Excretion and Tissue Distribution Studies in Chickens Fed H^3 Monensin (Na Salt)

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The rate and route of excretion of the coccidiostat, monensin (as the sodium salt), was determined. The monensin, tritiated by the Wilzbach technique, was given to chickens in feed. In one experiment, daily combined urine-fecal samples were collected. In a second experiment with surgically prepared chickens, urine and fecal samples were collected separately. Selected tissues were examined. Most of the administered radioactivity was excreted in the feces. Less than 2% was present in urine and tissues. At least 75% of the radioactivity of the whole tissue was associated with the tissue water, indicating that most of the tissue radioactivity was not monensin. Tissue levels of radioactivity declined rapidly after withdrawal of medication.

Monensin is a coccidiostat present as the principal factor of the antibiotic complex produced by a strain of *Streptomyces cinnamonensis* (Haney and Hoehn, 1968). When monensin as the sodium salt (hereafter referred to as monensin) is fed to chickens at recommended levels, it prevents mortality associated with coccidiosis and permits normal weight gains and feed efficiency. It is effective against the six major disease-producing species of coccidia in chickens (Shumard and Callender, 1968). The structure has been published (Agtarap and Chamberlain, 1968; Agtarap *et al.*, 1967).

During the development of monensin, experiments were conducted to determine excretion rates and routes.

MATERIALS AND METHODS

Tritiated Monensin. Different monensin samples were used in each portion of the study. Both samples were similarly labeled and purified. The monensin was tritiated by the Wilzbach process (Wilzbach, 1957) (New England Nuclear Corp.). Labile tritium was removed by dissolving the sample in methanol and removing the methanol. The monensin was placed on a silica gel column (Brinkmann 7729 silica gel). A silica gel–sample ratio of between 50 and 100 was used. Ethyl acetate was used as eluting agent. Ten- to 15-ml. fractions were collected. Thin-layer chromatography on silica gel G (ethyl acetate solvent), followed by autoradiography and liquid scintillation counting, together with microbiological assay (Donoho and Kline, 1968), were used as a guide in determining fractions to be combined. The characteristics of the final purified monensin samples were as follows:

Sample 1. Microbiological potency 751 μg. per mg.; 88.8% of radioactivity in monensin peak when sample was examined by thin-layer chromatography with silica gel GF–ethyl acetate 2X; specific radioactivity 36.8 microcuries (μCi.) per mg. [By microbiological assay and radiochemical measurements, the sample contained 10% of a B factor, a minor component in the antibiotic complex produced by *Streptomyces cinnamonensis* (Haney and Hoehn, 1968).] The monensin was diluted with carrier (90% monensin–10% B factor) to a specific activity of 16.5 μCi. per mg.

Sample 2. Microbiological potency 923 μg. per mg.; 92.8% of radioactivity in monensin peak; specific radioactivity 22.3 μCi. per mg. The sample contained less than 5% B factor.

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FEED

Experiment 1. A 312.8-mg. quantity of tritiated monensin of specific activity 16.5 μCi. per mg. (total radioactivity 5161 μCi.), dissolved in methanol, was added to 74.7 grams of solvent-extracted soybean meal. The methanol evaporated, and this premix was then diluted with a quantity of 24% protein chick starter ration such that a total weight of 700 grams resulted. The monensin concentration in this feed was 397 p.p.m. or 360 grams per ton. The radioactivity concentration was 7.37 μCi. per gram of feed [16.2×10^6 disintegrations per minute (d.p.m.) per gram of feed].

Experiment 2. A 416.9-mg. quantity of monensin of specific activity 22.3 μCi. per mg. (total radioactivity 9297 μCi.) and 106 mg. of light liquid petrolatum were dissolved in 5 ml. of methanol and mixed with 1.00 gram of Microcel E (calcium silicate, Johns Manville). This in turn was combined with 2.775 grams of soybean meal. After the methanol had volatilized, the premix was diluted with sufficient 24% protein chick starter ration to yield 7 pounds total. The monensin concentration in this feed using a microbiological purity value of 92.3% was 121 p.p.m. or 110 grams per ton. The total radioactivity concentration value was 2.72 μCi. per gram of feed (6.04×10^6 d.p.m. per gram of feed).

FEEDING AND SAMPLE COLLECTION

Experiment 1. Three Arbor Acres, strain 50, chickens 6 weeks old (two male, one female) were surgically prepared (Newberne *et al.*, 1957) so that the urine and feces could be collected separately. This was accomplished by suturing a plastic ring with nonabsorbent sutures around the ureters so that a rubber container could be attached for urine collection. The feces were collected in a plastic bag sutured around the posterior portion of the chicken. This bag also contained and protected the urine collection bag.

The three chickens were fed *ad libitum* for 3 weeks feed containing unlabeled monensin at 134 grams per ton to establish a metabolic equilibrium. Feed containing tritiated monensin at 360 grams per ton, approximately three times the recommended rate (110 grams per ton), was given for 2 days. The chickens were then again given feed containing unlabeled monensin at 134 grams per ton. One chicken was sacrificed at a withdrawal time from radioactivity of 0 days, a second at 2 days, and the third at 4 days.

Urine and feces were collected daily from each chicken. Breast muscle, liver, fat, kidney, spleen, lung, serum, pancreas, gall bladder, and heart tissure were obtained from the sacrificed birds. All samples were kept frozen until examined.

Experiment 2. Six 5-week-old chickens, three male and

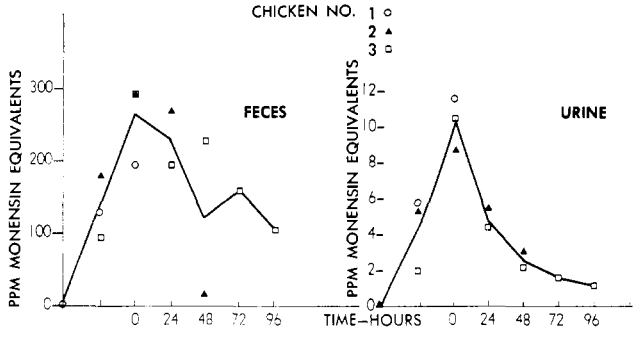

Figure 1. Excretion rate in feces and urine of H^3 radioactivity from chickens fed H^3 monensin (Na salt)

Experiment 1

three female, were used. Feed containing unlabeled monensin at 110 grams per ton was given for 21 days. Feed containing the tritiated monensin at 110 grams per ton was given for 7 days, followed by basal ration for 7 days. The chickens were permitted water and the designated feed *ad libitum*. Chickens were maintained in separate cages. Plastic sheets were placed in the bottom of the cages to collect urine-fecal samples for 24-hour periods. Samples from the 7-day period preceding the feeding of labeled monensin established the average control radioactivity level and its variation. Since no attempt was made to retain moisture in the urine-fecal samples during the collection period, some loss of water (including tritiated water) occurred. Fecal-urine samples were prepared for assay by blending them with a volume of water in milliliters equal to twice the sample weight in grams.

At the termination of the experiment, 7 days after the last tritiated feed was given, the chickens were killed. The whole carcasses were ground and stored separately in polyethylene bags. All samples were kept frozen until examined.

RADIOACTIVITY DETERMINATION

All counting for radioactivity was done with a 3000 series Packard Tri-Carb liquid scintillation spectrometer. Samples were usually counted for 10 minutes. Counting efficiencies were determined by internal standardization with tritiated toluene. Three scintillator solutions were used: (1) toluene containing 0.5% 2,5-diphenylozazole (PPO) and 0.01% 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene (dimethyl POPOP); (2) a solution composed of 100 ml. of toluene, 300 ml. of dioxane, 300 ml. of methyl Cellosolve, 7.0 grams of PPO, 350 mg. of dimethyl POPOP, and 56 grams of naphthalene (Bruno and Christian, 1961); (3) a solution composed of 500 ml. of dioxane, 300 ml. of methanol, 104 grams of naphthalene, 6.5 grams of PPO, and 130 mg. of dimethyl POPOP (Herberg, 1960; Kinard, 1957).

Urine samples were counted directly. Duplicate 0.5- or 1.0-ml. portions were added to 15 to 20 ml. of scintillator solution 2. Duplicate 0.5- to 1.0-gram portions of the wet feces samples were burned to CO_2 and H_2O in a quartz tube in a three-section electrically heated furnace (Lindberg Hevi-Duty furnace, Type 123–T-3). Water was condensed out of the combustion products stream and rinsed into counting vials with 15 to 20 ml. of scintillator solution 2. Duplicate 0.5- to 1.0-gram wet tissue samples were similarly burned. A small quantity of water (0.5 to 1.0 ml.) was added to dehydrated tissue samples (water had been removed to determine radioactivity) before combustion to obtain satisfactory recovery of radioactivity. Recovery studies with several tissues and various levels of added radioactivity showed a recovery by combustion of 93 to 99%.

DETERMINATION OF TRITIUM IN TISSUE WATER

Water was separated from some of the samples and counted for radioactivity to determine how much tritium was present as water. The water was separated by azeotropic distillation of the samples with toluene. Portions of the water were counted with scintillation solutions 2 and 3. Fifty-gram samples of feces from the six chickens from experiment 2, similar weights of the ground carcasses of the six chickens from the same experiment, and smaller weights of individual tissues from experiment 1 were investigated. Portions of all dehydrated tissues were burned, and burned products were counted for radioactivity.

EXAMINATION OF FECES SAMPLES AND GROUND CARCASSES FOR MONENSIN

Fecal-urine samples from chickens 1 and 2 of the second experiment were examined for tritiated monensin. Weights of samples ranging from 5 to 25 grams (chosen to give at least 1,000,000 disintegrations per minute starting radioactivity) were extracted with methanol-water (80 to 20). These extracts were in turn partitioned three times with CCl_4. (Monensin in the starting feces samples would be in the CCl_4 phase.) The CCl_4 phases were combined and evaporated to dryness and the residue was dissolved in methanol. Portions of all phases were examined for total radioactivity. Portions of the methanol solutions of the material from the CCl_4 phase were examined by thin-layer chromatography on silica gel G (250-micron layer) with three solvent systems: ethyl acetate 2X; ether–glacial acetic acid 99 to 1; and ethyl acetate–diethylamine 99 to 1. A tritiated monensin standard was run on each plate to designate a monensin zone for that plate. R_f values for monensin in the three systems varied between 0.5 and 0.6. The silica gel on the chromatograms was divided into 1-cm. segments, scraped into vials, and counted for radioactivity. A small volume of methanol (0.5 to 1.0 ml.) was added to obtain complete elution of radioactivity from the silica gel.

Sixty-gram samples of the ground carcasses from the second experiment were extracted with methanol. The methanol extracts were partitioned with 3 portions of CCl_4. (Monensin in the starting ground carcass samples would be in the CCl_4 phase.) The CCl_4 phases were combined and the solvent was evaporated. The residue, a clear yellow oil, was dissolved in hexane and applied to a silica gel column. The column was washed with $CHCl_3$ to remove fat. Monensin was then eluted with chloroform-methanol 95 to 5. The eluates were concentrated, examined for radioactivity, and then examined by thin-layer chromatography in the system silica gel G–CCl_4, benzene, ethylene glycol monomethyl ether 80:10:5.

DATA AND DISCUSSION

Experiment 1. The urine and fecal excretion data are given in Figure 1 with radioactivity calculated as parts per million of monensin. The urine, as collected, contained some solids. This was centrifuged and radioactivity determinations were made separately on solid and supernatant. The urine data shown are for the reconstituted urine.

Fecal and urinary excretion data were similar for the three chickens. Because of difficulties encountered in measuring feed consumption, no balance between intake and excretion was made in this experiment. The over-all recovery of

Table I. P.P.M. Radioactive Monensin Equivalents in Tissues in Chickens Given Tritiated Monensin (Na salt) in Feed

Experiment 1

	Fat	Heart	Lean	Liver	Kidney	Spleen	Lung	Serum	Pancreas	Gall Bladder
				Chicken 1, 0-Hour Withdrawal						
Wet tissue	2.32	2.64	2.16	7.66	10.14			1.84	6.97	
Aqueous fraction	0.02	1.90	1.85	2.14	2.15	1.97	1.34			
Dry tissue	3.26	0.37	0.14	3.38	4.76	2.75	1.82			
				Chicken 2, 48-Hour Withdrawal						
Wet tissue	0.44	1.26	1.12	1.47	1.54			0.63	0.95	38.4
Aqueous fraction	0.4	1.09	1.22	1.20	1.22	0.85	0.65			
Dry tissue	0.1	0.04	0.04	0.61	0.12	0.06	0.04			
				Chicken 3, 96-Hour Withdrawal						
Wet tissue	0.14	0.74	0.70	0.72	0.92			0.34	0.61	5.98
Aqueous fraction	0.08	0.55	0.64	0.65	0.56	0.07	0.68			
Dry tissue	0.05	0.02	0.03	0.19	0.09	0.04	0.04			

radioactivity from the three chickens together, however, was at least 67%. Of the radioactivity recovered in the urine and feces together, 99% was in the feces.

Some radioactivity was absorbed (Table I). For fat, heart, breast muscle, liver, and kidney tissue, radioactivity determinations were made both on the wet tissues and on water separated from the tissues and the resulting dehydrated tissues. Three separate determinations were made on the wet heart, breast muscle, liver, and kidney tissue.

In all tissues except fat, a large amount of the radioactivity was associated with tissue water. The radioactivity content of the tissue water for heart, breast muscle, liver, and kidney, calculated as parts per million of monensin, was nearly the same at a given withdrawal time: 0-hour withdrawal 2.01 p.p.m., 48-hour withdrawal 1.18 p.p.m., 96-hour withdrawal 0.60 p.p.m.

The widespread distribution of radioactivity in the water and its relative constancy in a given chicken indicate an equilibrium throughout the chicken's body. Since labile tritium had been removed before incorporation of the monensin into feed, some of the radioactivity could have resulted from metabolism of the monensin.

All tissues, whole or dehydrated, show a rapid decline of radioactivity with withdrawal. The small quantities of radioactivity remaining in the edible tissues at 96 hours do not constitute a detectable monensin residue. Other studies in which monensin was fed at 5 times the recommended rate show no detectable monensin (detection sensitivity 0.025 p.p.m.) in edible tissues at 96-hour withdrawal time (Donoho and Kline, 1968).

Experiment 2. The excretion data for the six chickens are given in Figure 2. The radioactivity present in the feces plus urine samples is calculated as monensin equivalents. The excretion pattern is similar for all six chickens. Differences are attributed to the time at which the monensin was consumed, the time at which excretion occurred, and normal biological variation.

Recoveries of radioactivity ranged from 51 to 71% (mean value 61%) (Figure 3). By the fourth day after the last H^3 monensin feeding, 92 to 98% of the total excreted had already appeared. At the time the birds were sacrificed, 7 days after the last labeled feed was given, the feces plus urine contained from 10,700 to 56,000 d.p.m. per gram. Average radioactivity content of fecal-urine samples during the period prior to administration of radioactivity was 20.0 d.p.m. per gram; range 12.2 to 25.7.

Initially, 0.5% of the feces radioactivity was associated with water. At the time of sacrifice of the chickens, the water radioactivity averaged 10% of the fecal-urine radioactivity.

Extraction of the fecal-urine samples with methanol-water removed from 94 to 98% of the radioactivity. When the methanol solutions were partitioned with CCl_4, the CCl_4 phase contained from 4 to 26% of the total radioactivity of the starting fecal-urine-samples (sample from first collection period, 26%; samples from other collection periods, 4 to 16%).

The monensin-zone radioactivity in the CCl_4 tissue extracts

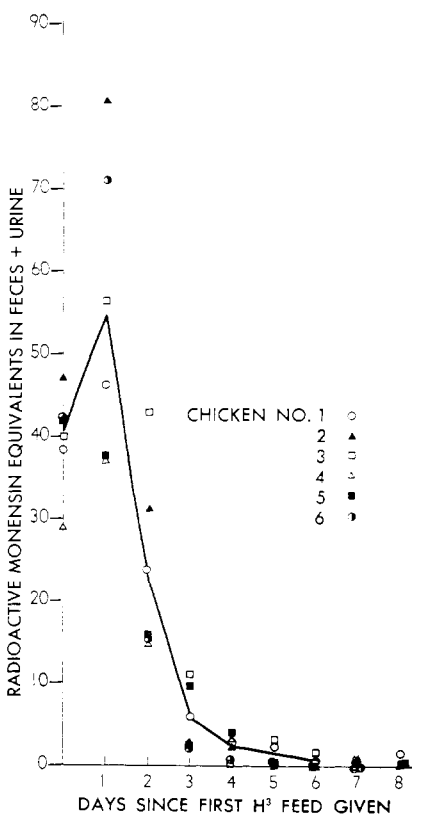

Figure 2. Excretion rate and radioactivity concentration in feces plus urine from chickens given H^3 monensin (Na salt) in feed

Experiment 2

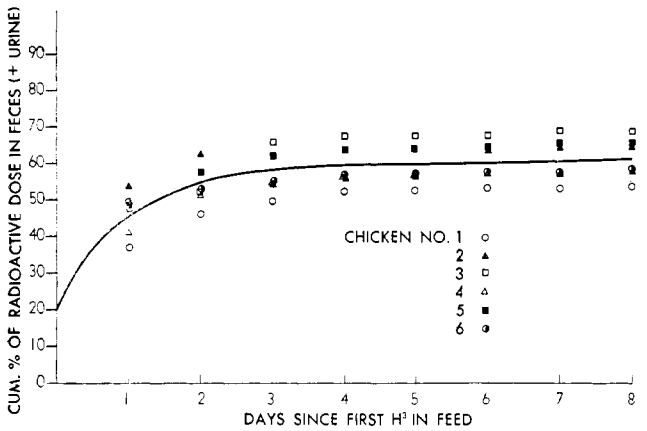

Figure 3. Cumulative per cent of radioactive dose excreted in feces plus urine after chickens received H^3 monensin (Na salt)

Experiment 2

Table II. Examination of CCl_4 Extracts of MeOH Extracts of Chicken Feces (+ Urine) in Three TLC Systems

P.p.m. radioactive monensin equivalents in monensin zone. Experiment 2

Days after 1st Labeled Dose	Ethyl Acetate–Diethylamine, 99:1	Ethyl Acetate, 2X	Ether–Acetic Acid, 99:1	Mean	Mean as % of Total Radioactivity
			Chicken 1		
0	2.400	3.064	3.581	3.015	8.0
2	0.781	0.986	0.804	0.851	3.5
4	0.013	0.017	0.028	0.019	0.7
6	0.011	0.018	0.020	0.016	1.7
8	0.013	0.017	0.037	0.022	2.0
			Chicken 2		
0	3.625	4.791	4.444	4.287	9.1
2	1.048	1.236	0.762	1.015	3.4
4	0.027	0.036	0.023	0.029	2.0
6	0.011	0.014	0.011	0.012	2.0
8	0.008	0.010	0.009	0.009	3.0

is shown in Table II for the three thin-layer systems used. The concentration of monensin-zone radioactivity decreased rapidly with time. Its per cent of the total fecal-urine radioactivity also decreased with time, although less rapidly.

The radioactivity in various fractions of the ground chicken carcasses is given in Table III. At least 75% of the radioactivity of the whole tissue was associated with the tissue water. The radioactivity remaining in the tissue after removal of water by distillation with toluene corresponded to an average value of only 0.023 p.p.m. monensin equivalents.

Table III. P.P.M. Radioactive Monensin Equivalents in Various Fractions of Ground Chicken Carcasses

Experiment 2

Chicken	Whole Tissue	% of Dose	Dehydrated Tissue	Tissue Extract Monensin, TLC Zone
1	0.229	1.50	0.023	0.0010
2	0.301	1.80	0.025	0.0006
3	0.246	1.95	0.027	0.0006
4	0.181	1.26	0.017	0.0007
5	0.252	1.39	0.025	0.0005
6	0.202	1.45	0.021	0.0007
Mean	0.235	1.56	0.023	0.0007

Only a portion of this residual radioactivity is monensin. Table III, column 5, gives the thin-layer monensin zone radioactivity of silica gel column eluates of tissue extracts (methanol, CCl_4). The whole ground carcasses contained 0.001 p.p.m. or less monensin.

CONCLUSIONS

Most of the administered radioactivity was excreted in the feces. Less than 2% was present in urine and tissues. At least 75% of the radioactivity of the whole tissue was associated with the tissue water, indicating that most of the tissue radioactivity was not monensin. Tissue levels of radioactivity declined rapidly after withdrawal of medication.

LITERATURE CITED

Agtarap, A., Chamberlin, J. W., "Antimicrobial Agents and Chemotherapy—1967," p. 359, American Society for Microbiology, Ann Arbor, Mich., 1968.
Agtarap, A., Chamberlin, J. W., Pinkerton, M., Steinrauf, L., *J. Am. Chem. Soc.* **89,** 5737 (1967).
Bruno, G. A., Christian, J. E., *Anal. Chem.* **33,** 1216 (1961).
Donoho, A. L., Kline, R. M., "Antimicrobial Agents and Chemotherapy—1967," p. 763, American Society for Microbiology, Ann Arbor, Mich., 1968.
Haney, M. E., Jr., Hoehn, M. M., "Antimicrobial Agents and Chemotherapy—1967," p. 349, American Society for Microbiology, Ann Arbor, Mich., 1968.
Herberg, R. J., *Anal. Chem.* **32,** 42 (1960).
Kinard, F. E., *Rev. Sci. Instr.* **28,** 293 (1957).
Newberne, P. M., Laerdal, O. A., O'Dell, B. L., *Poultry Sci.* **36,** 821 (1957).
Shumard, R. F., Callender, M. E., "Antimicrobial Agents and Chemotherapy—1967," p. 369, American Society for Microbiology, Ann Arbor, Mich., 1968.

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