The Presence of Residues in the Tissues of Rats Receiving Decoquinate Orally

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Male and female rats were medicated with decoquinate-3-^{14}C in their feed. Tissue samples were examined and a radioactive residue was found to have reached plateau level by the third day of medication. The radioactivity did not accumulate in any one tissue, but was present throughout the carcass. Levels of residue were higher in liver and kidney than in muscle and skin. Levels in liver tissue ranged from 0.3 to 0.8 ppm, while levels in muscle ranged from 0.1 to 0.4 ppm. Sex influence on the amount of radioactivity in any particular tissue was not clearly defined. The radioactive components of liver, kidney, skin, and muscle tissues were extracted with ethanol and analyzed by thin-layer chromatography. Decoquinate and three nondecoquinate components were detectable in each tissue extract. The R_{st} values for the nondecoquinate components indicated that two of the three were the same as those previously detected in chicken tissues. Decoquinate was the major component in each tissue, but in liver and kidney it accounted for less than 50% of the residue. The urinary excretion of radioactivity reached a plateau level after 2 days of continuous medication. A sex difference was evident in the urinary excretion pattern: approximately 12% of the administered radioactivity was excreted by the male rats, as compared to 6.4% excreted by the female rats.

Decoquinate [ethyl-6-(decyloxy)-7-ethoxy-4-hydroxy-3-quinolinecarboxylate] is a new compound effective for the control of coccidiosis in chickens (Ball *et al.*, 1968; Johnson *et al.*, 1968). Craine *et al.* (1971) administered single oral doses of decoquinate-^{14}C to chickens and found less than 2% of the radioactivity was excreted in urine. With continuous medication, the compound was readily eliminated through the feces. A high concentration of radioactivity in bile indicated that it might be a significant pathway of excretion. Only 2.6% of the decoquinate dose was converted to nondecoquinate metabolites by chickens. Filer *et al.* (1969) obtained a plateau of radioactivity in tissues of broiler chickens after administration of decoquinate-^{14}C through the feed. Using a fluorometric method of measure, Button *et al.* (1969) found residues in tissues of chickens medicated with decoquinate. In both cases a rapid disappearance of residue occurred when medication ceased.

When birds were medicated on successive days with decoquinate-3-^{14}C, Craine *et al.* (1971) obtained a plateau of tissue residues within 3 days. There was no accumulation of residue in any tissues examined. At plateau conditions the residues were extracted readily by homogenization with alcohols. By thin-layer chromatography, two nondecoquinate components in addition to decoquinate were detected in kidney, liver, and bile. In contrast, only decoquinate was present in chicken muscle, skin, and fat.

EXPERIMENTAL

Chemicals and Materials. Decoquinate-3-^{14}C (Filer *et al.*, 1969) was obtained from May & Baker Ltd. Particle size of the compound was reduced as described previously (Craine *et al.*, 1971). The compound had a specific activity of 0.61 μCi/mg and radiopurity of 99.9%, as determined by thin-layer chromatography.

The sources of other chemicals were as follows: reagent grade formic acid, glacial acetic acid, ammonium hydroxide, 1,4-dioxane, *n*-butanol, naphthalene, and absolute methanol, spectrophotometric grade chloroform (0.5% ethanol), J. T. Baker Co.; spectrophotometric grade toluene, Matheson Coleman and Bell; Soluene 100, Packard Instrument Co.; absolute ethanol, U.S. Industrials Chemical Co.; silica gel G with calcium sulfate as binder, Brinkmann Instrument Inc.; methoxyflurane, Pitman-Moore.

Dosage Preparation. The decoquinate-3-^{14}C (63.5 mg) was placed in a plastic bag containing 1800 mg of oiled corn meal, mixed to a uniform consistency by kneading and transferred to a 10-l. screw-cap plastic bottle. Rat chow meal was mixed with the decoquinate–corn meal mixture at a level so that each rat would receive about 0.4 mg of labeled decoquinate per day. To determine homogeneity, portions (5-g) of the meal were extracted with chloroform and radioactivity was determined in each extract.

Radioactivity Measurement. Radioactivity was measured in a scintillation spectrometer in glass counting vials using a colloidal silica gel suspension system (Green, 1970). Counting efficiency for individual samples was determined with an external γ source. Aliquots of urine were counted directly. Portions (1.0-ml) of the ethanolic extracts of tissues were placed in counting vials, which were heated in a water bath to evaporate the solvent. Portions of the extracted dry tissue residuals (50-mg) were weighed into counting vials. Soluene 100 (1 ml) (digestion fluid) was added and the vials were heated at 50° C for 8 hr. To reduce color a few drops of 50% hydrogen peroxide were added. After 15–20 hr, 1 ml of methanol and 15 ml of the counting system were added and the vials were counted.

Rat Treatment. Carworth rats (20 male and 20 female) weighing 80–90 g were maintained on a standard rat chow meal *ad libitum* for 10 days before experimentation to allow them to adjust to environmental conditions. Rats were selected for similar feed consumption as well as weight.

After the conditioning period, ten male and ten female rats were placed in individual metabolism cages. Feed consumption was recorded. Urine was collected for 24-hr periods and radioactivity in the urine was measured. On the third, fifth, seventh, ninth, and eleventh day after beginning medication, two male and two female rats were killed. Tissues were dissected out and radioactivity was measured.

In the dissection process, as much tissue and blood was obtained as possible. Each rat was removed from feed 4 hr before dissection and collection of tissues. A rat was placed under anesthesia by inhalation of methoxyflurane and secured to a dissection board. Deep anesthesia was maintained while skin was dissected from the abdominal wall area. An open-

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ing was cut in the abdominal wall followed by a cut in the diaphragm. Blood was removed by puncture of the exposed heart with a syringe, whereon the rat expired. The entire skin was removed from the rat. Where possible, subcutaneous fat was removed from the skin and fatty tissue was removed from the lower abdominal area. Muscle, liver, and kidney tissues were dissected out of the carcass. The skin was stretched and secured tightly to a board. The hair was cut off with scissors and the skin was brushed to remove adhering particles. Each tissue was frozen at once in crushed Dry Ice and stored in a sealed plastic bag at −25° C.

Tissue Extraction. A 15-g sample of frozen tissue was homogenized in a stainless steel cup of a Virtis "23" Homogenizer with 60 ml of absolute ethanol at medium speed for 1 min. Skin was thawed to a semisolid state and cut into small pieces before homogenization. The mixture was centrifuged at 680 × *g* for 20 min. The supernatant was decanted directly into a 500-ml separatory funnel. The residuals remaining after extraction were air-dried, digested, and counted directly to determine the remaining radioactivity.

The supernatant extract was combined with 100 ml of 2% hydrochloric acid and 20 ml of chloroform. The chloroform phase was separated and transferred to a round-bottomed flask. The aqueous phase was washed with chloroform. The chloroform extracts were combined and concentrated to dryness on a rotary evaporator. The residue was dissolved in 2–5 ml of absolute ethanol, assayed for radioactivity, and subsequently analyzed for radioactive components by the thin-layer chromatographic procedure. Samples of control rat skin and muscle were processed by the same procedures after the addition of standard decoquinate-^{14}C.

Thin-Layer Chromatography (tlc). Tlc adsorbent was silica gel G spread on glass plates (5 × 20 cm) with a Brinkmann High-Capacity Adjustable Applicator at a setting of 750 μ. Air-dried plates were activated at 110° 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 (Davies, 1963). Development was ascending in circular tanks (5.5 × 23 cm) at room temperature with two systems: (a) toluene–absolute ethanol–glacial acetic acid (5:1:1); and (b) *n*-butanol–concentrated ammonium hydroxide (5:1). Due to the high pigment and lipid content of the extracts under examination, primary development of the plates in chloroform or ether was necessary as a pretreatment.

Decoquinate was detected in all extracts examined. It was thus possible to calculate R_{st} rather than R_f using decoquinate as a standard (Kirchner, 1967). Thus $R_{st} = R_f$ of the component/R_f of the standard. To determine position of the decoquinate zone a duplicate plate was run with added standard decoquinate-^{14}C. Plates were examined under an ultraviolet light (254 and 366 nm) to locate fluorescent zones. 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).

Table I. The Amounts of Decoquinate-^{14}C Consumed by Each Rat

Rat #		Days on medication	Decoquinate in dose, mg	Decoquinate per day, mg
Male	1	3	1.60	0.53
	2	3	1.57	0.52
	3	5	2.57	0.51
	4	5	2.48	0.50
	5	7	3.73	0.53
	6	7	3.73	0.53
	7	9	4.97	0.55
	8	9	4.97	0.55
	9	11	5.80	0.53
	10	11	5.80	0.53
Female	11	3	1.38	0.46
	12	3	1.24	0.41
	13	5	1.66	0.33
	14	5	2.08	0.42
	15	7	2.76	0.39
	16	7	2.90	0.41
	17	9	3.34	0.37
	18	9	3.62	0.40
	19	11	3.84	0.35
	20	11	4.33	0.39

RESULTS

The Medication. The rat chow meal containing the decoquinate was homogeneous with respect to radioactive content. The theoretical content of activity in the feed mixture was 16.60 nCi/g and the actual amount was 15.88 nCi/g. Average deviation from mean for ten samples was 0.21 nCi/g.

The consumption of feed was uniform throughout the 11-day period. The decoquinate received by each rat is summarized in Table I. The amounts ranged from 0.50 to 0.53 mg/day for male rats and 0.37 to 0.43 mg/day for female rats.

Radioactivity in Tissues. Decoquinate-^{14}C consumed for 11 days did not induce an accumulation of radioactivity in any tissue of the rat. However, low levels of radioactivity were detected throughout the entire carcass of rats sacrificed on the third, fifth, seventh, ninth, and eleventh days of medication (Table II). In liver, kidney, and skin the levels of radioactive residue at 3 days were higher than any level at 5, 7, 9, or 11 days. The data indicated a plateau of residue had occurred by the third day of medication. In the case of muscle there appeared to be a plateau at 3 days also. However, very high values were obtained on day 5 which were not present on days 7, 9, and 11. These odd values are not explainable, but suggest contamination.

In the case of fat tissue, an extraction was not made because

Table II. The Average Radioactive Residue Present in Tissues of Rats Receiving Daily Doses of Decoquinate-^{14}C

Values are averages for two rats expressed as ppm equivalents of decoquinate

Days on medication	Liver		Kidney		Skin		Muscle	
	Male	Female	Male	Female	Male	Female	Male	Female
3	0.8	0.6	0.7	0.6	0.2	0.3	0.1	0.1
5	0.8	0.3	0.5	0.3	0.2	0.1	0.6	0.4
7	0.5	0.6	0.5	0.3	0.1	0.2	0.1	0.2
9	0.7	0.4	0.6	0.3	0.2	0.2	0.1	0.1
11	0.7	0.4	0.4	0.4	0.1	0.1	0.1	0.1

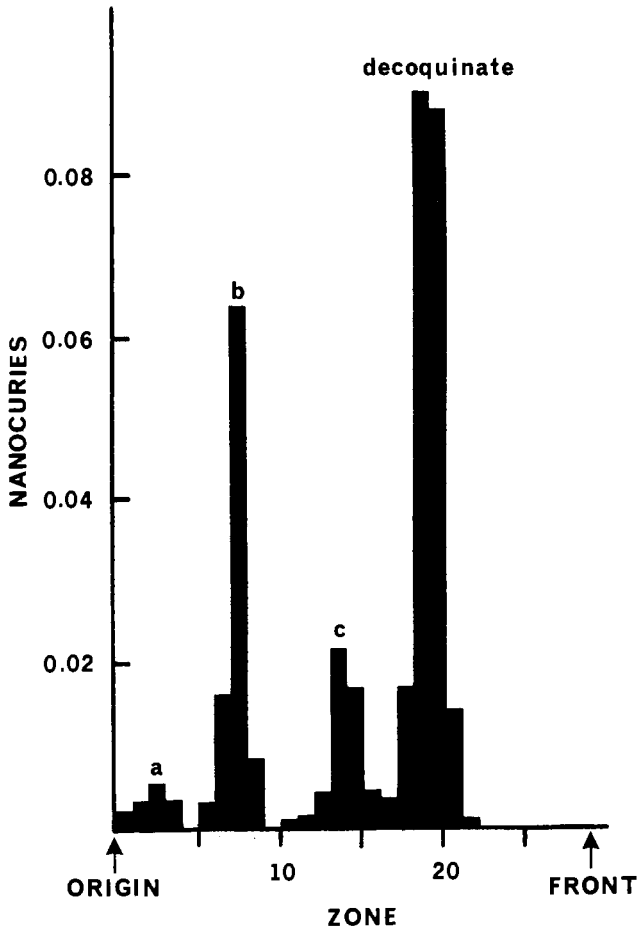

Figure 1. The radioactivity distribution on a thin-layer chromatogram of an extract of rat muscle. Development system: 1-butanol–ammonium hydroxide (5 : 1)

sufficient fat was not obtained from the rats for extraction and analysis. Where possible, portions of fat were digested and radioactivity was counted directly. Values obtained were 0.16, 0.56, and 0.53 ppm for fat obtained from rats on the seventh, ninth, and eleventh days, respectively.

Generally, the radioactivity in tissues was completely extracted by simple homogenization with ethanol. The only exception was the liver tissue which showed variable extractions ranging from 58 to 100%.

Chromatography. The extracts were all analyzed by tlc using both solvent development systems. Four radioactive components were detected in all tissue extracts with each development system. Resolution was better with the *n*-butanol–ammonium hydroxide than the other development solvent. One component exhibited the chromatographic properties of decoquinate, while the other three were designated unknowns a, b, and c. Figure 1 shows a chromatogram of an extract of rat muscle where the development system was 1-butanol–ammonium hydroxide (5:1). The chromatogram was a typical analysis of all extracts prepared. The position of decoquinate was determined by a second chromatography with decoquinate-*3*-^{14}C added.

The structure of these components is unknown and, for simplicity, we have calculated two fractions: (a) decoquinate and (b) nondecoquinate. The proportions present in individual tissues as decoquinate are summed in Table III. Decoquinate accounts for more than 50% of the residue in skin and muscle. In kidney and liver it accounts for less than 50% but it is still the major component in the residue.

Table III. The Amount of Decoquinate in Tissues Expressed as a Percent of the Total Radioactive Residue

Tissue	Decoquinate fraction, %
Liver, male	42
Liver, female	43
Kidney, male	42
Kidney, female	35
Muscle, male	72
Muscle, female	56
Skin, male	52
Skin, female	62

Table IV. Average R_{st} Values for Nondecoquinate Components in Rat Tissues

Solvent system: 1-butanol–ammonium hydroxide (5:1)

Component	Tissue			
	Liver	Skin	Muscle	Kidney
a	0.15	0.17	0.16	0.17
b	0.44	0.43	0.42	0.40
c	0.81	0.73	0.76	0.74

The possibility of modification of decoquinate during the course of extraction and tlc examination was discounted. In previous work (Craine *et al.*, 1971) extracts of chicken liver and kidney tissue were processed after addition of decoquinate-*3*-^{14}C. In eight replications an average recovery of radioactivity was 90.0%. Decoquinate was the only labeled component detected.

The R_{st} values of the three components were the same with each tissue. R_{st} values for the 1-butanol–ammonium hydroxide system are summed in Table IV.

Urinary Excretion. A substantial amount of the radioactivity consumed was excreted in the urine over the entire medication period (Table V). The urinary radioactivity reached a plateau level by the fourth day of medication. The percent of the dose excreted in the urine was markedly influenced by the sex of the animal; it was 12.0% for the males and 6.4% for the females. Furthermore, the vari-

Table V. The Average Amount of Radioactivity Excreted in the Urine of Rats Receiving Daily Doses of Decoquinate-^{14}C

No. of days on medication		No. of rats	Average urinary radioactivity	
			$\mu Ci \times 10^{-2}$	% of dose
Male	1	10	3.19	10.01
	2	10	3.77	11.83
	3	10	3.70	11.62
	4	8	3.98	12.47
	5	8	3.93	12.34
	6	6	3.95	12.21
	7	6	3.93	12.19
	8	4	4.11	12.71
	9	4	4.42	13.61
	10	2	3.22	10.19
	11	2	4.05	12.85
Female	1	10	1.00	4.22
	2	10	1.58	6.73
	3	10	1.70	7.21
	4	8	1.49	6.47
	5	8	1.57	6.85
	6	6	1.84	7.93
	7	6	1.71	7.70
	8	4	1.01	4.43
	9	4	1.48	6.62
	10	2	1.02	4.57
	11	2	1.57	7.10

ability of radioactivity excreted by individual male and female rats was small.

DISCUSSION

As with chickens (Craine *et al.*, 1971), orally-administered decoquinate-^{14}C induced a radioactive residue throughout the body of rats. In both cases there was no accumulation of residue in a specific tissue. For both species the plateau had occurred within 3 days after medication was started. It is of interest that a plateau of excreted urinary radioactivity occurred and was related to the tissue residue plateau.

The tlc examination of the tissue residues showed that decoquinate-^{14}C was metabolized to at least three other compounds by the rat. The rat apparently metabolizes decoquinate more extensively than chickens. First, only two of the three unknowns present in rat tissue were detected in chicken tissues. Second, the amount of radioactivity from a decoquinate-^{14}C dose excreted in urine is much less in chickens than in rats.

As in the case of chickens the urinary tract is not the major path for elimination of orally-administered decoquinate in the rat. However, a larger percentage of the decoquinate dose was excreted in urine by rats than by chickens. Chickens excreted less than 2% of the dose in the urine while male and female rats excreted 12 and 6%, respectively.

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LITERATURE CITED

Ball, S. J., Davis, M., Hodgson, J. N., Lucas, J. M. S., Parnell, E. W., Sharp, B. W., Warburton, D., *Chem. Ind.* 56 (1968).
Button, R. G., Muggleton, D. F., Parnell, E. W., *J. Sci. Food Agr.* **20,** 70 (1969).
Craine, E. M., Kouba, R. F., Ray, W. H., J. AGR. FOOD CHEM. 19, 1228 (1971).
Davies, B. H., *J. Chromatogr.* **10,** 518 (1963).
Filer, C. W., Hiscock, D. R., Parnell, E. W., *J. Sci. Food Agr.* **20,** 65 (1969).
Green, R. C., "Liquid Scintillation Counting," E. D. Bransome, Ed., Grune and Stratton, New York, N.Y., 1970, Chapter 19.
Johnson, C. A., Plank, H. E., Darling, M. A., *Poultry Sci.* **47,** 1685 (1968).
Kirchner, J. G., "Thin-Layer Chromatography," Techniques of Organic Chemistry series, Vol. XII, Interscience Publishers, New York, N.Y., 1967, p 8.
Snyder, F., Stephens, N., *Anal. Biochem.* **4,** 128 (1962).

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