preparing its acetoxy and methoxy derivatives; the derivatives were identified by comparing their infrared spectra with those of authentic compounds.

Metabolite III, which accounted for 3% of the radioactivity in the urine, and metabolite X, which accounted for 14% of the radioactivity in the feces, were not identified (Table VII). However, preliminary investigations indicated that metabolite III contained an intact isopropyl carbamate side chain, and that the ring structure was substituted in at least two positions. Additional studies are being conducted to characterize these two metabolites and the radio-labeled compound(s) which remained in the tissues and eggs from the chicken. Further studies are also needed to evaluate the toxicological and pharmacological properties of the metabolites of isopropyl carbanilate and to determine the fate of these compounds in the environment.

LITERATURE CITED

Budzikiewicz, H., Djerassi, C., Williams, D. H., "Mass Spectrometry of Organic Compounds," Holden-Day Inc., San Francisco, Calif., 1967, pp 118, 325, 342, 471, and 500.
Ennis, W. B., *Amer. J. Bot.* **35,** 15 (1948).
FAO/WHO, "Evaluations of the Toxicity of Pesticide Residues in Food," Rome, 1964.
Fales, H. M., Warren, K. S., *J. Org. Chem.* **32,** 501 (1967).
Feigenbaum, J., Neuberg, C. A., *J. Amer. Chem. Soc.* **63,** 3529 (1941).
Hoiberg, C. P., Mumma, R. O., *J. Amer. Chem. Soc.* **91,** 4273 (1969).
Holder, G. M., Ryan, A. J., *Nature (London)* **220,** 77 (1968).
Packard Technical Bulletin Number 18, Packard Instrument Company, 2200 Warrenville Road, Downers Grove, Illinois, 1969.
Paulson, G. D., *Poultry Sci.* **48,** 1331 (1969).
Paulson, G. D., Feil, V. J., *Poultry Sci.* **48,** 1593 (1969).
Paulson, G. D., Portnoy, C. E., J. AGR. FOOD CHEM. **18,** 180 (1970).
Paulson, G. D., Zaylskie, R. G., Zehr, M. V., Portnoy, C. E., Feil, V. J., J. AGR. FOOD CHEM. **18,** 110 (1970).
Paulson, G. D., Zehr, M. V., J. AGR. FOOD CHEM. **19,** 471 (1971).
Pollecoff, F., Robinson, R., *J. Chem. Soc.* **113,** 645 (1918).
Sullivan, L. J., Eldridge, J. M., Knaak, J. B., J. AGR. FOOD CHEM. **15,** 928 (1967).
Templeman, W. G., Sexton, W. A., *Nature (London)* **156,** 630 (1945).
Timson, J., *Pestic Sci.* **1,** 191 (1970).

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Tissue Residue Depletion of Sulfamerazine in Sheep

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Sulfamerazine was given orally to sheep at the recommended therapeutic dose of 132 mg per kg per day for 3 days. Sulfonamide residues were determined in muscle, liver, kidney, and fat at posttreatment days 0 (12 hr after the last dosing), 3, 5, 7, and 10. Residue depletion was most rapid between days 0 and 3 after the drug was withdrawn. Sulfonamide concentrations were below 0.1 ppm in liver, kidney, and fat by day 7, and in muscle by day 10. The data show that a 10-day withdrawal period is indicated for sulfamerazine in sheep given this therapeutic dosage.

Sulfamerazine is a chemotherapeutic agent which is of value in treating a number of infectious diseases of domestic animals. It is considered to be, along with sulfamethazine, the sulfonamide of choice in treating *Pasteurella* infections such as mastitis and pneumonia in sheep (Merck Veterinary Manual, 1967). Sulfamerazine is rapidly absorbed from the gastrointestinal tract and is excreted at a slower rate than its parent drug, sulfadiazine, thus allowing greater retention and prolongation of therapeutic action.

Although numerous studies have been conducted on sulfonamide concentrations in blood after therapy, tissue residue data have been essentially lacking. Sulfamethazine residues in edible tissues of swine at 7 days after treatment were less than 0.1 ppm when the drug, in combination with chlortetracycline and penicillin, was administered in the feed over a 14-week period (Messersmith *et al.*, 1967). In calf tissues, sulfamethazine residues were reduced to below 0.1 ppm by the eighth day after withdrawal when the drug was given as a drench at a dose of 99 mg per kg for 3 days; however, in tissues of breeder chickens, residues persisted at concentrations greater than 0.1 ppm at the tenth day after withdrawal, when the drug was given in the feed at 0.4% or in the drinking water at 0.1% (Righter *et al.*, 1971a). In swine treated with sulfathiazole at 330 mg per kg per day for 3 days, residues in edible tissues were depleted by 10 days withdrawal (Righter *et al.*, 1971b). A withdrawal period greater than 7 days was indicated in laying hens and cockerels administered sulfaquinoxaline at therapeutic (0.05%) or prophylactic (0.025%) doses (Righter *et al.*, 1970). In recent years, the sensitivity of methods for determining sulfonamides has been improved and smaller quantities of these drugs can be measured in body fluids and tissues. Because of increased concern over drug residues in edible tissues, more data are necessary to establish safe periods of drug withdrawal, in which tissue residues have reached what is considered a negligible amount, before treated animals are marketed. The purpose of this study was to determine residue levels of sulfamerazine in sheep tissues following a recommended dosage regimen.

MATERIALS AND METHODS

Thirteen 10-month-old lambs and one 22-month-old wether of mixed breeds were administered sulfamerazine orally at the recommended therapeutic dosage of 132 mg per kg of body weight per day (Merck Veterinary Manual, 1967). The drug was given in capsule form; a priming dose of 132 mg per kg was given initially and five consecutive doses of 66 mg per kg

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were given at 12-hr intervals. The capsules were dipped in corn oil to reduce trauma to the posterior oral cavity. Two lambs, one unusually heavy (81 kg) and one light (24 kg), were allotted to the initial withdrawal period (12 hr after the last dose). The remaining 12 lambs were dispersed equally by weight over the remainder of the withdrawal periods, which were 3, 5, 7, and 10 days posttreatment. Three 2-year-old wethers were used for control tissues. The animals were sacrificed by stunning them with a captive bolt pistol and severing the carotid arteries.

Representative samples (50 g or more) of muscle from the rump area, liver, kidney, and fat from the kidney area were taken and quick-frozen for sulfonamide assay. Blood samples (20 ml) were taken from the jugular vein of three lambs at 0, 12, 24, 36, 48, 60, and 72 hr during both the treatment and withdrawal periods of the study. The serum fraction was removed by centrifugation and quick-frozen for sulfonamide assay. When possible, urine samples were taken from the bladders of the lambs after sacrificing and were also frozen for subsequent analysis.

Tissues were analyzed for free sulfonamides according to the method of Tishler *et al.* (1968); the method is sensitive to about 0.1 ppm and was duplicated in our laboratory (Table I). (Felig and Westheimer (1968) have described a similar procedure with equal sensitivities using smaller sample sizes.) Blood serum and urine were analyzed by the method of Annino (1961), which is sensitive to about 10 ppm; this clinical procedure was modified in our laboratory by increasing the sample size to yield a sensitivity of 2 ppm (Table I).

Table I. Recovery of Sulfamerazine from Tissue and Blood Serum

Sample	Added, ppm	Found, ppm	Recovery, %[a]
	Tissue		
Control muscle	0.00	0.02	–
Fortified muscle	0.10	0.11	86
Control liver	0.00	0.03	–
Fortified liver	0.10	0.12	88
Control kidney	0.00	0.04	–
Fortified kidney	0.10	0.13	90
Control fat	0.00	0.02	–
Fortified fat	0.10	0.11	90
	Blood serum		
Control blood serum	0.0	0.84	–
Control blood serum	0.0	0.89	–
Control blood serum	0.0	0.91	–
Average control	0.0	0.88	–
Fortified blood serum	2.0	2.51	81
Fortified blood serum	2.0	2.59	85
Fortified blood serum	1.0	1.69	81
Fortified blood serum	0.5	1.08	40

[a] Corrected for control values.

Table II. Sulfonamide Residues in Tissues of Sheep Administered Sulfamerazine at the Therapeutic Dose Concentration of 132 mg/kg/day

Post treatment withdrawal day	Residues, ppm[a]			
	Muscle	Liver	Kidney	Fat
0	118.0	95.0	225.0	19.5
3	0.36	0.44	0.82	0.08
5	0.12	0.11	0.07	0.07
7	0.12	0.03	0.01	0.05
10	0.02[b]	0.03[b]	0.01[c]	—
Controls	0.02	0.03	0.05	0.02

[a] Values are averages of three animals and are corrected for mean control values which are included in the table. [b] One sample. [c] Two samples; individual values were 0 and 0.01.

Table III. Sulfonamide Concentrations (ppm) in Serum of Sheep both During and Following Administration of Sulfamerazine[a]

	Time of samples, hr								
	0	12	24	36	48	60	72	120	168
Dosing period	2.0	66	63	79	89	102	—	—	—
Withdrawal period[b]	99	73	46	9.7	5.4	2.7	2.0	2.0	2.0

[a] Values are averages of three animals and are corrected for mean control values. [b] Withdrawal time 0 is 12 hr after the last dose (72 hr after the first dose).

RESULTS

As seen in Table II, the sulfonamide levels in all tissues were markedly reduced between days 0 and 3 after drug withdrawal; more than 99% of the residues present at day 0 were eliminated from all of the tissues during this time. Residue levels were lowest in adipose tissue and highest in renal tissue at withdrawal day 0, and were below the method sensitivity of 0.1 ppm in adipose tissue at day 3, in renal tissue at day 5, and in liver at day 7. A sulfonamide level of 0.12 ppm, slightly above the method sensitivity, persisted in muscle at 7 days; however, the one muscle sample tested at day 10 contained only negligible amounts of residue. The amounts of sulfonamide were also negligible in the one liver and two kidney samples tested at withdrawal day 10.

Sulfonamide concentrations in blood were well above the minimum therapeutic level of 5 mg per 100 ml 12 hr after treatment began and continued to increase as the dosing continued (Table III). At the time the last dose was given (60 hr), the concentration had reached 102 ppm, but it then decreased rapidly after withdrawal of the drug. At 72-hr posttreatment, only background levels (2 ppm) were detectable.

DISCUSSION

Sulfonamide concentrations in serum at day 0 of withdrawal appear to be consistent with those in calves treated with sulfamethazine (Righter *et al.*, 1971a) and were depleted by 72 hr after withdrawal of the drug.

In agreement with results of previous sulfonamide residue studies in calves (Righter *et al.*, 1971a), swine (Righter *et al.*, 1971b), and poultry (Righter *et al.*, 1970), 90% or more of the tissue residues present at day 0 were eliminated by the third day of withdrawal and the residue levels were highest in renal tissue and lowest in adipose tissue. However, the persistence of residues in muscle through 7 days after withdrawal has not been observed with other sulfonamides in the other domestic animals. The amount of residue present in muscle at 7 days (0.12 ppm) was only slightly above the level of method sensitivity and that in the one muscle sample tested at 10 days (0.02 ppm) was well below the level of sensitivity; therefore clearance of the drug from muscle appears to be satisfactory at 10 days of withdrawal.

These data show that a 10-day withdrawal period is indicated for sheep given sulfamethazine at the dosage used.

LITERATURE CITED

Annino, J. S., "Standard Methods of Clinical Chemistry," Vol. 3, Association of Clinical Chemists, Seligson, D. E., Ed., Academic Press, New York, N.Y., 1961, p 200.
Felig, J., Westheimer, J., J. AGR. FOOD CHEM. **16,** 738 (1968).
Merck Veterinary Manual, Merck and Co., Inc., Rahway, N.J., 1967, p 513.
Messersmith, R. E., Sass, B., Berger, H., Gale, G. O., *J. Amer. Vet. Med. Ass.* **151,** 719 (1967).
Righter, H. F., Worthington, J. M., Mercer, H. D., *Amer. J. Vet. Res.* **32,** 1003 (1971a).
Righter, H. F., Worthington, J. M., Mercer, H. D., *J. Anim. Sci.* **33,** 797 (1971b).
Righter, H. F., Worthington, J. M., Zimmerman, H. E., Mercer, H. D., *Amer. J. Vet. Res.* **31,** 1051 (1970).
Tishler, F., Sutter, J. L., Bathish, J. M., Hagman, H. E., J. AGR. FOOD CHEM. **16,** 50 (1968).

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DDT Residue Depletion in Sheep Using Dietary Energy Restriction and Administration of Glucagon

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DDT residue depletion in sheep was studied using dietary energy restriction and treatment with glucagon. After 104 days DDT residues had decreased as follows: control, 12.8%; dietary energy restriction, 39%; dietary energy restriction + glucagon (1 mg/100 kg), 51.2%. Glucagon caused a 14% decrease in blood glucose after 6 days of administration. Dietary energy restriction and glucagon treatment produced a statistically significant ($p < 0.05$) reduction in DDT residues. However, a glucagon did not produce a statistically significant ($p < 0.05$) reduction over dietary energy restriction alone. Neither dietary energy restriction nor treatment with glucagon appears to offer a practical solution to the problem of DDT residues in meat animals.

Since DDT was introduced in 1942, the chlorinated pesticides have assumed an important role in the environment, but their persistent nature has led to widespread contamination of that environment. DDT residues have been demonstrated in soil (Durham, 1965), in water (Frazer, 1967), in plants and forage crops (King *et al.*, 1966), and in human tissues (Hoffman *et al.*, 1967).

Total diet studies made by the Food and Drug Administration indicate that the average diet in the United States today contains about 0.015 ppm of DDT and its analogs (Corneliussen, 1970). Animal protein sources contribute more than one-half of these residues (Cueto and Brown, 1958).

The significance of these DDT residues is not perfectly clear. DDT has been shown to be hepatocarcinogenic in rats (Fitzhugh and Nelson, 1947) and in rainbow trout (Halver *et al.*, 1962). Cueto and Brown (1958) found that DDE produced extensive adrenal cortical necrosis. Phillips and Hidiroglou (1965) reported that steers fed DDT-contaminated forage showed decreased liver vitamin A. However, high DDT levels in humans have not been shown to produce clinical changes (Durham, 1965; Hoffman *et al.*, 1967; Laws *et al.*, 1967).

Several methods to reduce pesticide residues in the meat animal have been attempted. These methods include dietary energy restriction (Wesley *et al.*, 1966) and feeding heptabarbital (Street *et al.*, 1966), thyroproteins (Bovard *et al.*, 1967), noncontaminated feed (Rumsey *et al.*, 1967; Fries *et al.*, 1969), phenobarbitol and charcoal (Cook and Wilson, 1970), or charcoal (Crookshank and Smalley, 1970). In a review of the literature, Laben (1968) reported other methods that have also been attempted.

This study was designed to investigate the role of fat mobilization in accelerating the depletion of DDT residues. Fat mobilization was initiated by dietary energy restriction and by the administration of glucagon (Foa, 1964; Hagen, 1961; Lipsett *et al.*, 1960; Salter *et al.*, 1962).

MATERIALS AND METHODS

Feeding Studies. Twenty-one adult ewes were used in the experiment. All animals were treated at the beginning of the experiment and at 25-day intervals thereafter with 8 g of Thiabendazole (Merck) to control internal parasites. Except for the treatment with glucagon or the period of stress by starvation, all animals were fed *ad libitum* a 12% protein ration and prairie hay. The animals were weighed at the beginning of the experiment, prior to and immediately following treatment/stress, and 30 days after treatment/stress.

To establish a level of DDT residue in the adipose tissue, all animals were dosed at the rate of 300 mg/kg (Rumsey *et al.*, 1967) with *p,p'*-DDT dissolved in corn oil. The dosage was divided into eight equal doses administered in 0.5-oz gelatin capsules over a 30-day period. Caudal fat samples were taken 30 days after administration of the last DDT dosage (60 days from beginning of the study), following treatment/stress, and 30 days thereafter. The animals randomly were divided into three groups of seven sheep and handled as follows.

The control group received a maintenance ration throughout the experiment and was not treated or stressed.

Group A (seven animals) was stressed 60 days after the experiment began by being placed on a low energy diet of cottonseed hulls for 2 weeks. Feed consumption was approximately 1 lb/head/day. Blood samples were taken every 4 days beginning on the day of placement on the low energy ration and ending 2 days after return to a normal ration. These blood samples were analyzed for the presence of ketone bodies.

Sixty days after the experiment began group B (seven ani-

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