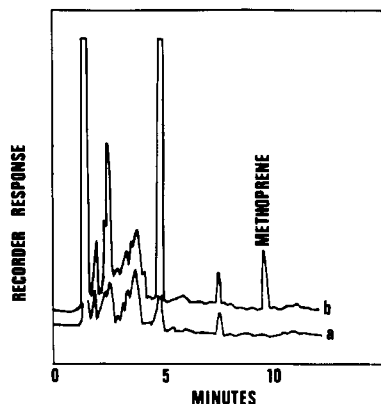

Figure 1. High-performance liquid chromatograms of (a) an extract of bovine fat and (b) an extract of bovine fat containing 0.008 ppm of methoprene.

adaptable to the determination of methoprene in a variety of animal tissues.

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Metabolism and Residues of ^{3}H- and ^{35}S-Labeled Ferbam in Sheep

^{35}S- and ^{3}H-labeled ferbam (Fermate) (ferric dimethyldithiocarbamate) was administered orally to 1 sheep, and only ^{35}S-labeled ferbam was administered to another sheep. By 76 h, 82% of the ^{3}H moiety and 23% of the ^{35}S moiety had been excreted in feces and urine. None of the radiolabeled metabolites co-chromatographed with available metabolic standards, dimethylamine and tetramethylthiuram disulfide. Tissues of slaughtered sheep contained radiolabeled materials.

The dialkyldithiocarbamates are an important class of fungicides. One of the representatives of this group, ferric dimethyldithiocarbamate (ferbam), is often applied to fruit crops, and because dried citrus pulp is used as a supplemental ration for livestock, ferbam residues may potentially be consumed by these animals. The maximum tolerance level for ferbam in citrus is 7.0 ppm. Because of this potential hazard, the authors investigated the excretion and deposition of residues of the sulfur and tritium moieties as well as that of the parent compound when the dithiocarbamate fungicide Fermate was administered orally to sheep.

EXPERIMENTAL SECTION

Chemicals. New England Nuclear Corportion, Boston, Mass., supplied the labeled ferbam and the metabolites, dimethylamine and TMTD (tetramethylthiuram disulfide). The ferric dimethyldithio-^{35}S-carbamate had a specific activity of 36 mCi/mmol, and that of ferric dimethyl-^{3}H-dithiocarbamate was 89.5 mCi/mmol.

Exposure and Sampling. Two Delaine-cross yearling ewes in good condition, each weighing 32 kg after being sheared, were conditioned in metabolism stalls for 7 days. During this pretreatment and the 76-h posttreatment period, the ewes were provided free choice a protein supplement, hegari, and water.

After preconditioning, sheep A was treated orally, via a gelatin capsule, with ferbam-^{35}S at 0.45 mg/kg of body weight, and also with ferbam-^{3}H equivalent to 0.74 mg/kg, or a total dose of 1.19 mg/kg of ferbam. The second ewe (sheep B) was treated orally with 14.5 mg of ferbam-^{35}S, equivalent to 0.45 mg/kg.

Retention catheters allowed separate collection of urine and feces. Blood and urine for each animal were collected at periodic intervals. The blood was heparinized, and the samples of blood and urine were quick-frozen for subsequent analysis. Samples of feces were also collected at intervals, freeze dried, ground in a CRC micro-mill, and frozen for later analysis. The ewes were electrocuted 76 h after treatment, and tissues were collected, ground in a Latapie grinder, and frozen for analysis. Bone was ground in a CRC micro-mill after the marrow had been removed.

TLC of Urine and Feces. Aliquots of whole urine, chloroform extracts of urine, urine after extracts, and chloroform and methanol extracts of feces were spotted on 250-μm silica gel GF thin-layer chromatographic plates and developed 15 cm in a solvent mixture containing benzene–methanol–acetic acid (12:2:1) and then in the second dimension in formamide–acetone (1:5). The plates were dried and placed under Ansco nonscreen x-ray film for 14 days. After the film was developed, appropriate gel regions, as indicated by the exposed film, were scraped and quantitated by liquid scintillation. R_f values of two of the potential ferbam metabolites, dimethylamine and TMTD (tetramethylthiuram disulfide), were determined nonradiometrically, but an analytical standard of the alkyldithiocarbamic acid, another metabolite, was not available for study.

Sample Preparations and Counting. Duplicate 100-mg samples of each tissue, other than blood, were digested for liquid scintillation counting (LSC) as described by Budd et al. (1968). Digestion time in the 130 °C sand bath varied from 3 to 5 h, depending on the tissue.

Table I. Total Micrograms of Radiolabeled Material in Urine and Feces of Sheep A Treated with 0.74 mg/kg Ferbam- ^{3}H and 0.45 mg/kg Ferbam- ^{35}S (Total Dose 1.19 mg/kg) and Sheep B Treated with 0.45 mg/kg Ferbam- ^{35}S

Time, h	Sheep A						Sheep B		
	Urine		Feces		Blood		Urine	Feces	Blood
	^{35}S	^{3}H	^{35}S	^{3}H	^{35}S	^{3}H	^{35}S	^{35}S	^{35}S
0.50							0.01		
0.75	0.01	0.61					0.05		
1	0.07	1.77				0.02	0.82		0.01
1.25	0.08	1.71				0.01	0.66		0.02
1.5	0.33	4.02				0.01	1.27		0.03
1.75	0.64	5.06			0.01	0.01	2.91		0.03
2	0.73	4.90	0.06	2.44	0.01	0.02	4.40		0.03
2.5	2.21	14.26			0.02	0.05	6.54	0.59	0.04
3	3.29	20.88	0.01	1.12	0.03	0.04	11.72	0.25	0.05
3.5	4.80	27.40			0.04	0.03	12.22	0.21	0.06
4	5.34	32.49			0.03	0.03	46.73		0.06
5	12.56	85.16	0.12	3.54	0.06	0.02	44.41		0.07
6	14.49	122.38	0.30	4.11	0.08	0.03	41.71		0.08
7	15.27	143.36			0.12	0.05	33.58	0.29	0.09
11	89.41	1105.91	3.77	17.43	0.18	0.06	235.55	56.83	0.12
15	98.97	1573.21	5.89	15.99	0.22	0.09	146.97	157.74	0.13
18	73.39	1402.86	17.04	100.61					
23	148.90	2663.21	345.67	1204.97	0.26	0.10	190.21	358.61	0.18
27	109.81	1776.00	277.68	721.10	0.30	0.10	224.75	173.15	0.15
31	83.16	1087.55	77.24	275.34	0.28	0.08	135.69	203.76	0.13
35	75.01	934.38	139.01	362.56	0.31	0.08			
39	96.81	783.20	59.24	210.56	0.27	0.02			
47	253.68	1319.92	248.47	735.59	0.25	0.07	597.14	307.62	0.09
51	98.85	460.50	105.82	335.12	0.25	0.08	71.76	64.47	0.08
55	141.16	370.61	130.35	288.31	0.22	0.07	62.14	16.65	0.07
71	301.32	586.86	128.40	322.23	0.18	0.07	105.48	28.26	0.07
76	105.01	133.32	72.44	156.66	0.17	0.08		18.71	0.06
Total	1735.30	14661.53	1611.51	4757.68			1976.72	1387.14	

Duplicate 100-μl aliquots of whole blood were prepared for LSC as described by Lindsay and Kurneck (1969). Urine was counted directly in the counting solution. After aliquots of the bone homogenates had been digested, suspensions of both bone and feces were counted in a gel scintillator solution as described by Turpin and Bethune (1967).

A Beckman LS-150 liquid scintillation spectrometer, Model 1694, was used for all counting. Efficiency on unquenched samples for ^{35}S was 67.5%, and samples were counted as long as necessary to obtain <3% counting error. Quench curves were made for urine, feces, whole blood, and each of the tissues, and corrections were made with both internal and external standards. Samples, other than whole blood, were counted in 10 ml of a scintillation solution containing 30.24 g of butyl PBD (2-(4-*tert*-butylphenyl)-5-(4-biphenylyl)-1,3,4-oxadiazole), 1.89 g of PBBO (2-(4-biphenylyl)-6-phenylbenzoxazole), and 3.78 l. of toluene. Twenty percent BBS (Bio-solv No. 3, Beckman Instrument Co.) and 10% water were added for counting urine. Samples for ^{3}H counting were prepared as described by Hunt and Gilbert (1972) and combusted in a Packard 300 oxidizer.

RESULTS

Seventy-six hours after treatment, sheep A had excreted about 12% of the ^{35}S radioactivity and 62% of the ^{3}H radioactivity in the urine (Table I). The 31- to 47-h collections accounted for the greatest eliminations of ^{35}S radioactivity, while the greatest elimination of ^{3}H radioactivity was in the 18- to 23-h collection. Sheep B excreted about 14% of the ^{35}S radioactivity in the urine, with the greatest elimination during the same period as that for sheep A (31 to 47 h). Although the rate of excretion in sheep B was faster than that of sheep A, the excretion pattern was very similar (Table I).

Sheep A eliminated 11% of the ^{35}S radioactivity and 20% of the ^{3}H radioactivity in the feces (Table I). The greatest elimination of radioactivity for each isotope was in the 18 to 23 h sample. Sheep B, which received only [^{35}S]ferbam, eliminated 10% of the ^{35}S radioactivity in the feces (Table I). The ^{35}S excretion pattern in sheep B was consistent with that of sheep A in that maximun elimination was seen in the collection 15 to 23 h posttreatment.

Table II. Total ppm of Radiolabeled Material in Tissues of Sheep A Treated with 0.74 mg/kg Ferbam- ^{3}H and 0.45 mg/kg Ferbam- ^{35}S (Total Dose 1.19 mg/kg) and Sheep B Treated with 0.45 mg/kg Ferbam- ^{35}S

Tissue	Sheep A		Sheep B
	^{35}S	^{3}H	^{35}S
Adrenal gland	0.696	0.373	0.423
Bone	0.630	0.073	
Bone marrow	0.413	0.121	
Brain	0.134	0.325	0.137
Fallopian tubes and ovaries	0.573	0.408	
Fat, kidney	0.056	0.093	
Fat, mammary	0.130	0.101	
Fat, omental	0.050	0.090	0.040
Gallbladder	0.809	1.100	
Heart	0.232	0.176	0.237
Intestine, large	0.760	0.333	
Intestine, small	0.576	0.315	
Kidney	0.722	0.811	0.765
Liver	0.659	3.378	0.988
Lung	0.680	0.350	
Muscle	0.144	0.220	0.114
Pancreas	0.516	0.360	
Pituitary gland	0.807	0.312	
Spleen	0.403	0.308	0.493
Thymus	0.310	0.555	
Thyroid	0.823	0.362	1.256

In sheep A the ^{35}S radioactivity was highest in the 35-h blood sample, and the ^{3}H radioactivity was highest in the 27-h blood sample (Table I). In sheep B, the ^{35}S radioactivity was highest in the 23-h blood sample (Table I).

All of the tissue samples from sheep A contained detectable radiolabeled residues (Table II). The thyroid,

gallbladder, pituitary gland, and large intestine had the highest levels of ^{35}S radioactivity equivalent to 0.82, 0.81, 0.81, and 0.76 ppm, respectively. This tissue sequence was not maintained by the ^{3}H residues; the liver had 3.38 ppm, gallbladder 1.10, kidney 0.81, and thymus 0.56 ppm equivalents. Of the tissues analyzed, the fats contained the lowest levels of radioactivity. Of the nine tissues from sheep B that were quantitated, the thyroid, liver, kidney, and adrenals had the highest levels of 1.26, 0.99, 0.77, and 0.49 ppm equivalents, respectively (Table II).

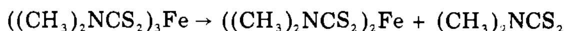
The two major nonpolar ^{35}S metabolites in urine (35–40% of the radioactivity in the chloroform extracts) were at R_f 0.64 and 0.72. Most of the remainder of the radioactivity was ^{3}H metabolites with R_f values of 0.48, 0.64, and 0.72. Less than 5% of the radioactivity was in the ferbam band area.

The two major nonpolar ^{35}S metabolites in feces (25 to 35% of the radioactivity in the chloroform extracts) were at R_f 0.64 and 0.72. Most of the rest of the radioactivity was ^{3}H metabolites at R_f values of 0.48 and 0.64, with no product at the 0.72 value.

Only four metabolites were found in the polar extracts of feces. The polar metabolites in the urine (60–70% of the radioactivity) resolved into five components, but no further attempts were made to chromatograph these products.

None of the above metabolites co-chromatographed with the available metabolite standards, dimethylamine and TMTD.

If the hydrogens are most stable and labeling in the dimethyl group represents a bonding area not readily exchanged with H^+ of water, the 82% elimination (62% in urine and 20% in feces) of the ^{3}H moiety indicates a molecular degradation of ferbam beyond the finding of Owens (1960), who speculated that ferbam operated through free radical rather than ionic mechanisms and proposed the following theoretical reaction:



$$((CH_3)_2NCS_2)_3Fe \rightarrow ((CH_3)_2NCS_2)_2Fe + (CH_3)_2NCS_2$$

The elimination of the ^{35}S moiety probably was not as CS_2 because the authors found no dimethylamine. Owens (1960) further concluded that ferbam retards citrate synthesis and indicated that the inhibition of enzymes resulted from complex formation with metals of the metal-containing enzymes or by interference in electron shifts between sulfhydryl or amino groups of the enzyme and substrate molecules. Owens (1969) and Weed et al. (1953) also found that the activity of the sulfhydryl-dependent enzymes was inhibited by ferbam. Our observations on the cellular distribution or "flooding" of the ^{35}S moiety through all the tissues of sheep support the conclusions of Owens (1969) and Weed et al. (1953) on the metabolic pathways of ferbam and its dithiocarbamic acid derivatives.

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Anti-Tumorigenic Effect of Maleic Hydrazide on Mouse Skin

Maleic hydrazide (MH; 1,2-dihydro-3,6-pyridazinedione), a tobacco sucker-control agent which has been detected in relatively high concentrations in cigarette smoke, was tested for tumorigenic activity on mouse skin. MH significantly inhibited the "initiation" phase of two-stage tumorigenesis caused by 7,12-dimethylbenz[*a*]anthracene (DMBA) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA). The inhibitory activity of MH resembled that of its parent compound maleic anhydride. At 5000 times its residue level in cigarette smoke, MH did not initiate tumor development on its own.

Maleic hydrazide (MH; 1,2-dihydro-3,6-pyridazinedione) is used extensively in agriculture as a herbicide and as a growth retardant to prevent suckering in tobacco plants. It is estimated that fully 80% of American grown tobacco is routinely treated with this chemical (Tso, 1972). The oral and parenteral toxicities of MH have been studied (Barnes et al., 1957; Nasrat, 1965) but the question of its carcinogenic properties is widely disputed among investigators (Dickens and Jones, 1965; Epstein and Mantel, 1968; Hunter et al., 1973).

Relatively high MH residues have been detected in tobacco and other crops (Ihnat et al., 1973), a factor which increased in importance when Liu and Hoffmann (1973), using sensitive assay techniques, found that over 1 μg, or about 4%, of the 30 μg of MH in an average American cigarette was transferred unchanged to the mainstream smoke, and thus was available to the smoker. Haeberer and Chortyk (1974) confirmed the presence of MH in cigarette smoke and also detected concentrations of up to 20 ppm in cigarette smoke condensate (CSC), commonly called "tar". These findings focus attention on the possible contribution of MH to the tumorigenicity of cigarettes.

Much of the experimental data on the tumorigenic activity of cigarettes are results from application of smoke condensate or its constituents to mouse skin. Salaman and Roe (1956) tested MH by the mouse skin bioassay and