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Distribution of ^{13}N in Rat Tissues Following Intravenous Administration of Nitroso-Labeled BCNU

Barry R. Freed[1], Roy L. McQuinn[2]*, Roy S. Tilbury[1]**, and George A. Digenis[2]

[1] Biophysics Laboratory, Memorial Sloan-Kettering Cancer Center, New York, NY 10021
[2] Division of Medicinal Chemistry, College of Pharmacy, University of Kentucky, Lexington, KY 40506, USA

Summary. *The concentrations of label in 16 major organs and tissues of pentobarbital-sedated normal male rats were measured at six time points ranging from 0.2 to 50 min after IV injection of the antitumor drug BCNU labeled in the nitroso position with cyclotron-produced nitrogen-13. Initial (12-s) concentrations in the lungs, kidneys, and heart were 41, 13, and 11 times the whole-body average, respectively. Time for clearance of the first 50% of the injected dose from the circulation was of the order of several seconds. Estimated first-pass extractions of 70% or more were noted in the heart, kidneys, brain, stomach, small intestine, muscle, fat, and bone. Washout of label from the heart and lungs was quite rapid, removing most of the initially extracted ^{13}N from these organs by 2 min after injection. Label concentrations in the kidneys exceeded those in all other tissues studied between 2 and 50 min. Secondary accumulations of ^{13}N were observed in muscle, skin, liver, small intestine, and fat. Label concentrations in a number of tissues closely paralleled the steadily decreasing concentration in blood for various intervals between 5 and 50 min. The results suggest that the toxic insult to lung tissue from IV administered BCNU is effected in a period of several minutes. They also suggest that intra-arterial administration of the drug would significantly raise the target/non-target dose ratio and lower the incidence of pulmonary toxicity.*

Introduction

BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea), the first nitrosourea to be employed in human cancer chemotherapy and a drug still in widespread use, remains the object of considerable experimental effort to determine its mechanism of action. Part of this effort has been devoted to measuring the distribution, lifetime and metabolism of the drug in vivo, using either ^{14}C-labeled or unlabeled BCNU. Although the nitroso moiety is indispensable to the biological activity of the drug [17], until recently [5, 16] there was no information available on its in vivo disposition. This report provides data on the distribution in rat tissues, as a function of time, of the nitroso nitrogen of BCNU labeled with the 10-min half-life cyclotron-produced radionuclide nitrogen-13, a positron emitter.

Reprint requests should be addressed to B. R. Freed at the above address

* *Present address:* Riker Laboratories, 3M Center, St Paul, MN 55144, USA

** *Present address:* Dept of Nuclear Medicine, MD Anderson Hospital and Tumor Institute, University of Texas System Cancer Center, Houston, TX 11030, USA

Materials and Methods

Nitrogen-13 was produced via the $^{16}O(p,\alpha)^{13}N$ reaction by the irradiation of water with 14.5 MeV protons from the Memorial Sloan-Kettering Cancer Center cyclotron (Model CS-15, Cyclotron Corp., Berkeley, CA, USA). The saturation yield of ^{13}N in this system is approximately 25 mCi/μA beam current (1 Ci = 3.7×10^{10} Bq). A typical irradiation produced 200–300 mCi of ^{13}N, mostly in the form of nitrate ion [22].

BCNU, labeled in the nitroso position with ^{13}N, or BCNU(^{13}NO) was prepared by the method of Pettit et al. [18]. Briefly, the irradiated water, after transfer to a beaker containing 1–3 mg Na_2CO_3, was evaporated to dryness on a hotplate. After cooling, the activity was dissolved in 1 ml 50 m*M* HNO_3 in glacial acetic acid and added to 200 mg Cu dust and 5 mg 1,3-bis(2-chloroethyl) urea. After reacting for 4–5 min, the solution was diluted with 5 ml H_2O and extracted with 1 ml $CHCl_3$. The chloroform extract containing the labeled BCNU was washed with saturated Na_2CO_3 solution, dried by passage through granular anhydrous Na_2SO_4, and evaporated to a dry residue under a stream of N_2. In this way, 3–20 mCi BCNU(^{13}NO) was produced in 35–40 min from the end of irradiation. Radiochemical yields, corrected for ^{13}N physical decay, were 20%–40%. Radiochemical purity, determined by radiomonitored thin-layer chromatography (Eastman silica gel plates eluted with $CHCl_3$), was greater than 99%. The specific activity of the final product ranged from 150 to 1,000 Ci/mol.

The tissue distribution measurements were carried out in normal male Sprague-Dawley rats weighing 333 ± 39 (SD) g, under sodium pentobarbital sedation (approx. 50 mg/kg IP). For injection, the labeled BCNU (dry residue) was dissolved in about 0.5 ml ethanol, which was then diluted with three volumes of saline. Each rat received 0.1–1 mCi BCNU(^{13}NO) in 0.2–0.5 ml of solution, injected into a femoral vein exposed by inguinal incision. The maximum BCNU dose given was estimated to be about 15 μmol/kg. Plastic syringes with a capacity of 1 ml and equipped with 26 gauge needles were weighed before and after injection to determine the net weight injected. Weighed aliquots of the injection solution were used as counting standards.

The rats were sacrificed at intervals of 0.2, 2, 5, 10, 30, or 50 min after injection by rapidly cutting open the chest and excising the heart. Whole heart, lungs, liver, spleen, stomach, small and large intestines, kidneys, testes, and brain were taken for radioassay, as well as specimens of pancreas, abdominal muscle, abdominal fat, skin (with hair), calvarium,

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and whole blood collected from the chest cavity. The 511 keV gamma rays produced by ^{13}N positron emission were detected in a Packard model 5986 gamma spectrometer equipped with a dual NaI(Tl) crystal/dual phototube scintillation detector, automatic sample changer, digital clock, and high-speed line printer. A wide energy band of 10–1150 keV, including both the single- and the two-photon peaks (the detector has greater than 2 π geometry), gave approximately 2.4×10^4 counts/s/μCi for ^{13}N. Net counts of tissue specimens and standards were corrected for the physical decay of ^{13}N to a common reference time, using a half-life of 10.0 min. Calculation of fraction of injected dose found in tissue was based on dilution by weight.

The results were expressed as percent of dose/whole organ, where measured, and in units of relative concentration, which is defined, for either a specimen of tissue or whole organ, as the fraction of dose found in the specimen or organ divided by the fraction of body weight contained in the specimen or organ. Use of this dimensionless unit avoids the artifact of dilution by body mass inherent in the commonly used percentage of dose/g or percentage of dose/kg and facilitates intercomparison of tracer concentrations among individuals and species of widely varying body size [26].

Order-of-magnitude estimates of the first-pass fractional extractions for BCNU in rat tissues were derived from the 12-s distribution data by comparing the label concentrations found here in tissue with microsphere-based measurements, drawn from the literature, of the distribution of cardiac output in rats. The assumptions and methods used have been described previously [6].

Results

The measured concentrations of ^{13}N in rat tissues (mean ± SEM) are given in Tables 1 and 2 (relative concentration and percentage of dose/whole organ, respectively). In some cases, the means are for one less than the number of rats studied per time point, due to loss of specimens or failure to take a complete set at dissection.

It should be noted that the 0.2-minute blood relative concentration value (Table 1) cannot be taken to be representative of the whole blood supply, since considerable arterio-venous concentration differences may well have been present at this sacrifice time. Although no attempt was made in this study to collect all the pooled chest blood consequent to cardiac excision, previous work shows that this mode of sacrifice releases blood into the chest cavity in the amount of approximately 3% of the body weight [6], that is, about half the blood weight of the rat [23]. Thus, the 0.2-min measurement represents blood containing about 13%–14% of the injected dose (percentage of dose = relative concentration × percentage of body weight). The blood relative concentration values found here at 5, 10, 30, and 50 min are quite similar to those reported in an earlier experiment with BCNU(^{13}NO) in a separate group of rats [5].

The entry 'small intestine (–contents)' in Table 1 refers to a small segment of the intestine from which the contents were mechanically extruded. At 0.2 and 2 min after injection the ratio of percentage of dose in the whole small intestine (with contents) to the relative concentration of these intestinal wall specimens was 2.70 ± 0.52 (SD, $n = 8$). This is close to the fractional body weight of the organ (~ 2.4%) in rats of this size [10], indicating that relatively little label was present in the intestinal contents at these time points. Subsequently, the average ratio increased with increasing time after injection, with large fluctuations in the individual values at 30 and 50 min, so that the specimens were no longer representative of the whole intestine with contents. The simplest interpretation of these results is that a substantial quantity of ^{13}N entered the contents, perhaps through the bile duct. Variation in proximity of the specimens to the entry point of the duct, coupled with adsorption of the label on the intestinal wall or incomplete removal of the contents, would account for the observed fluctuations.

The estimated first-pass extraction fractions for BCNU in rat tissues, obtained by the method referred to above, were as follows: heart, 0.9; kidneys, 0.8; brain, stomach, small intestine, 0.7; testes 0.6; pancreas, skin, 0.4; lungs, spleen, 0.2; total hepatosplanchnic (liver, spleen, pancreas, stomach and intestines), 0.6. Estimates could not be made by this method for the remaining tissues, either because of the lack of microsphere data (calvarium), or, in the case of abdominal muscle, fat, and to a lesser degree, large intestine, because the measured ^{13}N concentrations were anomalously high in comparison with microsphere-based measurements of blood flow to these tissues, a result also found previously for ^{13}N-ammonia [6]. Since the latter compound appeared to be nearly quantitatively extracted by most tissues of the rat on the first circulatory pass under experimental conditions essentially the same as those of the present study, it seems reasonable to estimate the BCNU extraction fractions for these remaining tissues by comparison of tissue ^{13}N concentrations found here for BCNU(^{13}NO) at 12 s after injection with those found for ^{13}N-ammonia at the same sacrifice time [6]. This yields the following extraction ratios (BCNU/ammonia): abdominal fat, 1.2; abdominal muscle, 0.9; calvarium, 0.8; large intestine, 0.5.

The time course of ^{13}N in the tissues studied was strikingly varied. The highest label concentrations at 12 s were observed in the lungs, kidneys, and heart. On a percentage-of-dose basis, the largest quantities of label were present initially in the lungs, muscle, and kidneys. The heart and lungs released their initially extracted label quite rapidly, so that by 10 min after injection they retained only 6% and 4%, respectively, of their 12-s concentrations. Net loss of label from the kidney was much slower, and between 2 and 50 min, renal ^{13}N concentrations exceeded those of all other tissues studied. Recirculated ^{13}N, supplied principally by the lungs, heart, and kidneys, as well as clearance of a considerable quantity of label present in blood at 12 s, contributed to subsequent accumulations in a number of tissues. The most rapid increases between 0.2 and 2 min occurred in the liver and skin. Concentration maxima were observed at about 2 min after injection in brain, pancreas, and muscle, at about 5 min in liver, testes, and calvarium, between 5 and 10 min in skin, and at about 30 min in fat. The musculature, skin, fat, and liver generally contained the largest fractions of dose between 2 and 30 min after injection. The fraction of dose in stomach and small intestine (both with contents) rose to relatively steady levels between 10 and 50 min, while that of the large intestine did not change significantly between 0.2 and 50 min.

The label content of most of the tissues studied decreased steadily after 5 min, reflecting a steady loss of ^{13}N into expired air and urine [5]. Label concentrations in a number of tissues paralleled the time course of label in blood for various intervals between 5 and 50 min. Table 3 lists tissue/blood ^{13}N concentration ratios, as means (± SD) of ratios for individual rats, with the data pooled for the time intervals over which the ratio

Table 1. Distribution of ^{13}N in rat tissue after IV injection of BCNU(^{13}NO): Relative concentration (mean ± SEM)

Time (min)	0.2	2	5	10	30	50
Blood	4.57 ± 0.25	1.60 ± 0.25	1.049 ± 0.084	0.645 ± 0.038	0.470 ± 0.016	0.389* ± 0.042
Heart	11.09 ± 0.94	1.96 ± 0.15	1.004 ± 0.086	0.664 ± 0.034	0.351 ± 0.019	0.310 ± 0.023
Lungs	41.4 ± 2.9	11.0 ± 2.8	3.95 ± 0.92	1.55 ± 0.31	0.766 ± 0.087	0.63 ± 0.13
Liver	1.31 ± 0.12	3.43 ± 0.29	3.76 ± 0.12	2.54 ± 0.17	1.474 ± 0.048	1.078 ± 0.064
Spleen	1.49 ± 0.25	1.19 ± 0.12	0.873 ± 0.037	0.583 ± 0.049	0.383 ± 0.025	0.332* ± 0.064
Pancreas	1.80 ± 0.20	1.94 ± 0.12	1.61 ± 0.17	1.02* ± 0.24	0.72* ± 0.20	0.71 ± 0.17
Small intestine (−contents)	1.77 ± 0.34	1.66* ± 0.21	1.43 ± 0.27	1.37 ± 0.28	2.76 ± 0.63	2.1 ± 1.0
Kidneys	12.8 ± 1.3	11.1 ± 1.5	8.50 ± 0.62	6.92 ± 0.45	4.63 ± 0.30	2.89 ± 0.40
Testes	0.37 ± 0.14	0.768 ± 0.062	0.878 ± 0.046	0.679 ± 0.040	0.414 ± 0.014	0.414 ± 0.059
Abdominal muscle	0.379 ± 0.047	0.564 ± 0.087	0.508 ± 0.041	0.449 ± 0.014	0.335 ± 0.009	0.275 ± 0.029
Abdominal fat	0.601 ± 0.084	1.080 ± 0.087	1.277 ± 0.087	1.58* ± 0.14	1.92* ± 0.14	1.555 ± 0.071
Skin	0.187 ± 0.022	0.449 ± 0.097	0.705 ± 0.057	0.684 ± 0.039	0.502 ± 0.022	0.421 ± 0.020
Calvarium	0.291 ± 0.034	0.334 ± 0.036	0.378 ± 0.071	0.204 ± 0.014	0.173* ± 0.030	0.165 ± 0.029
Brain	1.58* ± 0.19	1.69 ± 0.16	1.138 ± 0.057	0.587 ± 0.020	0.267* ± 0.015	0.213 ± 0.027
Rats/group (n)	(5)	(4)	(4)	(4)	(5)	(4)

* $(n-1)$

Table 2. Distribution of ^{13}N in rat tissue after IV injection of BCNU(^{13}NO): % Dose/whole organ (mean ± SEM)

Time (min)	0.2	2	5	10	30	50
Heart	3.30 ± 0.33	0.569 ± 0.030	0.298 ± 0.020	0.191 ± 0.021	0.0991 ± 0.0089	0.0914 ± 0.0081
Lungs	19.3 ± 2.2	5.7 ± 1.8	1.95 ± 0.40	0.79 ± 0.40	0.366 ± 0.037	0.326 ± 0.076
Liver	4.41 ± 0.31	11.80 ± 0.80	11.99 ± 0.62	8.51 ± 0.87	4.99 ± 0.19	3.42 ± 0.28
Spleen	0.357 ± 0.046	0.313 ± 0.039	0.200 ± 0.026	0.129 ± 0.004	0.094 ± 0.011	0.0709* ± 0.0078
Stomach (with contents)	0.550 ± 0.092	0.861 ± 0.038	0.675 ± 0.072	0.96 ± 0.24	1.09 ± 0.19	0.81 ± 0.15
Small intestine (with contents)	4.66 ± 0.85	3.77 ± 0.63	5.26 ± 0.74	7.02 ± 0.75	7.78 ± 0.88	7.21 ± 0.52
Large intestine (with contents)	1.61 ± 0.20	2.09 ± 0.22	1.63 ± 0.04	2.08 ± 0.43	1.72 ± 0.33	2.10 ± 0.52
Kidneys	10.25 ± 0.90	8.2 ± 1.1	6.70 ± 0.29	5.23 ± 0.47	3.33 ± 0.28	2.12 ± 0.31
Testes	0.36 ± 0.13	0.802 ± 0.046	0.850 ± 0.064	0.609 ± 0.035	0.375 ± 0.024	0.396 ± 0.042
Brain	0.92* ± 0.10	0.985 ± 0.095	0.600 ± 0.029	0.297 ± 0.010	0.152* ± 0.003	0.117 ± 0.015
Rats/group (n)	(5)	(4)	(4)	(4)	(5)	(4)

* $(n-1)$

Table 3. Tissue/blood ^{13}N concentration ratios

Time interval (min)	Tissue	Mean[a] ± SD	n
5–50	Testes	0.93 ± 0.12	16
	Spleen	0.85 ± 0.12	16
10–50	Skin	1.07 ± 0.15	12
	Muscle	0.693 ± 0.076	12
30–50	Fat	4.13 ± 0.71	7
	Lung	1.52 ± 0.37	8
	Heart	0.760 ± 0.079	8
	Brain	0.548 ± 0.087	7

[a] Mean of ratios for individual rats

appeared to be constant. In the testes, spleen, skin, muscle, and fat, this apparent equilibrium with blood followed a rise in the tissue/blood ratio. In the heart, lungs, and brain, it followed a decrease. Maxima in tissue/blood ^{13}N were observed only in the liver and kidneys, at about 10 min after injection in both tissues. The ^{13}N concentrations in these two organs seemed to decrease at quite similar rates between 5 and 50 min, giving a mean kidney/liver ^{13}N concentration ratio in this interval of 2.74 ± 0.56 (SD, n = 17).

Discussion

Although a number of studies have been published of the rate of clearance of IV BCNU from the whole blood or plasma of

rats, mice, and dogs [3, 11, 13, 14, 24], the rapidity with which *most* of an IV dose of BCNU is cleared from the blood of these species has yet to be pointed out. Investigators have fitted one or more negative exponential functions of time to the measured clearance curves, in order to characterize the process with various 'half-times', but appear to have overlooked the fact that at the earliest time points studied in each of these investigations only a small fraction of the injected dose remained in circulation.

Since the concentration of intact parent molecule cannot exceed that of the nitroso label, the measured concentrations of ^{13}N in tissue provide maximum values for the concentration of BCNU. Thus, the finding of 13%–14% of the injected dose in about one half the blood supply at 12 s after injection (a time meant to approximate a single circulatory pass to most tissues of the rat) shows clearly that the time for clearance of the first 50% of an IV dose of BCNU from rat blood is a matter of seconds rather than minutes. Plasma clearance studies in humans, in which BCNU was infused in relatively short time intervals of 1–3 min [19, 20], also show initial clearance rates that are consistent with extraction of a high order on each circulatory pass. Such rapid clearance has important clinical implications, in that a target/non-target dose advantage can be achieved by injecting the drug into the appropriate arterial supply. This principle appears to have been applied with some success to the treatment of metastatic brain tumors by means of BCNU infusions into the carotid or vertebral arteries [15].

Our finding of nearly 20% of the injected dose in the lungs at 12 s after injection, followed by a rapid washout of the label, is of particular interest in view of the well-documented severe pulmonary toxicity of IV BCNU in cancer patients [1, 25]. Pathological changes similar to those occurring in humans have also been found in the lungs of rats 4 months after a single large dose (62 μmol/kg) of BCNU administered IP [2]. Assuming that the presence of the nitroso group is essential to the observed toxicity, our data suggest that the toxic insult to lung tissue in the rat is effected over a period of a few minutes at most, setting in motion 'a lesion that progresses in the absence of the offending agent' [21]. That toxic effects can be induced by BCNU in a time interval of this order has been demonstrated by Frischer and Ahmad [7], who found 60%–90% inhibition of glutathione reductase activity in the erythrocytes, heart, lung, liver, spleen, kidney, muscle, and brain of mice at 10 min after IP administration of the drug.

The pulmonary extraction of IV BCNU in humans may be considerably higher than our results indicate for rats, given the much lower rate of perfusion of the human lung. In view of the generally high rate of tissue extraction of the drug, it is conceivable that intra-arterial administration, which would be expected to greatly reduce the fraction of dose delivered to the lungs, would thereby significantly reduce the incidence of pulmonary toxicity. The high initial concentration of ^{13}N found here in the rat heart also suggests the possibility of myocardial toxicity arising from IV BCNU therapy. While this has not been reported in humans, hemorrhagic lesions were found in the hearts of Rhesus monkeys after repeated IV doses of BCNU [4].

It was shown in a previous experiment that IV injection of BCNU(^{13}NO) in rats gave rise to a significant loss of label in expired air, amounting to a mean of 24% of the dose (range: 19%–30% of dose, 4 rats) at 40 min after injection [5]. (Extrapolation of the mean curve to 50 min, the time interval covered by the present study, gives a value of 26%–27% of dose.) While the volatile species was not identified, it seems most likely that it was molecular nitrogen, which is known to be quantitatively evolved by the aqueous decomposition of BCNU at physiological pH [17]. It was noted in the former report that the relatively slow rate of respiratory loss of ^{13}N was inconsistent with widely held notions of the rate of breakdown of BCNU in vivo and of the fate of the nitroso nitrogen. It was concluded that either the breakdown of BCNU in vivo is slower than is generally thought or that a large portion of the dose is metabolized in a manner that does not convert the nitroso nitrogen to N_2. The finding of a non-volatile ^{13}N-containing urinary metabolite (not identified), amounting to 7.91% ± 0.73% (SEM, n = 7) of the dose at 50 min after injection, demonstrated the presence of at least one such pathway [5].

Hill and co-workers have reported two metabolic pathways for BCNU in studies with mouse tissue. A microsomal enzyme in liver, requiring NADPH and O_2, denitrosated BCNU. This activity was also found in lung homogenate, but not in kidney, spleen, brain, muscle, intestine, or serum [9]. An enzyme in the soluble portion of mouse liver, requiring GSH, converted BCNU to a polar product which was tentatively identified as a glutathione conjugate no longer containing the nitroso group. The enzyme was also present in the lung and kidney, but not in the heart, spleen, brain, muscle, intestine, or serum [8]. Neither of these pathways would be expected to volatilize the nitroso nitrogen.

The data of Weinkam et al. [24], for the concentration of intact BCNU in the whole blood of pentobarbital-sedated rats after IV injection of 70 μmol BCNU/kg, converted to units of relative concentration, give mean values of 0.95, 0.58, 0.37, 0.24, 0.098, 0.078, and 0.039 at 2, 4, 6, 10, 20, 30, and 60 min, respectively. Comparison with our ^{13}N data indicates that the fraction of blood ^{13}N which represented labeled metabolite(s) was 41% at 2 min, 63% at 10 min, and 83% at 30 min. If the ^{13}N-labeled volatile lost in the breath [5] was indeed molecular nitrogen, then consideration of the rate of respiratory loss in relation to the magnitude of cardiac output in the rat shows that $^{13}N \cdot N$ could not have made up more than a few percent of blood ^{13}N at any given time. Thus, it seems likely that one or more non-volatile ^{13}N-containing BCNU metabolites accounted for most circulating ^{13}N within several minutes of injection.

Similarly, the rates of loss of ^{13}N from tissue observed in the present study were, in general, much too slow to be limited by rate of blood flow, so that labeled N_2 could not have been a large part of tissue ^{13}N at any given time. (In this context, it is worth noting that the quantity of label washed out of the cardiopulmonary system between 0.2 and 2 min after injection was several times the quantity previously found to be lost in the breath in this interval.) Thus, the close parallelism in ^{13}N concentration between blood and several tissues (see Table 3) suggests rapid exchange of some soluble ^{13}N-labeled species. It may be that the onset of these apparent 'equilibria' marked the point at which metabolite(s) predominated in tissue as well as in blood.

Further insight into these processes requires quantitative information on the time course of intact BCNU in tissues after IV administration. Unfortunately, the available data, those of Levin et al. [12] for rats (by HPLC analysis of CH_2Cl_2 extracts of tissues after injection of BCNU[chloroethyl-^{14}C]) and those of Kari et al. [11] for mice (by Bratton-Marshall assay of ether extracts using unlabeled BCNU) present a number of marked qualitative differences. While these differences may perhaps

be the product of interspecies variation, the likelihood of this source of disagreement is diminished by noting that the data of Kari et al. [11] for intact BCNU in mouse blood and those of Weinkam et al. [24] for rat blood (by chemical ionization mass spectrometry of hexane-ether extracts) agree quite closely (in units of relative concentration as defined herein) between 5 and 30 min after injection.

If, despite the differences referred to above, the intact BCNU concentrations reported for various tissues of mice [11] and rats [12] are assumed to be typical of the remaining tissues not assayed in each of these studies, then the time for breakdown of the first 50% of IV injected BCNU (that is, the time for mean tissue relative concentration of intact drug to fall to 0.5) is about 1−2 min in mice and 10−15 min in rats. The latter estimate, in conjunction with measurements of the rate of respiratory loss of ^{13}N from rats [5], implies that most of an IV dose of BCNU is metabolized via non-N_2-producing pathways, yielding one or more ^{13}N-containing metabolites that are more slowly eliminated in urine (and perhaps feces) than is N_2 in expired air.

With the possible exception of the lungs and heart in the first few minutes after injection, our data do not seem to indicate a special role for any particular tissue in the volatilization of the nitroso nitrogen of BCNU. This impression is reinforced by comparison of our ^{13}N data with measurements of ^{14}C in rat tissues after IV BCNU[chloroethyl-^{14}C], performed by Levin et al. [12] and by McQuinn [16]. (Despite the limited number of animals used in the last two studies, there was reasonable agreement between them for the ^{14}C relative concentrations of lung, liver, muscle, and brain, for various times between 2 and 40 min after injection. In kidney, however, for which the data from both ^{14}C studies showed the least fluctuation, the concentrations found by Levin et al. [12] were two to three times those found by McQuinn [16]. Our ^{13}N data for kidney were intermediate between the two). Sustained deficits of ^{13}N vs ^{14}C appeared only in blood and spleen (with reference to McQuinn's data) and it does not seem likely that these tissues could have accounted for most of the volatilized ^{13}N.

Intercomparison of the ^{13}N and ^{14}C data also raises an interesting possibility. Close agreement between concentrations of the two labels found at various times between 2 and 50 min in the lungs, liver, muscle, and fat suggests the presence of a species that contained the nitroso nitrogen and all four chloroethyl carbons of BCNU (which are randomly labeled in the ^{14}C preparation). If the disappearance of BCNU in vivo is as rapid as the data of Kari et al. [11] and Levin et al. [12] indicate, then a BCNU metabolite which retains the nitroso nitrogen is implied. Such a metabolite has yet to be identified.

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