Metabolites of Furazolidone in Urine of Chickens

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Abstract □ Colostomized chickens were medicated with single oral doses of the antibacterial furazolidone. An average of 7.5 ± 3.0% of the dose was excreted in the urine within 12 hr. as compounds measured by a procedure in which they react to form 5-nitrofurfural phenylhydrazone. Chromatographic analysis detected four metabolites containing a furan ring. Only one metabolite reacted to form 5-nitrofurfural phenylhydrazone. Elimination of the metabolites with a furan ring was complete in 12 hr. Only traces of furazolidone were detected by the chromatographic procedure.

Keyphrases □ Furazolidone—metabolites in urine, chickens □ Nitrofurans—furazolidone metabolites determined in urine, chickens

Furazolidone, *N*-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidinone, has found considerable use in the animal health field as an antibacterial (1). In general, the group of compounds known as nitrofurans are rapidly metabolized by birds and mammals. The degradation pathways vary from one compound to another, but some generalities do seem to exist. Metabolites closely related to the parent compound are excreted in the urine in many cases (2–4). Also, with some compounds, metabolism is so extensive that carbon atoms of the molecule appear in the normal body constituents (5, 6). Paul *et al.* (2) detected drug-related metabolites of nitrofurans in urine of laboratory animals by examining the UV absorption spectra. Tennent and Ray (3, 6) separated the drug-related metabolites of furazolidone in pig urine by ion-exchange chromatography. In the present work the methods of Tennent and Ray were used to determine the extent of metabolism of furazolidone and whether drug-related metabolites could be detected in urine of chickens medicated with the compound.

EXPERIMENTAL

Chemicals and Materials—The following were used: phenylhydrazine hydrochloride[1], reagent grade sulfuric acid[1], reagent grade hydrochloric acid[1], reagent grade sodium chloride[1], spectrophotometric grade toluene[2], reagent grade isopropanol[2], reagent grade aluminum oxide[3], 95% ethanol[4], practical grade 2,6-lutidine (redistilled)[5], 5-nitro-2-furoic acid[6], and furazolidone[7].

Dowex 1-X2, 200–400 mesh (chloride cycle), was cycled to the sulfate form with sulfuric acid, to the hydroxyl form with sodium hydroxide, and back to the chloride form with hydrochloric acid. Two buffers were used in the chromatographic development: Buffer A, pH 7.2, contained 0.3 *M* 2,6-lutidine and 0.05 *N* hydrochloric acid; and Buffer B, pH 6.6, contained 0.3 *M* 2,6-lutidine, 0.15 *N* hydrochloric acid, and 2.0 *M* sodium chloride.

[1] J. T. Baker Co.
[2] Matheson, Coleman and Bell.
[3] Merck and Co.
[4] U. S. Industrial Chemicals Co.
[5] Eastman Kodak Co.
[6] Prepared by R. E. Bambury.
[7] Hess and Clark, lot 9159.

Metabolism Studies—White Rock broiler chickens (1.7–1.8 kg.) were colostomized by a surgical technique similar to that of Rothschild (7). Urine free of feces was collected in rubber condoms taped over the cloacal vent. Within 3 days after surgery, the chickens were well adjusted for metabolic experimentation. Housed in individual cages, the birds consumed feed and water and moved about freely during experiments. Control of lighting kept the birds in complete darkness from 8 p.m. to 8 a.m.

Furazolidone was administered orally in a water suspension. For preparation of the dose, 60 g. of micronized furazolidone was encapsulated in partially hydrolyzed gelatin and mixed with 40 g. of sodium citrate. A 1% water suspension of the dry mixture containing 10.7 mg. of furazolidone/ml. was used. The suspension (2.0–3.8 ml.) was forced down the esophagus to the proventriculus by way of siliconized rubber tubing. Regurgitation of the drug did not occur. Ten colostomized chickens were held in individual cages, and control urine was collected for a 6-hr. period. A single dose of furazolidone at a level of 30 mg./kg. body weight was given to each bird. In Experiment I, urine was collected in 6-hr. periods for 24 hr. after dosage from one male and three female birds starved for 15 hr. before dosage. In Experiment II, urine was collected in 5-hr. periods for 20 hr. after dosage from six male birds starved for 24 hr. before dosage.

Urine from each collection period was removed to a centrifuge tube. Collection bags were washed with 3 ml. of water three times. The mixture was centrifuged at low speed. The clear supernate was decanted and the volume was measured. The amount of nitrofurfural moiety was determined on duplicate aliquots at once. The urine from all birds for each period was combined and divided into 25-ml. portions which were stored at −25°.

Analytical—Furazolidone and related nitrofurans react in acid with phenylhydrazine to form 5-nitro-2-furfuraldehydephenylhydrazone, which can be transferred to a solvent and measured spectrophotometrically (8). An aliquot of solution to be assayed (1.0 or 2.0 ml.) was diluted to 10 ml. in a centrifuge tube. One milliliter of a 1.5% solution of phenylhydrazine (prepared daily) was added with vigorous mixing, followed by addition of 1 ml. of 12.3 *N* hydrochloric acid with vigorous mixing. After 40 min. at 40°, the mixture was extracted with 5.0 ml. of toluene. Emulsions that developed with samples such as urine were broken by slow speed centrifugation. For purification, a 4.0-ml. portion of the toluene phase was mixed with 1 g. of aluminum oxide in a centrifuge tube. The 5-nitro-2-furfuraldehydephenylhydrazone adsorbed to the alumina, showing a red color which was a qualitative test for that specific hydrazone (9). To elute the hydrazone, isopropanol (0.4 ml.) was added to the mixture which was then centrifuged. Some impurities remained adsorbed on the alumina. The absorbance of the solution was determined at 437 nm. in a spectrophotometer[8] modified with a photometer[9]. A linear relation between absorbance and concentration was obtained in a range of 0.02–0.75 μmole of furazolidone. Values are expressed as micromole equivalents of furazolidone. Urine was collected from each bird before medication and was used as a control to correct analyses after medication. Absorbance values were 0.01–0.02 for 1.0 ml. of control urine.

Urine samples were chromatographed on columns (11 × 300 mm.) of Dowex 1-X2 in the chloride form. Methods were modifications of those of Tennent and Ray (6). Elution was accomplished with a gradient system similar to that of Peterson and Sober (10), using four chambers to raise sodium chloride concentration to 2.0 *M*. The first three chambers contained Buffer A and the fourth contained Buffer B. The columns were operated at room temperature in a darkened area with a flow of about 0.6–1.0 ml./min. The effluent was collected in 5-ml. fractions with a siphon device. The

[8] Beckman DU.
[9] Gilford.

Table I—Excretion of Compounds in the Urine of Chickens after Single Oral Doses of Furazolidone Measured by the Phenylhydrazone Assay Method

Experiment[a]	Bird Number	Sex	Furazolidone in the Dose, μmoles	—Equivalents of Furazolidone Excreted— First Period, μmoles	Second Period, μmoles	Total, μmoles	Total Amount Excreted, %
I	1	M	133	5.5	2.9	8.4	6.3
	2	F	120	3.5	1.1	4.6	3.8
	3	F	88	6.4	1.5	7.9	8.9
	4	F	97	7.7	0.3	8.0	8.2
II	5	M	157	3.7	1.7	5.4	3.4
	6	M	133	16.1	1.5	17.6	13.2
	7	M	181	15.3	0.9	16.2	9.0
	8	M	152	5.9	1.5	7.4	4.8
	9	M	109	7.7	3.2	10.9	10.0
	10[b]	M	167	9.3	3.8	14.2	8.5
Average							7.5

[a] Values are equivalents of furazolidone excreted in the urine in each collection period. In Experiment I, urine was collected in 6-hr. periods for 24 hr. In Experiment II, urine was collected in 5-hr. periods for 20 hr. [b] The false anus of bird 10 became plugged the night before the experiment and remained plugged throughout the experimental procedure.

absorbance of each fraction was measured at 320 and 375 nm. in the spectrophotometer modified with the photometer. Selected fractions or combinations were assayed by the phenylhydrazone procedure. Absorption spectra were determined in a recording spectrophotometer[10].

Uric acid was measured by a semiquantitative assay to determine the position of uric acid in the chromatographic elution pattern rather than to measure amounts present. The reagents of Benedict and Franke (11) were used. In a colorimeter tube, a 5.0-ml. aliquot of sample was mixed with 1.5 ml. of arsenophosphotungstic reagent and 0.3 ml. of sodium cyanide reagent. The tubes were shaken gently for 5 min., diluted with 5 ml. of water, and read in a colorimeter[11] at 520 nm. The measure was adequate in a range of 2–15 mcg./sample.

RESULTS

Each chicken administered furazolidone excreted urinary components which responded positively to the phenylhydrazone assay. The response indicated that the compounds contained the nitrofurfuraldehyde portion of the parent molecule. The micromole quantities excreted by individual birds, expressed as furazolidone equivalents, are summarized in Table I. The excretion was complete in 12 hr. since the assay procedure did not give a positive response in the urine of birds collected in 10–24 hr. after the dose was given. The exception was bird number 10 which excreted 1.1 μmoles in the third period (10–15 hr.). However, the false anus of that bird was closed through the entire experimental period and was considered unsatisfactory. An average of 7.5 ± 3.0% (*SD*) of the dose was excreted as these components from the nine birds within 12 hr. after dosage.

Chromatography—Portions of the urine samples from the two experiments were examined by the anion-exchange chromatography procedure. Eight major components were detected by monitoring the absorbance of the column effluent at 320 or 375 nm. Figure 1 is a typical chromatogram of urine after medication with furazolidone where absorbance of effluent is plotted against fraction number. The individual components detected are summarized in Table II.

Control urine contained three of the eight urinary components. Uric acid dominated all chromatographic patterns. The other two chromatographic bands of the control urine (Components 1 and 2) probably were mixtures. Control urine absorption at 320 nm. detected a component at about the position of Component 8. The absorption spectrum showed no bands above 300 nm.

The remaining five bands or components were induced by the medication. Four were compounds of unknown structure: 3, 5, 7, and 8. Two bands had strong absorption maxima at 415 nm. and were designated "415" metabolites. The properties of Component 4 were the same as those of furazolidone. Both had an absorption spectrum with a maximum at 358–360 nm. and both gave positive response to the phenylhydrazone assay. When authentic furazolidone was added to control urine and chromatographed, it eluted as a discrete band peaking at the same position as Component 4. With the phenylhydrazone assay, recoveries of furazolidone were in the range of 73–81%. The chromatography would detect furazolidone if as much as 0.5 μmole were eliminated in a collection period which would be about 0.5% of the dose under the conditions used.

The individual components separated by the chromatography were assayed by the phenylhydrazone procedure. Two components, the orange 415-A metabolite (7) and furazolidone (4), gave a positive reaction. None of the other metabolites in the column effluent reacted to form 5-nitrofurfural phenylhydrazone or any other detectable phenylhydrazone. Recoveries across the column based on the phenylhydrazone assay were only in the 50–72% range, and it is possible that other urinary components were present which were unstable and not detected by the chromatography.

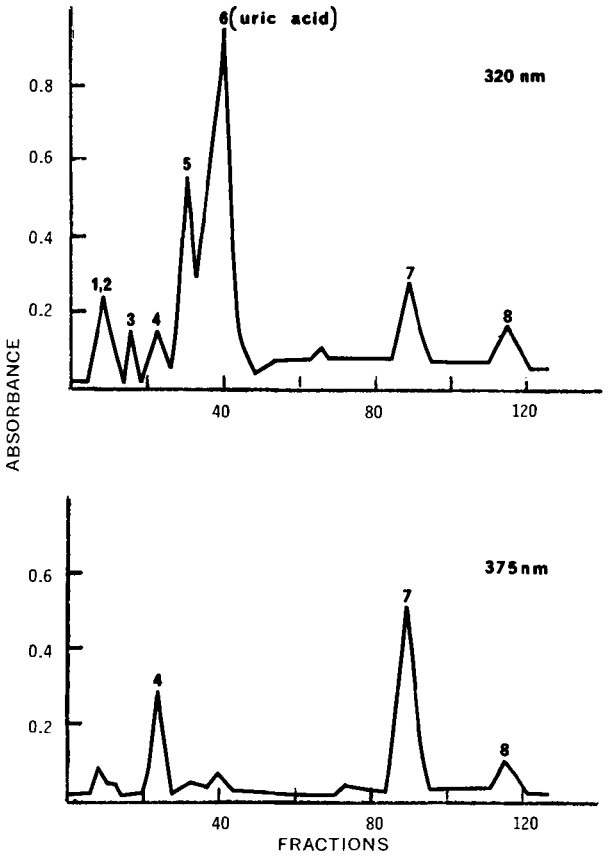

Figure 1—*Ion-exchange chromatogram of urine from a chicken medicated with furazolidone.*

[10] Beckman DK-1a.
[11] Spectronic 20.

Table II—Components of Chicken Urine after Medication with Furazolidone Detected by Anion-Exchange Chromatography[a]

Component Number	Peak of Component, Tube Number	Component and Properties
1	7	An unknown present in control urine which has no specific absorption bands above 300 nm.
2	9	An unknown present in control urine with an absorption band with a maximum at 380 nm.
3	16	An unknown metabolite with an absorption band with a maximum at 325 nm.
4	23	A component with the properties of furazolidone: has an absorption band with a maximum at 358 nm., responds positively to the phenylhydrazone assay, elutes at the same position as known furazolidone
5	28	An unknown metabolite with an absorption band with a maximum at 325 nm.
6	41	Uric acid detected by the colorimetric test
7	90	Designated the 415-A metabolite, which has orange color and an absorption band with a maximum at 415 nm. and responds positively to the phenylhydrazone assay
8	119	Designated the 415-B metabolite, which has yellow color and an absorption band with a maximum at 415 nm. and does not respond positively to the phenylhydrazone assay

[a] Refer to Fig. 1 for position of band peak.

Orange 415-A Metabolite—A combination of fractions containing the 415-A component from several columns was freeze dried. When the dry preparation was stirred with a small volume of water, the orange compound went into solution easily. The insoluble salt was filtered out on coarse fritted glass. A portion of the solution containing 439-mcg. equivalents by the phenylhydrazone assay was chromatographed. The component eluted in the same position. However, two other components appeared which probably were degradation products. The 415-A band assayed 309-mcg. equivalents by phenylhydrazone, giving a 71% recovery. The first degradation component had nonspecific absorption in the 300–450-nm. region, while the second compound had an absorption maximum at 316 nm.

The pH of a portion of the orange 415-A solution was adjusted to 3 with 1 *N* HCl. The orange color was extracted with butyl acetate. The extract was concentrated to dryness on a rotary evaporator, and the residue was dissolved in water. Chromatography of a portion showed that purification had occurred in the transfer to butyl acetate. A single chromatographic component appeared at the elution position of Component 7.

DISCUSSION

In agreement with previous work, the results here indicate that furazolidone is rapidly metabolized and eliminated by chickens. The present work also suggests extensive degradation of the molecule. Metabolites that retain the furan ring would have absorption bands with maxima above 300 nm. (2). Compounds with such spectra were detected by the methods used here. The present work indicates that: (*a*) metabolites containing the furan ring appear in the urine, but metabolism and elimination are rapid so they are not present 10 hr. after administration of furazolidone; and (*b*) metabolites that respond to the phenylhydrazone assay do not account for the major portion of the dose.

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ACKNOWLEDGMENTS AND ADDRESSES

Received February 28, 1972, from the *Biochemistry Department, Hess and Clark, Ashland, OH 44805*

Accepted for publication May 15, 1972.

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