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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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mals) was treated daily with glucagon at the rate of 1 mg/100 kg for 8 days. During glucagon treatment, these animals were on a low energy diet of cottonseed hulls. Blood samples for blood glucose determination were taken prior to glucagon treatment, three times during treatment (4 hr after glucagon injection), and 1 week after treatment was discontinued.

Ketone Body Determination. Blood samples were also tested for ketone bodies using Ketostix (Ames Company, Elkhart, Ind.) reagent strips. The strips detect 5–100 mg% ketone bodies.

Blood Glucose Determination. Blood glucose levels were determined by means of a Technicon AutoAnalyzer (Hoffman, 1937).

DDT Residue Determination. A 5-g fat sample was taken from the caudal region using aseptic surgical techniques. The extraction and cleanup procedure used was the method of Samuel (1966) as modified by Crookshank (1969), who demonstrated that the extraction step may be immediately followed by acetonitrile–hexane partitioning, thus by-passing the cleanup with dichloromethane (No. 5 cleanup). After acetonitrile–hexane partitioning was completed, the petroleum ether extract was analyzed by gas chromatography, without silicic acid cleanup. All solvents used were Fisher Pesticide Grade.

Gas Chromatography. The gas chromatograph was a Barber-Colman No. 5000 employing an electron capture detector with a tritium foil. A U-shaped 5.5-m × 5-mm i.d. column packed with Varaport No. 30, 100/120 mesh, coated with 5% DC 200 was used. Carrier gas was purified nitrogen with a flow rate of 120 ml/min. Column temperature was 200°C; detector temperature was 200°C; and injector temperature was 225°C. A 50-V DC power source was used with the detector being operated at 60% of the standing current at the plateau.

A Hamilton microliter syringe was used to measure 5-μl aliquots for injection. Standards of lindane, aldrin, dieldrin, *p,p'*-DDE, DDD, *o,p'*-DDT, and *p,p'*-DDT were analyzed and retention times were calculated relative to aldrin. Percent recovery was calculated by injecting known amounts of standards into untreated sheep fat followed by extraction, cleanup, and analysis.

RESULTS AND DISCUSSION

During the course of the experiment one animal from the control group and one from group A died as a result of anemia and heavy parasitism. The animals were old and remained in rather poor condition throughout the study, which possibly accounts for the variation in response.

Weight changes during the course of the experiment are shown in Table I. Some of the weight changes could have been due to changes in rumen fill. The weight loss shown by group A should have caused an increase in body fat metabolism. Weight changes during glucagon treatment have not been previously reported. It would appear that the carbohydrate deprivation of the low energy diet offset the hyperglycemic effect of glucagon, resulting in weight loss. This should have caused an increase in body fat metabolism in group B.

The stress created by low dietary energy intake was apparently insufficient to cause ketosis in group A since ketone bodies were not detected in blood samples from this group.

The rates and magnitude of the changes in the blood glucose levels of group B are shown in Table II. After six injections of glucagon the blood glucose level had decreased from 67.0 to 57.6 mg%. Using hypotheses testing this decrease was shown to be highly significant ($p < 0.01$). Campbell and Rastogi (1966) showed that glucagon caused a rise in blood glucose levels for 2 hr postinjection, followed by a decrease. This resulted from increased insulin secretion stimulated by the glucagon-induced hyperglycemia. Ideally, treatment with anti-insulin serum at the time of glucagon injection would have reduced this insulin secretion (Foa, 1964), but none was available for this experiment. The dosage rate of glucagon (1 mg/100 kg of body weight) was selected rather empirically by extrapolation from another study (Burtis, 1968), since no work on the use of glucagon to increase body fat metabolism in sheep has been reported. Possibly, increasing glucagon dosage to higher levels (2–5 mg/100 kg) would result in more significant reductions in DDT tissue residues.

Summation of the DDT residue changes by groups is shown in Table III. Statistical analysis was performed on the data to determine if the changes in the initial residue levels within each group were statistically significant.

The results were as follows: Control group, changes were

Table I. Means of Weight Changes Expressed in Kilograms

Time weight recorded	Control group	Group A, starvation	Group B, glucagon
Beginning of study	52	51.6	45.6
Beginning of treatment/ stress period	55.7	53.2	48.3
Treatment/stress period + 1 week	56.2	46.0	45.1
Treatment/stress period + 5 weeks	61.3	56.2	55.6

Table II. Blood Glucose Levels Expressed in mg% Taken 4 Hr after Glucagon Injection[a]

	Days from beginning of injection period[b]				
Animal no.	0	3	6	9	16
634	68.0	57.0	58.0	57.0	66.0
636	52.0	59.0	52.0	50.0	55.0
638	62.0	72.0	63.0	79.0	64.0
639	54.0	58.0	51.0	55.0	55.0
742	61.0	70.0	58.0	55.0	59.0
852	100.0	76.0	60.0	58.0	78.0
854	72.0	56.0	61.0	58.0	66.0
Means	67.0	64.0	57.6	58.9	63.2

[a] Glucagon was injected daily for 8 days at the rate of 1 mg/100 kg of body weight. [b] Initial blood sample (day 0) was taken prior to the first glucagon injection; others were taken 4 hr after injection of glucagon on that day.

Table III. Analysis of Total DDT Residue Changes Expressed in Parts per Million

	Control[a] group	Group A,[a] starvation	Group B,[a] glucagon
Initial residue levels	34.1 ±8.7	49.1 ±12.0	54.1 ±12.6
Treatment/stress period + 1 week	32.0 ±8.2	40.7 ±9.9	39.5 ±9.4
Final residue levels	29.7 ±7.4	30.0 ±7.4	26.4 ±5.9
Mean change	−4.4	−19.1	−27.7
Median	−4.16	−13.1	−26.2
Range	+8.5 to −21.1	+1.4 to −41.0	+7.7 to −48.7
Variance	15.66	12.22	11.70
Significance	$p < 0.1$	$p < 0.01$	$p < 0.005$

[a] Values are means ± the standard errors.

Table IV. Comparison of *p,p'*-DDT and *p,p'*-DDD Expressed as % of Total DDT[a]

	Control group	Group A, starvation	Group B, glucagon
Initial Level			
p,p'-DDT	64	67	66
p,p'-DDD	16	16	23
Final level			
p,p'-DDT	51	62	59
p,p'-DDD	33	25	21

[a] 88 % Recovery for DDD, 91 % for DDT.

Table V. Analysis of Variance between Groups

Source	df	Sum of squares	Mean squares	p <0.05
Among groups	2	1999	1000	
Within groups	16	5106	320	
Total	18	7105	F = 3.6337	Not significant

not significant ($p < 0.01$). Group A, changes were significant ($p < 0.01$). Group B, changes were significant ($p < 0.005$).

Significant amounts of *p,p'*-DDT were converted to *p,p'*-DDD as shown in Table IV. Analysis of variance was performed on the data by testing the hypothesis of equal means (Huntsberger, 1967) to determine if residue level changes between groups were statistically significant at $\alpha = 0.05$. The results are summarized in Table V.

It was concluded that glucagon is more effective than no treatment or by dietary energy restriction alone in reducing DDT residue levels. However, glucagon is not significantly more effective than dietary energy restriction alone in reducing these residues. The high cost of glucagon would tend to make DDT residue depletion by this method uneconomical. Neither dietary energy restriction nor treatment with glucagon appears to offer a practical solution to the problem of reducing DDT residues in meat animals.

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