Dietary Protein and DDT Toxicity[1]

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Recent investigations have shown that when weanling albino rats are fed for 28 days on a diet containing one third the normal amount of protein they become slightly more susceptible to the toxic effects of single oral doses of DDT (1) and carbaryl (2) than are controls fed diets containing normal amounts of protein. In similar experiments, weanlings fed for 28 days on a diet containing 13 percent of the normal amount of protein were found to have significantly lower values for the acute, oral LD_{50} of captan (3) and of lindane (4).

These studies demonstrated that pesticide toxicity can be augmented in protein-deficient albino rats but did not show the quantitative relationship between pesticide toxicity and the degree of protein deficiency. In the present work, the acute oral toxicity of DDT or Dicophane B.P., 1968, was determined in albino rats fed for 28 days from weaning on diets containing from 0 to 81% of protein as casein. During a feeding period of 28 days, weanling albino rats adapt to protein-deficient diets

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1 This project was supported by a grant from the World Health Organization.

Bulletin of Environmental Contamination & Toxicology,
Vol. 4, No. 5, 1969, published by Springer-Verlag New York Inc.

and growth rates in rats fed 26 to 27% casein are similar to those in rats fed a standard laboratory chow (5).

Methods

The experiments were performed upon weanling male albino rats of a Wistar strain weighing 50 to 60g. and obtained from Canadian Breeding Laboratories of St. Constant, Quebec, Canada. Group I consisted of 52 weanlings fed for 28 days on a diet containing 0% casein, 85% cornstarch, 8% hydrogenated cottonseed oil, 4% salt mix and 3% of an all vitamin mixture prepared after the formula of Hegsted and Chang (6) by General Biochemicals of Chagrin Falls, Ohio. Group II consisted of 51 weanlings fed a diet containing 3% casein and 82% cornstarch but otherwise of the same composition as the diet of group I. There were 55 weanlings in group III fed 9% casein and 76% cornstarch, 65 in group IV fed 27% casein and 58% cornstarch, and 50 in group V fed 81% casein and 4% cornstarch with other dietary items in each group as for the diet of group I.

At the end of the 28 days of feeding, the animals were placed in metabolism cages, one rat per cage, and given water but no food for 16 hours (overnight) to empty the stomach prior to oral administration of DDT. The DDT (WHO Technical Grade,Eastman) was dissolved in cottonseed oil and given by intragastric cannula in a constant volume of 20 ml. per kg. body weight for reasons reviewed by Boyd (7). DDT was given in a range of doses estimated from pilot tests to yield mortality rates from just above 0% to just

below 100%, each dose to 5 to 10 rats.

The animals were then returned to their metabolism cages with food and water available ad libitum. Cageside observations of clinical signs, semiquantitated in clinical units of 1+ to 4+, was made at daily, or shorter if indicated, intervals for 7 days. Gross pathology was recorded at autopsy on all animals which died. Details of method and of the statistical analysis of results have been reviewed by Boyd (7).

Results

During the dietary period of 28 days, the dietary death rate was 37% in group I, 18% in group II, 0% in group III, 3% in group IV, and 44% in group V. Most deaths occurred during the first 2 weeks on the purified diets. Data on body weight in survivors at the end of the dietary period are summarized in Table 1. Growth rate was significantly ($P<0.01$) lower in groups I,II,III and V than that in group IV fed normal amounts of protein. At 4 weeks after weaning, rats in group I and II looked like weanlings but had some signs similar to those seen in kwashiorkor reported by Boyd and De Castro (1). Animals in group V had a marked diarrhea, extensive soiling of the fur, some erosion of the skin over the tail from biting the tail, and hyperkinesia.

The clinical signs of intoxication to DDT were similar in all dietary groups. Most animals exhibited signs of stimulation of the central nervous system such as tremors, a Straub reaction, piloerection and convulsions. Some animals were listless and prostrate. Epistaxis,

hemodacryorrhea, diarrhea, soiling of the fur, anorexia and oligodipsia were present at 24 hours. Death followed convulsions or occurred in coma at a mean of 27 hours (range 10 to 71 hours) with no relationship between interval to death and the protein content of diet (see Table 1).

As indicated in Table 1, the LD_{50} of DDT progressively and significantly decreased with decrease in the amount of dietary casein below the normal percentage fed to animals of group IV. The estimated maximal LD_0 and minimal LD_{100} also progressively decreased at the same time. The calculated lethal doses of DDT in animals of group V fed 81% casein in their diet were significantly ($P<0.01$) lower than those in animals of group IV fed 27% casein in their diet.

TABLE 1

A summary of data on interval to death and lethal oral doses (mg./kg.) of DDT in albino rats fed for 28 days from weaning on diets containing increasing percentages of protein as casein.

% dietary casein	0	3	9	27	81
Body wt. before DDT, g. (mean ± S.D.)	42±3	48±5	80±12	180±18	104±15
Hours to death (mean ± S.D.)	19±5	29±8	41±14	24±9	20±6
Maximal LD_0	42	91	195	290	77
LD_{50} ± S.E.	81±17	165±34	327±42	481±13	130±41
Minimal LD_{100}	119	239	460	672	184

Signs of intoxication began to disappear in survivors at 24 to 48 hours. Recovery was accompanied by an increase in food and water intake and a diuresis.

Discussion

An appreciable number of animals fed diets containing 0% (group I), 3% (group II), and 81% (group V) casein in their diet died during the first two weeks on diet and before administration of DDT. The pesticide was therefore given to survivors of dietary death and not to a representative sample of the entire population. Since the diets fed to these three groups presumably eliminated the weaker animals, the LD_{50} in survivors may be higher than that which might have been found in a sample of the entire population had it been possible to use such a group. On the other hand, animals of group I were particularly susceptible to starvation and an additional 10% of them died during the overnight fast to empty the stomach before oral administration of DDT. Since DDT produced an extension of the decrease in food intake for 1 to 2 days more, some of the deaths following DDT in group I (and possibly in group II) may have been due to the stress of starvation. The usual explanation of increased susceptibility to toxic doses of pesticides in protein-deficient rats is that they have a deficiency in production of hepatic microsomal protein detoxifying enzymes, as noted by Boyd and Chen (4).

When weanling rats were fed a diet containing 81% of casein or three times the normal amount (group V), the diet itself produced signs

of toxicity including deaths and the survivors were very susceptible to the toxic effects of single oral doses of DDT. If feeding the high casein diet is postponed until some 4 weeks after weaning, it produces no obvious toxic effects in albino rats (8). Casein is practically non-lethal to adult rats but pre-digested or hydrolized casein can produce a dehydration syndrome and death from relatively small single oral doses (9). The type of protein is also of significance in assessing protein toxicity. Single doses of reconstituted spray-dried, raw egg white powder produce lethal toxicity in adult albino rats (10) and when the same egg white powder is given to adult albino rats in large amounts in the diet, it produces direct toxicity not due to biotin deficiency (8).

Summary

Albino rats fed for 28 days from weaning on diets containing progressively smaller percentages of casein become progressively more susceptible to the lethal effects of single toxic oral doses of DDT than are rats fed normal amounts of dietary casein (27%). When the amount of casein in the diet is increased to 81%, the LD_{50} also declines. The clinical signs of toxicity to DDT at the range of the acute oral LD_{50} were similar in animals of all dietary groups studied.

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