Metabolism of Furazolidone in Swine (35994)

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Studies in rats, dogs, chickens, and rabbits (1–5) show the rapid conversion of ingested nitrofurans to a limited number of structurally related metabolic products, not all of which have been positively identified. However, from work with isotopically labeled nitrofurans, (6) it is apparent that a considerable proportion of the dose is so extensively degraded that the fragments enter the normal carbon pool of the body and become widely distributed. Continuing investigation of this problem is important to an understanding of the fate of nitrofurans and other readily degraded compounds commonly used in the animal production industry. We present the results of a study of the metabolism and excretion of furazolidone in swine.

Methods. A 57 lb Yorkshire barrow maintained for the previous 3 weeks on feed containing 0.033% furazolidone was orally dosed with 39 μCi (1.25 mg) of furazolidone labeled with ^{14}C in the formyl carbon attached to the 2-position of the furan ring. Urine samples, collected when voided during the first 8 hr after dosing and at intervals thereafter, were filtered through glass wool, sampled for radioisotope counting, subdivided into 1–2 oz portions, and frozen. Feces were collected, air-dried and weighed. While under light ether anesthesia, the pig was sacrificed by exsanguination 48 hr after dosing, blood was collected with heparin, and tissue samples were taken and quickly frozen in Dry Ice.

Isotope counts were measured in a liquid scintillation spectrometer on duplicate preparations of each sample mixed with 15 ml of Diotol in the following proportions: urine, 0.2 ml and 0.8 ml of methanol; blood and bile, 0.1 ml and 0.9 ml of methanol (blood decolorized with H_2O_2); digestive tract contents, 0.5 ml of a homogenate in 1:1 DMF–methanol decolorized with H_2O_2; dried feces, 20 mg in 0.2 ml of water and 0.8 ml of methanol; tissues, 100 mg with 0.2 ml of water and 0.7 ml of methanol. All samples were counted for two 10 min periods, were fortified with ^{14}C-toluene, and were counted twice again. Counting efficiencies were 33–54% for liver, kidney, fat, muscle, blood, and bile; 44–50% for urine; 22–28% for feces and 8–30% for digestive tract. For measurement of isotopic carbonate, 2 ml samples of urine, serum, and bile were acidified with lactic acid as described by Moss (7) and the released CO_2 was trapped in Hyamine hydroxide and mixed with Diotol for counting.

Urine samples (10 ml volumes) were fractionated chromatographically on Dowex 1 $\times$ 2, 200–400 mesh. The resin (on the chloride cycle, and pretreated by conversion to the sulfate and back) was packed to a depth of 50 cm in 9 $\times$ 60 cm glass columns. Two buffer solutions were used for elution: 0.3 *M* 2,6-lutidine and 0.05 *M* HCl (pH 7.2); and 0.3 *M* 2,6-lutidine, 0.15 *M* HCl, and 0.6 *M* NaCl (pH 6.6). These entered the column through a 2-chambered gradient elution device. The first chamber, with 300 ml constant volume, drained into the column, was filled initially with the more dilute buffer, and was constantly stirred. The second chamber, which drained into the first, contained enough of the more concentrated buffer to allow collection of 350 eluate fractions with 3 ml volume. Flow rate was about 10 ml/hr. One milliliter of each fraction was mixed with 1 ml of methanol and 15 ml of Diotol and counted as described for urine samples.

Other columns were eluted with water alone to recover non-anionic labeled substances. The eluates from the latter, concentrated by lyophilization, were examined by two-dimensional paper chromatography using solvent systems described by Mizell and Simpson (8) and by McGreer *et al.* (9).

TABLE I. Isotope in Urine and Feces.

Sampling time (hr)	Urine Vol (ml)	Urine Total isotope[a]	Urine Labeled carbonate[a]	Feces Total isotope[a]
0.6	42	2.86	0.13	
1.6	75	20.82	1.38	
5.2	180	29.82	1.04	
8.5	220	6.70	0.26	0.21
12.5	220	2.63	0.01	
21.0	835	3.66	0.25	
21.5				0.21
24.0	175	0.56	0.03	0.33
27.5	300	0.72	0.07	
30.5				3.56
35.5	225	0.46	0.03	
46.5	1500	1.98	0.02	
48.0	295	0.28	0.02	7.72
Remaining in digestive tract				7.02
Total		70.49	3.24	19.05

[a] As % of dose.

Results. Table I presents the amounts of isotope found in urine and feces collected at various time intervals after dosing, and the amount of labeled carbonate in urine. The total recovery, 89.5% of the oral dose, agrees reasonably well with the results (Ray, unpublished data) of a similar test in rats: 81.5% in urine, 11.0% in feces, 3% in expired air, and 8.1% in the carcass. The pig excreted the isotope rapidly at first. A plot of the data indicates that half of the urinary isotope must have passed through the kidney during the first 2.6 hr. Later excretion was considerably slower and did not follow first order kinetics. Isotope concentrations in tissues taken from the pig at the end of the 48 hr experimental period were (dpm/g of tissue): kidney, 2105; liver, 1455; thyroid, 1415; bile, 860; blood, 345; muscle, 315; and fat, 135.

Figure 1 presents the elution pattern for radioactivity in urine collected at 1.6 hr after dosing. Total recovery of isotope from the column was more than 88%, and about 5% originally present as carbonate was apparently lost during fraction collection. There appear to be about 15 peaks in the pattern. We have calculated biological half-times of a number of the labeled substances from their depletion in chromatographic patterns of urine samples collected at various times after dosing, and have characterized some of the peaks by chemical tests and by chromatographic analysis of urine fortified with known nitrofuran derivatives.

The first large fraction, tubes 4–15, con-

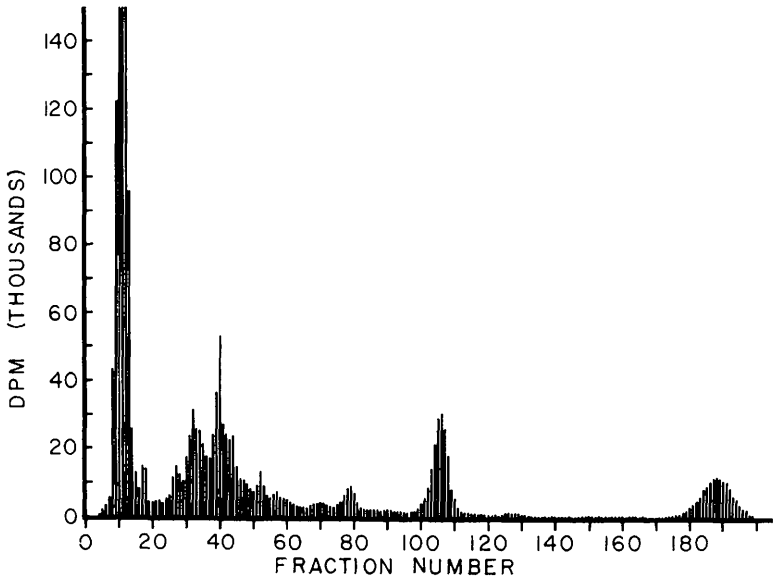

FIG. 1. Radioisotope concentrations (dpm/total fraction) in ion exchange fractionation of swine urine collected 1.6 hr after dosing with ^{14}C-furazolidone.

tained 43% of the radioactivity applied to the column. This peak, which could be eluted with water alone, gave 15 radioactive spots on paper chromatographic separation. The 5 most active were associated with Ninhydrin-positive areas. About 2/3 of the total radioactivity was lost during Ninhydrin treatment. Excretory half-time for the isotope in tubes 4–15 was 1.6 hr.

Furazolidone itself appears in tubes 38–41 of the chromatogram, as shown by fractionation of urine fortified with isotopic furazolidone. Fortification of urine with 5-acetamido-2-furyloxazolidinone increased absorbance at 330 nm in the region of tubes 42–50. Both the pure substance and these tubes gave a transient blue color with Riegler's uric acid reagent (10). Carbonate appeared in fractions 51–55. The peak with maximum at tube 79 is identified as nitrofuroic acid by fractionation of fortified urine. This substance accounted for 2% of the total radioactivity in the first three urine specimens, depleted with a half-time of 0.5 hr, and was not found in urine collected beyond 5.2 hr after dosing.

The peaks with maxima in tubes 106 and 188 have absorbance maxima at 415 nm and are readily apparent owing to their orange and yellow colors. They accounted for 8 and 6% of the total radioactivity in the first three urine collections, and were not found later than 5.2 hr after dosing. Their depletion half-times were 0.35 and 0.32 hr, respectively. The substance peaking at tube 106 reacted with phenylhydrazone to form 5-nitro-2-furfuralphenylhydrazone, but the substance peaking at tube 188 did not do so. Drug-related nitrofuran metabolites of this kind have been reported by other investigators (2, 4, 5).

Summary. Orally administered furazolidone is largely absorbed from the digestive tract by swine and excreted in the form of a large number of metabolic end products. Excretory half-times of products structurally related to furazolidone appear to be 30 min or less.

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