongrel dogs given a single i.v. injection of either ethylene-14C-labeled CCNU or cyclohexyl-14C-labeled CCNU are summarized in Table 5. Following injection of ethylene-14C-labeled CCNU, urinary excretion of radioactivity is somewhat more protracted during the first 24 hr as compared to excretion observed following administration of the cyclohexyl-14C-labeled drug. In the animal treated with cyclohexyl-14C CCNU, urine collected through a catheter during the first 6 hr contained approximately 10% of the dose as cyclohexylamine and less than 1% as N,N'-dicyclohexylurea. The remaining radioactivity (about 28% of the dose) could not be identi-
fied with a particular molecular species. In animals treated with CCNU labeled in either position, less than 0.1% of the radioactive dose was excreted as intact parent drug.

Charts 3 and 4 depict the plasma and concurrent CSF levels of intact CCNU and total radioactivity in 2 female mongrel dogs given a single i.v. dose of cyclohexyl-^{14}C-labeled CCNU and ethylene-^{14}C-labeled CCNU, respectively. With the cyclohexyl-labeled CCNU (Chart 3), radioactivity enters the CSF rapidly, but is only about 55% of the plasma level between 30 and 60 min after injection of the drug. Most of this radioactivity represents ether-nonextractable products of the parent compound since the initial rate of decline of the ether-extractable intact CCNU in both plasma and CSF is very rapid, with a half-life of about 5 min.

These results might be explained on the basis of plasma protein binding of the cyclohexyl portion of the CCNU molecule which would partially exclude its entry into the CSF. In dogs given the cyclohexyl-^{14}C-labeled CCNU, plasma protein binding of radioactivity of about 45% initially rose to about 60% by the end of 6 hr.

Approximately 0.5 hr following i.v. administration of ethylene-^{14}C-labeled CCNU in dogs, the level of radioactivity in the CSF was at least 3-fold greater than that of plasma. This radioactivity was almost entirely ether nonextractable since the ether-extractable parent CCNU in plasma and CSF was rapidly degraded during the first 10 min following injection of drug. The initial dip in plasma radioactivity with a subsequent second peak as was seen with BCNU (2) probably reflects the change in lipid solubility of the molecular species associated with the ^{14}C. It appears that the ethylene portion of the CCNU molecule has no difficulty in crossing the blood-brain barrier while the cyclohexyl portion, perhaps by virtue of its substantial plasma protein-binding and lipid-insoluble characteristics, is concentrated in the CSF to a much less extent. Of particular pertinence is the finding that, in dogs given the ethylene-^{14}C-labeled CCNU parenterally, no plasma protein binding of radioactivity was observed, which suggests that the binding occurs mainly with the cyclohexyl moiety. It is also apparent from the observation of the extremely short half-life of both the cyclohexyl- and ethylene-^{14}C-labeled CCNU in dog plasma that only a minute portion of the radioactivity in the CSF represented entry of intact parent compound. Indeed, this was the case when CSF radioactivity was analyzed for intact CCNU.

Monkeys. The urinary excretion and concurrent plasma and CSF levels of radioactivity following injection of cyclohexyl-^{14}C-labeled CCNU in the monkey were similar to the pattern obtained in the dog. In 1 of 2 monkeys studied (Chart 5), the plasma half-life of radioactivity after the initial fall-off was about 24 hr. The CSF levels of ^{14}C reached about 24% of the plasma radioactivity level at 2 hr and about 8% at 24 hr. Almost 90% of the radioactive dose had been excreted in the urine during the 5 days following drug injection.

In each of the 2 monkeys given a single i.v. injection of ethylene-^{14}C-labeled CCNU, there was a rapid rising appearance of radioactivity in the CSF, but there was also a more rapid disappearance of label when compared to a similarly treated dog. This is illustrated for 1 of the treated animals in Chart 6. As observed with the dogs and monkeys given the cyclohexyl-^{14}C-labeled CCNU, following the rapid initial falloff of radioactivity in the plasma, which probably represents mostly intact lipid-soluble parent compound, the drug undergoes biotransformation, resulting in release of a lipid-insoluble fraction...
The data from mice given CCNU p.o. suggest that the drug is probably degraded prior to and/or simultaneously with the absorption process since the radioactivity in plasma samples at early time intervals following drug dosage was almost entirely associated with nonparent molecular entities. The primary excretory route of the parent compound or its biotransformation products (or both) is through the kidneys, with biliary excretion and reabsorption from the gastrointestinal tract playing a predominant role. The cyclohexyl portion of the molecule is bound to plasma proteins, whereas the chloroethyl segment is not. The carbonyl and chloroethyl carbons are partially recoverable in expired CO₂ while the cyclohexyl carbon atoms are not. Thus, the cyclohexyl ring is probably not extensively degraded in vivo. Finally, in rodents, the parent compound and/or its biotransformation products do not appear to be localized selectively in any of the tissues or organs examined. In the monkey, however, retention of some fragment(s) of CCNU was observed 5 days following parenteral drug administration. Future studies will be pursued to determine whether retention of the bound radioactivity in the monkey liver is associated with a particular cell fraction or other tissue sites.

The above observations parallel those made in studies of the fate of BCNU in animals (2) and indicate that there is probably little difference between the physiological disposition of these 2 drugs which have similar antitumor potencies in experimental animal tumors. Recently, however, CCNU has shown a marked superiority over BCNU in the treatment of an experimental mouse ependymoblastoma (percutaneous). The availability of CCNU isotopically labeled in various positions of the molecule has afforded a clearer elucidation of in vivo fate of the different segments of the nitrosourea molecule. Therefore, on the basis of the results obtained here and from previous studies with BCNU in this laboratory (2, 10), the in vivo biotransformation of CCNU is envisioned as proceeding according to the in vitro decomposition scheme for BCNU proposed by Montgomery et al. (12). Following parenteral administration of a single dose of CCNU in animals, a small but measurable quantity of lipid-soluble parent compound enters the CSF before degradation occurs. The parent compound could then degrade similarly in blood, brain, and CSF, according to the suggested scheme (1) which involves initial rearrangement of the CCNU molecule to an oxazolidine intermediate which almost instantaneously cleaves to form ethylenediaminohydroxide and cyclohexyl isocyanate. Hydrolysis of the isocyanate would result in the amine. Indirect evidence for isocyanate formation was obtained in the present studies by identification of radioactive cyclohexylamine and N,N'-dicyclohexylurea in the urine of mice given cyclohexyl-labeled CCNU and ¹⁴CO₂ in expired air following administration of carbonyl-labeled CCNU. Radioactive cyclohexylamine and N,N'-dicyclohexylurea were also detected in the urine of a dog treated with cyclohexyl-¹⁴C CCNU. Cyclohexylamine and CO₂ would be expected to arise from hydrolysis of the isocyanate. The amine would then be excreted largely un-

into the blood stream which is characterized by the second peak. Here too, after the initial falloff of radioactivity, the plasma-¹⁴C half-life was about 24 hr.

One of the monkeys treated with ethylene-¹⁴C-labeled CCNU was sacrificed after 5 days, and the radioactivity was determined in the organs and tissues designated in Table 6. As was similarly found in studies with ¹⁴C-labeled BCNU (2), in contrast to the homogeneous specific activity of organs in the mouse at 24 hr following administration of ethylene-¹⁴C-labeled CCNU, the monkey showed appreciable concentration of radioactivity in the liver 5 days following treatment. Bile taken from the gallbladder also contained significantly high radioactive counts. Despite this, the intestinal radioactivity was considerably less.

**DISCUSSION**

These studies have established several important facts concerning the physiological disposition of CCNU in animals.

The parent compound is lipid soluble, but the major portion of the drug entering the CSF following parenteral administration is associated with its lipid-soluble degradation products. The chemical and radioactive half-lives of the parent compound are extremely short, being approximately 5 min in both plasma and CSF of dogs and plasma of mice during the initial disappearance phase.
changed in the urine which is in accordance with results of studies of the metabolic fate of cyclohexylamine in rabbits reported by Elliott et al. (4). The small amount of urea derivative isolated probably resulted from the interaction of the amine with free isocyanate. Access of the isocyanate and the amine into the CSF might be limited by the demonstrated plasma protein binding of the cyclohexyl moiety of CCNU. The results of the present experiments shed little or no light on the fate of the chloroethyl moiety. The recovery of a small portion of the chloroethyl group of CCNU in expired CO₂ does demonstrate 2-carbon metabolism. Furthermore, since it was demonstrated that the chloroethyl carbon atoms were not bound to plasma protein, it might be questioned whether the left side of the molecule is bound at all. However, the hydroxy diazoalkane or subsequent metabolic products may well be bound to some site other than protein. Indeed, this has been suggested as the mechanism of action of drugs with alkylating-like activity (19).

We still have not explained the carcinostatic activity or the delayed toxicity of these compounds for liver, kidney, or marrow. Certainly, the postulated intermediate biotransformation products cyclohexyl isocyanate, cyclohexylamine, and cyclohexylurea, both the latter being excreted in the urine, were all inactive against the L1210 mouse leukemia under our experimental conditions.

Also, on the basis of the infinitesimal detection of intact parent compound in the plasma of mice given CCNU p.o., it is questionable whether the parent compound is, indeed, the active carcinostatic agent. Since CCNU is equally active by the oral and parenteral routes against the murine leukemia L1210 (8), the activity most likely emanates from biotransformation product(s). The above biotransformation products represent only several of the numerous possible metabolic products of CCNU and further efforts will be made to isolate, identify, and determine the role of other chemical moieties in the antitumor effectiveness of CCNU and other nitrosoureas. Finally, the observed high levels of both urinary and biliary excretion of both sides of the CCNU molecule indicate that both liver and kidney are exposed to relatively high concentrations of metabolic degradation products which might be bound and adversely affect cellular function at a later period.

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