were added to give net final concentrations of 40 and 150 mg. per liter. In the case of the blank, the solution was diluted with the proper amount of water. The aliquots of the resulting systems were then treated with 84% sulfuric acid in the manner described. At both levels the average percentage recovery amounted to approximately 96%.

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Received for review October 9, 1957. Accepted April 22, 1958. Contribution No. 1147, Massachusetts Agricultural Experiment Station, University of Massachusetts, Amherst, Mass.

ANIMAL GROWTH STIMULANTS

The Metabolic Fate of Carbon-14–Labeled Trimethylalkyl Ammonium Stearate

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Trimethylhexadecyl ammonium stearate labeled with carbon-14 in the one position of the hexadecyl chain has been administered to rats orally, as a diet ingredient, and by injection intraperitoneally. This compound is highly insoluble in water and, therefore, appeared unlikely to be absorbed from the intestinal tract or metabolized by the body, when given by injection. However, the compound was absorbed from the intestinal tract of both species to a small extent, and the absorbed fraction was rapidly metabolized and excreted by way of the gastrointestinal tract, urine, and respiratory carbon dioxide. Absorption of the compound appeared to be higher in the chick, but concentration of the label in the tissues was similar in both species.

MANY CHEMICAL COMPOUNDS, which stimulate animal growth in controlled amounts, are toxic in large doses. To evaluate the biological effects of such compounds, feeding as well as metabolism experiments must be conducted to demonstrate the growth-stimulating effect at various levels of intake and to determine the ability of the animal to metabolize and excrete the compound, if it is absorbed, before it accumulates in the tissues to levels which could be toxic either to the animals involved or, indirectly, to man.

In these experiments, trimethylhexadecyl-1-C^{14}-ammonium stearate, which has chick and swine growth-stimulating activity (*1–3*), was administered orally or by injection to rats and chicks and the distribution of the radioactivity in the tissues and excreta was determined.

Materials and Methods

Experiments with Rats. In experiment I (Table I), 500 mg. of C^{14}–labeled trimethylalkyl ammonium stearate [Arquad stearate (Dynafac), Armour and Co.] was thoroughly mixed with each kilogram of a balanced synthetic diet. The diet contained sucrose, casein, and corn oil as sources of carbohydrate, protein, and fat, respectively. Two weanling male albino rats of the Sprague Dawley strain were fed on the above diet for periods of 22 and 25 days. The feces and urine were collected over the whole period and the respiratory carbon dioxide for the last 48 hours. At the end of the feeding period, the rats were killed with ether, the livers and intestines were separated from the carcasses, and the intestinal contents were added to the feces.

The radioactivity was measured by burning each sample with Van Slyke combustion liquid and collecting the carbon dioxide in an ionization chamber. The counts were made on a vibrating reed electrometer.

In experiment II (Tables II and III), the labeled Arquad stearate was injected. Preliminary experiments showed that Arquad stearate was much more readily absorbed when given intraperitoneally than when administered subcutaneously. Two male albino rats weighing 124 and 126 grams were injected intraperitoneally with 7 mg. of Arquad stearate suspended in 1.0 ml. of water. The rats were immediately placed in all-glass metabolism cages for 7 days. The respiratory carbon dioxide from rat I was collected in 12-hour fractions and the urine from rat II in 24-hour fractions. At the end of the seventh day, the rats were killed with ether, and the radioactivity was determined on the feces, urine, respiratory carbon dioxide, carcass, and liver.

In experiment III (Table IV), three male albino rats were fed C^{14}–labeled Arquad stearate at the level of 500 mg. per kg. of a synthetic diet for 7 days. The Arquad stearate was then withheld from the diet. One rat was killed at 0 hour, one after 48 hours, and one after 96 hours from withholding the compound; their carcasses were assayed for radioactivity.

Experiments with Chicks. Two 1-day-old Columbian crossed with New Hampshire chicks were given a nutritionally adequate corn-soybean meal diet for 5 weeks. At this point, 200 mg. of C^{14}–Arquad stearate were mixed with each kilogram of the diet. The chicks were placed in individual wire-bottomed cages in a ventilated hood. Food and water were provided *ad libitum*. The feces and urine of each chick were collected together for the first 4 days on the C^{14}–Arquad stearate diet. On the fifth day, both chicks were operated on to obtain a sample of urine uncontaminated with feces (*4*). This was accomplished on one chick. At the end of the seventh day on the C^{14}–Arquad stearate diet the chicks were sacrificed, feathered, and washed. The abdomen was split open by a midline incision and the liver and the gastrointestinal tract were removed, washed, and frozen. The radioactivity of the carcass and the organs examined was determined as before.

Results and Discussion

Table I shows the distribution of the

Table I. Fate of Orally Administered C^{14}–Arquad Stearate

(500 mg. per kg. diet)

	Rat I	Rat II	Average
Initial wt., g.	36.6	40.5	
Final wt., g.	99.0	127.0	
Experimental period, days	22	25	
Feed intake, g.	116.00	161.00	
Arquad intake, mg.	58.00	80.50	
C^{14} intake, μc.	26.216	36.225	
Recovery, %			
Feces	94.33	93.97	94.15
Urine	4.27	3.37	3.82
Carcass	0.33	0.28	0.30
Liver	0.09	0.06	0.08
Gut	0.07	0.02	0.04
CO_2	0.97	0.75	0.86
Total	100.06	98.45	99.25

Table II. Fate of Intraperitoneally Injected C^{14}–Arquad Stearate

(3.17 μc.)

	μc. C^{14}		Recovery, %	
	Rat I	Rat II	Rat I	Rat II
Feces	1.5400	1.5785	48.58	49.79
Urine	1.1140	1.2091	35.14	38.14
CO_2	0.0204	0.0581	0.64	1.83
Liver	0.0108	0.0219	0.34	0.69
Carcass	0.3836[a]	0.3789	12.10[a]	11.95
Gut	...	0.0243	...	0.77
Total	3.0688	3.2708	96.80	103.17

[a] For Rat I this figure represents carcass plus gut.

Table III. Excretion of C^{14}–Arquad Stearate

Period, Hours	Radioactivity, μc.	Recovery, %
Carbon Dioxide (Rat I)[a,b]		
0–12	0.0073	0.230
12–24	0.0040	0.126
24–36	0.0035	0.110
36–48	0.0040	0.126
48–60	0.0026	0.082
Total	0.0214	0.674
Urine (Rat II)[c]		
0–24	0.7462	23.54
24–48	0.1891	5.96
48–72	0.1061	3.35
72–96	0.0610	1.92
96–120	0.0363	1.15
120–144	0.0301	0.95
144–168	0.0403	1.27
Total	1.2091	38.14

[a] Specific activity of $BaCO_3$ too low to count from third day on.
[b] Fractionally collected every 12 hours during 7-day experiment.
[c] Fractionally collected every 24 hours during 7-day experiment.

Table IV. Residual Radioactivity in Carcasses of Rats Killed after C^{14}–Arquad Stearate Was Withheld from Diet

	Hours		
	0	48	96
Initial wt., g.	48	47	47
Final wt., g.	76	83	94
Feed intake, g.	51	43	49
Arquad intake, mg.	25.5	21.5	24.5
μc. intake	11.475	9.675	11.025
Carcass wt.	67.3	69.85	78.55
% recovery in carcass[a]	1.74	1.34	1.27

[a] Intestinal contents were removed.

Table V. Fate of C^{14}–Arquad Stearate Orally Administered to Chicks

(Approx. 200 mg. Arquad per kg. of diet)

	Chick I	Chick II
Initial wt., g.	434	421
Final wt., g.	511	484
Experimental period, days,	7	7
Feed intake, g.	259	242
Arquad intake, mg.	51.8	48.4
C^{14} intake, μc.	23.31	21.78
Carcass wt., mg.	345	355
Recovery, %		
Carcass	7.44	4.31
Liver	0.73	0.46
Gut	2.06	1.00
Gizzard	0.40	0.57
Crop	0.15	0.18
Feces	49.81	53.66
Gut contents	5.95	2.42
Total	66.54	62.60
Specific activity of		
Carcass tissue	3.9×10^{-3}	2.3×10^{-3} μc./g.
Liver tissue	6.8×10^{-3}	3.7×10^{-3} μc./g.
Bile	1×10^{-3}	μc./ml.

The specific activity of the uric acid was too small to count.

radioactivity in the tissues and excreta of the rats following oral administration of C^{14}–labeled Arquad stearate. The apparent absorption of the compound was slight, 94% of the radioactivity being recovered in the feces (Table I). When labeled Arquad stearate was injected (Table II), nearly one half of the injected radioactivity was recovered in the feces during 7 days following the injection. The true absorption of the orally administered Arquad stearate was therefore estimated to be about 10%. Of this absorbed activity, 50% was excreted by way of the gastrointestinal tract, 38% by way of the urine, 8% by way of the respiratory carbon dioxide, and 4% remained in the carcass tissues.

Table III shows that excretion of the label, by way of the respiratory carbon dioxide and the urine, was high during the first 24 hours following injection, dropped sharply the second day, then decreased gradually over the remaining 5 days of collection. Thus, during 7 days following injection of the labeled Arquad stearate, 87% of the injected radioactivity was excreted by way of the gastrointestinal tract, the urine, and the respiratory carbon dioxide; only 13% remained in the carcass tissues. Table IV shows that after withholding the labeled Arquad stearate from the diet, the small amount of radioactivity remaining in the carcass was being slowly excreted.

The distribution of the activity in the tissues and excreta of the chick following oral administration of labeled Arquad stearate (Table V) indicates that absorption of the compound was greater in the chick than in the rat. The uric acid recovered from one chick did not contain any measurable radioactivity. The unrecovered radioactivity, which amounted to about 30% of the administered dose, included losses in the respiratory carbon dioxide, feathers, and represented the difficulty of quantitatively collecting chick excreta.

These data show that in both species little of the compound is absorbed from the gastrointestinal tract and that which is absorbed can be metabolized both by oxidation to carbon dioxide and also by excretion both into the feces (presumably via the bile) and into the urine (by conversion to a water-soluble compound). One dimensional paper chromatography of the rat urine followed by radioautography of the developed chromatograms indicated the urinary excretion compounds to be different from the compound administered.

Acknowledgment

This work was supported in part by a grant-in-aid from Armour and Co., Chicago, Ill.

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Received for review December 23, 1957. Accepted April 10, 1958.