Effect of Dietary Supplements of Vitamins A, D, and E on Body Burdens of DDT in the Rat

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In a study of nutrient–pesticide interactions, the rate of depletion of DDT in adipose tissue of the rat was measured when the diets contained various levels of supplemental vitamin A, D, or E. The vitamin A status of the rat did not influence the rate of depletion or metabolism of DDT. Similarly, vitamin D and the supplementation of a nutritionally adequate diet (laboratory cubes) with vitamin E did not hasten the depletion of body burdens of DDT. The use of dietary supplements of fat-soluble vitamins does not appear to have practical significance for decreasing body burdens of DDT.

The reduction of body burdens of persistent organochlorine pesticides in man and domestic mammalian and avian species of economic importance has been the subject of many studies. The principles, in most cases, have been to decrease total body fat by modification of the diet, thus increasing excretion of the pesticide, or to alter metabolism of the contaminant by use of drugs. Regimens using thyroprotein and a low-energy ration (Miller, 1967) or periods of starvation followed by full feeding (Donaldson *et al.*, 1968) have a disadvantage from an economic standpoint in that an appreciable loss of body weight occurs.

Acceleration of the metabolic degradation of organochlorine pesticides has been accomplished by the use of drugs and other pesticides. Street (1964; Street *et al.*, 1966) demonstrated that threatment of rats with DDT caused significant reductions in dieldrin storage in the adipose tissue. This probably results from induction of hepatic mixed function oxidases, since barbiturates (Cueto and Hayes, 1965) evoke a similar response. More recently, Davies *et al.* (1969) observed, in a group of patients taking anticonvulsant drugs for more than 3 months, lower DDE levels in the blood than had been found in the general population. Phenytoin (diphenylhydantoin) had a potent action in reducing blood DDE levels and phenobarbitone was moderately active for this purpose. Samples of adipose tissue confirmed the findings that the burden of DDT-derived materials could be reduced or even eliminated.

Although some of the methods described above have potential for reduction of pesticide residues in animals, a more desirable approach would be one that did not decrease body weight or leave drug residues. Such an approach was suggested by Hironaka (1968) who demonstrated that although the feeding of a submaintenance ration to reduce body fat did not influence the rate of dieldrin elimination in cattle, the administration of vitamins A, D, and E appeared to reduce the residue half-concentration time. We have therefore studied the depletion of body burdens of DDT and metabolites in the rat in relation to dietary supplements of each of the above vitamins.

EXPERIMENTAL

The experimental design of all experiments was similar in that male Wistar rats were fed either semipurified diets or standard laboratory cubes (Maple Leaf Mills, Master Feeds Division, Toronto, Canada) and, following a specified period, all animals were dosed orally with p,p'-DDT in corn oil (25 mg/kg of body weight). A few days after pesticide dosing, initial control animals were killed, and liver, blood, and adipose tissue were removed and analyzed for DDT and metabolites. The remaining animals were placed at random into groups receiving diets supplemented with various

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Table I. Effect of the Level of Dietary Vitamin A on the Concentration of DDT and Metabolites in Adipose Tissue (Experiment 1)

Group	1	2	3	4
Number of animals	9	9	8	9
iu Vitamin A/kg diet	...	500	2000	40,000
Days on diet	0	34	34	34
Body weight (g)	188 ± 7[a]	256 ± 16	310 ± 16	320 ± 10
Liver weight (g)	...	13 ± 1	15.8 ± 0.5	13.3 ± 0.3
iu Vitamin A/g liver	...	23.6 ± 1.9	47.5 ± 3.4	1232 ± 134
Adipose tissue (ppm)				
DDT	117.2 ± 6.8	29.6 ± 2.8	21 ± 2.2	25.7 ± 2.3
DDD	7.6 ± 0.8	1.8 ± 0.1	1.9 ± 0.2	1.6 ± 0.1
DDE	6.3 ± 0.4	3.6 ± 0.5	3.0 ± 0.6	2.9 ± 0.3
Total	131 ± 7.3	34.9 ± 3.3	25.9 ± 2.7	30.2 ± 2.4
Metabolites as % of total	10.6	15.5	19.1	14.9

[a] ± standard error of the mean.

amounts of vitamins A, D, or E. Animals were killed after approximately 28 days on diet and pesticide residues were determined in the various tissues. DDT and metabolites in adipose tissue were determined by the following procedure: 200 mg of fat was blended with 100 ml of hexane and 50 g of anhydrous sodium sulfate for 10 min. The extract was filtered, the volume reduced to 10 ml, and 3 ml of concentrated sulfuric-fuming sulfuric acids (1:1) added to precipitate the fat. After mixing, the tubes were centrifuged and the hexane layer transferred to another test tube. Four grams of sodium sulfate–sodium carbonate (9:1) was added to the hexane extract and an aliquot was used for glc analysis for DDE, DDD, and DDT (McCully and McKinley, 1964). In blood, DDT and metabolites were determined by the method described by Dale *et al.* (1966). Total body fat and pesticide residues were determined in the total rat body minus the liver in experiment 3. Individual carcasses were freeze-dried for 7 days and mechanically ground to a fine powder. Crude fat was determined on a 2-g sample by the Offical Methods of Analysis (1965). DDT and metabolites were determined in an aliquot of the crude ether extract as described above for adipose tissue.

Experiment 1. Effect of Level of Dietary Vitamin A. Thirty-six weanling male Wistar rats were maintained on a standard vitamin A deficient diet for 36 days prior to dosing with DDT. The percent composition of the basal diet was vitamin-free casein, 18%; sucrose, 65%; salt mix (U.S.P. XIV), 4%; dried brewers yeast, 8%; and corn oil, 5%. The corn oil contained 60 mg of added DL-α-tocopherol acetate, 8000 units vitamin D_2, and 1.5 mg of menadione per 100 g of oil. All animals were dosed orally with *p,p'*-DDT in corn oil (25 mg/kg of body weight). Nine animals were killed 5 days after dosing and abdominal fat pads removed for DDT analyses. The remaining animals were divided into three groups and fed the above diet which was supplemented with 500, 2000, or 40,000 iu of stabilized vitamin A (A.N.R.C. Reference Standard) per kilogram of diet. These dietary levels of vitamin A represent 25% of the nutrient requirement, and 20 times the requirement level for the rat. After 37 days feeding, all animals were killed and liver and fat pads removed for analyses.

Experiment 2. Effect of Supplemental Vitamin D. Weanling male rats were maintained on stock cubes for 14 days, at which time average body weight was in excess of 100 g. All animals were dosed orally with a corn oil solution containing *p,p'*-DDT to give a dose of 25 mg DDT/kg of body weight. Twelve animals were killed 3 days after dosing and abdominal fat pads removed for DDT analyses. The remaining animals were divided into three groups and fed a semipurified diet to which was added 200, 400, or 4000 units of vitamin D (calciferol) per kilogram of diet. The composition of the basal diet was similar to that used in the previous experiment except that 6000 iu of stabilized vitamin A was added per kilogram of diet. The animals were fed the appropriate diet for 28 days following the DDT dosing, killed, and abdominal fat pads analyzed for DDT and metabolites.

Experiment 3. Effect of Supplemental Vitamin E. Thirty-five male rats (average body weight 133 g) were dosed orally with *p,p'*-DDT in corn oil at a level of 25 mg per kg of body weight. Three days after dosing, eight animals were killed and tissues removed. The remaining animals were divided into three groups and fed the standard ground cube diet to which was added 3% corn oil containing α-tocopherol at a level of 0, 60, or 600 mg/kg of diet. Tocopherol determinations were not made on the basal diet. These animals were killed 28 days after receiving the oral dose of DDT.

RESULTS

Experiment 1. The dietary levels of vitamin A were effective in producing animals at various planes of vitamin A nutrition as indicated by liver vitamin A stores and the decreased rate of growth by those animals consuming only 25% of the requirement (Table I). The oral dosing of the rats with *p,p'*-DDT produced body burdens of 131 ppm DDT and metabolites in the adipose tissue of which 10% of the total residue was composed of the metabolites DDD and DDE. Over the 37-day depletion period, although body weight increased by less than a factor of two, total residues in the adipose tissue decreased to approximately 20% of the initial values. Further confirmation of metabolism of DDT during this period is shown in the increase in the percentage of metabolites from an initial value of 10 to final average values between 15 and 19%. Analysis of variance indicated no significant effect of the level of dietary vitamin A from 500 iu/kg to 40,000 iu/kg on the final concentrations of *p,p'*-DDT, DDD, or DDE in the adipose tissue. However, treatment had a significant effect ($P = 0.05$) on the total residue. This effect was limited to a higher total residue in animals receiving 500 iu vitamin A/kg diet and no difference

Table II. Effect of Dietary Vitamin D on Tissue Levels of DDT and Metabolites (Experiment 2)

Group	1	2	3	4
Number of animals	10	9	9	9
Units vitamin D per kg diet	...	200	400	4000
Days on diet	0	28	28	28
Body weight (g)	113	235	257	248
Adipose tissue (ppm)				
DDT	109.7 ± 17.5[a]	31.6 ± 3.3	37.3 ± 8.5	40.4 ± 3.5
DDD	16.1 ± 1.8	3.3 ± 0.4	2.1 ± 0.6	3.3 ± 0.3
DDE	9.3 ± 1	3.9 ± 0.4	5.6 ± 1.7	5.1 ± 0.7
Total	135.1 ± 19.2	40.8 ± 5.1	45 ± 10.6	48.8 ± 4
Metabolites as % of total	...	17.6	17.1	17.2
Blood (ppb)				
DDT	...	10.4 ± 1.6	9.9 ± 1.5	12.5 ± 2.3
DDD	...	4.6 ± 0.9	6.9 ± 1.7	5.2 ± 1
DDE	...	2.8 ± 0.3	3.6 ± 0.8	5.2 ± 1.6
Total	...	17.8 ± 1.9	20.4 ± 3.4	23 ± 2.9

[a] ± standard error of the mean.

Table III. Effect of Dietary Vitamin E on Tissue Levels of DDT and Metabolites (Experiment 3)

Group	1	2	3	4
Number of animals	8	9	9	9
Vitamin E mg per kg diet	...	0	60	600
Days on diet	0	28	28	28
Body weight (g)	156 ± 6[a]	288 ± 13	298 ± 12	297 ± 8
Adipose tissue (ppm)				
p,p'-DDT	116.8 ± 9.3	33.3 ± 2.4	19.5 ± 1.5	19.9 ± 1.7
p,p'-DDD	10.6 ± 0.5	2.3 ± 0.4	1.5 ± 0.3	1.3 ± 0.2
p,p'-DDE	6.8 ± 0.5	3.7 ± 0.3	2.3 ± 0.1	3.3 ± 0.8
Total	134.2 ± 9.9	39.1 ± 2.7	23.2 ± 1.6	26.5 ± 2.9
Blood (ppb)				
p,p'-DDT	...	31.1 ± 2.1	25.8 ± 4.4	31.4 ± 2.4
p,p'-DDD	...	6 ± 0.4	3.6 ± 0.6	4.5 ± 0.6
p,p'-DDE	...	5.6 ± 0.5	5 ± 0.9	5.7 ± 0.5
Total	...	42.7 ± 2.6	34.4 ± 5.3	41.7 ± 3
Carcass				
Weight (g)	140 ± 8	248 ± 12	264 ± 15	270 ± 9
% Fat	6.1 ± 0.7	6.8 ± 1.6	8.1 ± 1.3	8.5 ± 0.9
Total fat g/carcass	8.6 ± 1.1	16.4 ± 3.4	21.3 ± 2.9	22.7 ± 2
DDT and metabolites ppm/g fat	134.8 ± 19.8	39.2 ± 5.3	24.2 ± 4.7	32.7 ± 4
% of original dose in carcass	34.5 ± 5.8	17.6 ± 1.4	14.5 ± 2.1	20.3 ± 1

[a] ± standard error of the mean.

between animals receiving either 2000 or 40,000 iu/kg diet. Of prime significance is the observation that the feeding of 20 times the vitamin A requirement to rats does not decrease the concentration of DDT or its metabolites in the adipose tissue of the rat.

Experiment 2. The average body weight of all animals at the time of initiation of feeding the experimental diets was 110 g (Table II). The pesticide residues in the adipose tissue at the end of the experimental feeding period thus reflect changes resulting not only from excretion but also to an increase in the amount of fat resulting from increased growth and maturity. Vitamin D supplementation did not influence the rate of growth, as demonstrated by the final body weight. The concentration of DDT and metabolites in the adipose tissue decreased to approximately 33% of the initial value and the level of vitamin D supplementation was without significant effect on the concentration of *p,p'*-DDT or the sum of DDT and metabolites. The metabolites (DDD and DDE) comprised approximately 17% of the total residue, and vitamin D supplementation did not alter this ratio. There was a significant difference between the DDE concentration in the blood of animals receiving 200 units of vitamin D in the diet compared to animals receiving 4000 units. However, this may be fortuitous since treatment evoked no other significant changes in the pesticide concentration in the blood.

Experiment 3. The animals used to study the effect of supplemental dietary vitamin E were heavier (Table III) than animals used in the previous experiment. Initial residues of DDT and metabolites following dosing with DDT at the rate of 25 mg/kg body weight were similar, however, in both experiments. After 28 days on diet with no vitamin supplementation (Group 2), the concentration of DDT and the total DDT and metabolites decreased to approximately 30% of the initial value, as was observed in the previous experiment. Vitamin E supplementation had a highly significant effect on decreasing the concentration of *p,p'*-DDT and the sum of DDT and metabolites in the adipose tissue. However,

there was no significant difference between the groups receiving either 60 or 600 mg of vitamin E per kilogram of diet. Treatment had no effect on the levels of the metabolites DDD or DDE. In blood, vitamin E supplementation decreased the concentration of DDD and was without effect on DDE. In all groups the concentration of DDT and metabolites was much higher than in the previous experiment, even though levels in the adipose tissue were similar. Since significant differences were found in the pesticide concentration of the adipose tissue, total body fat was extracted from six animals in each group. The total amount of fat per carcass and the percentage of fat in the carcass, although tending to be greater in animals receiving the vitamin E supplement, was not significantly different due to treatment. Similarly, treatment did not affect the concentration of DDT and metabolites per gram of body fat. However, the total DDT and metabolites per whole body and also the percent of the administered dose remaining in the whole body was slightly larger ($P = 0.05$) in animals receiving the highest vitamin E supplementation. Excellent agreement was observed between the concentration of pesticide in the adipose tissue and total body fat for animals in Groups 2 and 3, while in Group 4 higher residues were observed in body fat. None of the parameters measured gave indications that vitamin E supplementation increased the rate of depletion of DDT body burdens. Since there was a suggestion that residue levels were lower in the adipose tissue, the experiment was repeated (although not shown) using a shorter depletion period (19 days). Again concentrations of DDT in the adipose tissue were lower ($P = 0.1$) and the total DDT and metabolites were lower ($P = 0.01$) when the diet was supplemented with 60 or 600 mg of tocopherol/kg diet. However, extraction of the total fat from the carcass showed no effect of treatment on depletion of the body burdens of the total DDT and metabolites.

DISCUSSION

The nutritional status of the rat is known to modify both the metabolism and toxicological effects of pesticides. The dietary modifications studied have been mainly related to protein intake (Boyd and Krijnen, 1969; Krijnen and Boyd, 1970; Tinsley and Claeys, 1970), dietary fat (Sauberlick and Baumann, 1947), and limited studies on the interaction of vitamins and minerals with pesticides (Casterline and Williams, 1969; Clark *et al.*, 1967; Phillips, 1963; Radeleff, 1964; Tinsley, 1966). The report of Hironaka (1968) that large supplements of the fat-soluble vitamins appeared to decrease body burdens of dieldrin was, therefore, of interest not only from the immediate practical importance for decontamination but also from the basic standpoint of a vitamin–pesticide interaction. Extension of Hironaka's work was needed since it is not clear from present knowledge why a mixture of vitamins A, D, and E should increase excretion of dieldrin. Further, since vitamin A (Moore, 1957) and vitamin D (Forbes, 1967) can themselves be toxic at high levels, it would be advantageous to know if any of the vitamins in the mixture were effective at reasonable dose levels when fed singly.

The vitamin A requirement of the rat for growth and maintenance is generally accepted as 2000 iu/kg diet (NAS–NRC, 1962); thus in the present study the vitamin A intake was varied from $^1/_4$ to 20 times requirement. Decreased rate of growth was observed in the animals receiving the lowest dietary level of vitamin A, indicating suboptimum intake. In the group receiving the highest dietary intake, liver stores of vitamin A were 25 times those observed in animals receiving the requirement level, indicating excess vitamin A intake. The higher level, however, was not in the range considered to induce hypervitaminosis A since no effect was observed on growth. Higher total pesticide residue levels were observed in the adipose tissue of animals receiving 500 iu of vitamin A/kg diet. This probably reflects the lower rate of growth with a smaller absolute amount of adipose tissue relative to the other treatment groups rather than a decreased rate of depletion. The present study clearly demonstrates however that over a wide range of vitamin A intakes (from requirement to 20 times the requirement) the metabolism and excretion of *p,p'*-DDT and metabolites as indicated by residues in the adipose tissue is unaffected. It is known from recent surveys (Hoppner *et al.*, 1969) that the vitamin A reserves of Canadians vary widely; however, this should be without a detrimental effect on DDT metabolism. Furthermore, there does not appear to be any practical importance to supplementation of the diet with excess vitamin A to increase the rate of depletion of body burdens of DDT.

The animals used in the vitamin D and vitamin E experiments were not depleted of these vitamins prior to the administration of DDT. However, in the case of vitamin D, during the 28-day DDT depletion period, animals were fed a vitamin D deficient basal diet similar to that used in the British Pharmacopeial bioassay for vitamin D (Bliss and Gyorgy, 1951) supplemented with vitamin D at levels of 200, 400, and 4000 iu/kg. It is not possible to express these levels as a percentage of the daily requirement since the position of vitamin D in diets containing optimum levels of calcium and phosphorus is obscure and there is insufficient evidence to establish the vitamin D requirement. The range of supplements used would span the daily requirement and at the highest level be in excess. At these intake levels no significant effects of treatment were observed on the rate of depletion of body burdens of DDT as determined from concentrations in the adipose tissue. The ratio of metabolites of DDT to the parent compound was also not changed by vitamin D supplementation, further indicating no effect of treatment on the metabolism of DDT.

The use of vitamin E as a nutritional adjunct to increase the depletion of DDT body burdens, if effective, would appear to be a desirable approach since "no state or syndrome of hypervitaminosis E has been described, nor is there evidence that tocopherols *per se* exert any deleterious effect in animals or man" (Mason, 1954). Under the present experimental conditions supplementation of the diet with tocopherol did not alter the metabolism of DDT. Although it appeared that tocopherol supplementation decreased the concentration of DDT and metabolites in adipose tissue, extraction of the whole body showed that treatment did not increase the rate of DDT depletion. It should be emphasized that the basal diet used in this latter experiment was presumably nutritionally adequate in vitamin E and supplements equal to the growth requirement (60 mg/kg diet) and 10 times the requirement (600 mg/kg diet) was added.

Over the ranges of supplementation used in the present experiments there appears to be no grounds for suggesting the use of fat-soluble vitamins, therapeutically or prophylactically, for decreasing body burdens of DDT.

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