The Disposition of Decoquinate-^{14}C Administered Orally to Chickens

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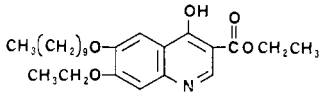
Single doses of decoquinate-3-^{14}C were administered orally to colostomized chickens. Minor amounts (0.6 and 1.1%) of radioactivity were excreted in the urine, but most of the radioactivity appeared in the feces or remained in the intestinal tract at 24 hr. Decoquinate-3-^{14}C was orally administered to intact chickens for 3 successive days. The radioactivity was readily eliminated through the feces with no apparent retention of the compound. The radioactive components of the excreta were solubilized by successive extractions with ethanol and formic acid and were analyzed by thin-layer chromatography. Only 2.6% of the radioactive dose was metabolized to nondecoquinate components. The concentration of radioactivity in the bile was at least 30 times that in urine and blood. Thus, the bile appears to be implicated in the elimination of the absorbed compound. Decoquinate-^{14}C given to chickens orally induced tissue residues throughout the bird body. When birds were medicated on successive days with decoquinate, a plateau of tissue residues occurred within 3 days. There was no accumulation of residue in any tissues examined. The residues were extracted by homogenization with alcohols. Decoquinate and another radioactive component were detected in liver and kidney. In bile, decoquinate and two other components were detected. Decoquinate was the only component present in muscle and skin/fat tissues.

Decoquinate [ethyl 6-(decyloxy)-7-ethoxy-4-hydroxy-3-quinolinecarboxylate (**1**)] is a new and effective compound used for the control of coccidiosis in broiler chickens (Ball *et al.*, 1968; Johnson *et al.*, 1968a,b; Challey and Johnson, 1968). Filer *et al.* (1969) obtained a plateau of radioactivity in tissues of broiler chickens after continuous administration of decoquinate-^{14}C through the feed. Using a spectrofluorometric method of measure, Button *et al.* (1969) found residues in tissues of chickens medicated with decoquinate. In both cases the level of residue in the tissues decreased rapidly after withdrawal of medication.



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The present work determined the routes of elimination of orally administered decoquinate by chickens, as well as the extent of conversion of the compound to metabolites. In addition, the possible disposition of decoquinate or metabolites to tissues was examined. Decoquinate is extremely insoluble in aqueous systems and was administered in these studies as a solid by way of capsules. Biological activity of the compound is increased by micronizing or reduction of particle size (Johnson, 1970). The particle size of the decoquinate-^{14}C used here was reduced to simulate products used commercially.

EXPERIMENTAL

Chemicals and Materials. Decoquinate-3-^{14}C (Filer *et al.*, 1969) was obtained from May and Baker, Ltd. To reduce the particle size, a solution of the compound in formic acid was forced into rapidly stirred water. The resulting suspension was freeze-dried to a powdery preparation which, by microscopic examination, was similar to micronized material. Two preparations were used: one with a specific activity of 0.26 μCi/mg and a second of 0.47 μCi/mg. Radiopurity was 97.0% as determined by thin-layer chromatography.

The sources of other chemicals were as follows: unlabeled decoquinate, May and Baker Ltd.; reagent grades of formic acid, ammonium hydroxide, 1-butanol, absolute methanol, and spectrophotometric grade chloroform (0.5% ethanol), J. T. Baker Co.; spectrophotometric grade toluene, Matheson Coleman and Bell; absolute ethanol, U. S. Industrials Chemical Co.; silica gel H with hydrated SiO_2, Brinkman Instruments Inc.; 50% hydrogen peroxide (certified), Fisher Scientific Co.; and NCS solubilizer reagent, Nuclear Chicago Corp.

Radioactivity Measurement. Radioactivity was determined with a scintillation spectrometer using glass-counting vials and a colloidal silica gel suspension system (Green, 1970). Counting efficiency for individual samples was determined by an internal standard technique. Highly-colored samples were treated with a few drops of 50% hydrogen peroxide prior to counting. Samples were mixed with 1 ml of methanol and 15 ml of counting mixture and counted.

Aliquots of 100 μl of the soluble portion of urine and 200-mg portions of the insoluble fraction of urine were counted directly. Aliquots (100 μl) of bile were counted directly. Aliquots (200 μl) of blood or portions of tissues (200 mg) were heated in counting vials with 1 ml of NCS solubilizer reagent at 60° C for 48 hr. Since the dry excreta and feces were heterogeneous, each material was extracted serially with solvents. Portions of extracts were heated in counting vials in a bath to evaporate the solvent. Radioactivity was determined directly on digests of the insoluble residuals remaining after extractions. Portions (20–25 mg) were slurried with 1 ml of NCS solubilizer in counting vials and heated at 60° C for 16 hr. With the decoquinate-^{14}C with specific activity of 0.26 μCi/mg the sensitivity of detection was 0.1 ppm in tissues.

Thin-Layer Chromatography (tlc). Tlc adsorbent was silica gel G. Plates (5 × 20 cm) were spread with a Brinkmann High-Capacity Adjustable Applicator at a setting of 750 μ. Air-dried plates were activated at 100° C for 2 hr and stored in a desiccator. To obtain uniform solvent fronts, a 1-mm strip of adsorbent was removed from each edge of the plates (Davis, 1963). Development was ascending in circular tanks (5.5 × 23 cm) at room temperature with one of three solvent systems: toluene–absolute ethanol–glacial acetic acid (5:1:1); *n*-butanol–ammonium hydroxide (5:1); or *n*-butanol–ammonium hydroxide (10:1) for 10 to 15 cm.

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Radioactivity on the plates was detected by a direct count of the silica gel from cross-sectional zones (0.5 cm) on each plate (Snyder and Stephens, 1962). About 3.0–10.0 nCi of radioactivity was applied for each chromatographic assay.

Bird Treatment, General. Commercial broiler chickens were maintained in metabolism cages without medication on a standard ration *ad libitum*. The dosage capsules were forced gently down the birds' esophagus to the proventriculus with a glass rod. Feces or excreta fell through openings in the cage floors to sheets of aluminum foil. Individual collections were lyophilized, ground to powders, and stored at −25° C. To terminate experiments, birds were exsanguinated by heart puncture with a 50-ml syringe. Digestive tracts were dissected out, divided into sections, lyophilized, and handled in a manner similar to feces and excreta. Tissues were dissected out of the carcass, were frozen in Dry Ice, and stored in plastic bags at −25° C. The subcutaneous fatty tissue adhered to the skin, which was referred to as skin/fat.

Dosage Preparation. Decoquinate was administered orally in gelatin capsules. In experiments A, B, and C, the decoquinate was weighed directly into capsules. In experiment D, decoquinate-^{14}C was kneaded with oiled corn meal to give a mixture which was easier to handle, was homogenous, and contained 0.0317 μCi/mg. Portions of the corn meal mix (33 mg) were loaded into capsules giving 2-mg doses of decoquinate. Distribution of the decoquinate through the meal mix was determined by extraction and count of radioactivity.

Experiment A, Colostomized Birds. White Rock broiler chickens weighing 1.7 to 1.8 kg were colostomized by surgical technique similar to that of Rothchild (1947). Urine free of feces was collected in rubber prophylactics 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. Two male birds assigned numbers 1 and 2 were colostomized at 6 weeks of age and used for experiment at 9 weeks of age. Normally, the artificial anus functions well for 3 to 4 weeks. With these two birds, malfunction began on the day selected for experimentation. Fecal material was loose, irregular, and difficult to recover.

Each bird was given a single dose of decoquinate-^{14}C (specific activity of 0.26 μCi/mg). Bird #1 received 43.5 mg of decoquinate (11.27 μCi of ^{14}C) while bird #2 received 35.7 mg of decoquinate (9.25 μCi). At 3-hr intervals postdosing, urine was removed from the collection bags, which were rinsed with 5 ml of water. Twenty-four hours after the dose was given, the birds were killed and appropriate samples obtained.

Radioactivity was determined in urine, blood, bile, feces, and the intestinal tract. Each urine sample was centrifuged at low speed. After decanting the supernatant, the insoluble material was washed twice by slurrying with water, centrifuging, decanting. The combination of supernatant and water wash was the soluble portion of urine. The washed insoluble fraction was lyophilized.

Experiment B, Single Dose Medication. Two male White Rock broiler chickens 7 weeks old weighing 1.5–1.6 kg assigned numbers 3 and 4 were placed in metabolism cages 4 days before the experiment to allow them to adjust to isolation conditions. Each bird received a capsule containing about 20 mg of decoquinate-^{14}C (specific activity of 0.47 μCi/mg) every 12 hr for 3 successive days. Bird #4 regurgitated the third capsule, which was not discovered until the following morning. The total excreta (mixed urine and feces) were collected for 12-hr periods and lyophilized. The birds were killed 4 hr after the last dose was given.

Radioactivity was determined in blood, bile, excreta, and the intestinal tract. The excreta and gastrointestinal contents were individually extracted and the extracts were analyzed by tlc. In the case of bird #3, numerous successive extractions were made with methanol or chloroform and finally with formic acid. The entire dry material was stirred magnetically with solvent in a closed Erlenmeyer flask for 1 hr at room temperature. The mixture was filtered through coarse fritted glass to give an extract and residual.

The dry materials from bird #4 were stirred magnetically with absolute ethanol for 2 hr at room temperature. The mixture was filtered on a coarse fritted glass to give the alcoholic extract. The residual was air-dried and then stirred with formic acid for 1 hr. Absolute ethanol was added and the mixture was filtered on coarse fritted glass to give the formic acid extract. The ethanolic extracts were analyzed directly by tlc. Solvent was removed from the formic extracts under vacuum on a rotary evaporator. The residual was extracted by slurry with ethanol, followed by centrifugation to remove insoluble material.

Experiment C, Multiple Dose Medication. Two male White Rock broiler chickens 7 weeks old weighing 1.5–1.6 kg were placed in individual metabolism cages 4 days before experiment for adjustment to isolation conditions. Each bird received a capsule containing 20 mg of decoquinate-^{14}C every 12 hr for 3 days. One bird regurgitated the third capsule, which was not discovered until the following morning. The birds were killed 4 hr after the last capsule was given.

Experiment D, Multiple Dose Medication. Nine male Leghorns which were 9 weeks of age weighed 0.7–1.1 kg. Each bird was medicated twice daily with a capsule containing 2 mg of decoquinate-^{14}C mixed with oiled corn meal. Three birds were medicated for 3 days, while a second and third group of three was treated in the same manner for 5 and 7 days, respectively. Birds were killed 4 hr after the last capsule was given, and liver and kidney tissues of each group were removed and combined for analysis.

Tissue Extraction Procedures. Two types of extractions were run. In the first, a 5-g sample of tissue was homogenized for 1 min with a Virtis "23" Homogenizer with 20 ml of methanol. The mixture was centrifuged at 680 × *g* for 10 min, and the supernatant was decanted. Twice the centrifuged solid was washed with 10 ml of methanol. The methanol insoluble tissue was dried in a vacuum oven at 40° C for 8 hr. The fat and skin samples were extracted with 10 ml of toluene for 30 min. After the toluene extract was removed, the residual tissues were dried in a vacuum oven at room temperature for 24 hr. Portions of these dry residuals were digested and counted by the same procedures used for wet tissues.

The second type of extraction was for isolation of material for tlc examination. A 15-g sample of tissue was homogenized for 1 min with 60 ml of absolute ethanol with a Virtis "23" Homogenizer. The mixture was transferred to a centrifuge tube, which was covered and centrifuged at 680 × *g* for 20 min. The supernatant was decanted directly into a 500-ml separatory funnel and mixed with 100 ml of 2% HCl and 20 ml of chloroform. After shaking, the chloroform phase was separated and transferred to a 500-ml round-bottomed flask. The aqueous phase was washed once with 15 ml of chloroform. The chloroform extracts were combined and concentrated to dryness on a rotary evaporator. The residual was dissolved in chloroform for tlc analysis.

Extraction of Bile. Bile was lyophilized and then slurried with 20 ml of chloroform. The mixture was filtered through a medium pore glass filter. The remaining residual was slurried with 10 ml of chloroform and filtered. The filtrates were combined and concentrated to dryness on a rotary evaporator. The residuals were dissolved in chloroform for tlc studies.

RESULTS

Experiment A, Colostomized Birds. URINARY EXCRETION. Only a small amount of the radioactivity from the ^{14}C-labeled decoquinate administered orally was excreted through the urinary trace of colostomized birds (Table I). The extent of absorption from the alimentary tract is unknown. Bird #1 excreted 1.1% of the radioactive dose in the urine, while bird #2 excreted 0.6% of the dose in the urine. Only traces of radioactivity were associated with the insoluble fraction of the urine.

FECES AND GI TRACT. Count of small portions of fecal material and gastrointestinal tract contents revealed heterogeneity of ^{14}C concentration. The remaining fecal material available was extracted, radioactivity in the extracts was measured, and total amounts in the original material were calculated assuming homogeneity. Extractions were simple slurry stirs with methanol or chloroform. The low levels of radioactivity remaining insoluble after extraction with methanol and chloroform were determined directly by count of alkaline digests.

The majority of the radioactivity administered was found in the feces and in the intestinal and cecal tracts (Table II). With bird #1, 66% of the dose was excreted in the feces and 12% remained in the intestinal tract at 24 hr. With bird #2, 42% of the dose was found in the feces and 23% remained in the intestinal tract. Total recovery of radioactivity was hindered by difficult fecal collection, preliminary difficulties in counting the dry preparations, and by heterogeneity of the dry samples.

At least 98% of the radioactivity in feces and gizzard contents was extracted. However, in the case of intestinal and cecal contents, the residuals contained 5–10% of the total radioactivity in the material.

Experiment B, Single Dose Medication. EXTRACTION OF RADIOACTIVITY. The excreta and intestinal tract contents from bird #3 were extracted successively with methanol or chloroform until the amount of radioactivity dissolved was minimal. Extraction was not complete but slurry with formic acid dissolved most of the remaining radioactivity. The low levels remaining after formic acid extraction were measurable by direct count of alkaline digests.

The radioactivity in the excreta is summarized in Table III as three fractions: (1) extracted with alcohol; (2) extracted with formic acid; and (3) residual. The fraction which required formic acid for extraction varied considerably, but was a significant amount in most of the materials. At least 99% of the radioactivity was extracted in each case. The cecal contents contained 9.92 μCi. The ethanol–formic acid sequence extracted 82% of that radioactivity. The intestinal contents contained 2.14 μCi. The ethanol–formic acid sequence extracted 99% of that radioactivity.

After completing the extractions with materials from bird #3, we became aware of the fact that transesterification of decoquinate occurs rapidly in the presence of methanol, producing the methyl ester analog (Kouba *et al.*, 1971). To avoid the transesterification, each material from bird #4 was extracted with ethanol, followed by an extraction with formic acid. Again the formic acid extracted fraction was a significant amount and the total amounts extracted were greater than 99%. The cecal contents contained 3.68 μCi. The ethanol–formic acid sequence extracted 85% of that radioactivity. The intestinal contents contained 3.04 μCi. The ethanol–formic acid sequence extracted 99% of that radioactivity.

Decoquinate was eliminated readily from these birds. Bird #3 received 54.9 μCi in the 3-day period and excreted 54.5 μCi or 99%. Bird #4 received 47.1 μCi and eliminated 41.7 μCi or 89%. These values do not include amounts of radioactivity in the gastrointestinal tract.

TLC OF EXTRACTS. After removal of the formic acid from formic extracts, the radioactive components were soluble in ethanol. Thus, more than 97% of the radioactivity excreted from bird #4 was solubilized and then was analyzed by tlc. The extracts from the excreta of the 24–36-hr period were not examined because of the low concentration of radioactivity.

Table I. The Amounts of Radioactivity (^{14}C) Excreted in the Urine of Colostomized Birds (Experiment A)

Collection period, hr	Bird #1, dose: 11.27 μCi	Bird #2, dose: 9.25 μCi
0–3	0.0154	0.0059
3–6	0.0239	0.0085
6–9	0.0303	0.0119
9–12	0.0151	0.0085
12–24	0.0431	0.0247
Total, 0–24	0.1278	0.0596

Table II. The Amounts of Radioactivity in Feces and Gastrointestinal Tract Contents of Birds from Experiment A

Bird #1 received 11.27 μCi and Bird #2 received 9.25 μCi

Material	Radioactivity	
	Extracted, μCi	In residual, μCi
Bird #1, feces	7.350	0.040
Intestinal contents	0.914	0.072
Cecal contents	0.270	0.029
Gizzard contents	0.053	0.000
Crop contents	0.001	0.000
Bird #2, feces	3.819	0.052
Intestinal contents	0.925	0.046
Cecal contents	0.050	0.062
Gizzard contents	0.111	0.001
Crop contents	0.002	0.000

Table III. The Amounts of Radioactivity in the Excreta of Birds from Experiment B

Each bird received 9.4 μCi every 12 hr for 3 successive days

Bird and collection period	Alcohol extracted, μCi	Formic acid extracted, μCi	Residual, μCi
Bird #3, 0–12 hr	3.52	0.23	0.005
12–24	12.33	0.77	0.026
24–36	6.58	0.85	0.040
36–48	10.97	1.82	0.114
48–60	6.78	0.60	0.018
60–72	9.54	0.24	0.006
Bird #4, 0–12 hr	3.92	0.67	0.039
12–24	6.70	3.70	0.176
24–36	0.17	0.19	0.006
36–48	5.90	3.70	0.244
48–60	4.76	3.64	0.008
60–72	6.73	1.15	0.002

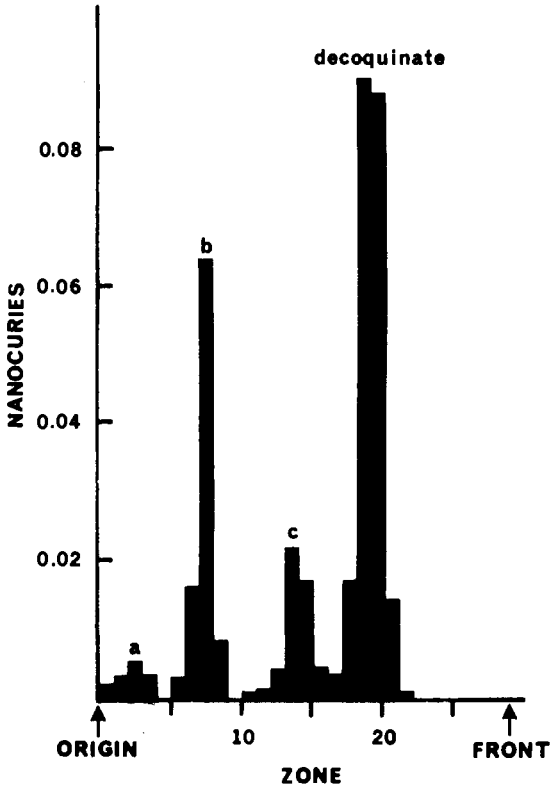

Figure 1. Radioactivity distribution on thin-layer chromatograms of excreta extracts from bird #4, Expt B. Development solvent: *n*-butanol–ammonium hydroxide (10:1)

All of the ethanolic extracts gave patterns similar to the chromatogram of the standard decoquinate-^{14}C used in the dose (Figure 1). A major component appeared with an R_f of approximately 0.9, which was fluorescent under ultraviolet light. A second chromatography of the extracts after standard decoquinate-^{14}C was added showed that the major component was decoquinate, as it moved as a single zone with the standard. The amount of decoquinate in the individual extracts ranged from 96 to 98% (Table IV).

All of the formic acid extracts gave patterns showing two distinct nondecoquinate components with low R_f (Figure 1). As in the alcoholic extracts, a major fluorescent component was detected. Again a second chromatography after addition of standard decoquinate-^{14}C showed that the major component was decoquinate since the two moved as a single zone. The decoquinate content of the formic acid extracts ranged from 78 to 89% (Table IV).

From the tlc analysis, the amounts of decoquinate in each extract and in each excreta were calculated. From these values the amount of decoquinate as a percent of total excreted radioactivity was calculated to be 94.2% (Table IV). The amount of nondecoquinate radioactivity in the dose was 3.2%, while the nondecoquinate in the excreta was 5.8%. Thus, only 2.6% of the decoquinate passing through the bird was converted or metabolized to nondecoquinate components. There was no increase in the extent of this metabolism with continued exposure to decoquinate.

Tlc of Control Extracts. To evaluate the extraction and assay procedures selected for examination of the radioactive components of the excreta, standard decoquinate-^{14}C was added to control excreta which was processed by the same methods. The radioactivity was recovered (97.3%) and the decoquinate had not degraded; the radiopurity of decoquinate in the alcoholic extract was 97.2% and in the formic extract was 96.0%.

Table IV. The Decoquinate Content in Excreta Extracts as a Percent of the Total Radioactivity in the Extracts from Bird #4, Experiment B

Excreta	Ethanolic extract, %	Formic acid extract, %	Total extracted radioactivity, %
1	97.8	78.4	95.0
2	97.2	89.0	94.3
4	97.2	89.2	94.0
5	96.6	88.3	93.0
6	97.2	82.5	95.0
Total			94.2

Bile Excretion. In both the colostomized birds of Experiment A and the intact birds of Experiment B the concentration of radioactivity in the bile on a volume basis was much higher than the level of radioactivity in blood or urine. Comparison of these levels implicate the bile in the excretion of decoquinate. In both experiments the bile was diluted about 50% with water in the process of removing bile from the gall bladder. Thus, counts were not precise values for the original bile.

At 24 hr after medication of colostomized birds, the bile of one bird contained 3.6 nCi/ml and the bile of the second bird contained 5.6 nCi/ml. Radioactivity in the blood was <0.1 nCi/ml. The urine samples collected in the period 12–24 hr after medication contained 0.49 nCi/ml for bird #1 and 0.26 nCi/ml for bird #2. Again dilution affects precision of these values. But the concentration 24 hr after medication was no greater than 0.1 nCi/ml. Thus the amount of radioactivity in bile was at least 25 times the level in urine or blood.

The blood and bile of intact birds of experiment B were obtained 4 hr after the last medication. The level of radioactivity in bile for each bird was 16.0 nCi/ml and again was less than 0.1 nCi/ml in the blood.

Experiment C, Multiple Dose Medication. Decoquinate-^{14}C given for 3 days did not induce an accumulation of radioactive residue in any tissue of the chicken. However, low levels of radioactivity were present throughout the entire carcass 4 hr after the administration of the last dose of decoquinate-^{14}C. Direct counts on digested portions of tissues are presented in Table V. The highest residue of 3 ppm was in the fatty tissue, whereas in liver, kidney, and skin/fat it was about 2 ppm. Residues were much lower in muscle tissues.

The radioactive residues of tissues were readily extracted by simple homogenization with alcoholic solutions. Portions of leg muscle, kidney, and liver tissue from Experiment C

Table V. Radioactive Residues Induced in Tissues of Birds by Medication with Decoquinate-^{14}C for 3 Days (Experiment C)[a]

Tissue, sample	Bird #5, ppm	Bird #6, ppm
Liver	2.0	2.1
Kidney	1.9	1.9
Heart	1.4	1.6
Breast muscle	0.2	0.2
Leg muscle	0.6	0.6
Fatty tissue	3.0	2.6
Pancreas	0.8	1.1
Spleen	0.5	0.4
Gonads	0.4	0.2
Skin/fat	1.9	1.3
Lung	0.2	0.3

[a] Each bird received a 20-mg dose every 12 hr and was killed 4 hr after the last dose. Values are average equivalents of decoquinate determined by direct count of at least two alkaline digests.

Table VI. The Extraction of Radioactive Residues from Skin/Fat of Birds Medicated with Decoquinate-^{14}C for 3 Days, Experiment C[a]

Tissue sample	Radioactivity: Methanol extracted, μCi × 10^{-4}	Radioactivity: Toluene extracted, μCi × 10^{-4}
1	61.7	1.8
2	61.0	2.7
3	26.3	3.1
4	25.7	4.5
5	72.1	4.1
6	95.5	6.5

[a] The residual tissues, after extraction with toluene, were free of radioactivity. Each bird received a 20-mg dose every 12 hr and was killed 4 hr after the last dose. The first extraction procedure was used.

Table VII. The Residues Induced in the Tissues of Chickens by Twice Daily Medication with 2-mg Doses of Decoquinate-^{14}C (Experiment D)[a]

Tissue	Radioactive residue in tissues: Day 3	Day 5	Day 7
Liver	1.4	1.9	1.5
Kidney	1.3	1.3	1.1
Leg muscle	0.2	0.2	0.2
Skin/fat	0.5	0.3	0.7

[a] Birds were killed 4 hr after the last medication. Values are equivalents of decoquinate measured by count of radioactivity extracted.

Table VIII. Extraction of Radioactive Residues Induced in the Tissues of Chickens by Twice Daily Medication with 2-mg Doses of Decoquinate-^{14}C (Experiment D)

Birds were killed 4 hr after the last decoquinate was given.

Tissue	Time of medication, days	Radioactivity in tissue: Before extraction, μCi	After extraction, μCi	Amount extracted, %
Liver	3	13.1	2.8	79
	5	13.2	1.5	88
	7	9.6	2.5	74
Kidney	3, 5, 7	11.9	3.5	71
Bile	3	24.0	3.4	86
		19.6	3.8	80
		29.7	5.5	81

were processed by the first extraction procedure. Radioactivity was not detected in the residual tissue after extraction, indicating complete extraction. The radioactive residue measured by extraction compared well with values by direct count. By extraction, liver values were 1.9 and 2.0 ppm for birds #5 and #6. Kidney values were 1.9 and 1.7 ppm, while muscle values were 0.5 and 0.3 ppm. Most of the radioactivity in skin and fatty tissue was extracted with methanol (Table VI). The remaining radioactivity dissolved in toluene. Again, radioactivity was not detected in the residual after extraction. In the case of skin, 85–95% of the radioactive residue was extracted with methanol and with fatty tissue, 96–98% into methanol.

Experiment D, Multiple Dose Medication. Nine birds were medicated twice daily with 2-mg doses of decoquinate-^{14}C. A group of three birds was killed every 2 days. Tissues were extracted with methanol and the amount of radioactivity was determined (Table VII). There was no accumulation of residue in the tissues examined. A plateau of residue was reached in kidney and muscle within 3 days and there was no increase in the following 4 days. Although the residue in liver at 7 days was essentially the same as at 3 days, a higher value appeared at 5 days. The values for skin/fat were irregular.

Table IX. The Amounts of Individual Chromatographic Components Appearing in Tissue Extracts of Chickens Medicated with Decoquinate-*3*-^{14}C

Values are percentages of the extracted radioactivity.

Tissue	Days on medication	Non-decoquinate component, %	Decoquinate, %
Liver, Experiment C	3	13	87
Liver, Experiment D	3	13	87
Liver, Experiment D	5	17	83
Liver, Experiment D	7	13	85
Kidney, Experiment C	3	38	62

Table X. The Amounts of Chromatographic Components Appearing in the Bile of Chickens Given Decoquinate-*3*-^{14}C

Values are percentages of extracted radioactivity.

Experiment	Days on medication	Nondecoquinate components: *a*, %	*b*, %	Decoquinate, %
D	3	55	21	23
D	5	54	27	19
D	7	51	21	26
C	3	77[a]		21

[a] The two components were not completely resolved.

Tlc Analysis of Extracts. Absolute ethanol extracted most of the radioactivity from the tissues (Table VIII). The extracts from Experiments C and D were analyzed by tlc using two solvent development systems. The toluene–ethanol–acetic acid resolved only two radioactive components, while the butanol–ammonium hydroxide resolved three components. Only data obtained with the latter system is presented in Figure 2, where the three components are designated a, b, and decoquinate.

Two radioactive components were detected in liver extracts. Figure 2A shows a typical chromatogram of liver extracts. A second chromatography with standard decoquinate-*3*-^{14}C added to the liver extract was made to ascertain the position of decoquinate in the chromatograms. By this method, decoquinate was shown to be the major component in liver extracts, accounting for 83–87% of the radioactivity (Table IX). There appeared to be no difference in the decoquinate content among the groups of birds medicated for 3, 5, or 7 days. Kidney extracts were shown to have the same two radioactive components that were found in liver. Decoquinate was the only component found in skin, muscle, and fat. Figure 2B illustrates a representative chromatogram of those tissues. The nondecoquinate components are not the radioimpurities from the standard decoquinate used in the dosage. In the tlc systems used the radioimpurities appear at the origin and are resolved from the nondecoquinate components of tissues.

Three radioactive components (Figure 3C) were detected in bile extracts. Decoquinate was not the major component, however, and amounted to only 19–26% of the extracted radioactivity (Table X). The nondecoquinate component of liver and kidney extracts appeared to be the same as one of the bile components.

In order to eliminate the question of possible chemical modification of decoquinate during the course of extraction and chromatography, standard decoquinate-*3*-^{14}C was added to liver, kidney, or bile extracts and processed in the same manner as medicated tissues. In eight replications an average recovery of radioactivity was 90.0%. In each tissue, decoquinate was the only labeled component present.

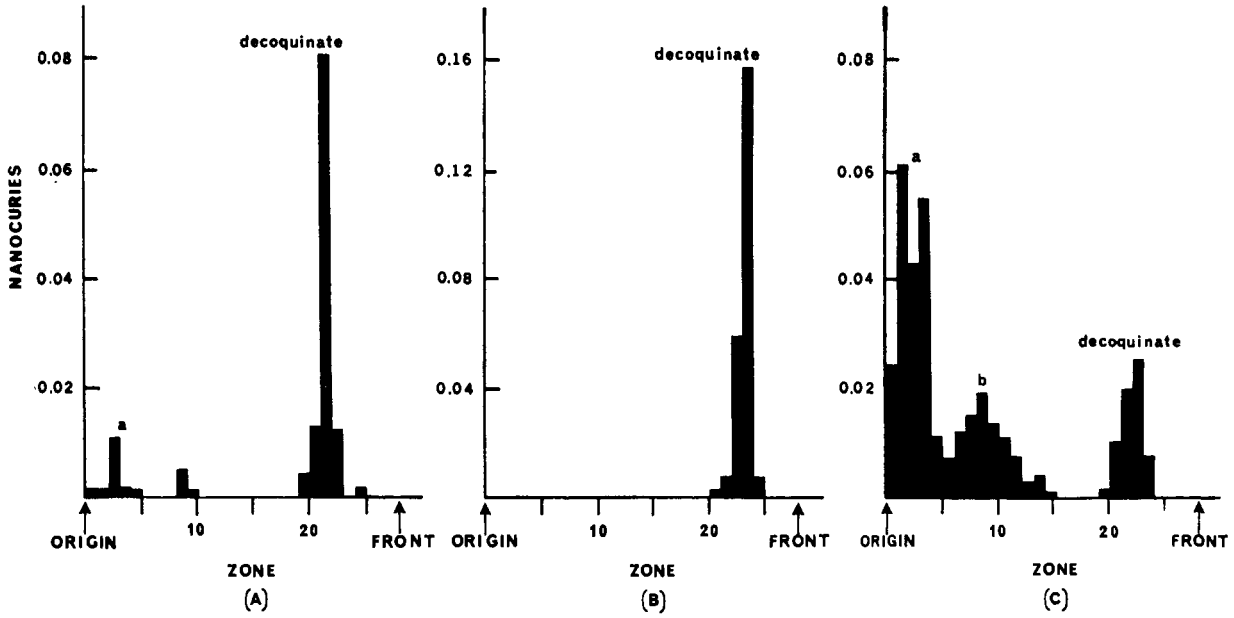

Figure 2. Radioactivity distribution on thin-layer chromatograms of tissue extracts. Development solvent: *n*-butanol–ammonium hydroxide (5 : 1). A. Typical liver extract. B. Typical muscle extract. C. Typical bile extract

DISCUSSION

The effective dosing procedure and the collection of urine from the colostomized birds gave firm quantitative data on urinary excretion. It is certain that less than 2% of the orally administered decoquinate was excreted in the urine. The difficulties which occurred in collection and assay did not allow similar conclusive observations on excretion in feces. It was certain, however, that large portions of the orally administered decoquinate were excreted in the feces.

Knowing the urinary excretion was minimal, we turned to examination of the total mixed excreta of intact birds making no attempts to separate urine and feces. The data from both intact birds of Experiment B showed that orally administered decoquinate was rapidly excreted from chickens. With bird #4 we were able to extract the radioactive components excreted and to measure the amounts of decoquinate present. The dose was 3.2% nondecoquinate while the material in the excreta was 5.8% nondecoquinate. It was thus possible to calculate that only 2.6% of the decoquinate dose administered was metabolized or converted to nondecoquinate components. Decoquinate is extremely stable chemically (Craine, 1970), thus this difference probably is significant. However, it is probably best to use the data to emphasize that decoquinate is only slightly metabolized by the chicken rather than make a claim as to the actual amount metabolized.

Two general possibilities seem to exist. Only small amounts of decoquinate may be absorbed from the gastrointestinal tract and urine and bile both may be major pathways for elimination of the compound and metabolites. A second possibility is that decoquinate is readily absorbed and eliminated primarily through the bile.

Medication with decoquinate induced residues throughout the carcass of chickens. It was apparent that the residues had reached a plateau in all major edible tissues after 3 days of medication. A plateau of residue is convincing with respect to kidney and muscle. In the case of liver the higher value at 5 days casts doubt. The differences are not large and the low levels of residue and limited amounts of tissue (liver and kidney) for analysis make it difficult to determine reproducibility and accuracy. The lack of plateau in skin/fat probably could be due to contamination of the skin with radioactivity from the excreta of the birds. A plateau within 3 days is in agreement with the results of Filer *et al.* (1969) where the dosing procedure, time of sacrifice after the last dose, and level of residue differed from the present work.

The data of Experiment B showed that decoquinate was not extremely metabolized to nondecoquinate components. In spite of this low metabolism, nondecoquinate was detectable in tissues of chickens medicated with decoquinate. The disposition between nondecoquinate and decoquinate also has reached a plateau within 3 days. The amount of nondecoquinate residue in liver, kidney, and bile did not increase as a result of medication beyond the 3-day period.

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