Certain Physiological Factors Affecting Organochlorine Pesticide Metabolism in Ovine Females

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Twenty-eight crossbred 2-year-old ewes were used to determine the effects of pregnancy and lactation on the body storage of DDT and dieldrin and on their elimination in milk. Pregnancy did not significantly affect total DDT degradation in ewes, but significantly affected dieldrin metabolism. Body fat from pregnant animals averaged higher in dieldrin concentration at parturition than did that of nonpregnant animals at a comparable sampling time. After parturition, DDT, DDT metabolites, and dieldrin decreased steadily with time. Lactation status significantly affected DDD concentrations and the total DDT:dieldrin ratio. Residues in body fat of lactating animals decreased faster than those of nonlactating animals. However, the rate of pesticide decrease was similar in nonlactating and nonpregnant animals. The concentration of DDT metabolites in milk fat was proportional to the total DDT concentration in body fat. However, dieldrin in milk fat was not correlated with dieldrin concentration in body fat.

Several physiological factors significantly influence the metabolism of organochlorine pesticides in farm animals (Braund *et al.*, 1968; Durham *et al.*, 1956; Kunze and Laug, 1953). However, most of the data available are from separate physiological factors, without considering their interactions during a given period of time. Little organochlorine pesticide excretion was noted in contaminated heifers between contamination and parturition (Braund *et al.*, 1969), but body fat pesticide residues have been shown to decrease in lactating dairy cattle (Laben *et al.*, 1965). There is a paucity of information regarding the effects of pregnancy, lactation, and lactation stage in ruminants on organochlorine pesticide metabolism. The objective of this study was to consider the effect of these factors and their interactions on DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane] and dieldrin metabolism in female ovines, between breeding and 10 weeks *postpartum*.

MATERIALS AND METHODS

Animals and Treatments. Twenty-eight crossbred 2-year-old, first-lamb ewes (*Ovis aries*) were used to determine the effects of pregnancy and lactation on the body storage of DDT and dieldrin and on their elimination in milk. Prior to breeding, the ewes were contaminated with 50 mg/kg of body weight of DDT and 50 mg/kg of body weight of dieldrin. The DDT and dieldrin were purified compounds administered in gelatin capsules, and were divided into five equal doses over a period of 3 weeks. After parturition, the ewes were divided into three groups: those that lambed and were to retain their lambs (parous and lactating, $n = 12$); those that lambed and whose lambs were removed and raised separately (parous and nonlactating, $n = 12$); and those that did not lamb (nonparous, $n = 4$). The ewes were fed a ration to meet the requirements for their physiological status as established by the National Research Council (1968).

Sampling. Body fat samples from the parous ewes were obtained by biopsy from the tailhead area at parturition (0 time) and at 5 and 10 weeks *postpartum*. Nonparous ewes were biopsied after all ewes had lambed (0 time), with subsequent biopsies at 5 and 10 weeks later. Milk samples were collected by hand-milking the lactating ewes at parturition (0 time) and at 2.5, 5, 7.5, and 10 weeks *postpartum*.

Pesticide Analysis. Duplicate pesticide analyses were carried out on milk and body fat samples, according to a method based on standard FDA (1969) procedures, which involved acetonitrile partitioning of an ether extract followed by cleanup on a Florisil column, and electron-capture, gas-liquid chromatography.

Statistical Analysis. Statistical analyses were by least-squares analyses of variance, which included: sampling time and pregnancy status; sampling time and lactation status as fixed main effects; and their two-way interactions (Table I). Ewe parturition weight was included as a continuous independent variable (Harvey, 1960). Duncan's multiple range test (Harter, 1960) was applied to sampling time means and significance was determined at the 5% probability level. Partial correlation coefficients were derived on an intragroup variance basis among individual observations for each sampling time. In deriving the correlations, ewe parturition weight was included as a continuous independent variable.

RESULTS AND DISCUSSION

Pregnancy. The least-squares means for ewe body fat concentrations of DDE, DDD, DDT, total DDT (DDD, DDE and DDT), dieldrin, and total DDT:dieldrin ratio (a value for comparing the relative storage-elimination of these pesticides), as affected by sampling time and pregnancy status, are presented in Table II.

Body fat DDE, DDD, and dieldrin concentrations, as well as the total DDT:dieldrin ratio, were significantly affected by pregnancy status. At first sampling (approximately 160 days after contamination) body fat DDE and dieldrin concentrations were greater in parous compared to nonparous ewes, but the reverse was true for amount of DDD and the total DDT:dieldrin ratio. Comparisons at subsequent sampling periods show DDE and DDD concentration decreases, reaching comparable levels in both parous and nonparous ewes. Total DDT values in parous and nonparous animals (20.3 *vs.* 19.8 ppm) at the first sampling were not significantly different. However, total DDT concentration in both ewe groups decreased with sampling time, but again the pregnancy effect was nonsignificant.

The effect of pregnancy on the dieldrin concentration was greater than was the effect of sampling time, suggesting that this particular physiological factor is more important than time lapse. The mean concentration of dieldrin in the body fat of parous ewes at parturition was 17.4 as compared to 8.2 ppm in nonparous animals sampled at a similar time after contamination. Not until the 10th week did the dieldrin concentration appear to be the same for both groups.

Some reports have indicated an interaction between DDT and dieldrin (Street, 1964; Street and Blau, 1966) when both pesticides are administered together. DDT ap-

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Table I. Outline of the Least-Squares Analysis of Variance Used for Separating the Effects of Pregnancy, Lactation, and Time on Residues of DDT and Dieldrin in Ewes

Source of variation	Degrees of freedom[a]	Degrees of freedom[b]
Total	28	24
MU	1	1
Pregnancy status (P)	1	
Sampling time (T)	2	2
P X T interaction	2	
Lactation status (L)		1
L X T interaction		2
Residual (error)	22	18

[a] Analysis for comparing data from parous (both lactating and nonlactating) and nonparous ewes. [b] Analysis for comparing data from parous, lactating and parous, nonlactating ewes.

parently induces dieldrin metabolism and excretion from the animal body. The results reported here suggest that this apparent interaction is somewhat confounded by pregnancy. Changes in the total DDT:dieldrin ratio could result from differences in metabolism induced by pregnancy. The ratio in ewes at parturition compared with the ratio in nonparous animals at a similar time after contamination (1.17 *vs.* 2.41) supports this suggestion. This trend continued throughout the trial.

Time and Lactation. The least-squares means for ewe body and milk fat concentrations of DDE, DDD, DDT, total DDT, dieldrin, and total DDT:dieldrin ratio, as affected by time after parturition and lactation status, are presented in Table III.

Time after parturition significantly affected all pesticide concentrations and the ratio of total DDT:dieldrin in ewe body fat. As time progressed, residue concentrations decreased, suggesting that part of the pesticide had been metabolized and/or removed from the tissue. Lactation status (lactating *vs.* nonlactating) significantly affected DDD concentration and the total DDT:dieldrin ratio in body fat.

Decline of total DDT and dieldrin in ewe body fat was similar for nonparous and parous, nonlactating ewes during the trial. The mean rate of decline per week was 3.5 and 6.5% for total DDT and dieldrin, respectively, for both groups. However, the weekly decline in lactating animals during the same time was 7.5% for total DDT and 10% for dieldrin. Thus, it appears that milk secretion was an important route of organochlorine residue elimination. The weekly decline of total DDT and dieldrin in milk fat was 6.5 and 16%, respectively. Laben *et al.* (1965, 1966) reported a decline in cow milk of 6.6% per week for cows which had a maximum milk fat DDT concentration of 2 ppm.

Table II. Least-Squares Means for Pesticide Concentration in Ewe Body Fat by Week of Sampling and Pregnancy Status[a]

Sampling time, week	DDE, ppm	DDD, ppm	DDT, ppm	Total DDT, ppm	Dieldrin, ppm	Ratio[b]
			Parous ewes			
0	14.1	1.9[c]	4.3[c]	20.3	17.4	1.17[c]
5	10.1[c]	1.7[c]	3.6[c,d]	15.4[c]	10.5	1.46[c,d]
10	8.9[c]	1.1	3.3[d]	13.3[c]	6.3	2.13[d]
			Nonparous ewes			
0	10.4[c]	3.9	5.5[c]	19.8[c]	8.2[c]	2.41[c]
5	9.9[c]	1.5[c]	3.6[c]	14.9[c]	6.4[c]	2.33[c]
10	9.5[c]	0.9[c]	3.7[c]	14.0[c]	4.3[c]	3.25[c]

[a] For parous animals 0 weeks was at parturition; for nonparous animals 0 weeks was 160 days after contamination. [b] Ratio = total DDT:dieldrin. [c,d] Means with the same superscript were not significantly different ($p > 0.05$).

Table III. Least-Squares Means for Pesticide Concentration in Ewe Body and Milk Fat by Time of Sampling and Lactation Status

Sampling time, week	DDE, ppm	DDD, ppm	DDT, ppm	Total DDT, ppm	Dieldrin, ppm	Ratio[a]
			Lactating ewes			
0	16.8	2.5[b]	4.7[b]	23.9	20.6	1.16[b]
5	10.7[b]	2.2[b]	4.0[b,c]	16.8	10.8	1.56[b]
10	8.5[b]	1.0	3.3[c]	12.7	3.7	3.39[b]
			Nonlactating ewes			
0	13.5	1.7[b]	4.3[b]	19.4	14.6	1.33[b]
5	10.4[b]	1.3[b,c]	3.7[b]	15.4[b]	10.2	1.51[b]
10	9.1[b]	1.1[c]	3.3[b]	13.5[b]	7.5	1.79[b]
			Milk fat			
0	16.0	0.6[b]	1.8[b]	18.4	10.9	1.69[b]
2.5	7.7[b]	2.6[c]	2.8[b]	13.1[b]	2.9[b]	4.48[c]
5	7.5[b]	1.4[b,c]	3.1[b]	11.9[b]	3.1[b]	3.80[b,c]
7.5	6.6[b]	1.6[b,c]	2.0[b]	10.1[b]	0.8[b]	12.51[c]
10	5.7[b]	1.4[b,c]	1.6[b]	8.7[b]	3.6[b]	2.44[b]

[a] Ratio = total DDT:dieldrin. [b,c] Means with the same superscript were not significantly different ($p > 0.05$).

The concentration of DDT metabolites in milk was proportional to the total DDT concentration in the body fat, as demonstrated by the significant correlation coefficient (0.62) between these two traits (Table IV). Each of the DDT metabolite concentrations in milk fat was proportional to its concentration in body fat. Laben *et al.* (1965) reported proportional concentration of DDT in milk as compared with DDT concentration in body fat. However, dieldrin in milk was not correlated significantly with dieldrin in body fat.

The decline of pesticide metabolites in milk fat was not steady. Concentration of DDT increased from 1.8 ppm during week 0 to 3.1 ppm during week 5, and then declined to 1.6 ppm during week 10. The change in dieldrin concentration in milk fat was also variable. However, total DDT concentration declined steadily throughout the 10 weeks of the trial. Laben *et al.* (1965) reported considerable day-to-day variation in cows' milk DDT metabolite concentration.

As time after parturition progressed, the total DDT:dieldrin ratio increased. In parous animals the ratio in body fat increased after parturition, suggesting a definite effect of pregnancy on the DDT:dieldrin interaction and also that this effect may disappear progressively with time or as residues diminish.

Correlations Among Pesticide Concentrations in Body and Milk Fat. The partial correlation coefficients among pesticide concentrations in ewe body and milk fat are presented in Table IV.

Dieldrin in milk fat was correlated −0.54 and −0.56 with DDT and total DDT concentration in milk fat, respectively. In body fat, dieldrin was correlated −0.63, −0.76, −0.64, and −0.86 with body fat DDE, DDD, DDT, and total DDT concentrations, respectively. Thus, these correlations certainly suggest dieldrin metabolism can be related to that of DDT, as reported by Street (1964).

LITERATURE CITED

Braund, D. G., Brown, L. D., Huber, J. T., Leeling, N. C., Zabik, M. J., *J. Dairy Sci.* **51**, 116 (1968).
Braund, D. G., Brown, L. D., Huber, J. T., Leeling, N. C., Zabik, M. J., *J. Dairy Sci.* **52**, 1988 (1969).

Table IV. Partial Correlations Among Pesticide Concentrations in Ewe Body and Milk Fat[a]

Item[b]	1	2	3	4	5	6	7	8	9	10	11	12
						Milk fat						
(1) DDE	1.00	−0.13	0.19	0.86[e]	−0.45	0.57[d]	0.74[e]	0.00	0.15	0.55[e]	−0.46	−0.06
(2) DDD			−0.29	0.27	0.03	−0.37	−0.14	0.08	−0.38	−0.20	0.01	−0.36
(3) DDT				0.37	−0.54[d]	−0.44	0.09	−0.21	0.01	0.02	0.30	0.02
(4) Total DDT					−0.56[d]	0.47	0.59[d]	−0.04	−0.05	0.62[e]	−0.28	−0.22
(5) Dieldrin						−0.77[e]	−0.09	0.19	0.13	0.03	−0.26	−0.04
(6) Ratio[c]							0.31	−0.10	0.33	0.31	−0.04	0.41
						Body fat						
(7) DDE								0.36	0.51[d]	0.92[e]	−0.76[e]	0.07
(8) DDD									0.30	0.56[d]	−0.63[e]	0.43
(9) DDT										0.75[e]	−0.64[e]	0.64[e]
(10) Total DDT											−0.86[e]	0.36
(11) Dieldrin												−0.30
(12) Ratio[c]												1.00

[a] Partial correlations coefficients were derived on intragroup variance basis; residual d.f. = 15; ewe parturition weight was included as continuous independent variable. [b] For identification, the item numbers across the top of the table correspond to those traits (listed by number in parentheses) down the left margin. [c] Ratio = total DDT: dieldrin. [d] $p < 0.05$. [e] $p < 0.01$.

Durham, W. F., Cueto, C., Hayes, W. J., *Amer. J. Physiol.* **187,** 373 (1956).
Food and Drug Administration, "Pesticide Analytical Manual," United States Department of Health, Education, and Welfare, Washington, D. C., 1969.
Harter, H. L., *Biometrics* **16,** 671 (1960).
Harvey, W. R., "Least squares analysis of data with unequal subclass numbers," United States Department of Agriculture, Agricultural Research Service, 1960, pp 20-28.
Kunze, F. M., Laug, E. P., *Fed. Proc.* **12,** 339 (1953).
Laben, R. C., Archer, T. E., Crosby, D. G., Peoples, S. A., *J. Dairy Sci.* **48,** 701 (1965).
Laben, R. C., Archer, T. E., Crosby, D. G., Peoples, S. A., *J. Dairy Sci.* **49,** 1488 (1966).
National Research Council, "Nutrient Requirements of Domestic Animals. No. 5 Nutrient Requirements of Sheep," 4th ed, revised, Washington, D. C., 1968.
Street, J. C., *Science* **146,** 1580 (1964).
Street, J. C., Blau, A. D., *Toxicol. Appl. Pharmacol.* **8,** 497 (1966).

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Certain Physiological Factors Affecting Organochlorine Pesticide Metabolism in Young Ovines Contaminated *in utero*

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Thirty crossbred lambs (15 males and 15 females), the progeny of ewes contaminated with DDT and dieldrin, were used to determine the effects of placental transfer, length of contamination, sex, and age on the body storage and elimination of these pesticides. Three lambs of each sex were sacrificed at birth. Similar numbers of both a suckling and an artificially raised group were sacrificed at 5 and 10 weeks of age. Placental transfer of the pesticides occurred, resulting in contamination of all newborn lambs. Suckling lambs accumulated significantly more DDT, DDT metabolites, and dieldrin because of constant exposure to pesticide through their mother's milk. Of the suckling lambs, females stored significantly more DDT and DDT metabolites than did males, but dieldrin storage was the reverse.

Placental transfer of organochlorine pesticides (Braund *et al.,* 1968; Finnegan *et al.,* 1949; Hathway, 1965) and residue elimination through the milk (Braund *et al.,* 1967; Brown *et al.,* 1966; Crosby *et al.,* 1967; Gannon and Decker, 1960; Laben *et al.,* 1965; Zweig *et al.,* 1961) have been reported. More information is needed regarding the metabolism of pesticides in young ruminants contaminated *in utero* and subsequently through their mother's milk and regarding the effect of certain physiological factors on the rate of deposition and elimination of residues. Sex affects deposition rate in rats (Durham *et al.,* 1956), but little or no information is available regarding sex effects on pesticide metabolism in ruminants. The purposes of this study were to ascertain placental transfer, and to determine the effect of length of contamination, sex, and age on the rate of deposition and elimination of DDT, DDT metabolites, and dieldrin in progeny of ovine females contaminated *prepartum.*

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