# *In Vivo* Loss of Tritium from Labeled Drugs: Determinations in Urine and Plasma

**Keyphrases** □ Tritium-labeled drugs—*in vivo* loss of tritium, urine and plasma analyses compared □ Radioactive labels—*in vivo* loss of tritium, urine and plasma levels compared □ Benzazocines, substituted—tritium labeled, used in study of *in vivo* loss of tritium, urine and plasma levels compared

*To the Editor:*

We wish to report an interesting observation that occurred during metabolic studies with tritium-labeled compounds.

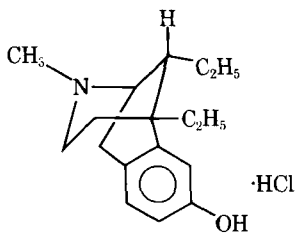
Frequently, tritiated compounds can be prepared more economically and with a higher specific activity than carbon-14-labeled compounds. The main disadvantage of using tritium is that the tracer can be lost from the molecule by exchange with the hydrogen atoms of the solvent, whereas a similar exchange between carbon-14 and carbon-12 is not possible. Even where no *in vitro* exchange is observed in water, urine, or plasma, an *in vivo* loss of label leading to problems of interpretation is still possible.

The *in vivo* stability of a tritium label is usually tested by distillation of urine specimens (1). Our results indicate that the distillation of plasma specimens provides an earlier and more apparent indication regarding the *in vivo* stability of the tritium label in an organic molecule.

During studies on the biological disposition of several tritium-labeled drugs, we found a considerably greater percentage of volatile radioactivity in plasma than in urine. The following example is illustrative.

The analgesic *l*-6,11α-diethyl-1,2,3,4,5,6-hexahydro-3-methyl-2,6-methano-3-benzazocin-8-ol hydrochloride (I)(2) was labeled by adding 3.0 g of tritiated water (3.0 Ci) to 3.4 g of I and 4.8 g of phosphorus pentoxide at −70°. The mixture was allowed to warm, and the resulting solution was heated at 100° for 4 hr. The reaction mixture was poured onto a mixture of ice and concentrated ammonium hydroxide, and the resulting precipitate was collected by filtration.

The solid was dissolved in ether, and the solution was washed with water, dried over sodium sulfate, and acidified with hydrogen chloride gas. Then the product was collected and recrystallized from 2-propanol to give 2.4 g of analytically pure (C, H, Cl, N) prisms. The NMR spectrum of the product of a similar experiment using




I

Table I—Urinary Excretion of Radioactivity in a Monkey Given 2 mg/kg ip of $^3$H-I

| Interval, hr | Excreted Radioactivity, % of Dose | | Distillable / Total × 100 |
|---|---|---|---|
| | Total | Distillable | |
| 0–6 | 48.8 | 0.05 | 0.1 |
| 6–24 | 14.5 | 0.35 | 2.4 |
| 24–48 | 3.2 | 0.84 | 26.3 |

deuterium oxide showed the deuterium to be in both positions *ortho* to the phenolic hydroxyl group of I. A similar acid-catalyzed exchange reaction also was reported (3).

A 2-mg/kg ip dose of tritiated I (specific activity 34 μCi/mg) was given to a Rhesus monkey, and the total radioactivity content of plasma and urine specimens was measured by direct counting[1] in Bray's solution (4) using the external standard method of quench correction. Volatile radioactivity was determined by lyophilization and counting. Two consecutive lyophilizations were performed to avoid contamination by mechanical carryover of nonvolatile material. The *in vitro* stability of the tritium label was demonstrated in control experiments by incubation at 37° in urine and plasma.

Urinary excretion of radioactivity is summarized in Table I. These results might suggest that the initial *in vivo* loss of label was not sufficiently fast to affect the validity of the experiment, at least during the first 24 hr after administration of I. However, a considerably greater proportion of distillable radioactivity was found in the plasma of the animal during that time interval (Table II).

Further analysis revealed that the discrepancy between urine and plasma could be resolved in terms of the concentrations of tritiated water. Since the weight of the monkey was approximately 3 kg, it received approximately 204 μCi of radioactivity. During the first 6 hr after dosing, the urine (volume of 25 ml) contained 0.05% of the dose or 0.1 μCi as distillable radioactivity. Thus, the concentration of distillable urinary radioactivity amounted to $4 \times 10^{-3}$ μCi/ml.

Since some time was necessary for absorption to occur from the injection site, the 4-hr time point was an acceptable approximation for the true midpoint of the interval between 0 and 6 hr. Distillable plasma radioactivity at 4 hr attained 0.102 μg/ml, expressed as unchanged I. This value corresponds to $3.4 \times 10^{-3}$ μCi/ml of distillable radioactivity.

Thus, at the early time interval, the results show good agreement between the concentrations of tritiated water in urine and plasma. The agreement was found in spite of the apparently large differences between the ratios of distillable to total radioactivity in urine and plasma (Tables I and II). One can rationalize the findings by assuming that the glomerular filtration and tubular

[1] Packard Tri-Carb model 3380 scintillation spectrophotometer.

Table II—Plasma Concentrations of Radioactivity in a Monkey Given 2 mg/kg ip of $^3$H-I

| Hours | Plasma Radioactivity[a], μg/ml | | Distillable / Total × 100 |
|---|---|---|---|
| | Total | Distillable | |
| 0.5 | 2.48 | 0.060 | 2.4 |
| 1 | 2.11 | 0.076 | 3.6 |
| 2 | 1.34 | 0.043 | 3.2 |
| 4 | 0.61 | 0.102 | 16.7 |
| 7 | 0.19 | 0.057 | 29.9 |
| 24 | 0.11 | 0.093 | 84.5 |
| 48 | 0.08 | 0.076 | 94.7 |

[a] Each value is the amount of administered drug that would give rise to the observed radioactivity.

reabsorption processes of the kidney could not distinguish tritiated water from unlabeled water. Therefore, tritiated water would have comparable concentrations in urine and plasma and would be excreted at the same rate as unlabeled water. The exact value for the half-life of water in the monkey is not known, but corresponding values of 3.5, 3.9, and 5.1 days have been reported for the rat, rabbit, and dog, respectively (5).

In contrast, I and/or its metabolites were rapidly excreted and their plasma levels fell below their concentration in urine. The result of these differences in the excretion rates of tritiated water and I was a greater ratio of distillable to total radioactivity in plasma than in urine.

The observed *in vivo* loss of the tritium label might be attributed to a tritium–hydrogen exchange, perhaps facilitated by some biological mechanism, or to a specific metabolic alteration of the molecule at the site of tritium substitution. To distinguish between these two mechanisms of *in vivo* instability, one must isolate the unchanged drug from urine or plasma and compare its specific activity with that of the administered drug. Comparable specific activities would suggest that the loss of label occurred in conjunction with metabolism at the site of tritium substitution. In contrast, a decrease of specific activity would indicate that the loss of label was caused partly or entirely by exchange.

The findings reported in this communication may have general applicability and serve as a warning to the uninitiated user of tritium. One must be careful in drawing conclusions from urinary data regarding the *in vivo* stability of the tritium label in a compound. Furthermore, our results strengthen the argument that when a substantial fraction of the label is lost, tritium should not be used as a tracer for the parent molecule.

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Received January 16, 1975.
Accepted for publication June 7, 1976.
The authors are indebted to Dr. Frank H. Clarke, Dr. Naokata Yokoyama, and Mr. Walter Munch, Ciba-Geigy Corp., for the preparation of tritiated I. The technical assistance of Mrs. Judith Zagarella is gratefully acknowledged.

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## GLC Determination of Pilocarpine

**Keyphrases** □ Pilocarpine—GLC analysis, rabbit aqueous humor □ GLC—analysis, pilocarpine, rabbit aqueous humor □ Ophthalmic cholinergic agents—pilocarpine, GLC analysis, rabbit aqueous humor

*To the Editor:*

Measurement of the transport of pilocarpine into the internal eye as well as information concerning its distribution and inactivation within individual intraocular structures depends on accurate determination of small quantities of this drug in biological fluids. Although many procedures have been used for this purpose, including those involving polarimetric, polarographic, volumetric, colorimetric, and kinetic techniques (1–6), all have been associated with problems of complexity, lack of specificity, or lack of sensitivity.

GLC coupled with electron-capture detection has been employed during the past 10 years for the determination of nanogram amounts of organic compounds exhibiting certain similarities to pilocarpine (7–9). A similar technique provided an ideal system for a sensitive new procedure for the reliable assay of pilocarpine.

Heptafluorobutyric anhydride[1], trimethylamine, and pesticide grade benzene[2] were utilized in the formation of the derivative of pilocarpine[3]. Heptafluorobutyric anhydride (10 μl) and 0.1 ml of trimethylamine (0.05 *M* in benzene) were added to 500 μg of pilocarpine hydrochloride in benzene (10). The reaction was performed in screw-capped vials[4]. After the sample was heated for 15 min at 50°, 1 ml of water was added and the vial was shaken for 1 min. One milliliter of aqueous ammonia (5%) was added, and the reaction mixture was shaken for an additional 5 min.

After centrifugation, the benzene layer was diluted to give a derivative concentration of 100 ng/ml. Then 1 μl (equal to 0.1 ng) was injected into a 1.2-m × 4-mm i.d. U-shaped column packed with 2% XF-1105[5] on Gas Chrom Q[6] (80–100 mesh) for GLC analysis. Separation of the derivative was achieved using a gas chromatograph[7] equipped with two electron-capture detectors, each containing a 150-mCi tritium foil.

The oven temperature was kept at 190°; the detector, inlet, and outlet temperatures were maintained at 200°.

[1] Pierce Chemical Co., Rockford, Ill.
[2] Fisher Scientific Co.
[3] Mallinckrodt Chemical Co.
[4] Lined with Teflon (du Pont).
[5] Now called OV-105.
[6] Ohio Valley Specialty Chemical, Marietta, Ohio.
[7] Packard model 7400.