Effect of Tetrachlorodibenzo-p-Dioxin on Growth Rate and the Synthesis of Lipids and Proteins in Rats

by H. M. CUNNINGHAM and D. T. WILLIAMS
Research Laboratories, Food and Drug Directorate
Tunney's Pasture, Ottawa

Chick edema disease was first observed in 1957 when thousands of broilers were lost (1) but it was not until 1966 that chlorodibenzo-p-dioxins were found to be the causative agent (2). One of the most toxic dioxins was 2,3,7,8-tetrachlorodibenzo-p-dioxin (3). The most prominent toxic effects in chickens are hydropericardium and hydroperitoneum (4,5,6) while in mammals the liver appears to be the main point of attack showing enlargement and fatty infiltration (6, 7). In one report, enlarged livers in rats were not accompanied by an increase in liver fat (8). The present experiments were conducted to determine if dioxins affect the ability of the liver to synthesize lipids and protein, and the dosage and time required to produce maximum effects.

MATERIALS AND METHODS

In each experiment 12 to 16 weanling male Wistar rats were allotted at random into two to four treatment groups. 2,3,7,8-tetrachlorodibenzo-p-dioxin (99% pure, courtesy of the Dow Chemical Company) was dissolved in corn oil and given by oral intubation at levels of 0 to 10 µg/kg of body weight. Both treated and control rats received corn oil at a level of 10 ml/kg. One to seven days later each rat received an intraperitoneal injection of 1.0 µc of 1-^{14}C-L-leucine and 10.0 µc of ^{3}H-sodium acetate. Exactly 1 hr later the rat was killed by a blow on the head, the liver was removed and 1-g samples were homogenized with 4.0 ml of 0.9% NaCl. The lipids were extracted by a procedure similar to that of Folch et al. (9) and the radioactivity was counted with a liquid scintillation counter (10). In some experiments the lipids were partitioned by thin-layer chromatography (10). The proteins were separated from the aqueous phase of the Folch extraction by centrifuging, washing twice with 5% trichloroacetic acid and twice with 2:1 methanol: ether (v/v). They were dried under nitrogen and 20 mg aliquots were solubilized in scintillation vials with 1 ml of Soluene (Packard Instrument Company) at 55°C in a shaking water bath. In some experiments lipids were also extracted from adipose tissue and proteins isolated from heart and skeletal muscle.

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RESULTS

Experiment 1. A comparison was made between levels of 0, 1.0 and 10.0 µg of dioxin per kg of body weight on the incorporation of labelled acetate and leucine into liver lipids and proteins. The dioxin significantly reduced body weight gain ($P<0.05$) and the radioactivity of the free fatty acids in the liver ($P<0.01$) along with reductions in other liver lipid fractions (Table 1). The specific activity of liver protein, on the other hand, increased ($P<0.05$) when dioxin was given. The 10.0 µg/kg dose level appeared to have a greater effect on most measurements than the 1.0 µg/kg level but the differences between them were not large.

TABLE 1

Effects of Dioxin on Liver Lipid and Protein Synthesis 3 days after dosage

	Oral dioxin dosage (µg/kg)			
	0	1.0	10.0	S.E.
Number of rats	4	4	4	
Initial weight, g	49.5	51.2	51.0	
Weight gain per day, g	3.1	0.4	0.2	2.1*
Liver lipids				
(DPM ^{3}H/mg liver)				
total lipids	23.66	15.46	11.46	4.25
triglycerides	3.47	2.09	2.17	0.53
free fatty acids	0.31	0.13	0.08	0.05**
diglycerides	13.95	10.68	6.63	2.67
phospholipids	5.91	2.58	2.66	1.26
Liver protein				
(DPM ^{14}C/mg protein)	234	330	330	28

*P < 0.05
**P < 0.01

Experiment 2. The 10.0 µg level of dioxin was again compared with a zero control over a 3-day period. It again had a similar effect on growth rate and the incorporation of labelled acetate and leucine into lipids and proteins but with more rats per group more of the differences were significant at the 1% level of probability (Table 2). The ^{14}C-leucine incorporation by the heart was not affected by dioxin but there was a significant reduction ($P<0.05$) in the incorporation of ^{3}H-acetate by epididymal adipose tissue.

TABLE 2

Effects of Dioxin on Lipid and Protein Synthesis in Liver Compared with that in Heart and Adipose Tissue 3 days after Dosage

	Oral dioxin dosage (µg/kg)		
	0	10.0	S.E.
Number of rats	8	8	
Initial weight, g	76.1	76.2	
Weight gain per day, g	2.25	0.36	0.35**
Liver lipids			
(DPM ^{3}H/mg liver)			
total lipids	15.62	6.92	1.86**
triglycerides	2.69	1.31	0.39*
free fatty acids	0.08	0.07	0.07
diglycerides	4.02	1.62	0.95
phospholipids	5.55	1.98	0.64**
Epididymal adipose tissue			
(DPM ^{3}H/mg lipid)	215.8	71.7	37.4*
Liver protein			
(DPM ^{14}C/mg protein)	166	223	6.9**
Heart protein			
(DPM ^{14}C/mg protein)	38	33	7.0

*P < 0.05
**P < 0.01

Experiment 3. The 10.0 µg level of dioxin was used to determine the number of days after dosing at which it would have the greatest effect on liver lipid synthesis (Table 3). There was a slight reduction in ^{3}H-acetate uptake by liver lipids (DPM/g of liver) at 2 days and a significant decline (P<0.05) at 7 days.

TABLE 3

Effect of Dioxin on Liver Lipid Synthesis at Varying Intervals After Dosage

	Days after giving 10 µg dioxin/kg				
	Controls	1	2	3	7
Number of rats	4	4	4	4	4
Initial weight, g	61.5	64.0	65.7	62.0	63.5
Weight gain per day, g	3.4	2.5	1.9	2.8	3.1
Total liver lipids (DPM/mg liver)	16.38	16.79	13.78	14.35	9.00*

*P < 0.05

Experiment 4. Graded levels of dioxin were used in this experiment to determine the smallest dosage that would have a noticeable effect on protein or fat synthesis 7 days later (Table 4). There was a progressive increase ($P<0.05$) in liver weight as the level of dioxin intake increased with a maximum increase of 21% at the 10 µg/kg level and a minimum of 9% at 0.1 µg/kg. There also was a trend towards reduced ^{3}H-acetate incorporation into liver and adipose tissue lipids with dioxin treatment but with only 4 rats per treatment the differences did not reach statistical significance. Part of the reduction in the incorporation of ^{3}H-acetate per g of liver could be accounted for by an increase in the size of the liver.

TABLE 4

Effects of Varying Levels of Dioxin on Lipid and Protein Synthesis 7 days after dosage

	Oral dioxin dosage (µg/kg)				
	0	0.1	1.0	10.0	S.E.
Number of rats	4	4	4	4	
Initial weight, g	63.2	64.2	69.2	65.7	
Weight gain per day, g	4.6	5.0	5.1	5.1	0.43
Liver weight, g	4.3	4.7	4.8	5.2	0.21*
Liver lipids, total (DPM ^{3}H/mg liver)	14.93	11.34	9.12	9.51	1.88
Epididymal adipose tissue (DPM ^{3}H/mg lipid)	322.3	125.2	56.3	138.0	86.0
Liver protein (DPM ^{14}C/mg protein)	186	168	153	143	17.0

Experiment 5. This experiment compared protein and fat synthesis at 3 and 7 days after dioxin dosage (Table 5). The dioxins had little effect on rate of body weight gain but liver weights were noticeably increased 3 days after treatment ($P<0.05$) and slightly increased after 7 days. The increase in liver weight was accompanied by a large increase in liver lipids which also was more apparent 3 days after dioxin treatment than after 7 days. The total incorporation of ^{3}H-acetate into liver lipids (DPM x 10^{3}/liver) was unaffected by dioxin but the increase in unlabelled liver lipids diluted the labelled acetate so that the specific activity of the liver lipids (DPM/mg lipids) was significantly ($P<0.05$) reduced.

Adipose tissue in the flank region of rats incorporated considerably less ^{3}H-acetate into lipids than epididymal tissue in earlier experiments but dioxin appeared to have a greater inhibitory effect on incorporation at 7 days than at 3 days after treatment, although neither of the differences reached statistical significance.

The protein concentration in the liver (mg/g) was slightly reduced 3 days after dioxin was given, apparently due to the large dilution of the liver with lipids. At 7 days the protein concentration in the liver was unaffected by dioxin. The incorporation of ^{14}C-leucine into liver protein was significantly ($P<0.05$) increased 3 days after dioxin treatment and only slightly increased after 7 days. The specific activity of skeletal muscle was not affected by dioxin treatment.

DISCUSSION AND CONCLUSIONS

Most of the animal experiments reported to date in the literature have used "toxic fats" or fractions of these fats containing mixtures of different chlorodibenzo-p-dioxins. The tetrachlorodibenzo-p-dioxin used in the present experiments was 99% pure and the highest dosage used (10 µg/kg) was close to lethal for when this amount was given orally to rats each day in a preliminary experiment they all died within 2 to 4 days. The lowest level in a single dosage that caused an increase in the liver weights of rats was 0.1 µg/kg. The dioxin had no effect on the rate of incorporation of ^{3}H-acetate into liver lipids but since large quantities of lipids accumulate in the liver it would appear that in some way the chemical restricts the transport of lipids out of the liver. This storage reached a maximum at about 3 days after dioxin was given and was accompanied by a significant increase in the incorporation of ^{14}C-leucine into liver proteins. Seven days after dioxin was given the incorporation of ^{14}C-leucine into liver proteins varied considerably between experiments being slightly higher than controls in Experiment 5 and slightly lower in Experiment 4. Weight gain per day showed an opposite trend being lower than controls in Experiment 5 and slightly higher in Experiment 4 but none of these differences was statistically significant. It is possible that the increased synthesis of liver proteins at 3-days was the result of an induction of liver enzymes (11) in response to the dioxin and that the time required for it to decline again varied slightly between experiments.

TABLE 5

Effect of Dioxin on Lipid and Protein Synthesis 3 and 7 days after Dosage

	3 days			7 days		
Dioxin oral dosage (µg/kg)	0	10	S.E.	0	10	S.E.
Number of rats	4	4		4	4	
Initial weight, g[1]	63.0	63.0	0.7	64.0	65.0	1.4
Weight gain per day, g	5.2	4.9	0.4	5.1	4.5	0.3
Liver weight, g	3.78	4.80	0.20*	4.03	4.65	0.30
Liver lipids						
mg/g liver	60	132	25	56	92	19
mg/liver	229	640	138	226	428	28**
DPM ^{3}H/mg liver	15.3	12.1	4.5	21.7	17.5	3.2
DPM ^{3}H/mg lipid	258	98	42*	394	190	62*
DPM $^{3}H \times 10^{3}$/liver	58	58	22	86	82	9
Adipose tissue, flank						
DPM ^{3}H/mg lipid	1.61	1.53	1.33	2.30	1.35	0.53
Liver protein						
mg/gm liver	277	241	14	252	252	13
mg/liver	1046	1157	66	1015	1169	83
DPM ^{14}C/mg protein	135	156	6*	144	152	8
DPM $^{14}C \times 10^{3}$/liver	141	178	7*	145	177	11
Skeletal muscle						
DPM ^{14}C/mg protein	42	42	2	40	39	2

[1] rats on 3-day treatment were dosed 4 days after initial weights taken and were then killed on the same day as 7-day rats.

*P < 0.05

**P < 0.01

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