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# CHARACTERIZATION OF THE URINARY METABOLITES OF 5-AZACYTIDINE IN MICE

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**Abstract**—The metabolism of 5-azacytidine, radiolabeled in the 4- or 6-position with carbon-14, was studied in mice. High pressure liquid chromatographic analysis of urine from mice given [4-$^{14}$C]- or [6-$^{14}$C]-5-azacytidine showed that six radioactive peaks were present and that peaks V and VI were the major peaks excreted. Similar analysis of urine from mice given the labeled compounds and tetrahydrouridine, a deaminase inhibitor, indicated that peaks I, II and V were deaminated metabolites. Peak V appeared to contain components with the 6-carbon absent, whereas peak VI contained components, in a 1:1 ratio, with the 6-carbon either present or absent. Co-chromatography of urine from treated mice with authentic standards indicated that peaks I, III, IV were 5-azauracil, 5-azacytosine and 5-azacytidine, respectively. These data, along with the characterization of peaks V and VI by gas–liquid chromatography and mass spectrometry, indicated that the metabolism of 5-azacytidine in mice is similar to that proposed by other investigators.

The cytidine analog, 5-azacytidine (5-azaCR) was first synthesized in 1964 [1], and since that time it has received considerable attention. It has antimicrobial [2, 3] and antineoplastic activity against experimental [4–6] and human [7–13] tumors.

The mechanism of action of the compound has been investigated extensively [2, 4, 14–24]; its mode of antitumor action, however, is not entirely clear. Based primarily on reports by the Czechoslovakian group [14, 24–27], Neil *et al.* [28] proposed a scheme for the metabolism of 5-azaCR, which included enzymic and non-enzymic degradative pathways. The identification of metabolites of 5-azaCR has been based primarily on the detection of its products from bacteria [27], rodent urine [14, 25], and hydrolytic studies *in vitro* [24, 29]. Detection was accomplished by physical methods such as paper chromatography, electrophoresis [14, 24] and ultraviolet absorbance [29], or by chemical methods [24] which rely on reagent reaction with selective chemical groups to yield characteristic colors.

In the present report, the metabolism of 5-azaCR from mouse urine was studied by utilizing high pressure liquid chromatography (h.p.l.c.), gas–liquid chromatography (g.l.c.) and mass spectrometry (m.s.) and by evaluating the effect of the antimetabolite, tetrahydrouridine (THU), on the overall metabolism of [4-$^{14}$C]-5-azaCR and [6-$^{14}$C]-5-azaCR.

## MATERIALS AND METHODS

5-Azacytidine (NSC 102816) and tetrahydrouridine (NSC 112907) were obtained from the National Cancer Institute, Bethesda MD. [4-$^{14}$C]-5-AzaCR, 50 mCi/mmole (radiochemical purity, 96 per cent), was supplied by the Monsanto Research Corp., Dayton, OH, through the National Cancer Institute. The [6-$^{14}$C]-5-azaCR, 9.5 mCi/mmole (radiochemical purity, 87 per cent), was purchased from Dr. J. Moravek of Nova Chemicals, Rosemead, CA. The radiochemical purity of the drugs was determined by h.p.l.c. Solvents used for chromatographic analysis were obtained from Burdick and Jackson Laboratories, Inc., Muskegon, MI. All other chemicals were reagent grade.

High pressure liquid chromatography was performed on a Waters model ALC-202/R-401W/UV liquid chromatograph (Waters Associates, Milford, MA). Samples were eluted from the column [250 × 4.2 mm i.d., packed with Merck LiChrosorb Si-60 5 $\mu$ (E. M. Laboratories, Inc., Elmsford, NY)] with chloroform–methanol–water–acetic acid (30:10:1.7:0.42, v/v from 0 to 22 ml and with methanol from 22 to 27 ml, at a flow rate of 1 ml/min. Recovery of radioactivity from the column ranged from 88 to 97 per cent. Radioactive samples were counted in Aqueous Counting Scintillant (Amersham/Searle Corp., Arlington Heights, IL) in a Searle Analytic Mark III liquid scintillation system, and appropriate fractions were combined, dried under a stream of nitrogen, and stored at −20° until needed.

Gas–liquid chromatography was performed in a Hewlett–Packard 5700A or a Varian 2800 gas chromatograph with a flame ionization detector and a 5ft × 1/8in or 6ft × 1/8in i.d. glass column containing 20% SE-30 on Gas Chrom Z or 1% SE-30 on Gas Chrom Q (Applied Science Laboratories, Inc., State College, PA), respectively. Radioactive components in the gas effluent were trapped by inserting a short disposable glass Pasteur pipette containing loosely packed glass wool into the externally heated gas outlet. The pippette was flushed several times with scintillation solvent and the washes and glass wool were counted for radioactivity as described above.

Mass spectrometry was performed on a DuPont 21-491 instrument interfaced with the Hewlett–Packard 5700A gas–liquid chromatograph and a Hewlett–Packard 2100A computer.

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Methanolysis of h.p.l.c. fractions was accomplished by heating the samples at 65° for 16 hr in methanolic HCl which was prepared by adding acetyl chloride (5 ml) and methanol (95 ml) (reagent kit, Applied Science Laboratories, Inc.).

Trimethylsilylation of h.p.l.c. fractions was carried out in Teflon-lined screwtop culture tubes with a 1:1 mixture of *N,O*-bis(trimethylsilyl)trifluoroacetamide (Regis Chemical Co. Chicago, IL) and pyridine (v/v). The sealed vessel was heated at 105° for 30 min, and samples of the mixture were injected into the gas chromatograph for g.l.c. or g.l.c.–m.s. analysis.

Blood, obtained from Beagle dogs by carotid puncture, was centrifuged in a table top clinical centrifuge at maximum speed for 5 min to separate the plasma from the other components. Plasma and 5-azaCR at the appropriate concentration were incubated at 37° for 10 min, and the mixture was deproteinized by the addition of 19 vol of absolute ethanol. The precipitate was separated from the mixture by centrifugation, and the supernatant fraction was dried under nitrogen and stored at −20° until needed.

Female $BDF_1$ mice (20 g mean weight) were given 5-azaCR (4-$^{14}$C or 6-$^{14}$C), 50 mg/kg (2.6–6.2 μCi), alone or in combination with THU, 10 mg/kg. Drugs were dissolved in saline and administered to animals i.p. The animals were housed in Roth metabolism cages and fed food and water *ad lib.* Air which passed through a 20 × 7 cm glass column packed with Ascarite and Drierite flowed through the system with the aid of a vacuum of approximately 125 mm Hg at the outlet of the cage. Air exiting from the system was passed through three successive KOH (8 M) solutions to trap expired $CO_2$ and after 8 hr aliquots of the KOH samples were counted by liquid scintillation to determine the amount of $^{14}CO_2$ present. The urinary collection vessel was encased with dry ice to minimize degradation of drug and metabolites during the 8-hr collection period. The urines were lyophilized and stored at −20° until needed or resuspended in 1.0 ml of distilled water and analyzed by h.p.l.c.

## RESULTS

*Effect of tetrahydrouridine on the metabolism of 5-azacytidine.* High-pressure liquid chromatographic analysis of urine from mice given [4-$^{14}$C]-5-azaCR showed six major fractions. Peaks I–IV were radioactive and u.v.-absorbent, and peaks V and VI were radioactive and non-u.v. absorbent (Fig. 1). Although Fig. 1 shows u.v.-absorbance associated with peak VI, previous studies showed evidence that the peak was not u.v.-absorbent.* In a recent study [30], we reported that THU, when administered with [4-$^{14}$C]-5-azaCR at concentration ratios of 0.01:1 to 2:1, was effective in increasing the urinary excretion of 5-azaCR (peak IV) by 2 to 6-fold, respectively, which was consistent with the mechanism of action of THU as an inhibitor of cytidine deaminase [31, 32]. Table 1 shows that 5-azaCR, labeled in either the 4- or 6-position and administered with THU to mice, resulted in a decrease in the levels of radioactivity in h.p.l.c. peaks I (about 4-fold) and II (about 3- to 7-fold). Peak IV increased approximately 6- and 4-fold when THU was administered with [4-$^{14}$C]- or [6-$^{14}$C]-5-azaCR, respectively. Peak V decreased approximately 3-fold with the co-administration of [4-$^{14}$C]-5-azaCR and THU, whereas the peak was absent or barely detectable with [6-$^{14}$C]-5-azaCR or [6-$^{14}$C]-5-azaCR and THU, respectively. There was a 2-fold decrease in the level of peak VI due to [6-$^{14}$C]-5-azaCR when compared to the level due to [4-$^{14}$C]-5-azaCR; both levels, however, increased about 2-fold when THU was given with either of the labeled drugs. Table 1 shows a 2-fold increase in the level of radioactivity in peak III when THU was co-administered with [4-$^{14}$C]-5-azaCR, but the levels are relatively small. These findings are supported by a similar study [30]. The table also shows that there was approximately a 3-fold increase in peak III with [6-$^{14}$C]-5-azaCR when compared to that with [4-$^{14}$C]-5-azaCR. This increase in radioactivity in the peak was probably due to the contaminant, [6-$^{14}$C]-5-azacytosine (13 per cent as determined by h.p.l.c.), in the stock preparation of [6-$^{14}$C]-5-azaCR.

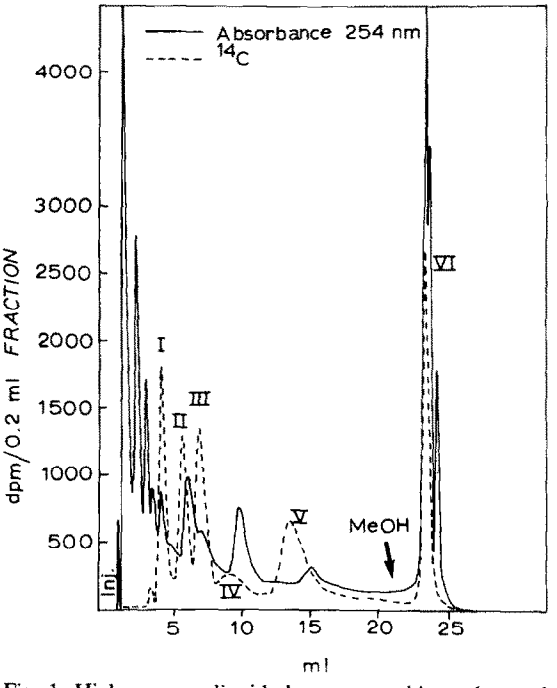



Fig. 1. High-pressure liquid chromatographic analyses of urine collected from $BDF_1$ mice after dosing with [4-$^{14}$C]-5-azaCR (3.7 μCi/mg), 50 mg/kg. The column was 15 cm × 4.2 mm i.d., packed with 5 μm silica. Eluents were chloroform–methanol–water–acetic acid (30:10:1.7: 0.42) (0–22 ml) and methanol (22–27 ml), flow rate 1 ml/min. $^{14}$C peaks are labeled I–VI, where IV is 5-azaCR.

* In earlier studies, peak VI was allowed to elute from the column prior to a methanol wash. Under these conditions u.v.-absorbance did not overlap with peak VI, and no radioactivity was eluted after the peak. Based on these findings, subsequent chromatographic runs were shortened by washing the column with methanol immediately after the elution of peak V.

Table 1. Effect of THU on the urinary metabolites of [4-$^{14}$C]- and [6-$^{14}$C]-5-azacytidine in BDF$_1$ mice*

| Treatment | High-pressure chromatographic fractions | | | | | | Per cent of administered dose |
|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | |
| [6-$^{14}$C]-5-azaCR | 28.9 ± 5.4 | 24.7 ± 3.2 | 16.0 ± 4.3 | 8.3 ± 2.8 | 0 | 8.8 ± 0.6 | 40.5 ± 11.4 |
| [6-$^{14}$C]-5-azaCR + THU | 7.2 ± 2.4 | 7.5 ± 0.9 | 20.4 ± 4.8 | 33.3 ± 3.9 | 4.6 ± 5.2 | 18.9 ± 4.8 | 41.4 ± 3.7 |
| [4-$^{14}$C]-5-azaCR | 23.2 ± 0.9 | 19.9 ± 6.0 | 4.6 ± 0.1 | 3.6 ± 2.0 | 26.5 ± 5.0 | 19.1 ± 1.8 | 45.0 ± 10.9 |
| [4-$^{14}$C]-5-azaCR + THU | 6.0 ± 1.9 | 2.7 ± 1.2 | 8.6 ± 1.7 | 21.4 ± 1.8 | 9.3 ± 1.2 | 47.9 ± 4.2 | 29.3 ± 11.8 |

* Conditions for dosing animals were the same as those shown in Table 2. The values shown in h.p.l.c. peaks I–VI represent the per cent recovery of radioactivity from the column (from three or more analyses, mean ± S.D.). The urine samples shown in Table 2 were used for analyses in these studies.

The per cent recovery of radioactivity in expired air and urine from mice dosed with [4-$^{14}$C]- or [6-$^{14}$C]-5-azaCR and with or without THU is shown in Table 2. Less than 1 per cent of the dose from mice given [4-$^{14}$C]-5-azaCR was recovered as $^{14}CO_2$, whereas approximately 20 per cent of the dose was recovered as $^{14}CO_2$ from mice given [6-$^{14}$C]-5-azaCR. These data indicate that the triazine ring of the molecule was cleaved at the number 6-carbon which the animals expired as $CO_2$. THU had no effect on the recovery of radioactivity in expired air or urine.

*Characterization of the urinary metabolites of 5-azaCR.* Co-chromatography of urine, from mice given 5-azaCR, with authentic samples of 5-azauracil (5-azaU), 5-azacytosine (5-azaC) and 5-azaCR indicated that h.p.l.c. peaks I, III and IV were, respectively, similar to the authentic standards. Peak II was not characterized.

Peak V was isolated from mouse urine by h.p.l.c. and derivatized with pyridine:bis (trimethylsilyl)trifluoroacetamide reagent. Gas–liquid chromatographic analysis of the derivatized sample showed a major radioactive peak which eluted at 50° and a minor radioactive peak which eluted at 110° when the oven temperature was programmed at 4°/min. Mass spectrometric analysis of the early g.l.c. radioactive peak indicated a molecular weight of 115 for the peak with a base ion at *m/e* 100 (Fig. 2). Derivatization of h.p.l.c. peak V with the same reagent and pyridine:bis(trideuteromethylsilyl)trifluroacetamide-$d_{18}$ reagent (50% deuterium), which contained nine deuterium atoms per mole, and analysis by g.l.c. showed a corresponding nine mass unit increase of the early g.l.c. peak (Fig. 3). This mass unit shift indicated the presence of a single trimethylsilyl (TMS) group and that the mass of the underivatized molecule was 43 (115 minus 72). Several compounds, e.g. cyanic acid, having this molecular weight were considered. Trimethylsilylation of a commercial preparation of the potassium salt of cyanic acid and analysis by g.l.c. showed a peak with g.l.c. properties and a m.s. fragmentation pattern identical to those of the early g.l.c. peak obtained from h.p.l.c. peak V. Similarly, analysis of the second g.l.c. peak from h.p.l.c. peak V indicated it was bis(trimethylsilyl)cyanamide with a molecular weight of 186 and the $M^+$-15 ion at *m/e* 171 (Fig. 4).

It appeared, however, that cyanate and cyanamide could have been produced during the derivatization of the h.p.l.c. fractions. For example, biuret and urea derivatized under the same conditions and analyzed by g.l.c.-m.s. indicated the following structures: TMS—OCN, TMS—NH—CO—NH—TMS and TMS—N=C=N—TMS. Biuret, urea or cyanate, however, did not co-chromatograph by h.p.l.c. with peak V, lending support to the indication that cyanate and cyanamide originated during the derivatization of h.p.l.c. peak V.

High-pressure liquid chromatography peak V was subjected to acid methanolysis which should have resulted in cleavage of any ribosyl linkage to yield the corresponding methyl glycoside. After trimethylsilylation of the mixture, g.l.c. analysis showed a major peak which eluted at 153°. Mass spectro-

Table 2. Effect of THU on the metabolism of [4-$^{14}$C]- and [6-$^{14}$C]-5-azacytidine in BDF$_1$ mice*

| Treatment | Per cent recovery of administered dose | | |
|---|---|---|---|
| | Urine | $^{14}CO_2$ | Total |
| [6-$^{14}$C]-5-azaCR | 47 ± 14 | 19 ± 4 | 67 ± 17 |
| [6-$^{14}$C]-5-azaCR + THU | 45 ± 5 | 21 ± 3 | 66 ± 10 |
| [4-$^{14}$C]-5-azaCR | 46 ± 11 | 0.5 ± 0.1 | 47 ± 12 |
| [4-$^{14}$C]-5-azaCR + THU | 31 ± 12 | 0.5 ± 0 | 31 ± 12 |

* Mice were dosed i.p. with THU, 10 mg/kg, immediately prior to 5-azaCR (2.6–6.2 μCi), 50 mg/kg, and urine and $CO_2$ were collected over an 8-hr period. Three or more animals were used for each experiment and each experiment was run in triplicate. The values shown are expressed as the per cent recovery of administered radioactivity (mean ± S.D.).

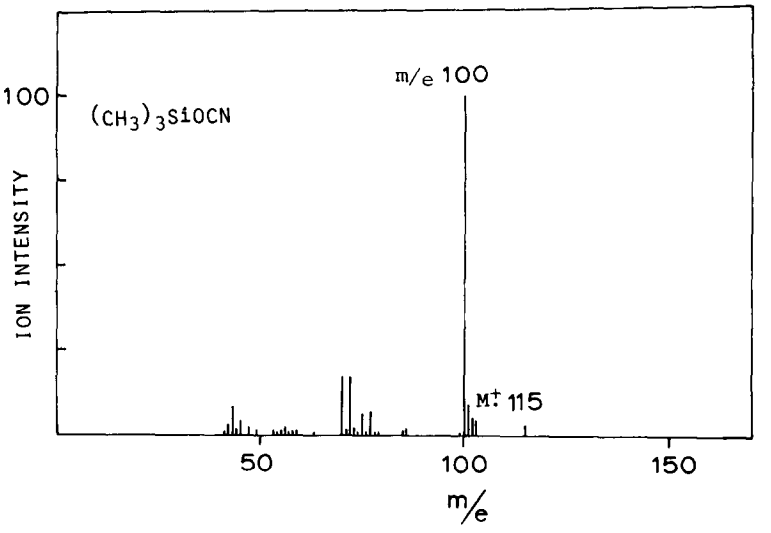



Fig. 2. Mass spectrum of trimethylsilyl cyanate (early g.l.c. peak). High-pressure liquid chromatography peak V, from urine of mice given [4-$^{14}$C]-5-azaCR, was derivatized for g.l.c.–m.s. analysis as described in Materials and Methods.

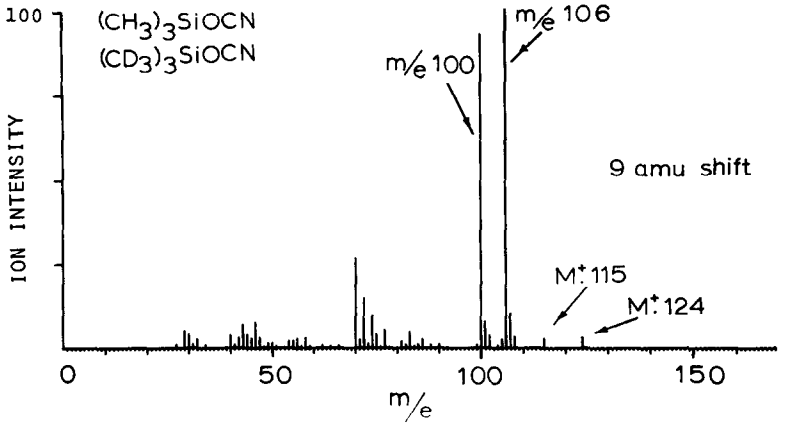



Fig. 3. Mass spectrum of trimethylsilyl cyanate (early g.l.c. peak). The conditions were the same as in Fig. 2 except that the derivatization mixture was 50% *N,O*-bis(trideuteromethylsilyl)trifluoroacetamide-$d_{18}$.

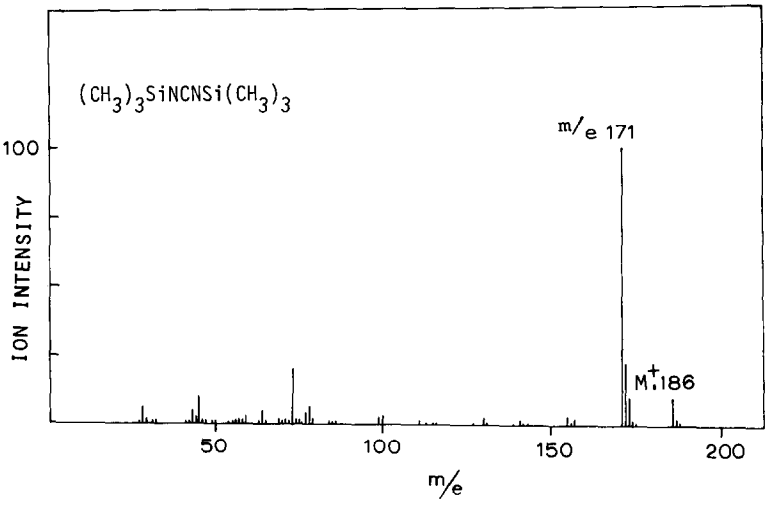



Fig. 4. Mass spectrum of bis(trimethylsilyl)cyanamide. The conditions were the same as in Fig. 2.

metric analysis of this peak showed a mass spectrum identical with methyl-α-D-ribofuranoside-2,3,4,*O*-trimethylsilyl ether.

[4-$^{14}$C]-5-AzaCR, when incubated with dog plasma, readily decomposed to give h.p.l.c. peak VI. The peak obtained in this manner co-eluted with peak VI from mouse, dog and human urines.* After acid methanolysis and trimethylsilylation, g.l.c. analysis of the non-radiolabeled species indicated the presence of ribose. Analysis of the volatile radiolabeled species by g.l.c.–m.s. indicated a single radioactive peak which was identical in its g.l.c. properties and mass spectral fragmentation with bis(trimethylsilyl)cyanamide. The fragmentation pattern was identical to the second g.l.c. peak seen in the g.l.c.–m.s. analyses of h.p.l.c. peak V (Fig. 4).

## DISCUSSION

Tetrahydrouridine was reported by others to increase the level of 5-azaCR in plasma of mice [28] and, in the present report, is shown to increase the urinary excretion level of 5-azaCR (peak IV) and to alter the profile of 5-azaCR equivalents in mouse urine. With these facts, we have used THU and 5-azaCR, labeled in the 4- or 6-position of the triazine ring, to study the metabolism of 5-azaCR and, in particular, to characterize the structure of h.p.l.c. peaks V and VI.

As seen in Table 1, THU was effective in decreasing the level of h.p.l.c. peaks I, II and V and increasing the level of peaks IV and VI. This effect by THU indicated that peaks I, II and V were deaminated-related compounds and that peaks IV and VI retained the 4-amino group. Co-chromatography of mouse urine with authentic standards of 5-azaU, 5-azaC and 5-azaCR indicated that peaks I, III and IV were these compounds, respectively. This evidence is supported by the detection of 5-azaU and 5-azaCR in mouse urine by Raska *et al.* [14] and 5-azaC as a degradation product of 5-azaCR *in vitro* [24]. Peak II was not characterized, primarily because of its instability. Based on the increase in this peak due to the influence of THU (Table 1) and its instability, it was anticipated that peak II may be 5-azauridine (5-azaUR, see Scheme B) which has been identified in *Escherichia coli* culture after incubation with 5-azaCR [27].

Peak III was not affected by THU, nor did its total level in urine change when the excretion of metabolites was quantitated over a period of time [30]. The peak was also seen in stock preparations of 5-azaCR. These data suggest that the presence of peak III in the metabolic profile was due primarily to its presence as a contaminant in the drug preparation.

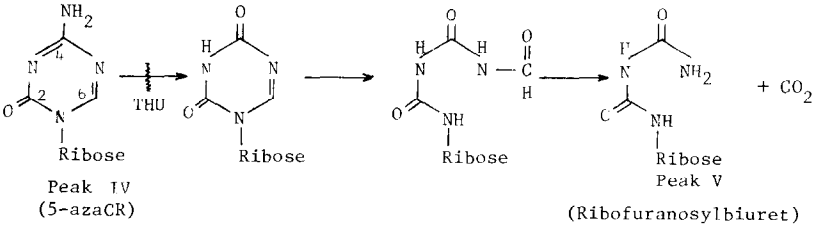
High-pressure liquid chromatography peaks V and VI are present in abundance in urine of mice and humans dosed with 5-azaCR, and they lack u.v. absorbance. Cleavage of the triazine ring of 5-azaCR during metabolism is supported by several experiments: (1) detection of $^{14}CO_2$ in expired air from mice dosed with [6-$^{14}$C]-5-azaCR (Table 2); (2) disappearance and reduction of radioactivity in h.p.l.c. peaks V and VI, respectively, when [6-$^{14}$C]-5-azaCR is substituted for [4-$^{14}$C]-5-azaCR (Table 1); and (3) non-u.v. absorbance of h.p.l.c. peaks V and VI (Fig. 1). Non-chromophoric compounds have been described as metabolites *in vivo* [14, 25] or degradation products of 5-azaCR *in vitro* [24].

High-pressure liquid chromatography peak V gave rise to cyanate and ribose (major components), and peak VI gave rise to cyanamide and ribose when analyzed by g.l.c.–m.s. Based on the work of others [14, 24, 27], it was expected that the above constituent parts of the compound would be linked through the number 2-carbon atom of the intact compound to ribose. The comparative profile of urinary metabolites from [4-$^{14}$C]- or [6-$^{14}$C]-5-azaCR (Table 1) indicated that the linkage between the ribose and cyanate was via the 2-carbon in peak V and that approximately 50 per cent of the components of peak VI linked via the 2-carbon between ribose and cyanamide.

High-pressure liquid chromatography peak V represented approximately 27 per cent of the urinary radioactivity when animals were dosed with [4-$^{14}$C]-5-azaCR and this peak decreased in animals co-administered THU. When [6-$^{14}$C]-5-azaCR was used, h.p.l.c. peak V essentially disappeared. Thus, Scheme A is proposed to explain the formation of peak V (ribofuranosylbiuret).

The percentage of h.p.l.c. peak VI in urine varied, depending upon the position of the radiolabel. With [4-$^{14}$C]-5-azaCR, h.p.l.c. peak VI represented about 19 per cent of the 5-azaCR equivalents in urine and increased to about 48 per cent in urine of animals that were treated with THU. When [6-$^{14}$C]-5-azaCR

* In a separate investigation, we used human cancer patients and Beagle dogs to study the metabolism of 5-azaCR. Urine from the patients and dogs given [4-$^{14}$C]-5-azaCR was analyzed by h.p.l.c. which showed that h.p.l.c. peaks I–VI were present in both cases. Furthermore, we showed that peak VI from either human or dog co-chromatographed with that from mouse urine.

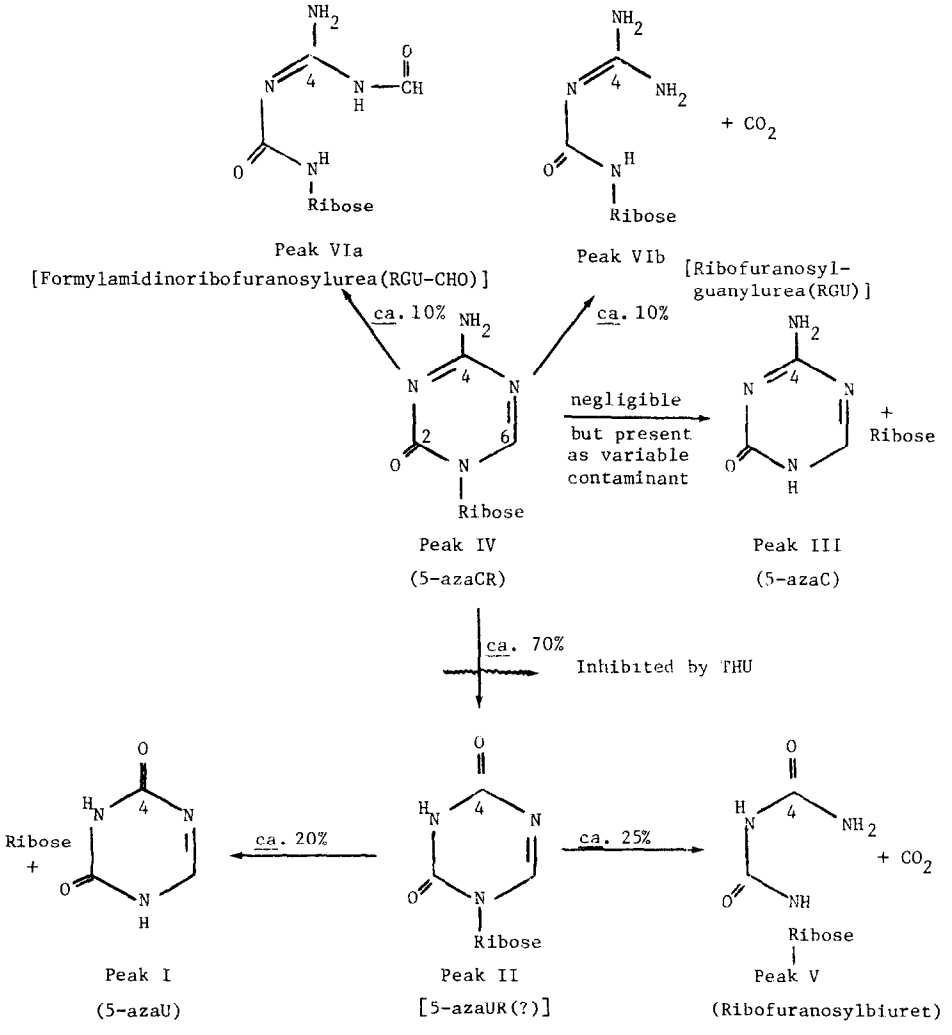



Scheme A.

was administered, the amount of h.p.l.c. peak VI was about half that found using [4-$^{14}$C]-5-azaCR and increased approximately 2-fold in THU-treated animals, which approximates the increase in h.p.l.c. peak VI using [4-$^{14}$C]-5-azaCR plus THU. These data indicate that the fraction (h.p.l.c. peak VI) may contain more than one component.

The components in peak VI may be: (1) 1-$\beta$-ribofuranosyl-3-guanylurea (RGU), to accommodate for the loss of the number 6-carbon; (2) RGU bound to contaminant biological material; (3) *N*-(formylamidino)-*N'*-$\beta$-D-ribofuranosylurea (RGU-CHO), the intermediate in the transformation of 5-azaCR to RGU; (4) formate; or (5) formate bound to contaminant biological material. Items 3–5 would account for the conservation of the radiolabeled carbon of the 6-position of 5-azaCR. Although formate was shown to be a hydrolytic product of 5-azaCR in aqueous solution *in vitro* [33], [$^{14}$C]-formate was not retained in our h.p.l.c. system. It is also doubtful that formate bound to contaminant material would be detected by the methods used in this study. In all likelihood, formate *in vivo* would become oxidized to $CO_2$, which we believe accounts for expired $^{14}CO_2$ (Table 2). We theorize further that RGU bound to contaminant biological material would not be detected by our method of sample preparation and analysis. The evidence in support of RGU can be seen from several experiments. For example, RGU has been shown to be a hydrolytic product of 5-azaCR *in vitro* with a 70 per cent yield [24] and was identified in the urine of mice treated with 5-azaCR [14]. RGU-CHO, the proposed intermediate in the decomposition of 5-azaCR to RGU, was indicated in studies *in vitro* by two separate reports [24, 29] and recently isolated as a hydrolytic product of 5-azaCR *in vitro* [34]. Therefore, we propose that peak VI is composed of RGU and RGU-CHO and that Scheme B represents a quantitative metabolic pathway for 5-azaCR in mice.

Since excretion of the radiolabeled species in urine (45 per cent) and expired air (20 per cent) represented about 65 per cent of the administered dose, another possible excretory pathway, e.g. feces, is




Scheme B.

indicated. In a previous study,* we showed that prior to 8 hr, little radioactivity was recovered in feces of mice; however, by 24 hr, about 15 per cent of the administered dose was recovered in feces, i.e. an 80 per cent cumulative excretion of radiolabeled equivalents. Based on the metabolic profile of [4-$^{14}$C]-5-azaCR (Table 1), approximately 20 per cent of the administered dose is metabolized to peak VI, divided equally between VIa and VIb, and about 70 per cent of the dose is metabolized and divided equally among the deaminated components (peaks I, II and V). The remaining radioactivity is accounted for by peaks III (5 per cent) and IV (4 per cent).

The modification of the metabolic pathway by THU supports the action of this analog as a deaminase inhibitor, since THU treatment resulted in a decrease (14–35 per cent) of the deaminated components and an increase (40 per cent) in the non-deaminated component (peak VI). There is, quantitatively, a similar pattern of urinary metabolites in humans and dogs. Therefore, the proposed Scheme B may also be applicable to most mammalian species.

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* In this study, mice were given [4-$^{14}$C]-5-azaCR and the radioactivity in feces was monitored over 24 hr. By 8 hr, only 5 per cent of the radioactivity was excreted, but by 24 hr the level of radioactivity increased to about 15 per cent of the administered dose.