Table II. Recovery of DAS and DAH from Feeds

Added (Mg./Lb.)	Recovered	Detns.
	Alfalfa	
2.5 (DAS)	2.1 ± 0.2	3
1.5 (DAH)	1.7 ± 0.2	2
2.5 (DAH)	2.5 ± 0.1	2
	Corn	
2.5 (DAS)	1.9 ± 0.1	2
40 (DAS)	40 ± 3.0	2
2.5 (DAH)	2.1 ± 0.1	2
	Soybean	
200 (DAS)	200 ± 4.0	9

Table III. Recovery of DAH and DAS in Milk and Eggs

Added, Mg.	Recovered	Detns.
	Milk, 50 ml.	
0.05 (DAS)	0.04 ± 0.01	4
0.25 (DAS)	0.25 ± 0.02	3
0.22 (DAH)	0.25 ± 0.02	3
	Eggs, 50 gm.	
0.35 (DAH)	0.35 ± 0.03	2
0.25 (DAH)	0.22 ± 0.02	2
0.35 (DAS)	0.33 ± 0.03	2

Samples of feed meal were prepared containing known amounts of DAS or DAH, and analyzed by extraction and paper chromatography. The results are tabulated in Table II.

In a further investigation, known amounts of DAS and DAH were added to known quantities of milk and eggs. Analyses of these products are listed in Table III.

Figure 1 shows curves for the concentrations of DAS and DAH on paper vs. the absorbance of the blue spots produced by the reaction of Folin's reagent with these compounds.

Figure 2 illustrates a paper chromatogram showing the migration of HEX, DAH, DES, and DAS.

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FEED ADDITIVES

Tissue Residues and Excretory Pathways of Orally Administered 2-C^{14}-Methimazole

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Methimazole residues were not detected (sensitivity 0.1 to 0.2 p.p.m.) in rat tissues 96 hours after daily administration of the isotopically labeled compound for periods ranging from 1 to 14 days. Excretory pathways appeared to be exclusively through the urine and feces. Approximately 80% of an oral dose was excreted in the urine within 24 hours, with the remaining 20% being excreted in the feces within 48 hours after administration. Total recovery ranged from 98 to 105%. No isotope activity was noted in expired carbon dioxide.

Methimazole (1-methyl-2-thiol imidazole) is a potent goitrogenic compound which, when properly fed to beef cattle, stimulates liveweight gains (*2*).

Chick bioassays employing a thyroidal iodine-131 uptake technique demonstrated the absence of methimazole residues in beef tissues of cattle previously fed this goitrogen (*5*). Ely *et al.* (*3*) working with a different goitrogen (thiouracil) found it to be both rapidly absorbed into the blood stream and essentially eliminated from the circulation of ruminants within a 24-hour period. Similarly in nonruminants, Paschkis *et al.* (*4*), Williams (*8*), and Williams and Chute (*9*) found thiouracil to be both rapidly absorbed from the gastrointestinal tract and rapidly excreted in the urine. The purpose of the present investigation was to characterize excretory patterns of orally administered 2-C^{14}-methimazole in the rat, and to study tissue residues following administration of this isotopically labeled goitrogen.

Experimental Procedure

Three trials were conducted with female rats to determine the excretory pathways of orally administered 2-C^{14}-methimazole. The isotope was administered via stomach tube at the rate of 0.5 mg. per pound of body weight (total dose approximately 1 μc.). After dosage, each rat was placed in a glass metabolism cage where feces, urine, and expired carbon dioxide were collected as outlined by Roth (*6*). Feces and urine samples were subjected to wet oxidation using a combination of a solid and liquid oxidant and an oxidation apparatus outlined by Aronoff (*1*). Feces or urine samples containing approximately 15 mg. of carbon were placed in the oxidation vessel to which 2 grams of solid reagent mixture of potassium iodate and potassium dichromate (10 to 1 ground together) and 10 ml. of a liquid reagent composed of 50 ml. of concentrated sulfuric acid, 50 ml. of sirupy phosphoric acid, and 1.5 grams of potassium iodate were added. The carbon dioxide produced was absorbed in a gas washer containing sodium hydroxide.

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Table I. Mean Per Cent Recovery of a Single Orally Administered Dose of 2-C^{14}-Methimazole

Excretory Path	Trial No.	Hours after Dosage 24	48	96
Urine	1	75.0	78.3	78.9
	2	83.3	88.3	89.5
	3	76.1	79.7	81.0
	Mean	78.1	82.1	83.1
Feces	1	3.5	17.5	18.8
	2	3.6	13.8	15.9
	3	3.4	16.8	20.5
	Mean	3.5	16.1	18.4
Expired CO_2	1, 2, 3	0.0	0.0	0.0

Table II. Methimazole Tissue Residues Following Oral Administration of 2-C^{14}-Methimazole[a]

Tissue	Daily Doses 1 Mean	1 X^b	7 Mean	7 X^b	10 Mean	10 X^b	14 Mean	14 X^b
Lean and fat	0.02	0.11	0.08	0.10	0.06	0.10	0.11	0.08
Bones	0.02	0.14	0.05	0.12	0.04	0.10	0.05	0.08
Blood	0.03	0.17	0.08	0.12	0.05	0.13	0.11	0.10
GI tract	0.05	0.14	0.15	0.10	0.08	0.11	0.12	0.08
Liver	0.07	0.15	0.11	0.12	0.11	0.11	0.16	0.09
Kidney	0.06	0.22	0.23	0.15	0.12	0.16	0.14	0.18
Heart	0.07	0.24	0.31[c]	0.15	0.05	0.23	0.09	0.18
Lungs	0.15	0.24	...	...	0.39	0.23	0.39	0.13
Urogenital tract	0.02	0.27	0.01	0.21	−0.02	0.23	...	...

[a] Mean of two animals except heart and lung tissues are from one animal. All values expressed as p.p.m. in wet tissue.
[b] Sensitivity ($P < 0.05$).
[c] Heart and lung tissue combined.

To this alkaline sodium carbonate solution, barium chloride was added so as to precipitate the carbonate as barium carbonate, which was filtered and plated in infinitely thick samples for counting. Also, portions of urine and feces samples were plated and dried, and their total activities compared with those recovered from oxidized samples. Consistently good agreement was obtained between the two techniques. A Nuclear (Model M-5) gas flow counting unit with manual sample changer was utilized in making all radioactivity measurements. Self-absorption corrections were applied to samples of less than infinite thickness. Conversion factors were calculated to convert activity at infinite thickness to specific activity per milligram, thus enabling determination of total radioactivity in each sample.

Tissue residue studies were conducted with a single oral dose of labeled methimazole as compared to daily doses for 7, 10, and 14 days. Again, daily dosage was via stomach tube at the rate of 0.5 mg. per pound of body weight. The rats were sacrificed 96 hours after receiving the last dose. This time period coincided with the cessation of excretion of labeled methimazole or metabolites. Upon sacrifice, the various tissues were separated and then dried in a vacuum desiccator over sulfuric acid. Lean, fat, and bone tissues were separated by subjecting the skinned, eviscerated carcasses to autoclaving for 30 minutes. Infinitely thick, dried tissue samples were plated in preference to barium carbonate from a wet oxidation-precipitation technique in an effort to obtain maximum concentration of labeled metabolites. Previously determined conversion factors were used to convert activity at infinite thickness to specific activity per milligram of plated material. Concentration of methimazole or metabolites in rat tissues was measured by reference to specific activity of the standard 2-C^{14}-methimazole.

All rats were fed Purina Lab. Chow throughout the experiments.

Results and Discussion

Results of the three trials studying excretory pathways of orally administered 2-C^{14}-methimazole are given in Table I. These data indicate that orally administered methimazole is rapidly absorbed and excreted via the urine. Concentration of radioactivity in the urine was greatest 6 to 12 hours after dosage. Nearly 80% of the total administered dose was recovered in the urine in the first 24 hours following dosage. This pattern was similar to that observed by Ely *et al.* (*3*) who reported that thiouracil orally administered to calves and goats was practically eliminated from the blood stream in 24 hours. Excretion of labeled methimazole or metabolites in this study was essentially complete 48 hours after dosing.

Twombly and Schoenewaldt (*7*) concluded that failure to detect any labeled respiratory carbon dioxide after administration of C^{14}-diethylstilbestrol indicated that diethylstilbestrol was not broken down far enough for permanent storage in the tissues. Failure in this study to detect any activity in expired carbon dioxide would similarly tend to indicate that methimazole was not broken down far enough for permanent accumulation in body tissues.

Table II presents a summary of the results for tissues of rats to which labeled methimazole had been administered for varying lengths of time. In general, the tissue values observed were quite small and insignificant since in most cases the sensitivities (0.1 to 0.2 p.p.m.) were larger than the actual values recorded. One exception was lung tissue which gave larger values in certain instances. These larger values may well have been the result of stomach tube contamination during dosage rather than the result of a metabolic residue since expired carbon dioxide revealed that the lungs were not an excretory pathway for methimazole.

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