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Effect of Dietary Variations upon the Toxicity of DDT to Rats or Mice.*

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The effectiveness of toxic agents in animals frequently depends upon the composition of the basal diet fed. Thus a high protein diet has been found to protect the liver against such diverse toxic agents as arsphenamine,[1] p - dimethylaminoazobenzene,[2] chloroform,[3] and selenized wheat.[4] Methionine is effective against several of these agents.[5] Another nutrient reported to modify toxicity is fat. When rats were fed trinitrotoluene (TNT) in diets high in protein or carbohydrates, little or no ill effects were observed, although definite toxic effects resulted when a high fat diet was fed.[6] On the other hand, mice fed dinitrotoluene (DNT) grew better and lived longer on a high fat diet than when the basal diet was nearly devoid of fat.[7] Vitamin C is reported to be of value in the prevention and treatment of toxic effects of TNT in munition workers[8] and to aid in the detoxification of aniline in rabbits and mice.[9] Chloronitrobenzene toxicity has been found to be augmented by fats and oils, as well as by alcohol,[10] and alcohol also increases the toxicity of dinitrotoluene.[7,10] These observations and the widespread use of DDT (2,2 bis(p-chlorophenyl) 1,1,1 trichloroethane) as an insecticide raised the question whether the toxicity of this compound in higher animals might not also vary with the composition of the basal diet fed.

Experimental. Methods. Most of the experiments were performed on young mice, but adult mice and both young and adult rats were also used in certain studies. The animals were kept in screened cages in groups of 3 to 6 and they were fed the synthetic or semi-synthetic diets listed in Table I, or similar diets in which the percentage of fat or protein was altered isocalorically at the expense of the carbohydrate. The animals were weighed periodically and the toxicity symptoms noted. The DDT used in these experiments was prepared by the method of Darling[11] and recrystallized twice from ethyl alcohol.

Results. In preliminary studies, DDT was incorporated into the yeast diet I and fed to young mice in graded concentrations ranging from 0.01% to 0.3%. At concentrations of 0.05% and over, the mice rapidly developed toxicity symptoms and died within 2 weeks or less. At the lowest concentration, 0.01% they survived for a 9-week period. At the intermediate concentrations, 0.03% and 0.04%, approximately one-half of the animals lived for 4 weeks, depending upon the concentration of the insecticide and the size of the mice. Older mice resisted the effects of the toxic agent somewhat better than younger ones. The symptoms of toxicity were similar

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TABLE I.
Composition of the Diets Fed.

Yeast diet (I)		Synthetic diet (II)	
	g		g
Crude casein	18	Crude casein	20
Wesson's salts[14]	4	Wesson's salts	4
Brewers yeast	8	Celluflour	2
Corn oil	5	Corn oil	5
Dextrin	70	Dextrin	69
			mg per 100 g of diet
		Pyridoxine hydrochloride	0.5
		Thiamine chloride	0.6
		Calcium pantothenate	2.0
		Riboflavin	0.6
		Nicotinic acid	1.0
		Choline chloride	100.0
		Inositol	50.0
		p-Aminobenzoic acid	30.0
		Cystine	100.0

to those described by others[12,13] and were characterized by hyperexcitability, followed by a jerking or retraction of the head, which developed into a general tremor that increased in intensity until there was complete loss of control, convulsions, and death. Alopecia was common in certain series. The compound did not exert any appreciable effect on the rate of growth until the tremors became sufficiently severe to interfere with the ability of the mice to eat. In general, rats responded to DDT like mice, although higher concentrations of DDT were necessary before deaths resulted in a comparable period of time: 0.15% of DDT was about as effective in a rat as 0.04% in the mouse.

The effect of DDT against either species depended upon the percentage of fat in the basal diet (Table II). Mice invariably developed toxic symptoms and died sooner when fed a diet containing 5% or more of fat than when fed a diet containing only 0.5% of fat; this was observed in both young and older mice and in series in which the concentration of DDT was 0.03% or 0.04% (Table II). Similar results were obtained with rats fed 0.15% of DDT (Table II). In other experiments, series of different fats were fed to mice at a 15% level in diets containing 0.03% or 0.04% of DDT. All fats tested appeared to increase the sensitivity of mice to DDT at this level. Mice fed a completely saturated fat, hydrogenated coconut oil, developed tremors and died at about the same time as those ingesting the moderately saturated fats, butter or lard, or the highly unsaturated fats, peanut oil or corn oil. The effect of the fat was not modified by the addition of 0.5% of cholesterol.

A reduction in the level of protein in the diet to 10% apparently decreased the resistance of the mice to DDT. This effect was noted whether the percentage of fat in the diet was high or low (Table II). When the level of protein in the diet was increased above 20%, the results were variable although some protective action against DDT appeared in certain series.

Discussion. The greater sensitivity of animals to DDT fed in a high fat diet as compared to one low in fat seems to be primarily due to a greater efficiency of absorption of DDT on the high fat diets. DDT is readily soluble in fat and tends to concentrate in the fat depots of animals exposed to this insecticide, *e.g.*, in rats,[15] cows,[16] and dogs.[17]

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TABLE II.
Effect of the Level of Fat and Protein in the Diet upon the Survival of Mice and Rats Fed DDT.
Diet II.

Casein, %	Corn oil, %	Number of animals alive at: 0 wk	1 wk	2 wks	3 wks	4 wks	5 wks	6 wks
		0.03% DDT, young mice.						
20	0.5	4	4	4	4	4	4	4
20	5	4	4	4	4	4	3	2
20	15	4	4	3	2	2	1	0
		0.03% DDT, young mice.						
20	0.5	5	5	5	5	3	3	
20	15	5	5	5	4	2	1	
10	15	5	5	4	1	1	0	
50	15	5	5	4	4	4	4	
		0.04% DDT, young mice.						
20	0.5	10	10	7	6			
20	5	4	4	1	1			
20	15	10	7	3	0			
10	0.5	6	5	1	0			
10	15	6	6	1	0			
		0.04% DDT, adult mice.						
20	0.5	4	4	4	4	4	4	4
20	5	4	3	3	3	3	2	2
20	15	4	4	4	4	4	3	2
20	25	4	3	3	2	1	1	1
10	15	4	3	0	—	—	—	—
50	15	4	3	3	3	3	2	2
		0.15% DDT, young rats.						
20	0.5	6	6	5	5	5		
20	5	6	2	0	—	—		
20	15	6	4	1	0	—		

More DDT accumulated in the fat of dogs when the compound was fed in corn oil solution than as a solid[17] and the symptoms of toxicity due to injected DDT were also more severe,[12,13] when an oil solution was used. Factors affecting the translocation of DDT within the body are probably more important in determining resistance to DDT than to other poisons. DDT does not appear to be rapidly detoxified in the body[18] and its association with fat may be an important mechanism for its removal from the general circulation. However, at least one metabolic derivative of DDT (di(p-chlorophenyl) acetic acid) has been identified in the urine of rabbits,[19] and fat may affect the speed with which such metabolites are formed. Dietary fat seems to influence the toxicity of certain nitro compounds.[6,7]

The mouse appears to be more sensitive to DDT than the rat, for on comparable diets tremors produced in mice fed 0.03% to 0.04% of DDT were similar to those in rats fed 0.10% to 0.15% of DDT. In other words the rat can tolerate 3 times the concentration tolerated by the mouse. However, the mouse consumes roughly 2 to 3 times as much food per unit of body weight as the rat and, hence, the amounts of ingested DDT per gram of body substance required for the production of tremors were quite similar in the two species. Indeed, it has been reported that the rat is somewhat more susceptible than the mouse to injected DDT or to large single oral doses.[12] Nevertheless, the present data indicate that the mouse has definite advantages as an animal for the bio-assay of foodstuffs exposed to the insecticide.

Summary. Mice fed 0.03% to 0.04% of DDT in a medium fat diet (5%) developed toxic symptoms and died; 0.10% to 0.15% of DDT in the same diet was necessary to

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produce a similar toxicity in the rat.

A reduction in the level of dietary fat to 0.5% decreased the toxicity of DDT in both species; when the diet contained 15% of fat, the symptoms of toxicity were aggravated. Several different types of fat were essentially equal in aggravating the toxicity.

On a low protein diet (10%) the toxicity of DDT was increased somewhat; whereas on a high protein diet (30%) the effects were variable.

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Endocrine Interrelationship and Spontaneous Tumors of the Adrenal Cortex in NH Mice.*

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Adenomas of the adrenal cortex appear in several inbred stocks of mice following gonadectomy.[1,2,3] In certain stocks, however, the phenomenon does not occur following removal of the primary sex glands.[3,4] In only one strain have similar adenomas been observed to appear spontaneously in non-castrate animals.[5] This is the NH stock† (Minnesota subline); tumors occur primarily in females. In these mice adenomas of the same type which develop spontaneously can be induced to appear precociously by gonadectomy.[6] The purposes of the current studies on this stock are to determine first, whether "physiologic castration" is responsible for the induction of spontaneous adenomas of NH females (spontaneous cortical tumors are very rare in males) and, second, to investigate the effect of gonadotrophic hormone on the secretion and development of these tumors.

The vaginal smear which is exhibited by mice with cortical adenomas is typical. The vaginal secretion is copious, relatively watery, and has a high cellular content. The cells are of two general classes—epithelial cells and leukocytes—these are approximately equally distributed. The epithelial cells may be classified as cornified and non-cornified. The ratio of cornified to non-cornified cells in the untreated tumor-bearing animal is 25 to 75.

The estrous cycle was studied in 6 inbred stocks of mice (CBA, dba, Balb, Strong A, F., NH). In general, it was found that the estrous cycle lengthened as the animals became infertile. This was observed to occur earlier in the NH stock than in any of the others except the F mice. Females of all stocks except the NH and F were cycling beyond one year of age. The F females (10 mice) exhibited a castrate smear by one year of age but did not develop cortical adenomas. If castrated early in life these mice showed only a minimum tendency towards adenoma development, suggesting that the end organ is not sensitive to the stimulus towards tumor formation. (CBA, dba, and Balb mice exhibit cortical adenomas within 3-5 months after gonadectomy). In most cases the NH females (43 mice) did not

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† This stock was obtained in the 8th inbred generation from Dr. L. C. Strong of the Yale University School of Medicine.

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