Nutritional Interactions in Dieldrin Toxicity

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The interaction of a nutritional and a toxic stress has been studied using dieldrin as the toxicant. A dietary level of 20 p.p.m. of dieldrin accentuates an essential fatty acid stress, because dieldrin interacts in the metabolism of polyunsaturated fatty acids. The transformation of linoleate to arachidonate is stimulated by dieldrin in male and female rats. In female rats dieldrin stimulates the transformation of oleic acid to 5,8,11-eicosatrienoic acid. A riboflavin deficiency results in a depressed growth rate in female rats subject to a dieldrin stress and appears to accentuate dieldrin toxicity. This nutritional stress results in an increase in the level of dieldrin in liver and an interaction of riboflavin in detoxication processes is postulated.

THE population in general is exposed to trace amounts of exogenous chemicals which exist in the environment. Certain segments of the population involved with the manufacture or utilization of these chemicals are exposed to a greater degree. The possibility that a nutritional stress might interact in these situations has not been explored very extensively. Such interactions could have implications in the use of pesticides in areas where the inhabitants are known to be existing on marginal diets. On the other hand, it is possible that plant operators could be provided with an additional margin of safety through careful attention to their nutritional status. The study of such interactions also provides information which will contribute to the understanding of the action of the toxicant and/or the nutrient.

Friedman (*1*) recently has summarized some of the information regarding the effects of nutritional variables on toxicity. Of particular significance to the work reported in this paper are the studies of Phillips (*9*) and Lee, Harris, and Trowbridge (*5*). Phillips has reported that, in the rat, DDT decreases the utilization of vitamin A and carotene and that rations low in protein accentuate dieldrin toxicity, and the combination of these two stresses reduces the levels of vitamin A in liver (*5*).

This paper summarizes studies of the interaction of toxic stress due to dieldrin with a nutritional stress due to marginal levels of essential fatty acids or riboflavin.

Materials and Methods

These interactions were studied by observing the performance of male and female rats raised from weaning on the following rations: a control, nutritionally adequate ration; a ration containing marginal levels of one nutrient; the control ration containing 20 to 30 p.p.m. of dieldrin; and the deficient ration containing 20 to 30 p.p.m. of dieldrin.

Litter-mate groups of four rats from our stock colony of Wistar strain animals were distributed on these rations. A total of eight (4♂ and 4♀) rats was used for each ration. Animals were weighed weekly and observed carefully for the appearance of any gross symptoms and the experiments were continued for 10 to 14 weeks, depending on the performance of the animals.

Nutritional variables were controlled using the semisynthetic ratios summarized in Tables I and II. Dieldrin and the fat-soluble vitamins were incorporated in corn oil. Percomorph oil was used to give levels of vitamins A and D of 12,250 and 1250 I.U. per kilogram of ration, respectively. Vitamin E as *d*-α-tocopherol acetate was added at a level of 50 I.U. per kilogram of ration. Analytical standard dieldrin was used.

A marginal level of riboflavin was obtained by reducing the level of this component in the vitamin mix. To obtain an essential fatty acid stress, the level of fat in the ration was raised to 15% and hydrogenated coconut oil was used in place of the corn oil. The hydrogenated coconut oil contained no detectable amounts of linoleic acid. The control ration contained 14% of the hydrogenated coconut oil and 1% of corn oil. In this experiment vitamin A acetate, calciferol, and *d*-α-tocopherol acetate were added to the ration in alcohol solution. A level of 20 p.p.m. of dieldrin was used.

Techniques used in this laboratory for the analysis of the fatty acids of liver lipids have been described (*6*). In analyzing liver tissue for dieldrin, the extraction procedure of Taylor, Rea, and Kirby was used (*11*). The extract was purified further by adsorption on a column of magnesium oxide–Celite (1 to 1) and elution with petroleum ether. The dieldrin was assayed by gas chromatography at 195° C. on a column of 5% QF_1 plus 5% Dow 11 on Chromasorb using an electron-capture detector.

Results and Discussion

Essential Fatty Acid Stress. No gross symptoms of essential fatty acid stress were induced by the feeding for a period of 10 weeks of a ration containing 15% saturated fat, nor did the addition of dieldrin have any additional effect. However, a study of the fatty acid composition of liver lipids indicated that an essential fatty acid–deficient state was being approached and that dieldrin accentuated this nutritional stress.

The fatty acid composition of liver lipids is given in Table III. Mean values and standard deviations are listed for those acids present in significant

Table I. Ration Composition

	Grams
Casein	220
Cerelose	680
Corn oil	50
Salts (HMW)	40
Vitamin mix	10
	1 kg.

Zinc added to ration at level of 6 mg./kg. as zinc acetate.

Table II. Composition of Vitamin Mix

	Grams
Thiamine hydrochloride	0.40
Riboflavin	0.80
Pyridoxine hydrochloride	0.50
D-Calcium pantothenate	4.00
Inositol	20.00
Menadione	0.40
Folic acid	0.40
Niacin	4.00
Choline dihydrogen citrate	424.00
Biotin	0.03
Vitamin B_{12}	0.02
Cornstarch to a total of	1000

Table III. Influence of Dieldrin Stress on Fatty Acid Composition of Liver Lipids from Rats Raised on Rations Deficient in Essential Fatty Acids

	% of Total Methyl Esters						
	16:0	16:1	18:0	18:1	18:2	20:3	20:4
Male							
Control	41.40 ± 5.79	2.10 ± 0.70	20.19 ± 4.71	22.59 ± 2.76	3.71 ± 0.52	*T*	7.66 ± 1.61
EFA deficient	31.11 ± 3.20	2.40 ± 1.22	14.91 ± 2.33	42.14 ± 3.92	0.57 ± ...	4.73 ± 0.75	1.52 ± 0.30
Control and dieldrin	34.92 ± 2.67	1.70 ± 0.32	23.86 ± 2.02	23.06 ± 2.98	3.44 ± 0.87	*T*	11.34 ± 1.72
EFA deficient and dieldrin	32.53 ± 3.10	3.95 ± 1.46	14.73 ± 2.41	41.42 ± 3.66	0.48 ± ...	4.42 ± 1.51	1.18 ± 0.73
Female							
Control	35.26 ± 1.72	1.25 ± 0.17	25.83 ± 4.13	23.29 ± 5.45	3.46 ± 0.51	*T*	8.85 ± 1.82
EFA deficient	30.29 ± 3.59	3.20 ± 0.83	19.27 ± 2.17	37.94 ± 2.11	0.62 ± ...	5.17 ± 1.08	2.17 ± 0.54
Control and dieldrin	30.84 ± 4.84	1.24 ± 0.20	29.95 ± 2.85	21.66 ± 2.11	3.25 ± 0.49	*T*	11.40 ± 0.67
EFA deficient and dieldrin	24.97 ± 6.56	2.81 ± 1.04	24.72 ± 3.76	33.73 ± 3.43	0.75 ± ...	8.97 ± 2.27	2.73 ± 0.96

quantities. The standard deviation of these observations is usually in the order of 10% of the mean value. However, much of this variation can be attributed to litter differences and can be accounted for in statistical analysis.

The ratio of triene to tetraene fatty acids has been suggested as an index of essential fatty acid stress (*4*). The only triene acid present in liver lipids to any extent is 5,8,11-eicosatrienoic acid (20:3), which is derived from oleic acid and the major tetraene acid is arachidonic acid (20:4). The fact that the 20:3/20:4 ratio is high (Table IV) in animals subject to the nutritional stress would suggest that a deficient situation is being approached but is not sufficiently acute to result in gross symptoms. In each of the eight litter-mate groups the combination of the dieldrin stress with the essential fatty acid stress produced an increase in the 20:3/20:4 ratio. This difference was statistically significant ($P < 0.01$) and would indicate that dieldrin accentuates an essential fatty acid stress. Further examination of these data indicates that in male rats the change in the 20:3/20:4 ratio induced by the dieldrin is due to a decrease in arachidonic acid. By contrast, the increase in this ratio induced by dieldrin in female rats is due to an increase in the level of 5,8,11-eicosatrienoic acid.

Studies by Mead and coworkers (*8*) have established that in rat liver linoleic acid is converted to arachidonic acid and oleic acid to 5,8,11-eicosatrienoic acid. The extent to which these transitions occur is indicated by the respective product-precursor ratios. The data in Table V show that the 20:4/18:2 ratio is increased by both the nutritional stress and the toxic stress. This response is observed consistently in all litter-mate groups and is statistically significant. The increase in this ratio induced by the nutritional stress is related to the low level of linoleic acid in the tissue. Previous studies in our laboratory (*6*, *12*) have demonstrated that when the level of linoleic acid in the tissue decreases, proportionately more of this acid is converted to arachidonic acid. The effect of dieldrin cannot be attributed to such a change, since the linoleic acid level is essentially the same in animals raised on the control and dieldrin rations. These data suggest that dieldrin increases the tendency of liver tissue to convert linoleic acid to arachidonic acid. The same response is not apparent in the nutritionally deficient ration; in males a small decrease is observed. The 20:4/18:2 ratio is not very precise under these conditions, since the linoleic acid level is low.

The degree to which oleic acid is converted to its derivative polyunsaturated fatty acid is indicated by the 20:3/18:1 ratio. This transition can be observed only in the deficient rations, and in female animals dieldrin almost doubles this ratio. Male rats did not respond.

The transformation of oleic acid and linoleic acid to their derivative acids involves a chain lengthening and a desaturation process. Stoffel (*10*) has established that the latter is microsomal in origin and requires NADPH and oxygen, which would classify this system with the mixed function oxidases (*7*). The dehydrogenation is probably effected by the combination of a hydroxylation and a dehydration.

Mixed function oxidases have been studied extensively in recent years, particularly the induction of these enzymes by exogenous chemicals. Dieldrin is active as an inducer of these systems (*2*) along with many other chemicals and thus its influence on the metabolism of polyunsaturated fatty acids may be another manifestation of the induction of microsomal oxidases. This induction results in a stimulation of the transformation of oleic and linoleic acids to their derivative acids.

Table IV. Influence of Dieldrin on Product-Precursor Ratios and Ratio of Triene to Tetraene Fatty Acids

	20:4/18:2	20:3/18:1	20:3/20:4
Male			
Control	2.07 ± 0.37[a]	...	...
EFA deficient	2.75 ± 0.63	0.114 ± 0.025	3.17 ± 0.59
Control and dieldrin	3.41 ± 0.71	...	...
EFA deficient and dieldrin	2.34 ± 0.43	0.109 ± 0.078	4.25 ± 1.29
Female			
Control	2.55 ± 0.22	...	...
EFA deficient	3.51 ± 0.75	0.136 ± 0.026	2.46 ± 0.56
Control and dieldrin	3.59 ± 0.70	...	...
EFA deficient and dieldrin	3.79 ± 1.11	0.265 ± 0.063	3.39 ± 0.59

[a] Mean and standard deviation.

Table V. Influence of Riboflavin Stress on Survival of Rats Subject to Dieldrin Stress

	Level of Ration Component		Survival		
Expt.	Dieldrin, p.p.m.	Riboflavin, mg./kg.	Male	Female	Length of Expt., Weeks
1	30	8.0	4/4	0/4	10
		3.2	4/4	1/4	
2	20	8.0	4/4	4/4	14
		3.2	4/4	3/4	
3	20	8.0	4/4	3/4	10
		2.4	4/4	2/4	

Table VI. Influence of Dietary Level of Riboflavin on Level of Dieldrin in Liver

	P.P.M. (Wet Weight) in Liver Tissue			
	Riboflavin Level, Mg./Kg.			
	Male		Female	
	8.0	2.4	8.0	2.4
	5.65	8.11	8.67	9.67
	5.66	9.18	9.83	12.20
	9.10	9.12	9.91	17.50
	9.46	11.30	...	...
Av.	7.47	9.43	9.47	13.12
	μg./Liver/100 G. Body Weight			
Av.	26.7	31.2	37.6	44.8

One contradiction is apparent and that is that the female rats show a more consistent response than males. Other studies (*3*) clearly show that the male rat shows a higher level of induction of microsomal oxidases. This discrepancy could be due to the fact that female rats are considerably more susceptible to dieldrin and possibly higher levels of dieldrin would be required to produce a comparable response in males. Another significant observation is that in these studies dieldrin does not stimulate the transformation of palmitic (16:0) acid to palmitoleic acid (16:1) nor the transformation of stearic acid (18:0) to oleic acid (18:1). One might conclude that the enzymes required for this desaturation are distinct from those involved in the production of polyunsaturated fatty acids.

Riboflavin Deficiency. In these studies it was not desirable to impose an acute nutritional stress, since the degree of the latter might override any interaction. Thus, in successive experiments the level of riboflavin was reduced progressively and the level of dieldrin held at 20 p.p.m. after the first experiment. A summary of the experiments is given in Table V.

Female rats proved to be more susceptible to dieldrin than male rats. Only one out of eight female rats survived for 10 weeks on a ration containing 30 p.p.m. (Experiment 1, Table V). Those rats which died when subjected to the combined stress, did so earlier in the experiment than those subject to the toxic stress alone. In Experiments 2 and 3 female rats subject to the combined stress showed lower growth rates than rats subject to the nutritional or toxic stresses alone. In general, the survival of female rats is poorer when subject to the combined stress.

Animals subject to the combined stress show the same gross symptoms of dieldrin toxicity as animals subject to the toxic stress alone. The animals became excitable and nervous and some of the females had convulsions. These observations would suggest that the nutritional stress of a lowered riboflavin intake accentuates the toxicity of dieldrin.

It is not possible to define the mechanism of this interaction, since the knowledge of the biochemical action of this toxicant is very meager. One possibility is that a nutritional stress could impair detoxication processes with a subsequent buildup of the toxicant in the tissues. With this possibility in mind dieldrin levels were assayed in liver tissue from animals of Experiment 2. The number of animals available was small, but the data indicate some significant differences which will be investigated further in more comprehensive experiments.

Analysis of variance of the data obtained (Table VI) showed that the level of dieldrin in the liver of female rats was significantly higher ($P < 0.025$) than in the liver of male rats. This observation is consistent with the fact that female rats are more susceptible to a dieldrin stress. If the data are considered as seven littermate pairs to account for the obvious variation due to litter, it is observed that a decrease in the level of riboflavin intake significantly increases ($P < 0.025$) the dieldrin level in the liver. Similar conclusions are obtained if liver size and body weight are taken into consideration in expressing dieldrin concentration in the liver. This observation would be consistent with the hypothesis that a riboflavin deficiency could limit the detoxication process.

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