Influence of Dietary Methionine on Dieldrin Metabolism in Rats[1,2]

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Dieldrin, an insecticide that controls a variety of agricultural pests throughout the world, resists degradation and has become a general environmental contaminant. Residues of dieldrin in plants, soils, and water can be a general public health problem, as well as being detrimental to fish and wildlife.

Recently, we (1) reported that young male rats, consuming high levels of dietary protein, had shown a reduced acute toxicity from dieldrin ingestion, together with an increased excretion of dieldrin metabolic products. Increasing dietary protein has been found to inhibit the acute toxicity of DDT (2) and a variety of xenobiotics (3) in rats. Tinsley and Claeys (4) have shown that increasing the levels of dietary methionine increases DDT buildup in liver tissue of weanling rats. This report describes our results on the influence of increased dietary levels of methionine on ^{14}C-dieldrin metabolism in the young male rat.

Materials and Methods

Weanling male rats of the Holtzman strain, 55-60 g, were housed separately, divided into 8 per group, and fed a basal semi-purified diet with either (in %): 0, 0.5, 1.0, or 2.0 DL-methionine added to the diets of each of the groups. The basal diet consisted of (in %): corn oil, 10.0; cellulose, 4.0; choline

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chloride, 0.2; vitamins, 1.0; minerals, 4.0; vitamin-free casein, 10.0; dextrose, 23.5; dextrin, 23.5; sucrose, 23.4. The 10% casein diet contained 0.21% methionine as analyzed by the Technicon Amino Acid Analyzer.

Randomly labeled ^{14}C-dieldrin, purchased from Nuclear-Chicago (specific activity, 72.4 mc/mM in benzene solution), was dissolved in corn oil, after the benzene was evaporated. At 5 weeks of age, 4 of the rats from each of the 4 methionine-treated groups, were orally dosed daily for 5 days with 1.25 μc of ^{14}C-dieldrin in 2.5 ml of corn oil/kg body weight. The urine and feces were collected daily and analyzed for total radioactivity in a Nuclear-Chicago Mark I liquid scintillation counter. After 1 week from the last dose, all animals were killed and the tissues were processed and counted for total radioactivity. Preliminary experiments showed no exhaled radioactive carbon from rats dosed with radioactive dieldrin.

Urine volumes were measured and an aliquot counted in a dioxane scintillation cocktail (4g POP, 0.5g POPOP, and 100g naphthalene per liter of dioxane). Feces and tissues were freeze-dried, ground to pass through a 20-mesh sieve, extracted with petroleum ether: isopropanol (4:1, v/v) and the extract counted in a toluene scintillation cocktail (4g POP, 0.5g POPOP per liter of toluene). About 30 mg aliquot of the fecal and tissue residues were digested in 2.0 ml of NCS (Nuclear-Chicago Solubilizer) and 0.2 ml water. After digestion, the samples were diluted to 15 ml with the toluene scintillation cocktail and counted. Efficiency of counting was approximately 75 to 80% as measured with an internal standard.

Results and Discussion

As seen in Table 1, body weight and food efficiency of young rats were optimal when the diet contained an additional 0.5% methionine. Rose, *et al.* (5) determined the methionine requirement of the weanling rat as 0.6% of the diet.

Methionine is the most toxic of the indispensable amino acids (6). It is evident from Table 1 that 2.0% dietary methionine produced rats with significantly lowered weight, together with a larger amount of food required per gram of weight gain. The young animal fed a low protein diet is very susceptible to adverse effects from excessive amounts of individual amino acids (6).

TABLE 1

Body Weight and Food Efficiency of 5-Week Old Rats Fed A Low Protein Diet with Added Methionine

Treatment	Body Weight (g)	Food Efficiency (g food/g wt. gain)
Basal Diet[1]	179.8 $\pm$ 3.0[2]	4.01
Basal + 0.5% Methionine	232.0 $\pm$ 3.4	3.08
Basal + 1.0% Methionine	206.2 $\pm$ 7.6	3.16
Basal + 2.0% Methionine	126.0 $\pm$ 3.7	4.23

1 10% casein diet contains .21% methionine

2 Mean $\pm$ SE

Figure 1 diagrams the total urine and fecal combined radioactive excretion of the rats dosed with ^{14}C-dieldrin. Although dietary methionine level was not directly correlated with total radioactivity excretion, the rats fed 2% methionine apparently metabolized and/or excreted dieldrin at a faster rate. A week after the fifth and last dose (day 11), 5% of the total dose was excreted in the urine of the rats fed the two highest levels of methionine, while only 3.5% of the total dose was metabolized and excreted via the urine of the rats fed either the basal diet or the diet containing 0.5% added methionine.

Korte and Arent (7) demonstrated that dieldrin metabolism occurs in mammals, producing many metabolic products. A variety of metabolites have been identified, and appear to result from oxidative attack on the carbon skeleton (8), or hydrative cleavage of the epoxide ring (7). Matthews and Matsumura (9) and Brooks and Harrison (10) have shown dieldrin is attacked and metabolized via a number of enzyme systems in liver microsomes.

Table 2 shows the amount of radioactive residue in specific tissues one week after the final oral dose of radioactive dieldrin. The epididymal adipose tissue, and the remaining tissues of the rats fed the highest level of methionine contain comparatively lower levels of radioactivity than the tissues of the animals fed the lowered amounts of methionine.

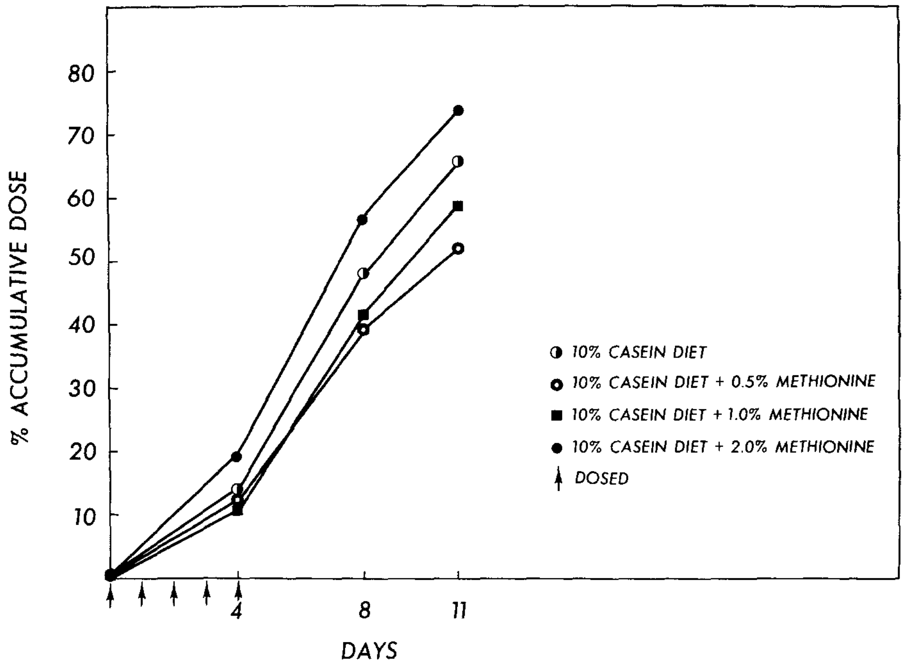

Figure 1. Accumulative excretion (as % of dose) of radioactivity in urine and feces of young male rats fed 4 dietary levels of methionine and orally dosed with ^{14}C-dieldrin.

The decreased amount of residual radioactivity in the tissues, presumably because of the increased excretion (Figure 1) of radioactive dieldrin metabolites in rats fed 2% methionine suggests that this amino acid induces microsomal enzyme induction. It is known that increased levels of dietary protein increase microsomal enzyme system activity, including the synthesis of the co-factor, cytochrome P-450 (11). Whether this increased activity occurs with ingestion of large amounts of any amino acid, as a general detoxication response, or whether methionine is a unique amino acid in inducing specific microsomal enzyme synthesis requires further investigations. These results add to the increasing evidence of the influence of dietary changes on the toxicity and metabolism of a variety of compounds (12).

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TABLE 2

Radioactive Residues in Tissues of Rats Dosed with ^{14}C-Dieldrin and Fed Increased Amounts of Methionine[1]

Treatment	Brain	Liver	Kidney	Adipose Tissue[3]	Remaining Tissues[4]	Total
Basal Diet	0.03 ± 0.01[2]	1.50 ± .15	0.35 ± 0.06	2.37 ± 0.14	29.21 ± 5.28	33.46
Basal + 0.5% Methionine	0.06 ± 0.02	1.20 ± .07	1.39 ± 0.49	3.19 ± 0.44	31.23 ± 6.78	37.07
Basal + 1.0% Methionine	0.05 ± 0.02	2.01 ± 0.28	1.63 ± 0.28	2.88 ± 0.52	31.66 ± 1.52	37.23
Basal + 2.0% Methionine	0.04 ± 0.01	1.37 ± 0.17	0.75 ± 0.17	1.32 ± 0.24	21.63 ± 3.75	25.01

1 All residue values expressed as the mean of the % of total dosage within each treatment

2 Mean ± SE

3 Excised epididymal fat pads

4 Includes skeleton, muscles, digestive tract, etc.

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