from birds maintained on *N*-^{14}CH-ronidazole; (3) radioactivity in the ampholyte fraction (from both tracers) was associated mostly (≃83%) with amino acids, with the remainder undoubtedly including other ampholytic substances; and (4) the acid fraction was shown to contain six or eight known carboxylic acids, which accounts for ≃70% of total radioactivity. This amounts virtually to complete accountability for the ronidazole molecule in terms of known substances.

Virtually all of the retained radioactivity is due to simple compounds commonly found in normal tissue or which are common dietary components.

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Effect of Testosterone on Metabolism of ^{14}C-Photodieldrin in Normal, Castrated, and Oophorectomized Rats

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Subacute levels (5 μg per day) of ^{14}C-labeled photodieldrin were administered orally to normal adult rats of both sexes and to rats that had been castrated or oöphorectomized within 3 days of birth. Urinary and fecal excretion patterns of ^{14}C activity were determined before and after administration of testosterone to all of the rats. Testosterone greatly enhanced excretion of ^{14}C-labeled metabolites in control females and sex hormone-deficient groups. The major metabolite found in the urine of these animals after testosterone treatment was ^{14}C-ketodieldrin, which was present in only trace amounts before the steroid was given. Testosterone did not affect the levels of ^{14}C-ketodieldrin excreted by control males. Tissue levels of ^{14}C activity in females and sex hormone-deficient males and females after 6 weeks of testosterone administration were comparable to those found in control males. The highest concentrations of ^{14}C activity were found in the kidneys of all four groups. High levels of labeled compounds found in biopsy samples of adipose tissue from females and sex hormone-deficient groups before testosterone administration were not evident at autopsy.

Dailey *et al.* (1970) recently reported a striking sex difference in the excretion, distribution, and storage of ^{14}C-photodieldrin (Figure 1) in rats. From three to ten times as much ^{14}C activity was retained by females as compared to males in all tissues monitored except in kidney, which appeared to be a major storage site in male rats. Adipose tissue was the major storage depot of ^{14}C activity in females. A definite sex difference was also apparent in the metabolites of ^{14}C-photodieldrin isolated from the urine of these rats, as was reported by Klein *et al.* (1970). The principal metabolite found in urine of males was ketodieldrin (Figure 1) but female rats excreted at least four very polar, nonvolatile metabolites, none of which were identified as ketodieldrin by the methods employed. Because of these findings, it became of interest to determine if the excretion, distribution, or storage of ^{14}C-photodieldrin, given in subacute doses, could be altered by administration of testosterone to normal rats and rats deficient in sex hormones.

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METHODS AND MATERIALS

The ^{14}C-photodieldrin, unlabeled photodieldrin, and ketodieldrin used in this study were part of the stock chemicals described by Dailey *et al.* (1970) and Klein *et al.* (1970). The instruments used to isolate, identify, and measure the metabolites and compounds of interest are listed in the same references and, in addition, a gas microcoulometer chromato-

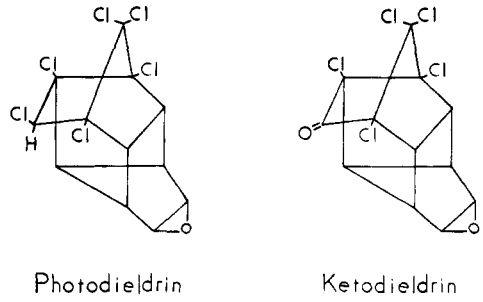

Figure 1. Structural formulas of photodieldrin and ketodieldrin

Table I. Average Weight of Rat Groups (Grams)

	Beginning of experiment	Tenth week	End of fifteenth week
Control male	350	460	515
Control female	215	305	350
Castrated male	280	390	450
Oöphorectomized female	225	325	375

graph, Model C-200A, described by Guiffrida and Ives (1969), was used.

Procedure. Twelve Osborne–Mendel rats of each sex were selected from our colony at birth. Six of the male rats were castrated during the first 24 hr after birth and six females were oöphorectomized within 3 days after birth, as described by Brouwer and Dailey (1971). The remaining six rats of each sex were subjected to sham procedures but any trauma to the sex organs was avoided; these rats constituted the control male and female groups.

After weaning, the rats were placed in individual metabolism cages and fed Purina rat pellet chow and water *ad libitum* until they reached the age of 4 months. At this time all groups were given daily oral doses of 5 μg of ^{14}C-photodieldrin dissolved in 0.25 ml of corn oil 5 days a week for a total of 15 weeks. At the beginning of the seventh week the rats were anesthetized with Surital (sodium 5-allyl-5-(1-methylbutyl)- 2- thiobarbiturate; Parke, Davis & Co.) (40 mg/kg intraperitoneally) and biopsies of adipose tissue were taken surgically from each rat. At the beginning of the tenth week each rat was given 5 mg of Depo-testosterone cyclopentylpropionate (The Upjohn Co.) in cottonseed oil intramuscularly, and this dose was repeated on the first day of each succeeding week through the fifteenth week. The average weights of each group of rats at the beginning of the study, at the tenth week, and at the end of the fifteenth week are shown in Table I. Procedures used for urine and fecal collection and for preparation and storage of samples, as well as urine, fecal, and tissue analyses, have been described previously (Dailey *et al.*, 1970).

Analysis of Urine. One-hundred milliliters of urine was blended successively with ethyl alcohol, ethylether, and petroleum ether (Klein *et al.*, 1968). The blended mixture was carefully transferred to a 1-l. separatory funnel and allowed to equilibrate. The lower layer (aqueous phase) was drained off; the volume varied from 185 to 192 ml. The ^{14}C activity was measured as described by Dailey *et al.* (1970). The volume of the upper organic layer also varied from 185 to 192 ml. A 100-ml aliquot of this organic phase (equivalent to about 55 ml of urine) was taken to dryness and dissolved in 20 ml of hexane (equivalent to about 2.75 ml of urine per milliliter).

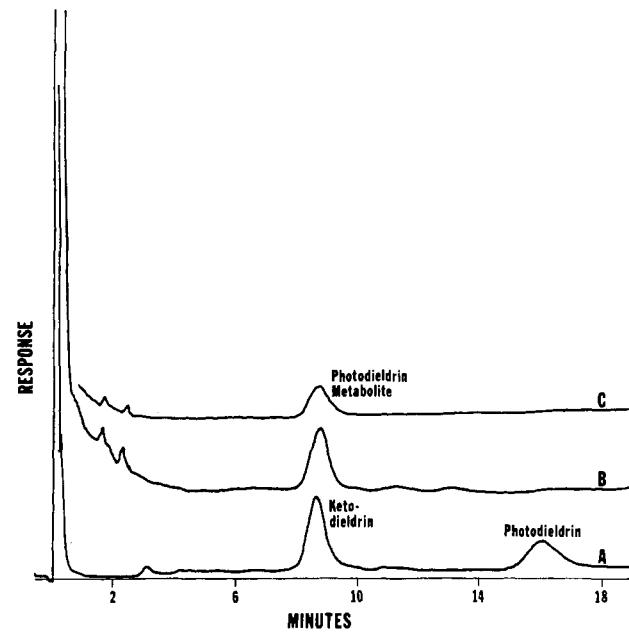

Figure 2. Glc analysis of urine collected during the tenth week of the experimental period. (A) Standard ketodieldrin and photodieldrin, 0.1 μg/ml, 3 μl injected. (B) Urine extract from female control rats; 1 ml equivalent to 10 ml of urine; 20 μl injected. (C) Urine extract from oöphorectomized rats; 1 ml equivalent to 5 ml of urine; 6 μl injected

Although this solution was not pure enough to yield accurate glc results when it was injected directly into the instrument, the tracings indicated that the urine samples under study contained very little or no photodieldrin. Aliquots of the solution were also examined for ^{14}C activity. The solution was then made sufficiently pure for reliable glc analysis by successive elution through activated Florisil (Klein *et al.*, 1968). Because the control female and sex hormone-deficient males and females excreted abnormally large quantities of ketodieldrin after testosterone administration, it was necessary to confirm the identity of the metabolite by means other than glc. About 3 l. of the urine from oöphorectomized females, collected between weeks 10 and 15 during testosterone administration, was pooled; the composite, according to glc analysis, was expected to contain about 100 μg of ketodieldrin. About the same volume of urine from control females collected between weeks 10 to 15 was also processed. Each urine pool was blended, extracted with acetonitrile, and further purified by solvent elution through activated Florisil (Klein *et al.*, 1968); glc analysis showed that the recovery of the metabolite was almost quantitative. These extracts were then used for mass spectral and infrared analysis to confirm that the metabolite from both groups of rats was identical to the ketodieldrin standard. Figure 2 shows the gas chromatographic pattern obtained from urine extracts from the control females and oöphorectomized females during the tenth week (the first week of testosterone administration). The photodieldrin metabolite had the same retention time as the ketodieldrin standard and no photodieldrin was detected.

Analysis of Kidney Samples. A 2- to 3-g sample was ground thoroughly in a mortar with 5 g of sand until dispersed and then with 5 g of anhydrous Na_2SO_4. The mixture was transferred to a stoppered 125-ml Erlenmeyer flask. The material remaining in the mortar was washed with three successive 25-ml portions of ethyl ether, which were added to the flask. After the suspension was shaken for 3 to 5 min, the

liquid was filtered and evaporated. The extraction proved to be quantitative. Without any other intermediate purification step, the residue was dissolved in petroleum ether and eluted through activated Florisil (Klein *et al.*, 1968). The final 5% acetone solvent contained both ketodieldrin and photodieldrin. Results obtained by glc were compared to those obtained by radiochemical measurements.

Analysis of Fat. A 2- to 2.5-g sample of fat was ground, leached with ethyl ether, and treated exactly as described above for the kidney samples. After all water was removed by evaporation on the steam bath, the residue was transferred to a 125-ml separatory funnel with 30 ml of hexane. The extract was shaken 3 min with 30 ml of acetonitrile (saturated with petroleum ether), the acetonitrile layer was transferred to a 1-l. separatory funnel, and the hexane layer was successively extracted with 10-ml and 5-ml portions of acetonitrile. The combined extracts were diluted with 500 ml of water and shaken vigorously with 100 ml of petroleum ether. The petroleum ether layer was removed, dried by filtering through anhydrous Na_2SO_4, and evaporated, and the residue was placed on an activated Florisil column. The purpose of this part of the work was to isolate and measure ketodieldrin and photodieldrin separately with a minimum of extraneous sample background. The column was washed with 50 ml of petroleum ether and 50 ml of 5% ethyl ether in petroleum ether, and both washings were discarded. The photodieldrin was then eluted with 75 ml of 15% ethyl ether in petroleum ether and the ketodieldrin was eluted with 75 ml of 20% ethyl ether in petroleum ether. Each eluate was analyzed separately by both glc and radiochemical measurements.

RESULTS AND DISCUSSION

None of the rats displayed any signs of toxic manifestations during the experiment. Hydronephrosis of both kidneys was found in one of the control males and one of the oöphorectomized females at autopsy. No other pathological conditions were observed macroscopically.

The male control rats consistently excreted a greater percentage of the administered dose of ^{14}C activity in their urine than either the female controls or the two sex hormone-deficient groups throughout the first 9 weeks of the study, with the exception of the seventh week, when all the animals were subjected to barbiturate anesthetization and adipose tissue biopsy (Table II). In that week the urinary excretion of ^{14}C activity by the female control and both sex hormone-deficient groups increased markedly; the fecal excretion of ^{14}C-labeled compounds increased in all of the animals. Urinary excretion of radio-labeled compounds by the male control group was not affected by the biopsy procedure. In the tenth week of the study, testosterone administration was begun and at this time a striking increase in urinary excretion of ^{14}C activity was observed in the castrated males and oöphorectomized females; fecal excretion of labeled compounds increased moderately. Only a slight increase in urinary excretion of ^{14}C compounds was seen in either the male or female control groups. In the second week of testosterone administration (eleventh week), the urinary and fecal excretion of ^{14}C activity by the female control group was increased noticeably. The excretion pattern of the male control group was not appreciably altered by testosterone administrations. Between the tenth and fifteenth weeks the urinary

Table II. Excretion of ^{14}C Activity; Percent of Administered Dose[a]

Week	Excreted in	Control male		Control female		Castrated male		Oöphorectomized female	
		Weekly	Cumulative	Weekly	Cumulative	Weekly	Cumulative	Weekly	Cumulative
1	Urine	19.6	19.6	2.7	2.7	7.2	7.2	3.0	3.0
	Feces	25.0	25.0	16.7	16.7	17.1	17.1	15.6	15.6
2	Urine	35.1	27.3	6.4	4.6	12.7	9.9	7.1	5.0
	Feces	29.9	27.4	29.2	23.0	26.6	21.9	31.7	23.6
3	Urine	37.5	30.7	7.8	5.6	14.9	11.6	9.0	6.4
	Feces	28.5	27.8	34.7	26.9	30.3	24.7	26.7	24.7
4	Urine	37.9	32.5	9.6	6.6	14.9	12.4	12.7	8.0
	Feces	30.0	28.3	33.8	28.6	35.8	27.5	38.8	28.2
5	Urine	40.3	34.1	10.8	7.5	15.3	13.0	13.3	9.0
	Feces	31.3	28.9	38.3	30.5	34.0	28.8	37.1	30.0
6	Urine	39.1	34.9	11.9	8.2	16.5	13.6	16.4	10.3
	Feces	31.3	29.3	41.8	32.4	38.5	30.4	41.1	31.8
7[b]	Urine	39.8	35.5	35.5	11.4	41.5	16.9	47.3	14.6
	Feces	53.5	32.2	74.0	37.3	69.4	35.0	80.9	37.7
8	Urine	40.5	36.0	15.0	11.8	19.2	17.1	18.0	15.0
	Feces	34.1	32.4	43.8	38.0	41.1	35.6	38.9	37.7
9	Urine	35.2	35.9	11.8	11.8	15.2	16.9	12.7	14.7
	Feces	35.5	32.7	41.5	38.4	34.2	35.5	33.7	37.3
10[c]	Urine	42.1	36.6	14.5	12.1	37.3	19.0	31.6	16.5
	Feces	35.3	33.0	46.2	39.2	40.9	36.0	41.2	37.7
11[c]	Urine	38.9	36.8	29.3	13.7	74.9	24.3	56.7	20.3
	Feces	37.1	33.4	55.8	40.8	47.3	37.1	43.2	38.2
12[c]	Urine	39.7	37.0	57.3	17.5	66.4	27.9	51.9	23.0
	Feces	35.9	33.6	54.5	41.9	45.2	37.8	34.1	37.9
13[c]	Urine	38.9	37.2	65.9	21.3	54.1	30.0	46.8	24.9
	Feces	36.8	33.9	47.7	42.4	35.2	37.6	37.9	37.9
14[c]	Urine	39.2	37.3	55.5	23.8	43.2	31.0	38.4	25.9
	Feces	38.6	34.2	41.0	42.3	36.9	37.5	34.3	37.6
15[c]	Urine	36.1	37.3	51.6	25.7	43.9	31.9	35.6	26.5
	Feces	35.1	34.3	42.7	42.3	33.7	37.3	33.4	37.3

[a] ^{14}C-Photodieldrin was administered 5 days a week for 15 weeks. [b] During this week, rats were anesthetized with Surital and a sample of adipose tissue was removed surgically. [c] 5 mg of Depo-testosterone cyclopentylpropionate in cottonseed oil was given intramuscularly on the first day of the week.

Table III. Tissue Levels (ppb) of ^{14}C Activity[a]

Tissue[b]	Control male	Control female	Castrated male	Oöphorectomized female
Fat (biopsy)	306	1780	1170	1420
Whole blood	5.9	10.8	6.1	6.6
Skeletal muscle	10.8	7.7	7.4	9.2
Brain	9.0	14.7	10.0	13.2
Spleen	12.0	20.1	12.0	15.3
Heart	13.1	15.8	16.4	13.7
Adrenals	38.9	67.5	37.8	55.4
Liver	116	136	117	114
Lung	280	299	274	164
Fat	249	383	252	335
Kidneys	1250	1530	1290	1020

[a] Calculation of values as ppb were based on the specific activity of the ^{14}C-photodieldrin administered. [b] The fat biopsy sample was obtained at the beginning of the seventh week. Testosterone administration was started at the beginning of the tenth week. All other samples were obtained at autopsy at the end of the fifteenth week.

and fecal excretions of labeled compounds by the female controls and the sex hormone-deficient groups reached a peak and began to return to levels near those reached by the male control group. At the end of the fifteenth week, the total cumulative excretion values (urine plus feces) for all four groups were nearly the same (Table II). Therefore it was expected that tissue storage levels in all the groups would be similar, and this was indeed the case (Table III). Analysis of the fat biopsy samples obtained before testosterone administrations were started showed the usual pattern of sex difference reported earlier by Dailey *et al.* (1970). Females stored nearly six times as much toxicant in their fat as males. Adipose tissue storage of ^{14}C activity by the castrated male group was almost four times as great as that observed in the control male group. After 6 weeks of testosterone injections, a striking decrease in fat storage of labeled compounds was found in the female controls and in both sex hormone-deficient groups. Storage in all the tissues assayed in these groups was roughly comparable to that found in the male control group. It should be noted that storage values used in this report are based on the concentration of ^{14}C activity in a weighed aliquot of tissue rather than on total storage in that tissue.

After testosterone administration, the highest tissue levels of ^{14}C activity were found in the kidneys in all four groups of rats. This confirmed the results of our previous report (Dailey *et al.*, 1970) for male rats and is in sharp contrast to the low kidney storage levels in normal female rats. In that study, which was conducted under almost identical conditions, normal female rats not receiving testosterone had the highest levels of ^{14}C activity in adipose tissue (about 2000 ppb) while only 150 to 160 ppb was found in their kidneys. Testosterone apparently alters both the excretory and tissue storage patterns of ^{14}C-photodieldrin by female rats and sex hormone-deficient rats of both sexes. The lack of testosterone in the castrated male rats (first 9 weeks) caused them to metabolize and excrete ^{14}C-photodieldrin and its metabolites in a manner similar to control females.

Solubility characteristics of the urinary metabolites from each group of rats were determined by extracting aliquots of the weekly pools with ethyl ether. ^{14}C activity in the ethyl ether extracts and the residue in the aqueous phase were determined; the results are shown in Table IV, which also includes the amount of ketodieldrin found in each of the ethyl ether extracts, as determined by glc. The amount of ketodieldrin found by glc was always less than that calculated from the ^{14}C measurement; apparently compounds other than ketodieldrin present in the solution were radioactive. During the first 9 weeks of the study, the male control rats excreted markedly higher levels of ethyl ether-soluble ^{14}C-labeled compounds than the other three groups; the average amount was 81.8% of the total ^{14}C activity of the unextracted urine (excluding the seventh week), compared with 37.4% for the female controls, 50.2% for the castrated males, and 39.2% for the oöphorectomized females.

During the first 9 weeks of the study only trace amounts of ketodieldrin were found in the urine of the female control group, but slightly higher levels were seen in the sex hormone-deficient groups. However, after testosterone administration was started in the tenth week, a marked increase was observed in the ethyl ether-soluble ^{14}C metabolites in the urine of the control females, castrated males, and oöphorectomized females (an average of 69.6, 82.1, and 80.4%, respectively, of

Table IV. Total Metabolite Content of Urine Fractions and Ketodieldrin Content of Ethyl Ether-Soluble Fraction (μg/l. of Urine)[a]

	Control male			Control female			Castrated male			Oöphorectomized female		
Week	Aqueous phase	Ethyl ether extract	Keto-dieldrin	Aqueous phase	Ethyl ether extract	Keto-dieldrin	Aqueous phase	Ethyl ether extract	Keto-dieldrin	Aqueous phase	Ethyl ether extract	Keto-dieldrin
1	4.5	24.1	20.0	2.6	1.3	0.8	3.1	6.0	3.2	2.3	1.4	1.5
2	7.2	44.3	30.1	6.4	4.1	1.0	6.1	9.7	5.9	6.1	3.3	2.1
3	7.2	43.8	32.2	7.6	4.8	1.1	5.7	6.1	5.8	6.1	4.9	3.5
4	9.4	41.4	32.2	8.4	6.1	1.2	6.5	6.3	5.3	5.7	6.3	2.5
5	9.8	37.0	32.2	8.5	7.8	0.6	6.9	5.0	4.4	8.3	2.4	2.6
6	9.1	45.0	35.0	11.0	6.9	0.4	10.8	10.6	4.9	12.0	7.6	2.0
7	9.6	33.3	30.7	32.1	5.7	0.4	30.2	8.2	3.7	48.2	8.8	2.8
8	8.4	34.0	30.8	11.7	4.9	0.8	9.0	5.3	3.0	11.2	6.5	2.1
9	8.1	31.0	23.8	11.2	4.8	0.8	11.0	8.9	3.5	9.7	7.1	2.1
10	10.5	39.0	31.8	13.1	8.4	5.8	13.5	41.1	32.5	14.2	26.7	16.5
11	11.0	39.9	30.9	16.2	20.6	11.3	17.5	84.7	67.4	13.6	69.1	32.5
12	9.8	47.4	41.0	18.5	58.2	19.1	14.3	75.5	58.0	12.0	59.4	42.0
13	9.8	49.0	35.2	18.4	76.3	40.9	13.1	63.5	45.6	9.0	50.2	35.6
14	10.4	49.0	36.0	13.9	66.3	36.0	8.6	49.0	47.1	8.2	39.0	31.7
15	8.5	40.6	29.4	11.3	55.8	23.4	10.6	50.1	49.0	9.1	44.6	31.2

[a] Calculations of total metabolites in the aqueous phase and ethyl ether extract were based on specific activity of ^{14}C-photodieldrin administered. Ketodieldrin was determined by glc of the ethyl ether extract.

the total ^{14}C activity in the urine). The radioactivity of the ether-soluble fraction (averaging 81.4% of the total activity) did not increase in the male control group after testosterone. A significant increase in ketodieldrin excretion was also apparent in the control females, castrated males, and oöphorectomized females after testosterone administration. There was not a noticeable increase in the urine volume in these three groups during the period of testosterone treatment.

During the seventh week of the experiment (the week in which the animals were anesthetized with a barbiturate compound and adipose tissues were taken surgically) marked increases in urinary and fecal excretions of labeled metabolites were observed in all but the male control group (Table II). As shown in Table IV, all of the increase in urinary excretion was due to water-soluble compounds; ketodieldrin was not increased. One possible explanation for this finding is that the barbiturate induced liver microsomal hydroxylating enzymes, resulting in an increased metabolism of mobilized fat stores of photodieldrin and/or its metabolites to highly polar hydroxylated compounds. Storage depots were probably involved since more than 100% of the administered dose of ^{14}C activity was excreted during that weekly period (Table II). Surgical trauma to adipose tissue is an unlikely explanation for the increased excretion, since storage of such polar compounds in adipose tissue would not be expected. However, surgical stress could have contributed to mobilization of the pesticide stored in the fat.

In order to study more fully the storage patterns of ^{14}C-photodieldrin and its metabolites in kidney, autopsy samples of renal tissues were extracted and the extracts were cleaned-up, chromatographed, and counted. Of the original tissue radioactivity, 78 to 98% was recovered in these extracts. Table V shows that the amount of ketodieldrin in the kidney was four to seven times the amount of photodieldrin. This finding was not unexpected since, after testosterone administration, ketodieldrin was being excreted into the urine in significant quantities by all the rats; presumably photodieldrin was being metabolized to ketodieldrin at a rapid rate after testosterone administration was begun.

Biopsy and autopsy samples of fat were also analyzed for their ketodieldrin and photodieldrin content (Table VI). Although control females and the sex hormone-deficient groups had excreted only small amounts of ketodieldrin in the urine, the biopsy samples of these groups contained high levels of the metabolite. Apparently an enzyme system capable of converting photodieldrin to ketodieldrin does exist in female and sex hormone-deficient rats, but for some unknown reason urinary excretion of ketodieldrin and photodieldrin is impaired.

Fat samples obtained at autopsy showed markedly decreased levels of both ketodieldrin and photodieldrin in all but the control male group (Table VI). Ketodieldrin levels in all four groups were nearly the same at autopsy but photodieldrin levels in the two male groups were roughly $^1/_3$ to $^1/_2$ those found in the female groups. The reason for this sex difference after testosterone treatment is not clear; however, it is possible that if testosterone administration had been continued for a longer period of time, this difference might have disappeared.

It is likely that the fat catabolic properties of testosterone played a role in the increase in urinary levels of ^{14}C-labeled compounds, including ketodieldrin. Fat mobilization would result in a decreased total storage capacity for lipid-soluble compounds. This fact, coupled with increased transport across the kidney tubules after testosterone, would account

Table V. Ketodieldrin and Photodieldrin Content (ppb) of Kidney Tissue at Autopsy[a]

Compound	Control male	Control female	Castrated male	Oöphorectomized female
Ketodieldrin (KD)	933	1250	802	869
Photodieldrin (PD)	136	N.D.[b]	198	135
KD/PD ratio	6.9	...	4.0	6.4

[a] Determined by glc. [b] Not detectable.

Table VI. Ketodieldrin and Photodieldrin Content (ppb) of Adipose Tissue[a]

Compound	Control male	Control female	Castrated male	Oöphorectomized female
	Biopsy samples			
Ketodieldrin (KD)	157	673	435	700
Photodieldrin (PD)	42	440	544	453
KD/PD ratio	3.7	1.6	0.8	1.5
	Autopsy samples			
Ketodieldrin (KD)	195	201	197	142
Photodieldrin (PD)	64	117	47	148
KD/PD ratio	3.0	1.7	4.2	1.0

[a] Determined by glc.

for a part of the increase in urinary excretion of ^{14}C activity, shown in Tables II and IV. A more important factor contributing to this finding, however, would be an increased rate of metabolism of photodieldrin mediated by testosterone administration. This is demonstrated by the fact that the decrease in ketodieldrin levels in the fat stores of control females and the sex hormone-deficient groups after testosterone administration (Table VI) is not nearly large enough to account for the increased amounts of ketodieldrin excreted in the urine of these animals during the 6-week period of testosterone treament (Table IV). Therefore testosterone not only facilitates the excretion of ketodieldrin into the urine, but also stimulates the metabolic conversion of photodieldrin to ketodieldrin, resulting in a greatly reduced tissue accumulation of the toxicants in rats.

The data suggest that the metabolism of photodieldrin can be accomplished by at least two different pathways in female and sex hormone-deficient rats: to water-soluble polar metabolites after barbiturate administration; and to ethyl ether-soluble metabolites (mostly ketodieldrin), a pathway which is enhanced after testosterone supplements. In male control rats the second pathway predominated and did not appear to be significantly altered by barbiturate under these experimental conditions. However, the first pathway might have been induced in male control rats if the barbiturate treatment had been continued over a longer period of time.

In the rat, sex differences in the storage of DDT and the influence of hormones have been studied by Durham *et al.* (1956). These investigators found that testosterone propionate or oöphorectomy decreased DDT storage in female rats, while testectomy increased DDT storage in males. The results reported here with photodieldrin are essentially in agreement with these observations.

The results of this study support the findings of Kato *et al.* (1962), who reported the effect of sex hormones on the metabolism of certain drugs in rats. They observed that castra-

tion decreased the high activity of the liver enzyme responsible for breakdown of carisoprodol in male rats, but spaying did not modify the enzyme activity in female rats. Treatment with testosterone increased the enzyme activity in hormone-deficient rats of both sexes.

The concept of stimulation of separate metabolic pathways by testosterone and barbiturate has been discussed previously by Gillette (1963), who noted that phenobarbital and methyltestosterone produced additive stimulatory effects on aminopyrine *N*-demethylation and concluded that two different pathways were involved. The results of this study support such a viewpoint for the metabolism of photodieldrin in rats.

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Inhibition of Biological Activity of Cholecalciferol (Vitamin D_3) by *o,p'*-DDT or *p,p'*-DDT in Rachitic Cockerel

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Considerable evidence suggests that chlorinated hydrocarbons cause thin eggshell formation, apparently in part through an altered calcium metabolism of adult birds and thus reproductive failure in raptors. This report concerns a comparative study of some of the known biological effects of cholecalciferol (vitamin D_3), a major steroid regulator of Ca^{2+} metabolism, in *o,p'*-DDT [1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane], or *p,p'*-DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] treated and untreated rachitic chicks. It was found that *o,p'*-DDT or *p,p'*-DDT treatment decreased the normal cholecalciferol (CC) mediated increase in intestinal calcium absorption, when assayed *in vivo* or *in vitro*, bone calcium resorption, and intestinal alkaline phosphatase activity. It is suggested that *o,p'*-DDT or *p,p'*-DDT interference in some of the biological responses to CC may furnish a partial explanation for the thin eggshell formation and thus reproductive failure of some raptors such as the peregrine falcons, brown pelicans, bald eagles, and sparrow hawks.

A number of recent reports in the technical literature (Ratcliffe, 1967; Porter and Wiemeyer, 1969; French and Jefferies, 1969; Hickey, 1969; Robinson, 1970; Edwards, 1970) and the public news media (Bengelsdorf, 1969; Moser, 1971) have focused attention on some of the ecological hazards that result from the widespread occurrence of chlorinated hydrocarbons, such as DDT and its major metabolite DDE, in the biosphere. The insecticide commonly used is technical grade with a composition of 79% of *p,p'*-DDT [1,1,1-trichloro-2-bis(*p*-chlorophenyl)ethane] and 20% of *o,p'*-DDT [1,1,1-trichloro-2-(*p*-chlorophenyl)-2-(*o*-chlorophenyl)ethane]. These compounds have been suggested as the possible cause for altered calcium metabolism and subsequent avian reproductive failures. It has also been shown that dietary dieldrin and/or DDT presence in a variety of avian species leads to avian reproductive failures (Edwards, 1970).

Raptors in Britain, including the peregrine (*Falco peregrinus*), sparrow hawk (*Accipiter nisus*), and golden eagle (*Aquila chrysaetos*), have reproduced less successfully with the increased use of chlorinated hydrocarbons (Ratcliffe, 1958, 1960, 1967) and egg breakage in the nest seems to be a major part of the phenomenon. Contamination by dieldrin has been suspected of causing increased egg breakage in shag (*Phalacrocorax aristotelis*) populations in Britain (Potts, 1968).

In the United States, declining populations of bald eagles (*Haliaetus leucocephalus*) and ospreys (*Pandion haliaetus*) and extirpated populations of peregrines have been related to decreases in eggshell weight (Hickey and Anderson, 1968). High levels of DDT and its metabolites in the eggs of herring gulls (*Larus argentatus*) have also been correlated with increased egg cracking (Ludwig and Tomoff, 1966) and decreased eggshell thickness (Hickey and Anderson, 1968).

A captive population of American sparrow hawks (*Falco sparverius*), given a diet containing both dieldrin and DDT,

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