Liver Enlargement Induced by the Herbicide 2,4,5-Trichlorophenoxyacetic Acid (2,4,5-T)

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Feeding of the herbicide 2,4,5-T causes the liver to enlarge while the weight of other organs (kidney, spleen) and the weight of the intact young rat are unaffected. Increases in relative liver fresh weight (and dry weight) were dose dependent and could be observed after feeding 2,4,5-T at 10 mg/day per animal for 1–2 days (total intake 167–334 mg/kg). Enlargement was associated with substantial increases in total RNA and total protein per liver. These increases were not restricted to any particular subcellular fraction, but appear to represent a general induction of RNA and protein synthesis. Total DNA content per liver was not affected by the herbicide while the DNA and RNA content per gram of fresh liver was reduced. The enlargement response was reversible on the removal of the herbicide from the diet and appears not to be directed toward the synthesis of 2,4,5-T metabolizing enzyme activities. The activity in 2,4,5-T fed rats of enzymes sensitive to known hepatotoxins suggests that 2,4,5-T does not have a strong hepatotoxic activity. The herbicide demonstrates activity similar to the structurally related drug chlorophenoxyisobutyrate (CPIB) which like 2,4,5-T induces liver enlargement and stimulates hepatic RNA and protein synthesis while inducing extensive self-metabolism.

The phenoxyacetic acids, 2,4-D and 2,4,5-T, were introduced as selective herbicides at the end of World War II following publication of wartime research on their growth regulatory and herbicidal activities. Despite extensive research the mechanism of action of these hormones in plants is not well known. Even more poorly understood are the biochemical effects of the phenoxyacetic acids as they relate to the health and welfare of animals and humans (Cherry, 1970).

Serious outbreaks of porphyria cutanea tarda (an acquired defect in hepatic porphyrin metabolism) and occupational chloroacne, both associated with the production of 2,4,5-trichlorophenol and 2,4,5-T, have been reported on numerous occasions (Hofman, 1957; Kimmig and Schulz, 1957; Poland et al., 1971). More recently, it was demonstrated and confirmed that 2,4-D, 2,4,5-T as well as 2,3,7,8-TCDD (tetrachlorodibenzo-*p*-dioxin) (a very toxic manufactural impurity of 2,4,5-T) are each capable of causing birth defects in laboratory animals (Courtney et al., 1970; Courtney and Moore, 1971; Khera and McKinley, 1972; Neubert et al., 1973). Concern has been expressed that the extensive use of 2,4,5-T contaminated with TCDD as a defoliant in Vietnam might have increased the incidence of birth defects in humans.

Research into auxin herbicide activity in animals has concentrated on toxicity studies, metabolism studies, and screening for impaired fertility effects and for teratogenicity. There is little literature relating to the biochemical activity of 2,4-D and 2,4,5-T in animals, and the few studies that have been published largely ignore what is known concerning the biochemical activity of these compounds in plants (Cherry, 1970).

A study from this laboratory demonstrated liver enlargement as a result of feeding 2,4,5-T to growing animals (Chang et al., 1974) at a rate of 2000–5000 mg/kg total over a period of 4–7 weeks. In other studies lesser quantities (250–800 mg/kg total over 5–10 days) failed to induce liver enlargement in adult rats (Courtney, 1970; Courtney and Moore, 1971). Since enlargement must be preceded by a number of significant biochemical events we have further investigated the biological activity of 2,4,5-T in the rat.

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Table I. Fresh Weights and Dry Weights of Livers, Kidneys, and Spleens from 7-Day Control and 2,4,5-T Fed Animals[a]

Organ	Wt of organ (mg) per 100 g body wt		
	Control	2,4,5-T	(2,4,5-T/control) × 100
Liver, fresh	3048 ± 68	3891 ± 87	127.7
dry	910 ± 23	1166 ± 27	128.1
Kidneys, fresh	941 ± 12	938 ± 18	99.8
dry	204 ± 3	200 ± 4	98.2
Spleen, fresh	219 ± 12	214 ± 10	97.6
dry	51 ± 3	50 ± 2	98.2

[a] Figures in the table represent average (± standard error) of individual measurements on 12 control and 12 2,4,5-T treated animals. As noted in the Experimental Section animals used in this experiment were older than those of all other experiments.

We report that relatively low concentrations of 2,4,5-T dramatically increase the liver to body weight ratio and result in a number of significant biochemical changes within the liver.

EXPERIMENTAL SECTION

Animals. Long-Evans rats were used in all experiments. Shortly after birth litters were thinned to six male animals. All results excepting one set of experiments (Table I) were obtained using animals within the litter weighing 68–72 g each at 28–32 days of age. Animals were individually housed under a regulated cycle of 12 h light–12 h darkness. Animals were maintained on a commercial rat chow (Allied Mills, Inc., Chicago, Ill.) and were provided water ad libitum. For all analyses and determinations at least four animals were used.

Herbicide Administration. Analytical standard grade 2,4,5-T (sample AGR 86187, Dow Chemical Co., Midland, Mich.), containing no detectable 2,3,7,8-TCDD, at a sensitivity of 0.05 ppm was used in all experiments. The herbicide was given orally as part of the animal food supply and was mixed at a concentration of 10 mg per 6 g of rat chow. Control animals each received 6 g of chow, while experimental animals each received 6 g of chow containing 2,4,5-T. Food was given daily at 4:30–5:00 p.m. for a period of 1–11 days and animals were sacrificed 18 h after the final feeding. In one set of experiments animals received a control or 2,4,5-T containing diet daily for 6 days and were then given free access to nontreated food for a period of

8 additional days before sacrifice.

Organ Weight Determination. Animals were weighed, stunned, and decapitated. Livers, kidneys, and spleens were excised, cleansed of extraneous material, and placed in cold sucrose–TKM (0.25 M sucrose, 50 mM Tris-Cl (pH 7.8)–25 mM KCl–5 mM $MgCl_2$). Organs were rinsed, blotted dry, weighed, placed in a drying oven at 95°C for 7 days, and reweighed. Animal body weight (weight of intact animal less the weight of the blood and liver) was also recorded.

Preparation and Fractionation of Liver Homogenates. Fresh tissue was minced and homogenized using 20 strokes (1800 rpm) of a motorized Potter-Elvehjem (P.E.) glass to Teflon homogenizer at a ratio of 1:2.5 liver to cold sucrose–TKM. Homogenates were forced through four layers of cheesecloth and duplicate 5.0-ml portions were sequentially fractionated at 4°C as follows.

Nucleic acids and proteins were precipitated by adding cold perchloric acid (PCA) to 5% concentration and were pelleted on centrifugation. The precipitate was washed twice with 5-ml aliquots of 1.5% PCA (by grinding to form a fine suspension in a P.E. homogenizer) and recentrifuged.

Pellets obtained were each taken up in 15 ml of 0.30 N NaOH (using a P.E. homogenizer) and RNA was hydrolyzed on incubation at 37°C for 2 h. Each incubation was then made 5% in PCA, chilled, and centrifuged. The resulting pellet was washed twice with 1.5% PCA utilizing a P.E. homogenizer and the washes added to the initial supernatant. Aliquots of the combined supernatant were used to determine liver RNA content.

Pellets containing DNA and protein were ground to a fine suspension in 15 ml of 1.5% PCA in a P.E. homogenizer and the suspension was incubated to 90°C for 45 min to hydrolyze DNA. Samples were acidified to 5% PCA and protein precipitates were collected on centrifugation. The protein pellet was washed twice again utilizing a P.E. homogenizer with cold 1.5% PCA and the supernatants pooled with the initial DNA supernatant. Aliquots of the DNA hydrolyzates were used to estimate DNA. Protein pellets were taken directly into Folins A solution for protein content estimation.

Preparation of Subcellular Fractions. Fresh tissue was homogenized at a ratio of 1:2.5 liver to 0.25 M sucrose–extraction buffer (EB) (EB = 50 mM Tris-Cl (pH 7.8)–15 mM KCl–10 mM $MgCl_2$–5 mM 2-mercaptoethanol), filtered, and centrifuged at 1475*g* for 10 min. The supernatant fraction was removed and treated with α-amylase (60 units/ml for 15 min at 4°C) (Gamulin et al., 1972) and aliquots were diluted to 10 ml with 0.25 M sucrose–EB and made to 0.5% diethyl pyrocarbonate (Weeks and Marcus, 1969) using 1.0 M Tris to buffer carbonic acid formation. Polysomes were released from attached membranous material by addition of deoxycholate to 1.3%, and 1.15 ml of the preparation was mixed with 8.0 ml of 0.25 M sucrose (enzyme grade) and centrifuged at 105500*g* for 4 h to yield a total ribosome pellet. The liver nucleotide content of total ribosome supernatants was measured after soluble RNA and protein were removed by PCA precipitation. All pellet fractions (1475*g* pellet, total ribosomes, and soluble RNA–protein) were individually homogenized in 0.25 M sucrose and suitable aliquots of each fraction were fractionated for RNA and protein according to the procedure previously described.

Assay of Glucose-6-phosphatase and Lysosomal Acid Phosphatase (Enzymes Indicative of Normal Liver Function or of Hepatotoxicity). Glucose-6-phosphatase (G-6-Pase) was assayed in the supernatant fraction obtained on centrifuging 5% liver homogenates (in 50 mM imidazole-Cl; pH 6.5) at 10000*g* for 10 min. Assay mixtures were 3.0 ml in volume and contained in micromoles: imidazole-Cl, pH 6.5, 110; glucose 6-phosphate, 5.0; $MgCl_2$, 10.0; and 0.50 ml of enzyme. Reactions were carried out at 37°C for 15 min and terminated by addition of trichloroacetic acid (Cl_3CCOOH) to 5% concentration. Protein precipitates were removed by centrifugation and the inorganic phosphate content of the entire supernatant was estimated by the method of Allen (1940).

To determine acid phosphatase (A-Pase) activity 250-mg portions of fresh liver were homogenized in 20 ml of cold water and diluted to 40 ml with water. Four milliliters of 1% Triton X-100 was added to release acid phosphatase from membranes (Meijer and Willighagen, 1963). The reaction mixture contained 10 ml of 0.20 M sodium acetate buffer (pH 4.8), 2.0 ml of liver homogenate, and 2.0 ml of substrate solution (sodium β-glycerophosphate, 50 mg/ml). Incubation was for 1 h at 37°C and inorganic phosphate released was measured in 2.0-ml aliquots from before and after incubation (Allen, 1940).

Estimation of 2,4,5-T Content of Liver. Animals fed a control or a 2,4,5-T containing diet for a period of 3 or 6 consecutive days were used. Exactly 24 h after the last feeding animals were sacrificed, and the livers were homogenized in 9 vol of 0.10 M potassium phosphate buffer (pH 7.4). The homogenate was filtered through cheesecloth and 2.0-ml aliquots were added to 35-ml glass tubes containing 2.0 ml of 3.0 N HCl and 10 ml of hexane-2.5% isoamyl alcohol (Courtney, 1970). The herbicide was extracted into the organic phase and the phases were separated on centrifugation (2500*g*, 5 min). Aliquots (5.0 ml) of supernatant organic phase were transferred to tubes containing 5.0 ml of 0.1 M KOH. The 2,4,5-T was extracted into the aqueous phase and the mixture centrifuged at 800*g* for 5 min. The organic phase was removed by aspiration and the absorbance of the aqueous phase was measured at 288 nm against a suitable blank. Normally samples containing 2,4,5-T were read against a blank prepared from liver homogenate from 3- or 6-day control fed animals.

Protein and Nucleic Acid Estimations. Protein content was estimated by the method of Lowry et al. (1951) using bovine serum albumin as standard. RNA and DNA were determined by the orcinol and diphenylamine reactions, respectively (Dische, 1955; Burton, 1956) using yeast RNA and calf-thymus DNA as standards.

RESULTS

Preliminary experiments indicated that animal weight (or animal weight gain) is not significantly affected by feeding 2,4,5-T. For example, the weight gain of 2,4,5-T fed animals was 96 ± 3% of control weight gain (individual measurements on 12 each control and treated animals fed 7 days). In addition, the data of Figure 1b indicate that 2,4,5-T has little or no effect on animal body weight. Therefore, organ weights were calculated on a weight per 100 g body weight (relative organ weight) basis for most experiments.

It was found that when 2,4,5-T was fed for 7 days there was no change in the relative fresh weight or dry weight of either the kidneys or the spleen (Table I). However, in these experiments, increases (of equal magnitude) in the relative fresh and dry weight of the liver were obtained. Moreover, when the dry weight percentage of livers from control and treated animals, as well as similar data for kidneys and spleens, were compared the dry weight percentage for organs from control and treated animals proved to be identical in each case. Such data indicate that

Table II. Effect of Feeding 2,4,5-T for 3 or 6 Consecutive Days on the DNA, RNA, and Protein Composition of the Liver[a]

	mg of component/g fresh weight			mg of component/g liver		
	Control	2,4,5-T	(2,4,5-T/ control) × 100	Control	2,4,5-T	(2,4,5-T/ control) × 100
			Three Days			
DNA	4.72 ± 0.28	3.57 ± 0.14	75.9 ± 1.6	10.39 ± 0.67	9.93 ± 0.33	96.1 ± 2.9
RNA	14.85 ± 0.24	13.82 ± 0.07	93.1 ± 2.0	32.67 ± 0.98	38.49 ± 0.73	118.0 ± 5.7
Protein	185.58 ± 3.47	185.90 ± 2.32	100.2 ± 0.4	408.17 ± 2.08	517.81 ± 13.51	126.9 ± 1.9
			Six Days			
DNA	5.15 ± 0.16	3.60 ± 0.06	70.3 ± 2.5	11.68 ± 0.23	11.93 ± 0.56	102.2 ± 4.4
RNA	13.06 ± 0.42	11.40 ± 0.37	87.3 ± 2.1	29.65 ± 0.87	37.60 ± 1.52	126.8 ± 3.3
Protein	185.01 ± 1.78	186.09 ± 3.60	100.6 ± 0.7	421.01 ± 18.64	616.33 ± 42.82	146.1 ± 1.7

[a] Values given in the table indicate the average (± standard error). Of three experiments for a total of nine control and nine 2,4,5-T treated animals.

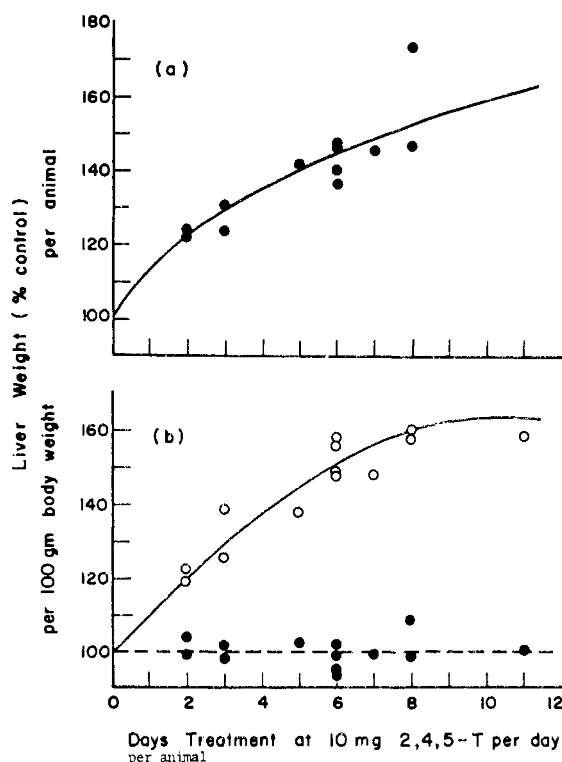

Figure 1. (a) Liver weight as a function of daily 2,4,5-T treatment. Each point on the graph was obtained by dividing the average weight of four livers from 2,4,5-T fed animals by the average weight of livers from four control animals. (b) Liver weight per 100 g body weight as a function of daily 2,4,5-T treatment. For each point on the graph determinations were made on four livers: liver weight per 100 g body weight as a percent of control (○); body weight as a percent of control (●).

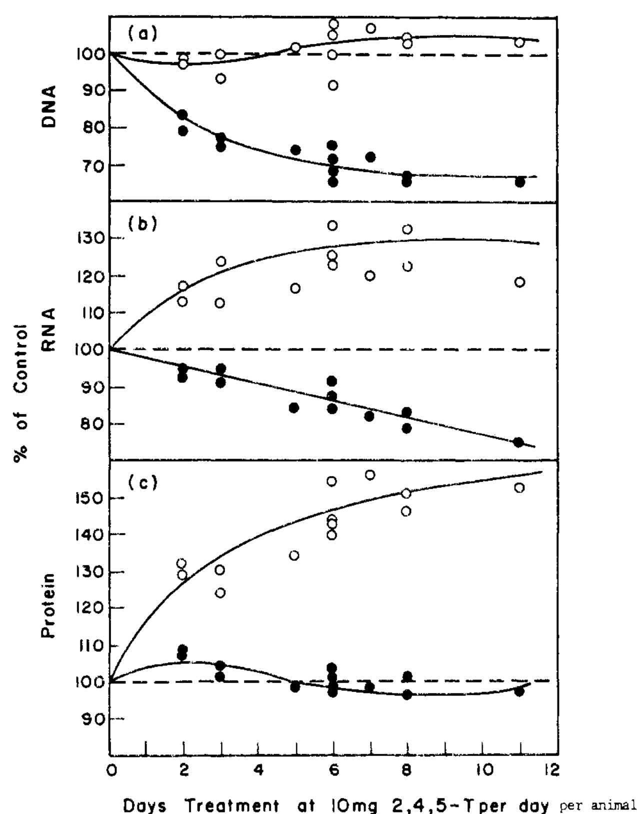

Figure 2. (a) DNA composition of livers from 2-11 day control and 2-11 day 2,4,5-T fed animals: DNA per liver (○); DNA per gram of liver (●). (b) RNA composition of livers from 2-11 day control and 2-11 day 2,4,5-T fed animals: RNA per liver (○); RNA per gram of liver (●). (c) Protein composition of livers from 2-11 day control and 2-11 day 2,4,5-T fed animals: Protein per liver (○); protein per gram of liver (●).

enlargement is not merely the result of a herbicide induced uptake of water by hepatocytes.

Liver Enlargement as a Function of 2,4,5-T Dosage. At this point we were interested in determining the relationship between 2,4,5-T dosage (number of days at 10 mg per day per animal) and the degree of liver enlargement. Increases in liver weight of approximately 20% were obtained in response to two doses of 2,4,5-T, and continued feeding resulted in liver weights 150% of control after 6 to 7 days (Figure 1a). Conversion of these data to a relative liver weight basis, as expected, gave similar increases (Figure 1b). These results suggest that a single 10-mg quantity of 2,4,5-T would increase relative liver weight about 10%.

DNA, RNA, and Protein Composition of Livers from Animals Fed 2,4,5-T. Results of two sets of experiments are presented. Figure 2 demonstrates the DNA, RNA, and protein content (as percent of control) both on a per liver and per gram of liver tissue basis as a function of repeated dosing with 2,4,5-T. Similar composition data (in terms of milligrams of DNA, RNA, or protein ± standard error) for experiments of 3 and 6 days duration are presented in Table II.

There was no 2,4,5-T induced change in the quantity of DNA per liver although DNA per gram of liver was reduced to 80 and 65% of control after 2 and 8 days, respectively (Figure 2a). These results as well as the data of Table II suggest that enlargement is due to cellular hypertrophy and not the result of increased cell division.

RNA content per gram of liver decreased to 93 and 87% of control on feeding 2,4,5-T for 2 and 8 days, respectively (Figure 2b). Total RNA content per liver, however, was increased to 115–120% of control (2 days) and 120–126% of control at 6 days.

The quantity of protein per gram of liver remained near

Table III. Effect of Feeding 2,4,5-T for 3 or 6 Consecutive Days on the Protein Content of Subcellular Fractions Isolated from Crude Liver Homogenates[a]

Fraction	Protein mg/g F.W.		(2,4,5-T/control) × 100	
	Control	2,4,5-T	g of liver F.W.	Liver
		Three Days		
Crude homogenate	185.58 ± 3.47	185.90 ± 2.32	100.2 ± 0.4	126.4 ± 1.9
1475 *g* pellet	134.41 ± 2.54	137.85 ± 4.18	102.4 ± 1.3	130.6 ± 0.5
Soluble	56.39 ± 0.65	58.56 ± 0.01	103.9 ± 1.2	132.5 ± 2.6
Total ribosomes	30.87 ± 0.77	31.48 ± 0.15	102.1 ± 2.1	130.1 ± 3.7
		Six Days		
Crude homogenate	185.01 ± 1.78	186.09 ± 3.60	100.6 ± 0.7	146.1 ± 1.7
1475 *g* pellet	142.45 ± 2.82	137.76 ± 4.14	96.8 ± 3.2	140.4 ± 2.2
Soluble	46.97 ± 1.67	47.35 ± 2.03	100.8 ± 2.9	146.5 ± 5.3
Total ribosomes	33.64 ± 0.49	29.95 ± 1.34	88.1 ± 2.9	127.7 ± 1.9

[a] Values given in the table indicate the average (± standard error) of three experiments for a total of nine control and nine 2,4,5-T treated animals.

Table IV. Effect of Feeding 2,4,5-T for 3 or 6 Consecutive Days on the RNA Content of Subcellular Fractions Isolated from Crude Liver Homogenates[a]

Fraction	RNA mg/g F.W.		2,4,5-T/control × 100	
	Control	2,4,5-T	g liver F.W.	liver
		Three Days		
Crude homogenate	14.85 ± 0.24	13.82 ± 0.07	93.1 ± 2.0	118.0 ± 5.7
1475 *g* pellet	6.76 ± 0.09	6.20 ± 0.09	91.7 ± 0.6	116.1 ± 2.5
Soluble	1.19 ± 0.04	1.27 ± 0.06	106.3 ± 1.2	134.5 ± 2.3
Total ribosomes	6.21 ± 0.15	6.78 ± 0.17	109.3 ± 5.3	139.4 ± 8.0
Nucleotide (OD units at 260 nm)	66.18 ± 3.37	68.90 ± 2.84	104.2 ± 1.0	131.9 ± 2.2
		Six Days		
Crude homogenate	13.06 ± 0.42	11.40 ± 0.37	87.3 ± 2.1	126.8 ± 3.3
1475 *g* pellet	4.44 ± 0.16	3.72 ± 0.10	83.8 ± 2.2	121.7 ± 1.7
Soluble	1.06 ± 0.06	1.05 ± 0.05	99.8 ± 2.2	144.9 ± 2.2
Total ribosomes	6.46 ± 0.30	5.72 ± 0.39	88.6 ± 2.9	128.4 ± 3.9
Nucleotide (OD units at 260 nm)	67.81 ± 0.55	73.45 ± 1.34	108.3 ± 1.3	157.3 ± 1.2

[a] Values given in the table indicate the average (± standard error) of three experiments for a total of nine control and nine 2,4,5-T treated animals.

100% of control in experiments of from 2 to 11 days duration (Figure 2c, Table II). Consequently, large increases in the quantity of protein per liver (125–130 and 145–150% of control) resulted in feeding 2,4,5-T for 2–3 and 7–8 days, respectively. From the data of Table II we calculate that 5.8 mg of RNA and 110 mg of protein (3-day experiment) and 7.9 mg of RNA and 195 mg of protein (6-day experiment) accumulate within the liver as a result of the 2,4,5-T treatment.

Subcellular Distribution of RNA and Protein Accumulated in Response to 2,4,5-T. When 2,4,5-T was fed 3 days the protein content per liver was increased to 127% of control. The 1475*g* pellet, the soluble fraction, and the total ribosome fraction contained 131, 133, and 130% of the protein found in similar fractions from control animals (on a per liver basis) (Table III). Similar studies with animals fed 2,4,5-T 6 days yielded values equal to 146, 140, 147, and 128% of the protein found in the following control fractions: crude homogenate, 1475*g* pellet, soluble RNA, and total ribosome.

Per liver increases in RNA were also observed in all subcellular fractions examined. The RNA content of crude homogenates after 3 days was 118% of control. Increases were largest in the total ribosome, soluble RNA, and nucleotide fractions (139, 135, and 132% of control, respectively), while the smallest increase was observed in the 1475*g* pellet (Table IV). When 2,4,5-T was fed 6 days similar data were obtained, the largest increases being in the soluble RNA and nucleotide fractions.

The increases in RNA and protein content resulting from feeding 2,4,5-T are thus not restricted to a particular subcellular fraction but appear to be the result of an induction of general RNA and protein synthesis.

Nature of the Enlargement Response. Liver enlargement may be a physiological (hyperfunctional) or a hepatotoxic response. It is known that reversible enlargement of the liver occurs as a passive expansion to accommodate storage material or as a response to an increased workload when adrenal corticoid secretion (gluconeogenesis) is increased (Chester-Jones and Bellamy, 1964; Morita and Kamei, 1962; Silber and Porter, 1953) or to a lesser extent when thyroid activity is stimulated. A nonreversible dose-dependent hepatotoxic enlargement is known to result when animals are fed macromolecules such as iron dextran (Golberg, 1966) or polyvinylpyrrolidone (PVP) (Meijer and Willighagen, 1963).

We have attempted to characterize 2,4,5-T induced increase in relative liver weight by determining the reversibility of enlargement and by assaying two enzymes, G-6-Pase and lysosomal A-Pase, whose activities are known to be drastically altered as a result of feeding hepatotoxic compounds (Golberg, 1966; Meijer and Willighagen, 1963). G-6-Pase is a key enzyme in the conversion of liver glycogen to blood glucose and inhibition of this enzyme activity suggests hepatotoxicity, especially when drug metabolizing enzymes are not induced (Golberg, 1966). Nonreversible enlargement in response to macromolecular toxins is associated with large increases in lysozomal A-Pase activity (Meijer and Willighagen, 1963).

In our studies animals fed 2,4,5-T 6 days (i.e. to a relative

Table V. Changes in Liver and Body Weights of Animals Fed 2,4,5-T and Then Returned to a Control Diet[a]

	Control	2,4,5-T	(2,4,5-T/control) × 100
Initial animal wt, g[b]	69.0 ± 1.62	68.71 ± 1.27	99.6
Final animal wt, g[c]	107.7 ± 5.74	114.50 ± 3.73	106.3
Liver wt, g	4.53 ± 0.22	5.03 ± 0.17	111.0

[a] Figures in the table represent average (± standard error) of individual measurements on 12 control and 12 2,4,5-T treated animals. [b] Animal weight at the beginning of experiments. [c] Weight of animal after receiving control or 2,4,5-T treated food at 6 g per day for 6 days, and then allowed to consume control food freely for an additional 8 days.

liver weight of 145–155% of control) (Figure 1) and then allowed to consume freely untreated food for 8 days had a relative liver weight (at sacrifice) of 106% of control (Table V). These data indicate that enlargement is largely reversible after the herbicide is withdrawn from the diet.

Enlarged livers had lower levels of G-6-Pase activity both on a liver weight (33% inhibition) and on a specific activity (28% inhibition) basis (Table VI). In addition A-Pase activity was not increased when 2,4,5-T was fed. A-Pase activity was inhibited 11% per unit liver weight and 14% based on specific activity. These experiments indicate enlargement is reversible and not of the type induced by toxic macromolecules. The moderate inhibition of G-6-Pase activity may be indicative of a degree of hepatotoxicity especially since only a slight 2,4,5-T metabolizing activity can be induced by prior treatment with the herbicide (Courtney, 1970).

Liver Concentrations of 2,4,5-T. The herbicide 2,-4,5-T appears not to accumulate in the organs of the rat but is rapidly excreted (as 2,4,5-T) in the urine (Courtney, 1970; Fang et al., 1973). Liver homogenates from non-2,4,5-T-treated animals do not metabolize 2,4,5-T in vitro and only traces of metabolic activity can be induced by feeding 2,4,5-T daily for 5 days before in vitro assay (Courtney, 1970). However, in these studies the relative liver weight was not increased by feeding 2,4,5-T and it has been suggested by others that increases in the relative liver weight are indicative of increased microsomal drug metabolism activity (Golberg, 1966).

Since extensive enlargement of the liver in response to 2,4,5-T (in the rat) has been reported only in studies from this laboratory we wanted to determine the quantity and form of the herbicide found in enlarged livers. The "herbicide" extracted from livers of animals fed 6 days had a spectrum identical with that of authentic 2,4,5-T (Figure 3). In addition no other ultraviolet absorbing peaks (possible breakdown products) such as 2,4,5-trichlorophenol (absorbance max 310 nm) and 2,4-D (absorbance max 283 nm) appeared in scans between 220 and 320 nm,

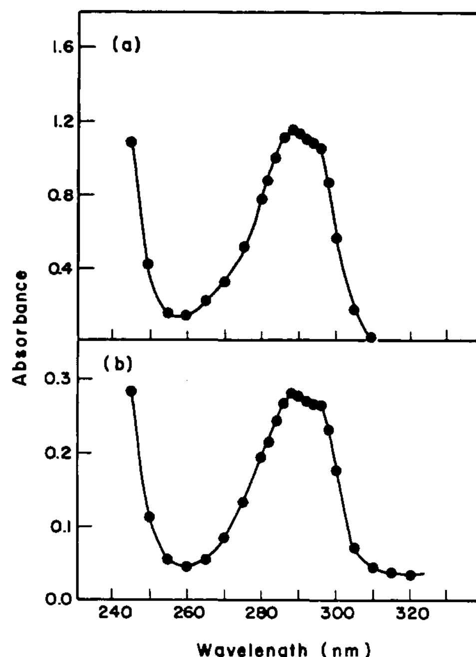

Figure 3. Ultraviolet absorption spectrum of: (a) authentic 2,4,5-T; concentration was 128 μg/ml and 0.1 M KOH was used as the reference blank; (b) "2,4,5-T" extracted from the livers of animals fed 2,4,5-T for 6 days.

suggesting that the extracted material is essentially all unaltered 2,4,5-T.

Animals fed 2,4,5-T for 3 consecutive days and sacrificed 24 h after the last feeding contained 145 ± 28 μg of 2,4,5-T per g of liver (fresh weight) or 461 ± 94 μg of 2,4,5-T per g of liver (Table VII). After 6 days livers contained nearly twice as much 2,4,5-T both on a per gram of liver (266 ± 24 μg) and on a liver (867 ± 153 μg) basis. The quantity of 2,4,5-T remaining in the liver 24 h after the final feeding represents 1.54% (3 day) and 1.45% (6 days) of the total herbicide intake.

These studies clearly demonstrate that 2,4,5-T induces enlargement of the liver of the young growing Long-Evans rat in a dose-dependent fashion. The enlargement was associated with substantial increases in total liver RNA and total protein, although the RNA content (as well as the DNA content) per gram of fresh liver was reduced. Total liver DNA content was not altered by feeding 2,-4,5-T, while both RNA/DNA and protein/DNA ratios were increased. These data suggest that enlargement is due to cellular hypertrophy.

Since enlargement is reversible on removal of 2,4,5-T from the diet and since there is only a moderate inhibition of G-6-Pase activity we believe the enlargement is not the result of herbicide toxicity alone, although a limited degree of hepatotoxicity cannot be ruled out.

Although 2,4,5-T is a poor inducer of 2,4,5-T metabolizing enzymes very large amounts of RNA and protein

Table VI. Glucose-6-phosphatase and Acid Phosphatase Activity of Liver Homogenates from Control and 2,4,5-T Fed Animals[a]

		μmol of P_i released per min			
		G-6-P-ase		Acid P-ase	
	Liver wt, g per 100 g body wt	Per g of liver	Per mg of protein ($\times 10^{-2}$)	Per g of liver	Per mg of protein ($\times 10^{-2}$)
Control	4.00 ± 0.10	2.06 ± 0.04	2.67 ± 0.08	8.83 ± 0.18	4.40 ± 0.05
2,4,5-T	5.04 ± 0.08	1.39 ± 0.09	1.92 ± 0.15	7.82 ± 0.25	3.79 ± 0.11
(2,4,5-T/control) × 100	126.0	67.3	72.4	88.6	86.1

[a] Values in the table are the average (± standard error) of individual measurements on four control and four 2,4,5-T animals. Animals received 2,4,5-T daily for 3 days.

Table VII. 2,4,5-T Content of Livers from Animals Fed a Diet Containing 2,4,5-T for 3 or 6 Days[a]

	2,4,5-T per g of liver fresh wt, μg	2,4,5-T per liver, μg	Total 2,4,5-T fed, μg	Liver 2,4,5-T content as % of total dose
3-day feeding	145 ± 28	461 ± 94	30 000	1.54
6-day feeding	266 ± 24	867 ± 153	60 000	1.45

[a] Values in the table are the average (± standard error) of three animals assayed individually for both 3- and 6-day experiments.

are produced as a response to the herbicide suggesting that little if any of the induced synthesis is directed to the production of 2,4,5-T metabolizing enzymes. Many of the results obtained in this study suggest the herbicide may have activity similar to chlorophenoxyisobutyrate (CPIB). This compound, structurally related to 2,4,5-T, decreases both liver cholesterol and circulating plasma cholesterol. The 2,4-D induced uptake of [^{14}C]acetate and [^{3}H]palmitate by L cells observed by Kolberg et al. (1972) could be related to a "CPIB-like" activity of auxin herbicides (Spector and Soboroff, 1971).

Both 2,4,5-T and CPIB reversibly increase relative liver weight, and each stimulates RNA synthesis, without extensive stimulation of self-metabolism. CPIB does not increase the relative liver weight of adrenalectomized–gonadectomized animals suggesting CPIB exerts its influence via the regulation of levels or activities of endogenous hormones produced by these organs (Golberg, 1966). Our studies suggest that 2,4,5-T may act in a similar fashion.

ACKNOWLEDGMENT

Samples of 2,4,5-T were gifts from the Dow Chemical Company.

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Received for review August 13, 1974. Accepted November 6, 1975. This research was supported in part by a grant (ROESO0551-03) from the U.S. Public Health Service. Journal Paper 5989 of the Purdue Agriculture Experiment Station.