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HEPATIC INDICES OF THYROID STATUS IN RATS TREATED WITH 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN*

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Abstract—The functional thyroid status of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-treated rats is unknown. Therefore, activities of certain thyroid-responsive enzymes were examined in the livers of adult male Sprague–Dawley rats 1 week after treatment with TCDD (6.25, 25 or 100 μg/kg). Activity of the thyroid-responsive flavin L-glycerol-3-phosphate dehydrogenase (per mg mitochondrial protein) was decreased slightly in livers of TCDD-treated rats, while that of succinate dehydrogenase remained unchanged. In contrast, activities (per mg supernatant protein) of three thyroid-responsive NADP-dependent cytosolic enzymes, malic enzyme, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, were increased by TCDD treatment in a dose-dependent manner. Lactate dehydrogenase (activity per mg supernatant protein) was also augmented slightly 1 week after TCDD administration. Liver mass was increased by TCDD treatment in a dose-dependent manner, but DNA content per liver was similar at all doses examined. Total hepatic protein, expressed per liver or mg hepatic DNA, was increased in TCDD-treated rats when compared to their pair-fed counterparts. The decreased activity of the mitochondrial L-glycerol-3-phosphate dehydrogenase, in contrast to the increased activities of the supernatant enzymes, malic enzyme, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, is not consistent with a shift in functional thyroid status following TCDD treatment.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD‡) is the prototype for certain halogenated aromatic hydrocarbons which effect a similar pattern of toxicity [1, 2]. Treatment of rats with TCDD decreases circulating levels of L-thyroxine (T_4) while either decreasing, not affecting or increasing those of 3,5,3′-triiodo-L-thyronine (T_3) [3–6]. The divergent effects of TCDD on T_4 and T_3 plasma concentrations in rats and the lack of information on thyroid hormone-modulated responses in these animals have caused uncertainty regarding their functional thyroid status. The presence of goiter [3] and depressed circulating levels of total and free T_4 [3–6] in TCDD-treated rats led Rozman and coworkers to propose that the animals are hypothyroid [7, 8]. On the other hand, the proposition by McKinney and associates [9] suggesting that TCDD acts as a potent and persistent T_4 agonist raises the possibility of TCDD-treated rats being hyperthyroid. Recently, administration of TCDD to rats was found to increase the hepatic activity of malic enzyme, a thyroid-responsive enzyme [10, 11]. From this effect, Roth *et al.* [10] have suggested that TCDD-treated rats are hyperthyroid.

The present investigation is the second segment of a three-part study from our laboratory [6, 12], the purpose of which is to determine the functional thyroid status of TCDD-treated rats at the level of the target tissue. Circulating levels of thyroid hormones, thyroid histology and a number of thyroid hormone-modulated responses have been evaluated in rats 1 week after vehicle or TCDD treatment [6]. The rationale for this protocol was that, if altered thyroid status contributes to the development of overt TCDD toxicity, then thyroid dysfunction should be present 1 week after TCDD administration because overt toxicity is present at this time [6, 13, 14]. TCDD doses ranging from nonlethal to lethal have been investigated in order to compare potential dose-related effects on thyroid function to those associated with overt toxicity. Since TCDD treatment results in a dose-dependent decrease in feed intake [15], vehicle-treated rats pair-fed to individual TCDD-treated animals have been used to determine whether an alteration in a thyroid hormone-modulated response is secondary to hypophagia. Findings in our laboratory have indicated that rats given doses of TCDD sufficient to cause overt toxicity remain functionally euthyroid in spite of severe alterations in circulating levels of thyroid hormones [6, 12]. This conclusion is based on the comparison of basal meta-

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‡ Abbreviations: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; T_3, 3,5,3′-triiodo-L-thyronine; T_4, L-thyroxine; TES, sodium *N*-tris(hydroxymethyl)-methyl-2-aminoethanesulfonic acid; PMS, phenazine methosulfate; INT, *p*-iodonitrotetrazolium violet; and ANOVA, analysis of variance.

bolic rate, thyroid histology, atrial sensitivity to the chronotropic and inotropic effects of the β-adrenoreceptor agonist (−)-isoproterenol, ventricular pyruvate kinase and citrate synthase activities and cardiac ventricular weights in TCDD-treated rats, their pair-fed counterparts, and a group of animals with unlimited access to feed.

This second segment of our on-going study of the functional thyroid status in TCDD-treated rats investigates the activity of certain thyroid-responsive hepatic enzymes in rats following administration of TCDD. Hepatic activities of both mitochondrial L-glycerol-3-phosphate dehydrogenase [L-glycerol-3-phosphate: (acceptor) oxidoreductase; EC 1.1.99.5] and cytosolic malic enzyme [L-malate:$NADP^+$ oxidoreductase (decarboxylating); EC 1.1.1.40] are increased in hyperthyroid rats but decreased in hypothyroid animals [16–19]. Activities of two NADP-dependent dehydrogenases of the pentose phosphate pathway [glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate:$NADP^+$ 1-oxidoreductase; EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (6-phospho-D-gluconate:$NADP^+$ 2-oxidoreductase; EC 1.1.1.43)] in liver are also affected by thyroid status in a manner similar to those of L-glycerol-3-phosphate dehydrogenase and malic enzyme [20]. The activities of these thyroid-responsive enzymes, as well as those of constitutive (non-hormone dependent) enzymes, will be compared in TCDD-treated rats, their pair-fed counterparts, and a group of vehicle-treated rats with unlimited access to feed. Thereby, further insight as to the functional thyroid status of TCDD-treated rats should be achieved.

METHODS

Chemicals. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD; > 99% pure) was obtained from the Dow Chemical Co. (Midland, MI). Mazola corn oil was purchased locally. Cytochrome *c*, sodium pyruvate, and L-glycerol-3-phosphate were obtained from the Boehringer Mannheim Corp. (Indianapolis, IN), where succinic acid and phenazine methosulfate (PMS) were from the Aldrich Chemical Co. (Milwaukee, WI). Coomassie blue G-250 was acquired from the Pierce Chemical Co. (Rockford, IL). The remaining substrates and cofactors necessary for the assay of liver enzyme activities, as well as crystalline bovine serum albumin, calf thymus DNA, and diphenylamine, were purchased from the Sigma Chemical Co. (St. Louis, MO). All other chemicals were of reagent grade.

Animals and treatments. Adult (275–300 g) male Sprague–Dawley rats, purchased from Harlan Sprague–Dawley (Indianapolis, IN), were individually housed in stainless steel cages with stainless steel mesh floors in a room maintained at 22 ± 1° and lighted from 6:00 a.m. to 6:00 p.m. All rats were entrained to a 14-hr feeding schedule where ground feed (Purina Rat Chow no. 5012, Ralston Purina Co., St. Louis, MO) was available from 6:00 p.m. to 8:00 a.m. and tap water was available *ad lib.* The chow was presented to the animals in glass screw-capped feeders (Hazleton Systems, Aberdeen, MD) fastened to the inside of the cages, which prevented feed spillage [15]. Rats were acclimated to the 14-hr feeding schedule for at least 5 days prior to initiation of the experiment.

Following the acclimation period, a pair of rats was matched based on similar body weights. A single dose of TCDD (6.25, 25 or 100 μg/kg, p.o.) was administered to one of these animals. Twenty-four hours following the dosing of its TCDD-treated partner, the weight-matched counterpart was given the equivalent volume of vehicle (acetone/corn oil, 5/95, v/v; 2 ml/kg, p.o.). These vehicle-treated rats received the same amount of feed that their TCDD-treated counterparts consumed during the previous 14-hr feeding period and were designated as pair-fed animals. A group of vehicle-treated rats allowed unlimited access to feed during the 14-hr feeding period was also examined.

Rats were exsanguinated by decapitation 7 days post-treatment between 1:00 and 2:30 p.m. A thoracotomy was then performed, the heart was removed [12], and a portion of liver was frozen by a freeze-stop technique [21]. The frozen tissue was weighed and ground to the consistency of find sand in the presence of liquid nitrogen with a mortar and pestle precooled to the temperature of dry ice. The frozen liver powder was stored in cryogenic vials under liquid nitrogen until the time of assay. The remaining liver tissue was excised and weighed. Total liver weight represents the combined weights of these two portions.

Preparation of subcellular fractions. Liver powders were fractionated according to a modification of the method described by de Duve [22]. One gram of rat liver was assumed to have a volume of 1 ml of 0.25 M sucrose, and was homogenized in 9 vol. of 0.25 M sucrose. A nuclear pellet was separated following centrifugation of the homogenate at 600 *g* for 10 min. The resulting supernatant fraction was centrifuged at 12,000 *g* for 10 min and yielded the mitochondrial pellet. The supernatant fraction from the 12,000 *g* spin was centrifuged at 100,000 *g* for 30 min and yielded the microsomal pellet. The supernatant fraction from the 100,000 *g* spin was used for the assay of the "supernatant" enzymes. All operations were carried out at 4°. Each particulate fraction was suspended in 0.25 M sucrose in a volume which was $\frac{1}{6}$ of the starting homogenate and repelleted at the same *g* force. This second supernatant was then combined with the original supernatant obtained for the respective fraction and subjected to the next higher *g* force to obtain the subsequent fraction. All particulate fractions were finally suspended in a volume of 0.25 M sucrose equal to that of the starting homogenate. To obtain a more homogeneous suspension, the nuclei and mitochondrial pellets were agitated briefly on a Vortex mixer, while the microsomal pellet was mechanically suspended with a Tissumizer (Tekman Co., Cincinnati, OH).

In the assay of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, homogenates of liver powders were prepared as described above. However, the particle-free supernatant was obtained directly from centrifugation of the whole homogenate at 100,000 *g* × 30 min.

Assay of enzyme activity. Enzyme activity was determined at 30°. All absorbances were measured with a 1 cm path in a Beckman DU-7 spectro-

photometer. For the assay of malic enzyme, lactate dehydrogenase, succinate dehydrogenase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, activity was determined with the Water Regulated Kinetics System.

Lactate dehydrogenase activity (L-lactate:NAD^+-oxidoreductase; EC 1.1.1.27) in the supernatant fraction was assayed with a reaction mixture containing 50 mM TES [sodium *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid], 0.1 mM dithiothreitol and 0.2 mM NADH [23]. After recording the baseline, the reaction was initiated by adding sodium pyruvate in an end concentration of 1 mM and the change in absorbance was determined at 340 nm.

Malic enzyme activity in the supernatant fraction was assayed with a reaction mixture containing 50 mM TES (pH 7.4), 4 mM $MnCl_2$, 0.2 mM NADP and 0.12 mM dithiothreitol [24–26]. After recording the baseline, the reaction was initiated by adding L-malic acid (disodium salt) in an end concentration of 0.5 mM. Change in absorbance was then measured at 340 nm.

The activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in the supernatant fraction were assayed with a reaction mixture containing 120 mM Tris buffer (pH 8.0), 7.5 mM $MgCl_2$ and 0.9 mM NADP [27]. Enzyme-containing supernatant fraction was added to each of two cuvettes. After recording the baseline at 340 nm, the reaction was initiated by adding a mixture of glucose-6-phosphate and 6-phosphogluconate in an end concentration of 2.0 and 0.6 mM, respectively, to the first cuvette, while 6-phosphogluconate alone was added to the second cuvette. The activity in the first cuvette minus that of the second gave the activity for glucose-6-phosphate dehydrogenase, whereas the activity for 6-phosphogluconate dehydrogenase was determined by the change in absorbance in the second cuvette alone.

Succinate dehydrogenase [succinate: (acceptor) oxidoreductase; EC 1.3.99.1] activity in the mitochondrial fraction was determined in a total reaction volume of 2 ml containing 10 mM potassium phosphate (pH 7.4), 10 mg/ml bovine albumin, 1 mM KCN and 5 mM sodium succinate [28]. To an assay mixture of 1.8 ml containing the enzyme, cytochrome *c* (2 mg in 0.2 ml H_2O) was added to initiate the reaction. Change in absorbance was measured at 550 nm against a blank reaction that contained all the above components except succinate.

L-Glycerol-3-phosphate dehydrogenase in the mitochondrial fraction was assayed using PMS and *p*-iodonitrotetrazolium violet (INT) as artificial electron acceptors [16]. The reaction mixture contained 125 mM phosphate buffer (pH 7.5), 5 mM KCN and 150 mM L-glycerol-3-phosphate in 0.02 ml. Enzyme-containing solution (0.04 ml) was added to the reaction mixture and preincubation was carried out for 10 min at 30°. Then, PMS–INT solution (0.04 ml), prepared by dissolving 5 mg PMS and 8 mg INT in 2 ml of 50 mM phosphate buffer (pH 7.5), was added. The reaction mixture was protected from light during the assay. The incubation was stopped after 15 min by adding 0.02 ml of 10% (w/v) trichloroacetic acid solution followed by 1 ml of 95% (v/v) ethanol, and the reaction mixture was centrifuged to remove the precipitate. Absorbance was read at 500 nm.

Enzyme activity. A unit of enzyme activity was defined as a μmole of substrate consumed or product generated per min under conditions given. Enzyme activity has been presented on a primary unit basis (i.e. g wet weight). Several other bases, including those of protein, DNA and total liver, were also utilized in the expression of enzyme activity.

Liver protein and DNA. Protein was determined by the dye-binding method of Bradford [29] using crystalline bovine serum albumin as the standard. For the determination of DNA, a liver homogenate (10%, w/v) was prepared in water by mechanically disrupting the tissue (2 × 15 sec; Tissumizer) with a 30-sec pause between bursts. Samples were cooled in an ice-water mixture during the homogenization procedure. Following extraction of interfering lipids [30], DNA was estimated by a micro-adaptation of the procedure described by Richards [31] using calf thymus DNA as the standard.

Statistical analysis. The main effects of treatment and dosing were tested by two-way analysis of variance (ANOVA) through the use of unweighted cell means [32]. Significance of differences between TCDD-treated rats and their pair-fed counterparts was detected by pairwise comparison [33]. Effects of dosing within the TCDD-treated or pair-fed regimens, including comparison to the vehicle-treated group of rats with unlimited access to feed, were tested by one-way ANOVA. This was followed by evaluation of contrasts using Scheffé's method [34]. Linear function was examined, following significance of a dosing effect, by testing for trends using orthogonal coefficients [33]. The computations were performed with a VAX-750 computer using BMDP [33]. All significance levels were set at $P < 0.05$.

RESULTS

Body weight and feed intake. TCDD-treated rats and their pair-fed partners exhibited a similar, dose-related reduction in body weight in comparison to the vehicle-treated group with unlimited access to feed (Table 1). Body weights of the TCDD-treated rats and their pair-fed counterparts were similar at day 7 post-treatment except at the lowest dose used (6.25 μg/kg), where rats receiving TCDD were significantly heavier. Feed intake on day 7 following treatment was also depressed in a dose-dependent manner following administration of TCDD (Table 1). At the highest dose of TCDD (100 μg/kg), this reduction amounted to approximately 50% of the amount consumed by the vehicle-treated group with unlimited access to feed. Rats pair-fed to their TCDD-treated counterparts had comparable feed intakes.

Liver mass, DNA and protein. TCDD-treated rats exhibited marked hepatomegaly by day 7 post-treatment (Table 1). At this time, absolute and relative liver weights of TCDD-treated rats were significantly greater than those of pair-fed animals and of vehicle-treated rats with unlimited access to feed. Absolute liver weight was increased similarly in TCDD-treated rats at all doses tested. Yet, one-way ANOVA among the various TCDD doses indicated a signifi-

Table 1. Effects of TCDD treatment on body weight, feed intake and liver weight*

TCDD dose (μg/kg)	Treatment	Body weight (g)	Feed intake (g/14 hr)	Liver weight	
				Absolute (g wet wt)	Relative (g wet wt/100 g body wt)
0	Unlimited-fed	323 ± 6†	22.7 ± 0.9†	11.4 ± 0.4†	3.5 ± 0.1†
6.25	TCDD	312 ± 5‡, ¶	19.8 ± 1.4‡	13.9 ± 0.4‡, ¶	4.4 ± 0.1‡, ¶
	Pair-fed	301 ± 4‡	18.3 ± 1.4‡	9.8 ± 0.5‡	3.2 ± 0.1†, ‡
25	TCDD	293 ± 4§	15.4 ± 1.4§	13.9 ± 0.6‡, ¶	4.7 ± 0.2‡, ¶
	Pair-fed	293 ± 4‡	15.4 ± 1.4‡	8.9 ± 0.5‡, §	3.0 ± 0.1‡
100	TCDD	273 ± 4‖	10.9 ± 0.9‖	13.1 ± 0.4‡, ¶	4.8 ± 0.1‡, ¶
	Pair-fed	274 ± 4§	11.0 ± 1.0§	8.1 ± 0.3§	3.0 ± 0.1‡

* Values in the table were determined 7 days after administration of TCDD or vehicle. Vehicle-treated rats were allowed unlimited access to feed during the 14-hr feeding period or were pair-fed to a TCDD-treated rat. Each value represents the mean ±SE of eight to fourteen animals.

†–‖ Mean value in a column not followed by the same superscript are significantly different from other dose levels in the same treatment group (TCDD or pair-fed) using Scheffé's methodology ($P < 0.05$). The unlimited-fed group serves as the 0 dose level for both treatment groups.

¶ Significantly different from pair-fed group at the same dose level by pair-wise comparison ($P < 0.05$).

cant linear trend to increase relative liver mass when expressed per unit of body weight. In contrast, rats pair-fed to TCDD-treated animals exhibited a significant linear decrease in both absolute and relative liver weights.

Hepatic DNA concentration (mg/g wet weight) increased linearly in the pair-fed animals as the dose of TCDD received by their TCDD-treated counterparts increased (Fig. 1). The increase in DNA concentration in the livers of rats pair-fed to rats treated with 100 μg/kg TCDD was significant when compared to rats with unlimited access to feed. Also, hepatic concentration of DNA in the corresponding group of pair-fed rats was significantly higher than that in their TCDD-treated partners at doses of 25 and 100 μg/kg. In TCDD-treated rats at all doses examined, the concentration of DNA per gram wet liver was similar to that found in the unlimited-fed group. When differences in liver mass were taken into account (Table 1), the total DNA per liver in the pair-fed rats and their respective TCDD-treated partners was similar (Fig. 1). Total liver DNA content increased across the doses investigated in both TCDD-treated and pair-fed rats, yet this trend was statistically significant only with pair-feeding.

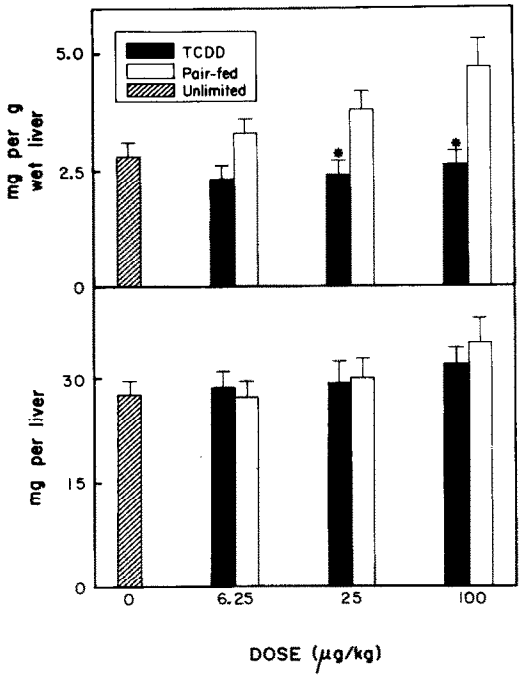

Fig. 1. Effects of TCDD treatment of hepatic DNA concentration and content in rats. Livers were removed on day 7 following administration of either TCDD or vehicle. Vehicle-treated rats were allowed either unlimited access to feed or were pair-fed to individual TCDD-treated rats. The height of each bar and associated vertical line represents the mean and SE of four to six animals. An asterisk indicates a significant difference between a TCDD-treated group and its respective pair-fed group ($P < 0.05$).

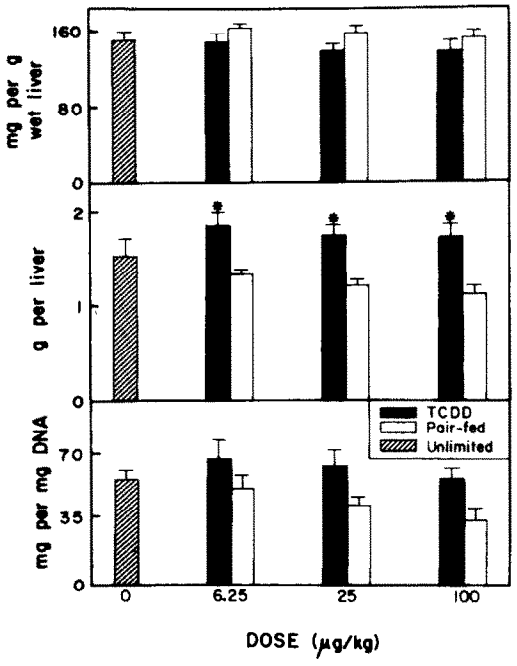

Fig. 2. Effects of TCDD treatment on hepatic protein concentration and content in rats. Other conditions were as in Fig. 1.

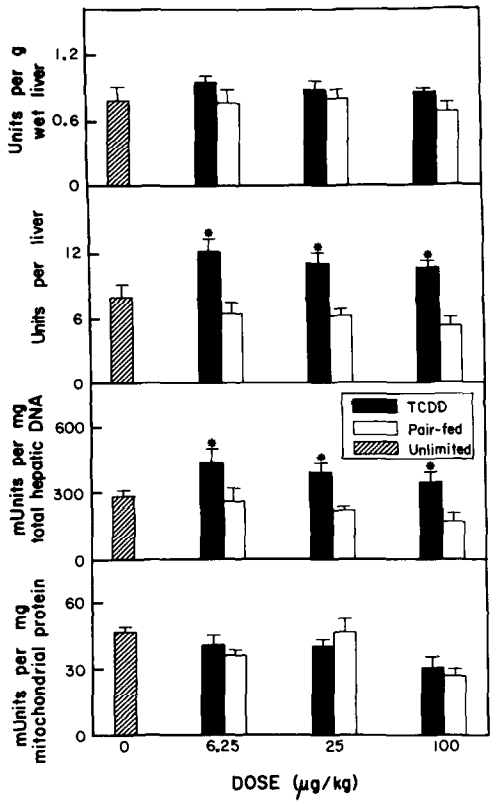

Fig. 3. Effects of TCDD treatment on hepatic succinate dehydrogenase activity in rats. Other conditions were as in Fig. 1.

Hepatic protein concentration (mg/g wet liver) was similar in all treatment groups (Fig. 2). As a result of a diminution of liver mass (Table 1), a significant linear decrease in total protein per liver of pair-fed rats with increasing TCDD dose in the treated counterparts was detected by one-way ANOVA (Fig. 2). Total protein content was significantly greater in the livers of TCDD-treated rats at all dose levels than in their pair-fed counterparts by pair-wise comparison following two-way ANOVA. When total hepatic protein was expressed per mg DNA, one-way ANOVA indicated a significant linear decrease in the pair-fed rats as the dose of TCDD received by the treated counterparts increased (Fig. 2). Liver protein expressed on a DNA basis was higher in the TCDD-treated rats than in their pair-fed counterparts (Fig. 2), and this overall treatment effect was statistically significant when tested by two-way ANOVA. Despite these significant differences between TCDD-treated and pair-fed rats in total liver protein (Fig. 2), the percent distribution of protein in the various subcellular fractions was similar when liver homogenates were fractionated according to the scheme presented in Methods (data not shown).

Mitochondrial enzyme activities. Succinate dehydrogenase activity in an isolated mitochondrial fraction, expressed per g wet liver, was similar in all treatment groups (Fig. 3). When expressed per total liver, the activity of hepatic succinate dehydrogenase in the various groups of pair-fed rats was similar to that observed in the unlimited-fed group. However, the activity per liver in the TCDD-treated rats was

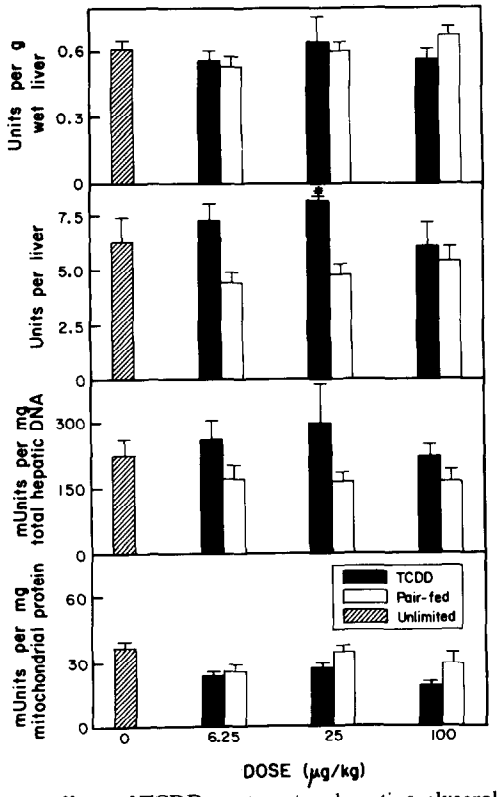

Fig. 4. Effects of TCDD treatment on hepatic L-glycerol-3-phosphate dehydrogenase activity in rats. Other conditions were as in Fig. 1.

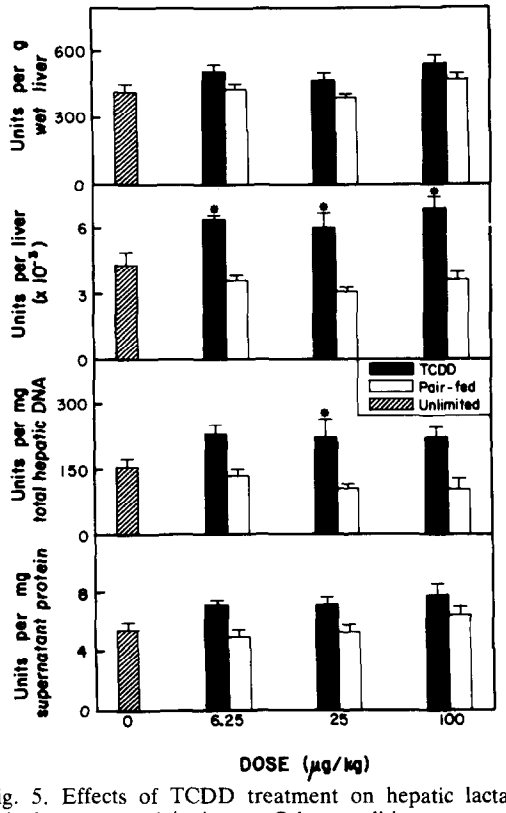

Fig. 5. Effects of TCDD treatment on hepatic lactate dehydrogenase activity in rats. Other conditions were as in Fig. 1.

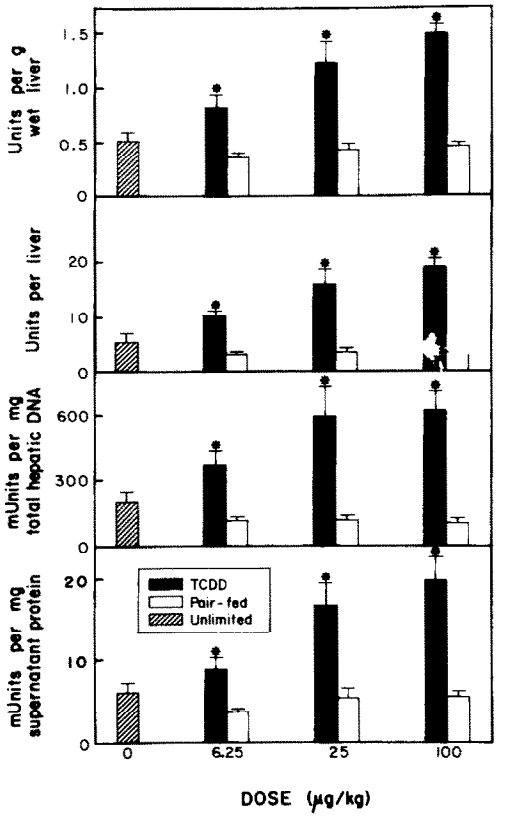

Fig. 6. Effects of TCDD treatment on hepatic malic enzyme activity in rats. Other conditions were as in Fig. 1.

elevated significantly in comparison to their pair-fed counterparts at all doses examined (Fig. 3). A linear decrease in the activity of succinate dehydrogenase per mg total hepatic DNA in pair-fed animals, as the dose of TCDD received by their treated counterparts increased, was demonstrated in the one-way ANOVA. In the TCDD-treated rats, its activity was also elevated when expressed on a DNA basis in relation to that found in pair-fed partners at all doses tested (Fig. 3). When succinate dehydrogenase was expressed per mg mitochondrial protein, a reduction in activity was found in both the TCDD-treated rats and their pair-fed counterparts at the 100 μg/kg dose. However, only the latter difference was statistically significant in comparison to the unlimited-fed rats with Scheffé's method.

L-Glycerol-3-phosphate dehydrogenase activity per g wet liver was similar in all treatment groups (Fig. 4). However, when activity was expressed per liver, taking into account changes in hepatic mass (Table 1), L-glycerol-3-phosphate dehydrogenase activity was increased in livers from TCDD-treated rats in relation to their pair-fed partners at doses of 6.25 and 25 μg/kg, with the difference being statistically significant at the latter dose (Fig. 4). L-Glycerol-3-phosphate dehydrogenase activity expressed on a DNA basis was also higher in TCDD-treated rats than in their pair-fed counterparts (Fig. 4), and this overall treatment effect was statistically significant when tested by two-way ANOVA. However, two-way ANOVA indicated that its activity per mg mitochondrial protein was significantly lower in TCDD-treated rats than in their pair-fed partners. The activity of L-glycerol-3-phosphate dehydrogenase per mg mitochondrial protein in TCDD-treated rats decreased significantly in a linear trend across doses. When compared to rats with unlimited access to feed, the reduction in hepatic activity of this enzyme in rats treated with 6.25 and 100 μg/kg was significant by evaluation of contrasts using Scheffé's method.

Supernatant enzyme activities. Lactate dehydrogenase activity determined in a high-speed supernatant of liver homogenate and expressed per g wet liver was increased in TCDD-treated rats when compared to their pair-fed partners, and this overall treatment effect was statistically significant in the two-way ANOVA (Fig. 5). When expressed per total liver, its activity in TCDD-treated rats was augmented, and the magnitude of the increase over that of the respective pair-fed counterparts was similar at all TCDD doses examined. In the two-way ANOVA, lactate dehydrogenase activity per mg total hepatic DNA was found to be augmented by TCDD treatment, and pair-wise comparison to respective pair-fed partners indicated this difference to be significant at the 25 μg/kg dose (Fig. 5). In the TCDD-treated rats, the activity per mg supernatant protein of hepatic lactate dehydrogenase was also elevated, and a significant linear trend was detected across the doses examined.

Malic enzyme activity exhibited a dose-related increase in livers from TCDD-treated rats when expressed per g wet liver. Whether expressed on the

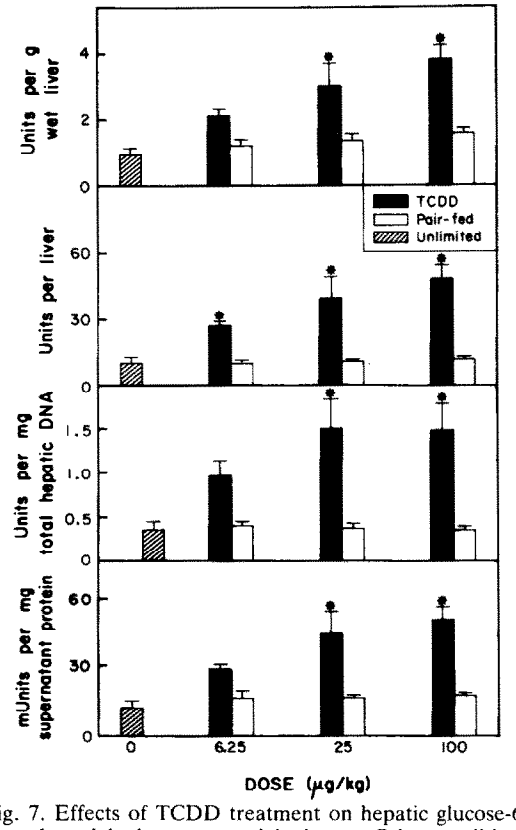

Fig. 7. Effects of TCDD treatment on hepatic glucose-6-phosphate dehydrogenase activity in rats. Other conditions were as in Fig. 1.

basis of total liver, mg DNA, or mg supernatant protein, its activity was also elevated in the rats receiving TCDD (Fig. 6). Activity of malic enzyme in livers from rats pair-fed to the TCDD-treated animals was not significantly different from that observed in livers of vehicle-treated rats with unlimited access to feed no matter how activity was expressed.

Hepatic glucose-6-phosphate dehydrogenase activity was increased by TCDD treatment in a dose-related manner (Fig. 7). This response of the enzyme to the administration of TCDD was similar whether its activity was expressed on the basis of g wet liver, total liver, mg DNA or mg supernatant protein. The activity of glucose-6-phosphate dehydrogenase in rats pair-fed to TCDD-treated animals was not only similar across the various doses examined, but was also comparable to the activity observed in vehicle-treated rats with unlimited access to feed.

The activity of 6-phosphogluconate dehydrogenase in liver was also augmented by administration of TCDD in a dose-dependent manner (Fig. 8). Whether the enzyme activity was expressed on the basis of g wet liver, total liver, mg DNA or mg supernatant protein, the response to the doses of TCDD examined was similar. The activity of 6-phosphogluconate dehydrogenase in livers obtained from rats pair-fed to TCDD-treated animals decreased in a linear manner that was statistically significant irrespective of whether its activity was expressed on the basis of g wet liver, total liver, mg DNA or mg supernatant protein.

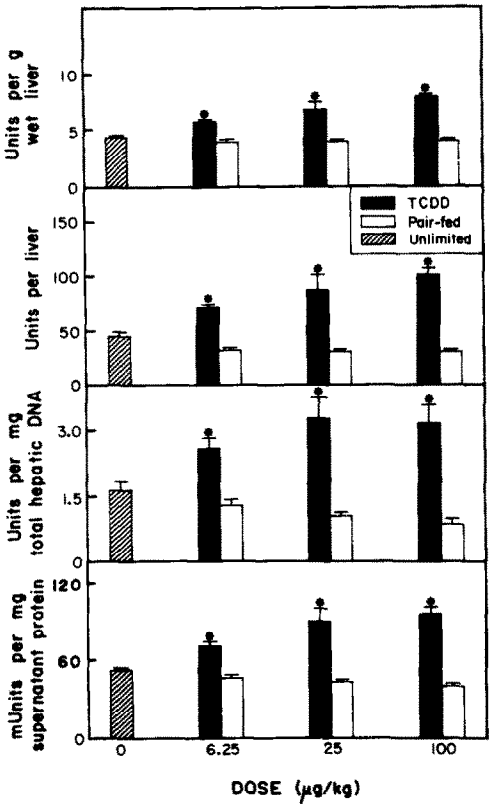

Fig. 8. Effects of TCDD treatment on hepatic 6-phosphogluconate dehydrogenase activity in rats. Other conditions were as in Fig. 1.

DISCUSSION

Assessment of a possible alteration of thyroid status by TCDD treatment of rats is complicated by the toxic effects of this compound. For instance, TCDD is known to elicit a wasting response that is caused by a reduction in feed intake [13–15, 35]. To account for alterations in the activities of thyroid-responsive hepatic enzymes that are secondary to reduced feed intake and body weight rather than a primary effect upon thyroid status, vehicle-treated animals pair-fed to TCDD-treated counterparts were examined. In the present study, pair-fed vehicle-treated rats lost nearly the same amount of weight as their TCDD-treated partners so, in this regard, pair-feeding accomplished its purpose. While TCDD treatment resulted in hepatomegaly, the liver mass of pair-fed rats was decreased. These divergent effects were shown to be due to hypertrophy and atrophy of the hepatocyte, respectively, since total hepatic DNA content remained unchanged. Similar findings have been reported by our laboratory following TCDD treatment of mature Sprague–Dawley rats [36]. To account for these differences in liver size between the experimental groups, activities of both constitutive and thyroid-responsive hepatic enzymes were expressed on the basis of g wet liver, total liver, mg DNA and mg supernatant or mitochrondrial protein.

Activities of the constitutive enzyme, succinate dehydrogenase, and the thyroid-responsive enzyme, L-glycerol-3-phosphate dehydrogenase, in an isolated mitochondrial fraction were increased in TCDD-treated rats relative to their pair-fed counterparts when expressed per liver or per mg DNA. These differences in enzyme activity were the result of differences in liver mass between the two groups. When succinate dehydrogenase activity was expressed per mg mitochondrial protein, its activity in TCDD-treated and pair-fed animals was similar. On the other hand, the activity of L-glycerol-3-phosphate dehydrogenase was decreased significantly in livers from TCDD-treated rats in comparison to their pair-fed counterparts. Also, the activity of L-glycerol-3-phosphate dehydrogenase per mg mitochondrial protein in the livers of TCDD-treated rats decreased in a linear manner across the doses examined. This finding could be interpreted as a shift in the functional thyroid status of these animals towards a hypothyroid state. A marked reduction in hepatic L-glycerol-3-phosphate dehydrogenase activity is found in hypothyroid rats [16, 18]. Other signs which might suggest hypothyroidism in TCDD-treated rats include a slightly reduced basal metabolic rate [6], elevated serum TSH concentrations [3, 6] and goiters [3]. Other effects of TCDD such as decreases in serum T_4 concentration, free T_4 index, feed intake, growth, hepatic Na^+, K^+-ATPase activity and increased serum cholesterol concentrations [3–6, 13–15, 37, 38] are also found in hypothyroid rats [39–42]. However, the increased activity of the cytosolic malic enzyme in livers of TCDD-treated rats strongly argues against a functional shift towards hypothyroidism in these animals.

Activity of the constitutive cytosolic enzyme, lactate dehydrogenase, was increased in TCDD-treated

rats relative to their pair-fed counterparts when expressed per liver or per mg DNA. This augmentation of lactate dehydrogenase activity was associated with the increase in both liver mass and total protein content in TCDD-treated animals, and the increases were similar at all TCDD doses used. In contrast to lactate dehydrogenase, activities of the three cytosolic enzymes (malic enzyme, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase), which are thyroid-responsive and NADP-dependent, were found to be increased by TCDD treatment in a dose-dependent manner. When considered in isolation from other effects of TCDD treatment on thyroid hormone-modulated responses, the increases in thyroid-responsive cytosolic enzyme activities could be interpreted to mean that there is a functional shift towards hyperthyroidism in TCDD-treated rats [10]. The increase in hepatic malic enzyme activity in TCDD-treated rats in the present study is similar in magnitude to that reported for rats made hyperthyroid by T_3 or T_4 treatment [18, 19, 43]. Also, the augmentation of the activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in the livers of TCDD-treated rats in the present study is similar to that reported in rats made hyperthyroid by chronic T_3 treatment [20]. However, the overall pattern of thyroid hormone-modulated responses in TCDD-treated rats does not support the contention that TCDD-treated rats are hyperthyroid. In particular, it is difficult to resolve the discrepant responses of hepatic L-glycerol-3-phosphate dehydrogenase and malic enzyme activities to TCDD treatment. The activities of both enzymes are decreased by hypothyroidism but increased by hyperthyroidism [16–19], yet in TCDD-treated rats the activity of L-glycerol-3-phosphate dehydrogenase fell whereas that of malic enzyme rose. Other arguments against hyperthyroidism are that basal metabolic rate is decreased by TCDD treatment [6] and that various cardiac indices of thyroid status are not altered in a way which would suggest that the animals are hyperthyroid [12]. The activities of two cardiac enzymes, pyruvate kinase and citrate synthase, and heart ventricular weight are not affected by TCDD treatment [12]. In hyperthyroid rats, the activities of these two ventricular enzymes are increased along with ventricular weight [44–47]. Therefore, the lack of effect of TCDD on these thyroid-responsive parameters further mitigates again a functional shift towards hyperthyroidism in rats following TCDD treatment.

The NADPH generated by malic enzyme and the pentose phosphate pathway provides reducing equivalents for hepatic monooxygenation. The supply of NADPH is not rate-limiting for monooxygenation in livers of normal rats, but may become rate-limiting following monooxygenase induction [48–50]. Monooxygenase inducers of the 3-methylcholanthrene [51] and phenobarbital [52, 53] type enhance $NADP^+$-dependent enzyme activity in rat liver, and the increased demand for NADPH associated with induction of monooxygenases has been proposed to be the impetus for induction of the $NADP^+$-dependent enzymes [54, 55]. The dose of TCDD needed to maximally induce rat microsomal monooxygenases has been reported to be lower [56] than that found to increase $NADP^+$-dependent enzyme activities in the present investigation, yet in other studies it was found to be similar [57]. Thus, the increase in $NADP^+$-dependent enzyme activities might be secondary to monooxygenase induction by TCDD. However, it is entirely possible that this effect of TCDD is not initiated by an augmentation of NADPH utilization by the hepatocyte but rather by an interaction of the compound with gene expression. That is, TCDD might induce these $NADP^+$-dependent enzymes by modulating the expression of a functional gene unit [20, 58, 59] which simultaneously codes for the synthesis of all three enzymes. This seems reasonable because TCDD and other positive effectors as well as negative effectors of these enzymes alter their activities in parallel [20, 58, 59].

In summary, the pattern of thyroid-responsive hepatic enzyme activities in livers of rats treated with TCDD was not compatible with a functional shift in thyroid status. This was particularly true when the results were interpreted in the context of other effects of TCDD on thyroid-modulated responses.

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REFERENCES

1. E. E. McConnell, in *Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products* (Ed. R. D. Kimbrough), p. 109. Elsevier/North-Holland Biomedical Press, New York (1980).
2. A. Poland and J. C. Knutson, *Ann. Rev. Pharmac. Toxic.* **22**, 517 (1982).
3. C. H. Bastomsky, *Endocrinology* **101**, 292 (1977).
4. C. L. Potter, I. G. Sipes and D. H. Russell, *Toxic. appl. Pharmac.* **69**, 89 (1983).
5. K. Rozman, G. A. Hazelton, C. D. Klaassen, M. P. Arlotto and A. Parkinson, *Toxicology* **37**, 51 (1985).
6. C. L. Potter, R. W. Moore, S. L. Inhorn, T. C. Hagen and R. E. Peterson, *Toxic. appl. Pharmac.* **84**, 45 (1986).
7. K. K. Rozman, in *Banbury Report 18: Biological Mechanisms of Dioxin Action* (Eds. A. Poland and R. D. Kimbrough), p. 345. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1984).
8. T. L. Pazdernik and K. K. Rozman, *Life Sci.* **36**, 695 (1985).
9. J. D. McKinney, J. Fawkes, S. Jordan, K. Chae, S. Oatley, R. E. Coleman and W. Briner, *Environ. Hlth Perspect.* **61**, 41 (1985).
10. W. Roth, P. A. Bank and S. D. Aust, *Toxicologist* **6**, 41 (1986).
11. C. K. Kelling, L. A. Menahan and R. E. Peterson, *Toxicologist* **6**, 308 (1986).
12. C. K. Kelling, L. A. Menahan and R. E. Peterson, *Toxicologist* **6**, 312 (1986).
13. R. W. Moore, C. L. Potter, H. M. Theobald, J. A. Robinson and R. E. Peterson, *Toxic. appl. Pharmac.* **79**, 99 (1985).
14. B. J. Christian, S. L. Inhorn and R. E. Peterson, *Toxic. appl. Pharmac.* **82**, 239 (1986).
15. M. D. Seefeld, R. E. Keesey and R. E. Peterson, *Toxic. appl. Pharmac.* **73**, 311 (1984).
16. Y. P. Lee and H. A. Lardy, *J. biol. Chem.* **240**, 1427 (1965).

17. W. R. Ruegamer, G. H. Newman, D. A. Richert and W. W. Westerfeld, *Endocrinology* **77**, 707 (1965).
18. J. W. Young, *Am. J. Physiol.* **214**, 378 (1968).
19. J. H. Oppenheimer, E. Silva, H. L. Schwartz and M. I. Surks, *J. clin. Invest.* **59**, 517 (1977).
20. C. N. Mariash, F. E. Kaiser and J. H. Oppenheimer, *Endocrinology* **106**, 22 (1980).
21. A. Wollenberger, O. Ristau and G. Schoffa, *Pflügers Archs. ges. Physiol.* **270**, 399 (1960).
22. C. de Duve, *Science* **189**, 186 (1975).
23. C. N. Statham, S. P. Szyjka, L. A. Menahan and J. J. Lech, *Biochem. Pharmac.* **26**, 1395 (1977).
24. S. Ochoa, in *Methods in Enzymology* (Eds. S. P. Colowick and N. O. Kaplan), Vol. 1, p. 739. Academic Press, New York (1955).
25. R. Y. Hsu and H. A. Lardy, in *Methods in Enzymology* (Ed. J. M. Lowenstein), Vol. 13, p. 230. Academic Press, New York (1969).
26. K. K. Yeung and R. J. Carrico, *Analyt. Biochem.* **74**, 369 (1976).
27. D. Rudack, E. L. Chisholm and D. Holten, *J. biol. Chem.* **246**, 1249 (1971).
28. D. E. Green, S. Mii and P. M. Kohout, *J. biol. Chem.* **217**, 551 (1955).
29. M. M. Bradford, *Analyt. Biochem.* **72**, 248 (1976).
30. J. N. Livingston, P. Cuatrecasas and D. H. Lockwood, *J. Lipid Res.* **15**, 26 (1974).
31. G. M. Richards, *Analyt. Biochem.* **57**, 369 (1974).
32. G. A. Milliken and D. E. Johnson, *Analysis of Messy Data. Vol. I: Designed Experiments.* Lifetime Learning Publications, Belmont, CA (1984).
33. W. J. Dixon, M. B. Brown, L. Engleman, J. W. Frane, M. A. Hill, R. I. Jennrich and J. D. Toporek, *BMDP Statistical Software.* University of California Press, Berkeley, CA (1981).
34. J. Neter and W. Wasserman, *Applied Linear Statistical Models.* Richard D. Irwin, Homewood, IL (1974).
35. C. K. Kelling, B. J. Christian, S. L. Inhorn and R. E. Peterson, *Fund. appl. Toxic.* **5**, 700 (1985).
36. B. J. Christian, L. A. Menahan and R. E. Peterson, *Toxic. appl. Pharmac.* **83**, 360 (1986).
37. R. E. Peterson, B. V. Madhukar, K. H. Yang and F. Matsumura, *J. Pharmac. exp. Theor.* **210**, 275 (1979).
38. P. W. Albro, J. T. Corbett, M. Harris and L. D. Lawson, *Chem. Biol. Interact.* **23**, 315 (1978).
39. E. C. Persike, *Endocrinology* **42**, 356 (1948).
40. R. Pitt-Rivers and J. R. Tata, *The Thyroid Hormones.* Pergamon, New York (1959).
41. T. J. Layden and J. L. Boyer, *J. clin. Invest.* **57**, 1009 (1976).
42. N. L. Eberhardt, J. W. Apriletti and J. D. Baxter, in *Biochemical Actions of Hormones* (Ed. G. Litwack), Vol. 7, p. 311. Academic Press, New York (1980).
43. R. A. Freedland, E. H. Avery and A. R. Taylor, *Can. J. Biochem.* **46**, 141 (1968).
44. M. Beznak, R. A. Paterson and J. R. E. Valadares, *Br. J. Pharmac.* **44**, 333P (1972).
45. K. M. Baldwin, P. J. Campbell, A. M. Hooker and R. E. Lewis, *Am. J. Physiol.* **236**, C30 (1979).
46. T. Tanaka, H. Morita, H. Koide, K. Kawamura and T. Takatsu, *Basic Res. Cardiol.* **80**, 165 (1985).
47. D. Siehl, B. H. L. Chua, N. Lautensack-Belser and H. E. Morgan, *Am. J. Physiol.* **248**, C309 (1985).
48. R. G. Thurman, D. P. Marazzo, L. S. Jones and F. C. Kauffman, *J. Pharmac. exp. Ther.* **201**, 498 (1977).
49. L. A. Reinke, F. C. Kauffman, S. A. Belinsky and R. G. Thurman, *J. Pharmac. exp. Ther.* **213**, 70 (1980).
50. R. G. Thurman and F. C. Kauffman, *Pharmac. Rev.* **31**, 229 (1980).
51. F. C. Kauffman, R. K. Evans, L. A. Reinke, S. A. Belinsky, C. Ballow and R. G. Thurman, *Biochem. Pharmac.* **29**, 697 (1980).
52. E. Bresnick and H. Y. Yang, *Biochem. Pharmac.* **13**, 497 (1964).
53. F. C. Kauffman, R. K. Evans and R. G. Thurman, *Biochem. J.* **166**, 583 (1977).
54. W. Kunz, G. Schaude, H. Schimassek, W. Schmid and M. Siess, *Proc. Eur. Soc. Study Drug Toxicity* **7**, 138 (1966).
55. R. Schulte-Hermann, *CRC Crit. Rev. Toxic.* **3**, 97 (1974).
56. A. Poland and E. Glover, *Molec. Pharmac.* **10**, 349 (1974).
57. G. W. Lucier, O. S. McDaniel, G. E. R. Hook, B. Fowler, B. R. Sonawane and E. Faeder, *Environ. Hlth Perspect.* **5**, 199 (1973).
58. G. Weber, R. L. Singhal, N. B. Stamm, M. A. Lea and E. A. Fisher, in *Advances in Enzyme Regulation* (Ed. G. Weber), Vol. 4, p. 59. Pergamon Press, New York (1966).
59. D. M. Gibson, R. T. Lyons, D. F. Scott and Y. Muto, in *Advances in Enzyme Regulation* (Ed. G. Weber), Vol. 10, p. 187. Pergamon Press, New York (1972).