Tissue Residues of Arprinocid in Chickens. 1. Depletion of Residues in Tissues of Chickens Fed Carbon-14-Labeled Arprinocid

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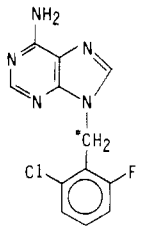
Chickens were fed diets containing 60–80 ppm of the anticoccidial agent arprinocid starting at 3–9 days of age and ending at 49–60 days of age. In these experiments the chickens were fed ^{14}C-labeled drug during the last 4 or 14 days or the total 44 days of the dosing. Of the edible tissues, liver retained the largest radioactive residue after withdrawal of drug from the diet. These residues consisted of two pools, a rapidly depleting pool and a persistent, slowly depleting pool. The persistent pool represents about 15% of the total residue at drug withdrawal and about 100% of the residue 5 days after drug withdrawal. The results of the 4-day pulse were used to estimate the steady-state level of residues due to the persistent pool. The estimated value was in good agreement with that found on lifetime exposure to radioactive drug.

Arprinocid [MK-302; 9-[(2-chloro-6-fluorophenyl)-methyl]-9*H*-purin-6-amine; I] is of interest for control of



*Site of ^{14}C label

I

Arprinocid (MK-302)

coccidiosis in broiler chickens (Kilgore et al., 1978; Miller et al., 1977; Olson et al., 1978; Tamas et al., 1978). Tissue distribution studies with the ^{14}C-labeled compound showed that of the edible tissues liver contained the largest radioactive residue. At 3–5 days after drug withdrawal, radioactivity was equivalent to about 0.1–0.3 ppm, expressed as arprinocid (Olson et al., 1976).

In actual use arprinocid would be administered daily in poultry feed at levels of 0.006% (60 ppm). To design a meaningful tissue residue experiment with radioactive drug, one must establish the length of the period of dosing with nonradioactive drug and the length of the period for dosing with radioactive drug. In a series of five experiments, the dosing with nonradioactive drug was followed by a 4- or 14-day pulse of labeled drug. In a sixth experiment, only radioactive drug was administered for 44 days. The effect of the dose level, duration of the radioactive pulse, and the withdrawal period on the tissue residue was noted.

MATERIALS AND METHODS

Arprinocid. Arprinocid, obtained from the Merck Sharp & Dohme Research Laboratories sample collection, was synthesized by reaction of 2-chloro-6-fluorobenzyl chloride with adenine. The product was greater than 98% in purity and contained about 1.0–1.2% of the 3-substituted adenine. (The commercial product contains less than 0.005% of the 3 isomer.)

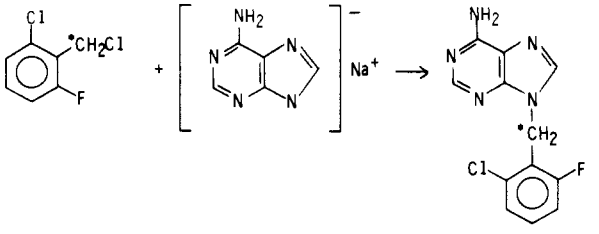
Animal Handling. Sex-separated Hubbard–Hubbard chickens were weighed and sorted into groups for each experiment. The experiments were usually initiated with more than the required number of chickens. At the onset of the ^{14}C-labeled arprinocid portion of an experiment, three males and three females, selected as the most uniform in weight, were used for each group. A group of control birds were fed nonmedicated diet throughout the entire experiment. Details of the animal handling are given in Table I.

After the chickens were fed for 4, 14, or 44 days with ^{14}C-labeled arprinocid, the birds in all groups were weighed and a nonmedicated diet was substituted for the medicated diet. The control birds were slaughtered at the end of the treatment period while the treated birds were maintained on a drug-free diet and slaughtered according to the schedule in Table I. Following bleeding by cardiac puncture, samples of liver, kidney, muscle, and skin/fat were taken from each bird for total radioactive residue analysis.

For measurement of any $^{14}CO_2$ produced, single chickens weighing approximately 500 g were dosed by gavage with a solution of ^{14}C-labeled arprinocid at a level of 10 mg/kg. The birds were kept in metabolism units for 6–48 h. During this period the exit gas was passed through traps containing 4 N sodium hydroxide. Radioactive CO_2 retained by the caustic solution was determined by acidification of aliquots and trapping the evolved $^{14}CO_2$ in Hyamine containing phoshor.

A control experiment in which $^{14}CO_2$ was generated by slowly adding acid to a solution of $NaH^{14}CO_3$ contained in a glass dish in the metabolism unit gave a recovery of 95% of $^{14}CO_2$.

Radiochemical Methodology. *Synthesis and Purity of the ^{14}C-Labeled Arprinocid.* The ^{14}C(*C)-labeled compound was prepared in our laboratories by Dr. R. L. Ellsworth. The synthesis is the subject of another publication. The last step in the sequence involves the reaction



Arprinocid (MK-302)

The chemical purity of the labeled material was determined by comparison of the ultraviolet absorption and HPLC properties of the labeled compound and a reference sample of nonradioactive arprinocid.

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Table I. Feeding and Slaughter Schedule of Chickens Fed Arprinocid in Their Diets

run	drug concn in feed, %	age of birds, days, for cold drug start	cold drug end	^{14}C-labeled drug start	^{14}C-labeled drug end	groups (3 M and 3 F)[b]	slaughter, days after drug withdrawal
1	0.008	3	46	46	50	9	0, 1, 3, 5, 6, 7, 8, 9
2[a]	0.008	3	47	47	51	6	0, 5, 6, 7, 8
3	0.006	9	49	49	53	6	0, 4, 5, 6, 7
4	0.007	9	49	49	53	6	0, 4, 5, 6, 7
5	0.007	3	46	46	60	6	0, 3, 5, 7, 9
6	0.007			5	49	7	0.25, 1, 3, 5, 7, 9

[a] Added 0.005% Roxarsone and 50 g/ton Bacitracin. [b] Includes one control group.

Table II. Recovery of Radioactivity Added to Tissue Samples. Replication and Accuracy. Specific Activity of ^{14}C-Labeled Arprinocid (1.2 μCi/mg)

added spike: liver, μg/g	homogenate,[a] μg/g	homogenate,[a] dpm/g	radioactivity found, dpm/g ± 3σ′[b] run 1 part A	run 1 part B	run 2 part A	run 2 part B	av 1	av 2	grand av
1	0.25	658	641.4 ± 57.2	649.2 ± 58.7	648.8 ± 59.8	682.8 ± 60.0	645.3	665.8	655.6
0.5	0.125	329	280.3 ± 41.3	365.6 ± 46.6	344.4 ± 45.5	325.2 ± 44.6	322.9	334.8	328.9
0.25	0.063	165	152.7 ± 33.8	156.8 ± 35.0	179.6 ± 36.3	158.6 ± 34.9	154.8	169.1	162.0
0.125	0.031	82	71.5 ± 29.5	90.7 ± 30.3	85.9 ± 29.9	66.0 ± 28.8	81.1	76.0	78.5
0.063	0.016	41	57.2 ± 26.9	30.6 ± 25.4	22.3 ± 24.8	34.4 ± 25.7	43.9	28.4[c]	36.2
0.031	0.008	21	18.4 ± 23.5	10.3 ± 23.5	27.0 ± 25.0	33.7 ± 25.5	14.3[c]	30.3	22.3
0.016	0.004	10	6.2 ± 22.3	15.1 ± 23.8	19.2 ± 24.5	4.5 ± 23.0	10.6[c]	11.9[c]	11.2

[a] Homogenate: 1 part of liver plus 3 parts of water. [b] σ′ based on radiochemical statistics. [c] Values lower than estimated detection limit. Standard deviation of blanks for four samples = 7.27 dpm; detection limit = 29.1 dpm; liver equivalent = 0.089 μg/g.

The radiochemical purity (>99%) was determined by thin-layer chromatography by using Brinkman silica G fluorescent plates developed with chloroform–methanol–NH_4OH (45:5:0.15). The thin-layer plate was sectioned, and the radioactivity content of each individual section was determined. No single impurity accounted for more than 0.5% of the total radioactivity.

The labeled arprinocid used in run 6 was also analyzed by reverse-phase HPLC with a μBondapak C_{18} column (Waters Associates) with a mobile phase of 0.01 M H_3PO_4 (pH 3.6)–MeOH (65:35) at a flow rate of 1.1 mL/min. Detection was by UV at 260 nm (LDC Spectromonitor II). Quantitation of radioactive species was by collection of 1-min fractions followed by liquid scintillation counting of the fractions. The specific activity of the dosed drug was 1.2 μCi/mg.

Total Radioactivity Determination. (*1*) *Preparation of Samples.* Each tissue sample was removed from the sacrificed bird, homogenized, and frozen until assayed.

Samples of tissues from chickens fed only the nonmedicated diet (which were used to calculate background dpm) and from chickens on ^{14}C-labeled drug were prepared in the same way and analyzed at the same time.

The samples were prepared for combustion as follows.

(*a*) *Liver and Muscle.* Ten grams of thawed ground tissue was mixed with water to a total weight of 40 g and blended in a Virtus homogenizer for about 2 min. A sample of the homogenate of about 0.5 g was accurately weighed into a combustion cup, air-dried, and burned.

(*b*) *Kidney.* Samples were handled as for liver and muscle except that the total kidney sample (8–10 g) was used and water was added to a total weight 4 times the kidney weight.

(*c*) *Skin/Fat.* Samples of thawed ground tissues of about 0.15–0.20 g were accurately weighed into the combustion cup without homogenization.

(*2*) *Combustion and Counting.* Measurements of radioactivity were made by using Intertechnique scintillation spectrometers with punched tape outputs. The tissue and plasma samples were combusted for total ^{14}C radioactivity by using a Packard Model 306 sample oxidizer. The $^{14}CO_2$ produced in the combustion was trapped in a phosphor mixture consisting of 8 mL of Carbo-Sorb and 13 mL of Permafluor V. Background tissue blanks were obtained from control birds from the same experiment for each set of tissue types being assayed. Quenching corrections were obtained by the external standard method on all samples and blanks.

Each tissue sample was analyzed in duplicate. Samples were reanalyzed in those cases where the replicate analysis was unsatisfactory.

The standard deviation, σ′, due to radiochemical statistics was determined as follows:

$$\sigma' = (\sigma_{background}^2 + \sigma_{sample}^2)^{1/2}$$

in which

$$\sigma_{sample} = (\text{total count of sample})^{1/2}/(\text{total time counted in minutes})$$

and

$$\sigma_{background} = [\text{total background count (all samples)}]^{1/2}/(\text{total time counted in minutes})$$

The total background count was obtained from the total count of replicate samples from six unmedicated birds run at the same time.

Tissue samples were reassayed in those cases where the original duplicate values differed by more than 3σ′ from their mean and also differed by more than 5% from their mean. These differences indicated an error in the experiment due to some factor other than radiochemical statistics.

(*3*) *Detection Limit Determination.* The detection limit was determined by using the standard deviation of six replicate control tissue samples and the efficiency of radioactivity recovery of radioactive spike at or near the detection limit. For those experiments the detection limit was taken as the net dpm equivalent to 4 times the standard deviation of tissue blank corrected for radioac-

Table III. Calculations of Radioactivity Detection Limits. Samples of Tissues Taken from Chickens Fed a Nonmedicated Diet

		liver		kidney		muscle		skin/fat	
no.	sex	dpm	av	dpm	av	dpm	av	dpm	av
1	M	50.92		57.07		55.00		55.08	
		49.24	50.08	53.02	55.05	47.42	51.21	45.79	50.44
2	M	54.43		50.62		46.58		53.50	
		52.03	53.23	53.28	51.95	47.28	46.93	53.15	53.33
3	M	51.54		50.12		51.80		50.63	
		55.88	53.71	54.22	52.17	45.45	48.63	45.30	47.97
4	F	53.64		52.39		47.41		45.03	
		52.99	53.32	49.14	50.77	51.49	49.45	55.69	50.36
5	F	54.46		52.74		47.51		51.52	
		48.67	51.57	52.34	52.54	51.20	49.36	54.10	52.81
6	F	50.31		48.61		50.77		59.16	
		49.71	50.01	54.11	51.36	52.92	51.85	47.86	53.51
$\bar{X}$			51.99		52.31		49.57		51.40
SD			1.67		1.48		1.78		2.19
sp act., dpm/μg			2605		2605		2548		2540
av sample wt, g			0.132		0.137		0.139		0.144
recovery of spiked control, %[a]			100		97		90		92
detection limit, ppm			0.019		0.017		0.022		0.026

[a] Average of four replicates spiked at a residue level of about 0.1 ppm.

Table IV. Summary of Total Radioactive Residues in Chicken Tissues after Dosing with $^{14}C(CH_2)$-Labeled Arprinocid, Expressed as Drug Equivalent to Radioactivity (ppm)

dose in diet, ppm		withdrawal, days										detection limit, ppm
		0	0.25	1	3	4	5	6	7	8	9	
80	run 1: 4-day radioact pulse											
	liver	3.44		0.42	0.17		0	0	0	0	0	0.12
	kidney	2.48		0.20	0.09		0	0	0	0	0	0.05
	muscle	0.44		0	0		0	0	0	0	0	0.05
	skin/fat	0.25		0	0		0	0	0	0	0	0.03
80	run 2:[a] 4-day radioact pulse											
	liver	2.68					0.12	0.10	0.08	0.06		0.04
	kidney	2.20					0.06	0.04	0.04	0		0.03
	muscle	0.32					0	0	0	0		0.04
	skin/fat	0.16					0	0	0	0		0.04
60	run 3: 4-day radioact pulse											
	liver	2.22				0.12	0.11	0.09	0.06			0.06
	kidney	1.75				0.05	0.05	0	0			0.05
	muscle	0.25				0	0	0	0			0.07
	skin/fat	0.16				0	0	0	0			0.05
70	run 4: 4-day radioact pulse											
	liver	3.13				0.14	0.10	0.08	0.06			0.04
	kidney	2.29				0.08	0.05	0	0			0.05
	muscle	0.38				0	0	0	0			0.02
	skin/fat	0.23				0	0	0	0			0.03
70	run 5: 14-day radioact pulse											
	liver	2.99			0.33		0.25		0.16		0.12	0.03
	kidney	2.29			0.15		0.11		0.07		0.06	0.03
	muscle	0.33			0		0		0		0	0.03
	skin/fat	0.23			0.02		0		0		0	0.02
70	run 6: 44-day radioact feeding											
	liver		1.47	0.57	0.42		0.29		0.20		0.15	0.02
	kidney		0.97	0.30	0.16		0.09		0.07		0.05	0.02
	muscle		0.08	0	0		0		0		0	0.02
	skin/fat		0.11	0.04	0		0		0		0	0.03

[a] Diet also contained Roxarsone and Bacitracin.

tivity recovery. Under these conditions there is less than a 2.5% chance that either a residue-free sample would be found positive or a positive sample would not be detected.

$$\text{detection limit } (\mu g/g) = \frac{4SD \text{ of control tissue in dpm}}{(\text{dpm/mcg})(\text{sample weight, g})(\text{fraction recovered})}$$

RESULTS AND DISCUSSION

Radiochemical Methodology. *Metabolic Stability of the ^{14}C Label.* The metabolic stability of the ^{14}C label was studied in vivo by administration of a single dose of ^{14}C-labeled drug to chickens and determining the amount of $^{14}CO_2$. Liberation of the labeled atom from either the adenine or benzyl moiety of the drug would result in a single carbon fragment. In vivo all single carbon compounds yield substantial quantities of CO_2. Collection of the $^{14}CO_2$ from the three different chickens at time intervals of 0–6, 0–24, and 0–48 h gave only 0.11, 0.25, and 0.16% of the radioactivity dosed. Thus, the labeled atom is not readily degraded to single carbon fragments which

Table V. Total Radioactive Residues in Tissues from Chickens on Lifetime Feeding of Arprinocid at 0.007% in Their Diets. ^{14}C-Labeled Arprinocid Fed for Final 14 Days. Expressed as Drug Equivalent to Radioactivity (ppm)

	days off		liver		kidney		muscle		skin/fat	
group	drug	sex	assay	av	assay	av	assay	av	assay	av
2	0	F	2.446		2.114		0.241		0.176	
			2.458	2.452	2.049	2.082	0.266	0.254	0.160	0.168
		F	3.102		2.030		0.356		0.275	
			3.013	3.058	2.135	2.083	0.314	0.335	0.244	0.260
		F	3.752		2.938		0.372		0.248	
			3.755	3.754	3.069	3.004	0.367	0.370	0.269	0.259
		M	2.762		2.629-R		0.298		0.285	
			2.770	2.766	2.022-L	2.326	0.398	0.348	0.249	0.267
		M	3.677		2.728		0.406		0.265	
			3.732	3.705	2.812	2.770	0.425	0.416	0.267	0.266
		M	2.251		1.498		0.263		0.151	
			2.203	2.227	1.423	1.461	0.263	0.263	0.155	0.153
$\bar{X}$				2.994		2.288		0.331		0.229
SD				0.636		0.550		0.063		0.053
3	3	F	0.343		0.192		0.005		0.033	
			0.310	0.327	0.170	0.181	−0.012	−0.004	0.025	0.029
		F	0.395		0.117		0.008		0.035	
			0.371	0.383	0.153	0.135	0.014	0.011	−0.014	0.011
		F	0.278		0.147		0.018		0.026	
			0.251	0.265	0.140	0.144	0.028	0.023	0.018	0.022
		M	0.333		0.180		0.021		0.017	
			0.387	0.360	0.151	0.166	0.004	0.013	0.015	0.016
		M	0.245		0.146		0.006		0.030	
			0.327	0.286	0.172	0.159	0.015	0.011	0.038	0.034
		M	0.366		0.123		0.008		0.028	
			0.374	0.370	0.139	0.131	−0.004	0.002	0.019	0.024
$\bar{X}$				0.332		0.153		0.009		0.023
SD				0.048		0.019		0.009		0.008
4	5	F	0.178		0.104		0.018		0.016	
			0.148	0.163	0.114	0.109	−0.006	0.006	0.015	0.016
		F	0.196		0.110		−0.003		−0.002	
			0.214	0.205	0.120	0.115	−0.005	−0.004	0.008	0.003
		F	0.334		0.141		−0.002		0.006	
			0.294	0.314	0.143	0.142	−0.005	−0.004	0.004	0.005
		M	0.251		0.120		−0.012		0.018	
			0.283	0.267	0.131	0.126	0.009	−0.002	0.005	0.012
		M	0.184		0.087		0.010		−0.006	
			0.199	0.192	0.036	0.062	0.007	0.009	0.016	0.005
		M	0.336		0.124		−0.013		0.022	
			0.334	0.335	0.102	0.113	0.002	−0.006	0.018	0.020
$\bar{X}$				0.246		0.111		0		0.01
SD				0.070		0.027		0.006		0.007
5	7	F	0.189		0.068		0.007		0.004	
			0.177	0.183	0.071	0.070	−0.025	−0.009	0.019	0.012
		F	0.157		0.081		0.016		0.008	
			0.144	0.151	0.047	0.064	0.004	0.010	0.010	0.009
		F	0.131		0.079		0.009		0.006	
			0.133	0.132	0.062	0.071	−0.012	−0.002	0.018	0.013
		M	0.144		0.079		−0.002		0.008	
			0.163	0.154	0.074	0.077	0.008	0.003	0.000	0.004
		M	0.167		0.077		0.015		0.008	
			0.153	0.160	0.041	0.059	−0.006	0.005	0.007	0.008
		M	0.173		0.054		−0.001		0.000	
			0.187	0.180	0.075	0.065	0.009	0.004	0.001	0.001
$\bar{X}$				0.160		0.067		0.002		0.008
SD				0.019		0.006		0.007		0.005
6	9	F	0.131		0.075		0.005		−0.014	
			0.134	0.133	0.050	0.063	−0.005	0	0.016	0.001
		F	0.102		0.049		−0.009		0.030	
			0.154	0.128	0.070	0.060	0.007	−0.001	−0.006	0.012
		F	0.077		0.063		0.008		0.016	
			0.090	0.084	0.064	0.064	−0.013	−0.003	0.011	0.014
		M	0.137		0.056		−0.003		0.021	
			0.137	0.137	0.091	0.074	0.012	0.005	0.006	0.014
		M	0.127		0.041		−0.008		0.012	
			0.149	0.138	0.063	0.052	−0.002	−0.005	0.007	0.010
		M	0.127		0.048		0.007		0.018	
			0.109	0.118	0.040	0.044	−0.025	−0.009	0.006	0.012
$\bar{X}$				0.123		0.059		−0.002		0.010
SD				0.021		0.010		0.005		0.005

Table VI. Total Radioactive Residues in Chicken Liver, Expressed as Drug Equivalent to Radioactivity (ppm). Average of Six Birds[a]

	days postdose									
run	0	0.25	1	3	4	5	6	7	8	9
1	3.44		0.42	0.17		0.11	0.10	0.07	0.09	0.05
2	2.60					0.12	0.10	0.08	0.06	
3	2.22				0.12	0.11	0.08	0.06		
4	3.13				0.14	0.10	0.08	0.06		
5	2.99			0.33		0.25		0.16		0.12
6		1.47	0.57	0.42		0.24		0.20		0.15

[a] Includes all observed radioactivity values: some are below the detection limit.

would enter the endogenous pool of the chickens. The label site was considered satisfactory for residue experiments.

Tissue Analysis and Detection Limits. The radioactivity analyses involved the combustion of tissue samples (skin/fat) or of water homogenates (liver, kidney, or muscle) and counting, with efficiency corrections. The applicability of this methodology, including all of the manipulation, the adequacy of the phosphor used, and the accuracy of the efficiency correction procedures were determined by spiking liver tissue homogenates from control birds with [^{14}C]arprinocid at levels of 0.016–1.0 μg/g of liver at 2-fold dilution steps. Two sets of duplicate analyses of these samples were carried out and gave the data contained in Table II.

Table II illustrates several points. First, the procedure of running duplicate analyses and repeating those which fall outside of the $\pm 3\sigma'$ (based on radiochemical statistics) or ±5% of the mean assay value appears satisfactory. This allows the discarding of unacceptable data which could result from faulty combustion, aliquoting, or contamination. In this experiment none of the 28 values was outside these limits. Second, the method of determining the detection limit has some limitations. In run 2 the sample spiked with 0.063 μg/g was judged as below the detection limit which was 0.044 μg/g or 29.1 dpm.

It is interesting to note that values for samples spiked well below the "detection limit" are reasonably close to their expected value. Indeed, the average of the four replicates is nearly identical with the expected value throughout the entire range of the experiment. For that reason all observed values from tested birds were used in the kinetic calculations below.

Table III contains the results of the tissue analysis of the control animals from run 6 and is typical of the data obtained in all other runs. The method of determining the detection limit for each tissue type is illustrated. Recoveries generally ranged from 88% to 100% and thus have only a small influence on the estimated detection limit.

Level of Total Radioactive Residues in Various Tissues. The results of the total radioactivity analysis are found in Table IV which contains the averaged assays of tissues from each group of six birds. If the average is below the detection limit, zero is reported.

Table IV clearly shows that although all the tissue samples from the "on-drug" birds contain residues, no residue is found in muscle tissue after only 1-day withdrawal. Skin/fat contains very low levels of radioactivity. Residues are detectable in kidney tissue but were at low levels by the third day after drug withdrawal. Liver contains substantially greater quantities of radioactive residues which persist for longer periods of time than in any other tissue.

All of the analytical data from run 5 (14-day radioactive pulse) are contained in Table V. Statistical analysis indicated that there was no statistically significant difference between sexes and that there was a statistically significant variation among individual chickens.

Table VII. Regression Analysis of Radioactive Residues in Livers of Chickens Fed ^{14}C-Labeled Arprinocid

	run					
	1	2	3	4	5	6
dose, ppm	80	80	60	70	70	70
pulse, days	4	4	4	4	14	44
est pool size, ppm						
rapid[a]	3.14	2.30	1.92	2.83	2.44	
slow[b]		0.3[c]			0.55	0.68
half-life of slow pool, days		3.3[c]			4.08	4.01

[a] Determined by subtraction of the estimated persistent pool from the observed on-drug value. [b] Determined from values obtained from withdrawal times, 3 days and later. [c] Runs 1-4 combined excluding day 8 from run 1.

Nature of the Liver and the Duration of the Radioactive Pulse. The liver tissue residues from all runs are given in Table VI. As a first approximation these residues may be considered to consist of a "rapidly depleting pool" and "persistent pool", although in fact each pool in turn may consist of a multiplicity of components. The rapidly depleting pool consists of relatively low molecular weight substances (Carlin et al., 1982). The slowly depleting pool was observed to disappear at a nearly uniform rate over the interval of days 3–9. Because the variation between experiments did not seem greater than the variation within each experiment, all of the data from day 3 and later from the four runs with 4-day radioactive pulse were combined to estimate the pool size and half-life of the persistent pool by using regression analysis of the first-order depletion. As indicated in Table VII, the persistent pool size was estimated by extrapolation to be about 0.3 ppm on drug.

The rapidly depleting pool for each run was determined from the observed on-drug level less the persistent pool level. These values are given in Table VII. The on-drug level of the rapidly depleting pool appears related to the dose in that the average value was the highest in one group dosed at 80 ppm and the lowest in the group dosed at 60 ppm. Intermediate levels were estimated for those groups dosed at 70 ppm. Because of the drug was administered in the diet and because the level declines rapidly after drug removal, the on-drug levels are influenced by the period since the birds last ingested the medicated diet. In any event, the total pool size is 6–10 times that of the persistent pool.

The disappearance rate of the rapidly depleting pool is indicated by the 6- and 24-h values in runs 1 and 6. If one assumes an initial level of about 2.6 ppm, the depletion rate corresponds to a half-life of 6–7 h. Since a 4-day pulse is sufficient to achieve 90% of the steady-state level of substances having a depletion rate equivalent to a half-life of 1.2 days, the 4-day radioactive pulse is sufficient to

achieve maximum levels of this pool. For any substance following first-order kinetics, 90% of the steady-state level is achieved when the dosing period is 3.3 times the half-life.

Because of the apparent rapid depletion of this pool, the on-drug level is influenced by the drug consumption in the time period immediately before termination of the experiment. After 3 days the level of drug residues in this pool is probably too low to contribute significantly to the total residue.

The half-life of the persistent pool appeared to be 3.3 days based on the 4-day radioactive pulse data. This pulse is only 1.2 half-lives and hence is of insufficient duration to achieve a steady-state level for this pool. The data from the 4-day radioactive pulse experiments were used to estimate both the duration of the radioactive pulse required to achieve 95% of the steady-state level and the size of the persistent pool.

In these experiments the animals were exposed to nonradioactive drug for a prolonged period before introducing the radioactive dose. The tissues therefore would have achieved a steady residue level. During the pulse period radioactive residues are replacing the nonradioactive residues already present in the bird. Thus, during the 4-day pulse interval, the nonradioactive residue would deplete 1.2 half-lives to a level of 43% of the initial level. Since the radioactivity reflects only 57% of the total pool, the anticipated persistent residue would be about 0.53 ppm at zero withdrawal. Similarly, it can be estimated that about 14 days of radioactive pulse would be required to displace 95% of the nonradioactive residue with the radioactive residue. For this reason an experiment using a 14-day pulse was carried out. The estimated on-drug level of the persistent pool was found to be 0.55 ppm, thus corresponding closely to that predicted. The apparent half-life, however, appeared to be about 4.1 days instead of 3.3 days determined from the 4-day pulse. This suggests that the persistent residue pool present after a 4-day pulse consists of substances with half-lives both longer and shorter than 3.3 days and that a pulse period greater than 14 days could be required to attain 95% of the steady-state level. Determination of the probable residue level due to the persistent pool using a 4.1-day half-life gave an estimated on-drug level of 0.6 ppm.

When a lifetime exposure to radioactive drug was carried out, the estimated value of the persistent residue (Table VII) was found to be about 0.68 ppm. This level may be influenced by even a trace amount of the radioactivity incorporated into the endogenous metabolism of the host.

In these experiments, the half-life of the persistent pool did not change appreciably (3.3–4.1 days) as the length of the radioactive pulse was increased. Thus, any single experiment of any pulse length can be used to estimate the steady-state level of the persistent pool. The slight change in the half-life also is an indication that only a small fraction of the drug is converted to endogenous residues since such residues would have a longer half-life by virtue of reincorporation (Von Wittenau, 1967).

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LITERATURE CITED

Carlin, J. R.; Rosegay, A.; Wolf, F. J.; Jacob, T. A. *J. Agric. Food Chem.* **1982**, following paper in this issue.

Kilgore, R. L.; Bramel, R. G.; Brokken, E. S.; Olson, G.; Cox, J. L.; Leaning, W. H. D. *Poult. Sci.* **1978**, *57*, 907.

Miller, B. M.; McManus, E. C.; Olson, G.; Schleim, K. D.; Van Iderstine, A. A.; Graham, D. W.; Brown, J. E.; Rogers, E. F. *Poult. Sci.* **1977**, *56*, 2039.

Olson, G.; Jacob, T. A.; Wolf, F. J.; Green, M. L.; Alvaro, R. F.; Van Iderstine, A. A. *Poult. Sci.* **1976**, *55*, 2074.

Olson, G.; Tamas, T.; Smith, D. A.; Weppelman, R. M.; Schleim, K.; McManus, E. C. *Poult. Sci.* **1978**, *57*, 1245.

Tamas, T.; Olson, G.; Smith, D. A.; Miller, B. M. *Poult. Sci.* **1978**, *57*, 381.

Von Wittenau, M. S. *J. Sci. Food Agric.* **1967**, *18*, 608–609.

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Tissue Residues of Arprinocid in Chickens. 2. A Common Derivative Approach for the Analysis of Residues in Liver

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Residue analysis of tissues taken from chickens administered ^{14}C-labeled arprinocid [9-[(2-chloro-6-fluorophenyl)methyl]-9*H*-purin-6-amine] showed that liver is the edible tissue containing the highest level of radioactivity after drug withdrawal. Less than 10% of this residue was assayed as either unchanged drug or known metabolities. Solvent extractions carried out by using homogenates of liver samples taken from birds on drug through 5 days after drug withdrawal showed that most of the radioactivity could not be separated from the insoluble fraction. Strong acid hydrolysis converted 88–74% of the respective residues to a readily extractable compound identified as 2-chloro-6-fluorobenzylamine. Through analysis of this amine, both drug and unknown (but drug-related) metabolites can be quantitated—regardless of whether the residue is solvent extractable or covalently bound to cellular macromolecules. Direct assay of the free base 2-chloro-6-fluorobenzylamine has serious limitations; however, the dansyl derivative proved to be an ideal compound and serves as the basis of the analytical assay of arprinocid-derived residues.

As described in the preceding paper (Jacob et al., 1982), tissue distribution studies with ^{14}C-labeled arprinocid [9-[(2-chloro-6-fluorophenyl)methyl]-9*H*-purin-6-amine (Figure 1)], a new coccidiostat (Kilgore et al., 1978; Olson et al., 1978) showed that liver is the edible tissue containing the highest level of radioactive residue. This residual radioactivity was equivalent to 0.1–0.3 ppm, expressed as

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