# Urinary Excretion of Biotin and Metabolites in the Rat[1] (37085)

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The metabolism of the vitamin, *d*-biotin, in the rat has recently been investigated by Lee *et al.* (1). When carbonyl-labeled biotin is injected intraperitoneally (ip) at the mg level, the radioactivity is almost completely excreted as intact biotin within one day, though the rate of excretion is less when the amount of biotin given is decreased to the μg level, especially in animals previously depleted of the vitamin. However, not all of the injected radioactivity was recovered within a few days.

In addition to the recovered vitamin, small amounts of several biotin metabolites, namely, the *d*- and *l*-sulfoxides, bisnorbiotin (two-carbon shorter side chain), and a neutral ketone were isolated from rat urine (1), but tetranorbiotin (four-carbon shorter side chain), which also accumulates in the culture medium from a biotin-degrading bacterium (2), was not detected.

The present study was made to determine the excretion rates in the rat of biotin and some of the more commonly occurring metabolites.

*Materials and Methods.* *d*-Biotin was purchased from Hoffmann-La Roche, Inc. The radioactive compounds used were exclusively ureido carbonyl[$^{14}$C]-labeled, with specific radioactivities, in dpm/mg, as follows: biotin, 20,240; bisnorbiotin, 20,100; tetranorbiotin, 20,440; biotin *d*-sulfoxide, 20,700; biotin *l*-sulfoxide, 20,500; and biotin sulfone, 20,450. The [$^{14}$C]biotin was supplied by Amersham/Searle Corp. The [$^{14}$C]catabolites formed by β-oxidation of the valeric acid side chain of [$^{14}$C]biotin were isolated from culture filtrates of a pseudomonad grown on the carbonyl-labeled biotin as described by Iwahara *et al.* (2). The sulfoxides and sulfone were prepared from carbonyl-labeled biotin by oxidizing with peroxide according to Melville (3). All radioactive compounds were dissolved in 0.1 *M* sodium phosphate buffer, final pH 7.4.

Young, male, Sprague–Dawley rats (250–310 g), which were obtained from Chordata Corp., were fed, for six weeks, a biotin-free, avidin-containing test diet[4] (4), which additionally contained 1% succinyl sulfathiazole. These animals were then divided randomly into six groups of five rats each. Each rat was given a single ip injection of 0.5 mg/100 g body weight of one of the six radioactive compounds. A control group of rats, maintained on a biotin-sufficient diet,[5] was given an injection of 0.5 mg/100 g body weight of radioactive biotin. Urine, feces, and expired $CO_2$ from each rat were collected separately in an all-glass, closed, rat metabolism cage system.[6] Assay of radioactivity from

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[4] Purchased from Nutritional Biochemicals Corp., St. Louis, Mo.; for composition of this diet, see Rubin *et al.* (4).

[5] Rat-Mouse Pelleted Diet from Agway, Inc., Specialties Division, Syracuse, N.Y.

[6] Purchased from Delmar Scientific Laboratories, Chicago, Ill.

samples was done according to Yang and McCormick (5). Isolation of radioactive compounds from samples of urine followed the method of Yang *et al.* (6). The identification of recovered radioactive compounds was accomplished with paper chromatography as described in previous work (1).

*Results.* No significant radioactivity was excreted through feces or expired $CO_2$. The major excretion occurs in the urine. The urinary excretion rates of biotin from both biotin-sufficient and biotin-deficient rats, which have been injected ip with milligram amounts of the vitamin, are similar to each other. However, there are notable differences if one compares the initial excretion rates of biotin with certain of the metabolites, although all six compounds tested were almost completely excreted within one day.

The differences among the urinary excretion rates of biotin and analogues from rats are presented in Table I. The initial excretion rates of tetranorbiotin, biotin sulfoxides, and biotin sulfone are similar and significantly faster than those of biotin and bisnorbiotin, which latter two behave similar to each other. In each case, the radioactive compounds recovered from urine evidenced but one major radioactive peak upon column (Dowex 1-X2, formate) and one spot upon paper chromatograms. Positions for elution and mobilities were identical to the original injected compounds. Thus, none of these compounds, when injected at the milligram level, appeared to be metabolized very extensively, even though some small fraction is. This relatively small amount becomes quite apparent when only microgram amounts of the vitamin are given (1).

TABLE I. Urinary Excretion Rates of Biotin and Biotin Metabolites from the Rat.[a]

| Compound | % Urinary radioactivity recovered after injection | | |
|---|---|---|---|
| | 0–3 hr | 3–6 hr | 6–24 hr |
| | Biotin-sufficient diet | | |
| Biotin | 84.3 ± 5.1 | 9.5 ± 2.2 | 2.7 ± 0.4 |
| | Biotin-deficient diet | | |
| Biotin | 83.8 ± 6.3 | 9.3 ± 2.7 | 2.6 ± 0.4 |
| Bisnorbiotin | 85.8 ± 2.2 | 10.4 ± 0.6 | 2.2 ± 0.0 |
| Tetranorbiotin | 96.4 ± 0.2 | 2.9 ± 1.2 | |
| Biotin *d*-sulfoxide | 97.4 ± 1.7 | 1.4 ± 0.4 | |
| Biotin *l*-sulfoxide | 97.4 ± 1.1 | 1.5 ± 0.2 | |
| Biotin sulfone | 97.8 ± 1.2 | 1.4 ± 0.2 | |

[a] Values shown are means ± SD.

*Discussion.* The urinary excretion rates of biotin, bisnorbiotin, tetranorbiotin, biotin sulfoxides, and biotin sulfone from the biotin-deficient rats are all of the same magnitude, but clear differences do exist. The urinary excretion rate of biotin appears to be the same from the biotin-deficient as from the control rat. Hence, the lowered initial excretion rate of such relatively large amounts (milligram level) of biotin, which is less than that of most of the metabolites, is not likely due to greater need (microgram level) of the vitamin in the biotin-deficient rat. The similar excretion rate of bisnorbiotin, which presumably cannot be incorporated into biotin-dependent enzymes, to biotin alone argues against a special need for retaining the larger amounts of the vitamin. The fact that each of the present metabolites is excreted at least as quickly and completely as biotin minimizes the possibility that the major part of the urinary radioactivity not recovered within a few days after microgram amounts of biotin are injected, as in the previous work (1), might be accounted for by any single one of these five biotin metabolites, rather than by several of them. The relatively fast and complete excretion of biotin and its metabolites at the milligram level may suggest that an active transport mechanism for reabsorbing these compounds in the renal tubule of the rat may not be so important as a simple passive absorption and/or that excretion is facilitated. This is further supported by examining the differential excretion rates of these compounds: biotin sulfone $\geqq$ biotin sulfoxides $>$ biotin, and tetranorbiotin $>$ bisnorbiotin $\geqq$ biotin. This behavior at least generally parallels the water solubilities.

Oxidation of the thioether sulfur in biotin expectedly leads to increased polarities and greater water solubilities in the sulfoxides and sulfone, just as does shortening the hydrophobic side chain in the bisnor- and tetranor-compounds.

Finally, the urinary excretion rates of biotin and its metabolites from the rat are also consistent with the rates of uptake of these compounds by the bacterial cell, where the sulfone > sulfoxides > biotin (7, 8).

*Summary.* Approximately 95% of an ip dose of 0.5 mg/100 g body weight of [$^{14}$C] biotin is excreted by the young, male rat within 24 hr; 84% is excreted within the first 3 hr. The rate and extent of excretion of bisnorbiotin is essentially the same. The more water-soluble tetranorbiotin, both *d*- and *l*-sulfoxides of biotin, and biotin sulfone are excreted even more rapidly, with 96–98% of the injected dose appearing in the urine within the first few hours.

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