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EFFECTS OF PERFLUORODECANOIC ACID ON HEPATIC INDICES OF THYROID STATUS IN THE RAT

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Abstract—Perfluorodecanoic acid (PFDA) alters the circulating level of thyroid hormones, but the physiological significance of this change at the target tissue remains to be defined. To this end, the activities of thyroid-responsive hepatic enzymes were examined in adult male rats 1 week after treatment with a single dose of PFDA (20, 40 or 80 mg/kg). Since PFDA treatment caused a dose-related reduction in feed intake, vehicle-treated rats pair-fed to their counterparts receiving PFDA were used to determine if any of the PFDA-induced alterations in enzyme activity were secondary to hypophagia. Following the administration of PFDA, L-glycerol-3-phosphate dehydrogenase, a liver mitochondrial enzyme sensitive to thyroid status, exhibited a modest increase in activity, whereas that of succinate dehydrogenase, a constitutive mitochondrial marker enzyme, was similar in both PFDA-treated rats and their pair-fed counterparts at all dose levels examined. Activity of cytosolic lactate dehydrogenase was also augmented modestly in livers of rats receiving PFDA. In contrast, activity of cytosolic malic enzyme, a thyroid-responsive enzyme, was increased markedly in PFDA-treated rats. Hepatic activity of glucose-6-phosphate dehydrogenase, which also responds to alterations in thyroid status, exhibited a modest increase with 20 and 40 mg/kg PFDA but was similar in both PFDA-treated rats and their pair-fed counterparts at the 80 mg/kg dose level. Absolute and relative liver mass was elevated in PFDA-treated rats at all dose levels in comparison to the appropriate vehicle-treated pair-fed animals. Total hepatic content of DNA was maintained in PFDA-treated rats at all dose levels, whereas a significant decrease in liver DNA was found in the vehicle-treated rats pair-fed to animals receiving 80 mg/kg PFDA. Following administration of PFDA, protein content per total liver was similar to that of their pair-fed counterparts. Thus, the pattern of activity of thyroid-responsive hepatic enzymes was not compatible with a functional shift toward a lessened thyroid status in rats treated with PFDA.

Perfluorocarboxylic and perfluorosulfonic acids have been used in a variety of industrial applications, primarily for their surfactant properties [1, 2]. Perfluorodecanoic acid (PFDA)† is representative of these compounds, and it is one of the most extensively studied compounds of this group in terms of toxicity. Acute toxicity of PFDA in rats is characterized by hypophagia, body weight loss, and delayed lethality [2]. Additional effects of PFDA treatment include thymic and testicular atrophy [2].

PFDA treatment in the rat is also associated with a rapid-onset, severe depression in serum T_4 concentration [3]. The concentration of T_3 in rat serum has also been reported to be decreased 1 week after treatment with PFDA in comparison with vehicle-treated rats allowed *ad lib.* access to feed [3]. PFDA also results in hypothermia and bradycardia 1 week after treatment, and Langley and Pilcher [3] suggested that these effects may be reflective of a hypothyroid state secondary to an alteration in the circulating level of thyroid hormones.

The present study extends the functional characterization of thyroid status in PFDA-treated rats to include thyroid-responsive hepatic enzymes. Since PFDA results in a dose-dependent decrease in feed intake [2, 3], vehicle-treated rats that were pair-fed to individual PFDA-treated rats have been used to determine whether potential alterations in thyroid-modulated responses by PFDA are secondary to hypophagia. Hepatic activities of 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] have been used extensively as a measure of a thyroid hormone effect by other investigators [4–7]. The activities of these enzymes, as well as of two hexose monophosphate pathway enzymes, 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), are increased in hyperthyroid rats but decreased in the hypothyroid state [8]. As the activities of these enzymes in liver are sensitive to alterations in thyroid status, evaluation of their hepatic activities would be an index of an effect of PFDA that resulted in an altered thyroid state at the cellular level.

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† Abbreviations: PFDA, perfluorodecanoic acid; T_4, L-thyroxine; T_3, 3,5,3′-triiodothyronine; and ANOVA, analysis of variance.

METHODS

Chemicals. PFDA, propylene glycol, succinic acid and phenazine methosulfate (PMS) were obtained from the Aldrich Chemical Co. (Milwaukee, WI). Cytochrome *c*, sodium pyruvate and L-glycerol-3-phosphate were purchased from the Boehringer Mannheim Corp. (Indianapolis, IN); Coomassie blue G-250 was from the Pierce Chemical Co. (Rockford, IL). The remaining substrates and cofactors necessary for the assay of liver enzyme activity, as well as crystalline bovine 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 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 5:00 a.m. to 5:00 p.m. All rats were entrained to a 15–16 hr feeding schedule where ground chow (Purina Rat Chow No. 5012, Ralston Purina Co., St. Louis, MO) was available from 5:00 p.m. to 8:30 a.m. and tap water was available *ad lib*. The chow was provided to the animals in glass screw-capped feeders (Hazleton Systems, Aberdeen, MD), fastened to the inside of the cages, which prevented feed spillage. Rats were acclimated to the feeding and lighting schedule for at least 1 week prior to the initiation of the experiment.

Following the acclimation period, sets of two rats each were matched, based on similar body weights, for pair-feeding. Then, a single dose of either PFDA (20, 40 or 80 mg/kg, i.p.) or an equivalent volume of vehicle (propylene glycol/water, 1/1, v/v; 1 ml/kg, i.p.) was administered. Twenty-four hours after the dosing of its PFDA-treated partner, the pair-fed rat was given the equivalent amount of vehicle. Pair-fed rats received the same amount of feed that their PFDA-treated counterparts consumed during the previous 15–16 hr daily feeding period. Vehicle-treated rats, allowed unlimited access to feed during the 15–16 hr feeding period, were also investigated.

Rats were exsanguinated by decapitation 7 days post-treatment between 1:00 p.m. and 4:00 p.m. Following exsanguination, a portion of liver was frozen by the freeze-stop technique [9], weighed, and ground to the consistency of fine 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. According to the scheme in Fig. 1, frozen liver powders were homogenized and fractionated [10]. 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. All operations were carried out at 4°. Each particulate fraction was suspended in 0.25 M sucrose in a volume which was 1/6 of the starting homogenate and repelleted at the same *g* force. This second supernatant fraction was then combined with the original supernatant of the respective fraction and subjected to the next higher *g* force to obtain the subsequent fraction. All pellets 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 fractions were agitated briefly on a Vortex mixer, while the microsomal pellet was mechanically sus-

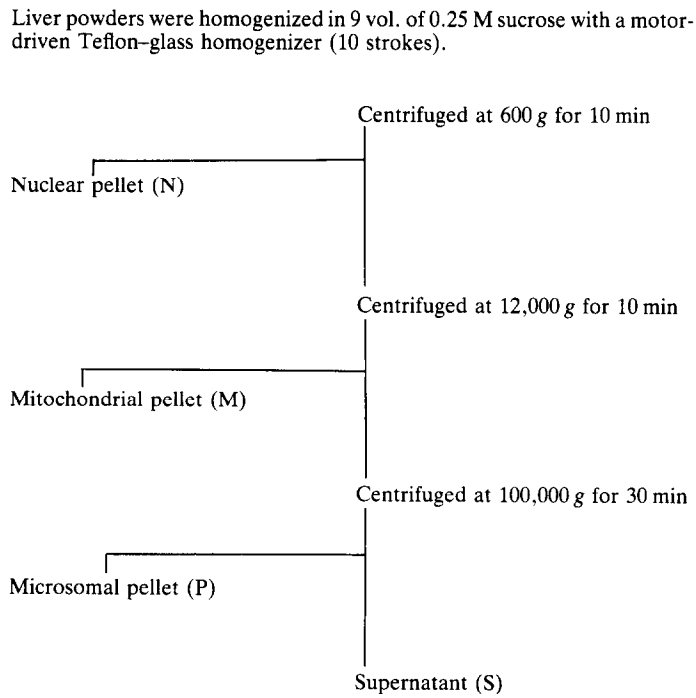

Fig. 1. Scheme for fractionation of rat liver homogenates.

pended with a Tissumizer (Tekman Co., Cincinnati, OH).

Determinations. The assay of lactate dehydrogenase (L-lactate:NAD^+-oxidoreductase; EC 1.1.1.27), malic enzyme, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, succinate dehydrogenase [succinate:(acceptor) oxidoreductase; EC 1.3.99.1] and L-glycerol-3-phosphate dehydrogenase, as well as the determination of protein and DNA, have been described recently in detail [11].

Statistical analysis. The main effects of treatment and dosing were tested for significance by two-way analysis of variance (ANOVA) through the use of unweighted cell means [12]. Significance of the differences between the PFDA-treated and pair-fed groups was detected by pairwise comparison [13]. Effect of dosing within the PFDA-treated and pair-fed groups, including comparison to the vehicle-treated group of rats with unlimited access to feed, was tested by one-way ANOVA. This was followed by evaluation of contrasts using Scheffé's method [14]. Linear function was examined, following significance of a dosing effect, by testing for trends using orthogonal coefficients [13]. The computations were performed with a VAX-750 computer using BMDP [13]. All significance levels were set at $P < 0.05$.

RESULTS

Body weight and feed intake. PFDA treatment resulted in a dose-dependent reduction in body weight (Table 1). Feed intake was also depressed in a dose-related manner 7 days after treatment with PFDA, and the difference between the amount of feed consumed by the PFDA-treated animals and that consumed by the vehicle-treated rats with unlimited access to feed was significant at the 40 and 80 mg/kg dose level (Table 1). The feed intake of the pair-fed groups was comparable to that of their PFDA-treated counterparts at each dose level.

Liver mass, DNA and protein. Livers of PFDA-treated rats were markedly enlarged by day 7 post-treatment (Table 1). Following the administration of PFDA, absolute liver weight was significantly greater than that of the pair-fed animals at all doses examined. In comparison to the rats with unlimited access to feed, the absolute liver weights of PFDA-treated animals were elevated only at doses of 20 and 40 mg/kg. Following significance of a dosing effect, a linear trend in relative liver weight in PFDA-treated rats was demonstrated. Relative liver weight was also higher in PFDA-treated animals at all doses than in either the respective pair-fed rats or the unlimited-fed group (Table 1). Rats pair-fed to their PFDA-treated counterparts exhibited significant dose-related decreases in both absolute and relative liver weight.

With PFDA-treated rats at all doses examined, hepatic DNA content per total liver was similar to that in vehicle-treated rats with unlimited access to feed (Fig. 2). Hepatic DNA concentration (mg/g wet weight) was unchanged in the pair-fed treatment groups at the 20 and 40 mg/kg dosing level but was elevated at the 80 mg/kg level, in comparison to the group with unlimited access to feed (Fig. 2). At the 80 mg/kg dose level, in the PFDA-treated animals, the concentration of DNA per g wet liver was also lower than that observed in their pair-fed partners. When differences in liver mass were taken into account (Table 1), however, DNA content per total liver in the pair-fed treatment groups decreased as feed intake decreased. With the pair-fed group at the 80 mg/kg dosing level, hepatic DNA content was significantly lower than that of either their PFDA-treated partners or vehicle-treated rats with unlimited access to feed.

Hepatic protein concentrations (mg/g wet liver) were similar in the vehicle-treated rats, whether pair-fed or unlimited-fed. Yet the concentration was decreased slightly in the PFDA-treated animals at 40 mg/kg (Fig. 3). This PFDA-induced reduction in hepatic protein concentration was significant when contrasted to either the pair-fed counterpart or the group with unlimited access to feed. The protein

Table 1. Effect of PFDA treatment on body weight, feed intake, and liver weight*

PFDA dose (mg/kg)	Treatment	Body weight (g)	Feed intake (g/15–16 hr)	Liver weight	
				Absolute (g wet wt)	Relative (g wet wt/100 g body wt)
0	Unlimited-fed	345 ± 5†	24.5 ± 0.4†	12.8 ± 0.3†	3.72 ± 0.05†
20	PFDA	338 ± 4†	25.2 ± 0.5†	16.1 ± 0.3‡‖	4.76 ± 0.05‡‖
	Pair-fed	336 ± 5†	23.0 ± 0.6†‡	12.0 ± 0.3†‡	3.55 ± 0.05†‡
40	PFDA	304 ± 10‡	19.8 ± 2.0‡	15.7 ± 0.5‡‖	5.08 ± 0.07§‖
	Pair-fed	323 ± 10†	19.7 ± 1.9‡	11.0 ± 0.7‡	3.43 ± 0.17‡
80	PFDA	235 ± 8§	3.2 ± 1.4§	12.1 ± 0.6†‖	5.14 ± 0.12§‖
	Pair-fed	252 ± 10‡	3.2 ± 1.4§	6.2 ± 0.6§	2.43 ± 0.17§

* Values in the table were determined 7 days after administration of PFDA or vehicle. Vehicle-treated rats were allowed unlimited access to feed during the daily 15–16 hr feeding period or were pair-fed to a PFDA-treated rat. Each value represents the mean ± SE of eight determinations, except in the unlimited-fed group which contains sixteen estimations.

†‡§ Mean values in a column not followed by the same superscript are significantly different from other dose levels in the same treatment group (PFDA 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 ($P < 0.05$).

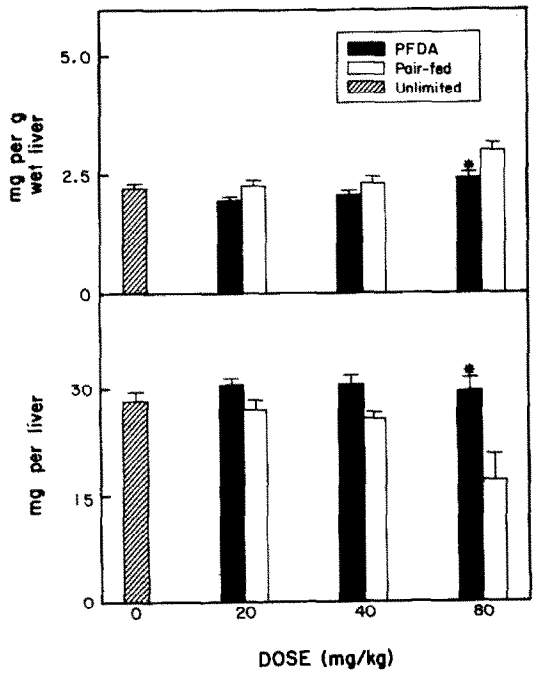

Fig. 2. Hepatic DNA in PFDA-treated rats. Livers were removed on day 7 following administration of either PFDA or vehicle. Vehicle-treated rats were allowed either unlimited access to feed or were pair-fed to individual PFDA-treated rats. The height of each bar and associated vertical line represents the mean and SE of four animals. An asterisk indicates a significant difference between a PFDA-treated group and its respective pair-fed set at a given dose ($P < 0.05$).

content per total liver, however, was similar in the rats with unlimited access to feed and the PFDA-treated animals at all dose levels. Due to the decrease in liver mass (Table 1), a dose-related decrease in protein content per liver in the pair-fed rats was detected in the one-way ANOVA. Hepatic protein content in the pair-fed rats at the 20 and 80 mg/kg dose levels was significantly lower than that observed in the PFDA-treated rats. When hepatic protein was expressed per mg DNA, the significance of the dosing effect was demonstrated in the one-way ANOVA for both PFDA-treated rats and their pair-fed counterparts. A linear decrease across dose levels was detected in both PFDA-treated and pair-fed rats, and this reduction was significant ($P < 0.05$) for both groups at the 80 mg/kg dose level in comparison to the group with unlimited access to feed. The percentage distributions of protein in the mitochondrial and supernatant fractions of liver homogenates were also similar in all experimental groups (data not shown).

Mitochondrial enzyme activity. Succinate dehydrogenase activity in the isolated mitochondrial fraction (Fig. 1), expressed per g wet liver, was depressed slightly in both PFDA-treated rats and their pair-fed counterparts (Fig. 4). However, one-way ANOVA indicated that the trend towards a reduction in activity with increasing dose level was only significant in the pair-fed animals. When expressed per liver (Fig. 4), activity of succinate dehydrogenase was similar in the unlimited-fed group and the PFDA-treated and pair-fed animals at the 20 and 40 mg/kg dose levels. Succinate dehydrogenase activity per

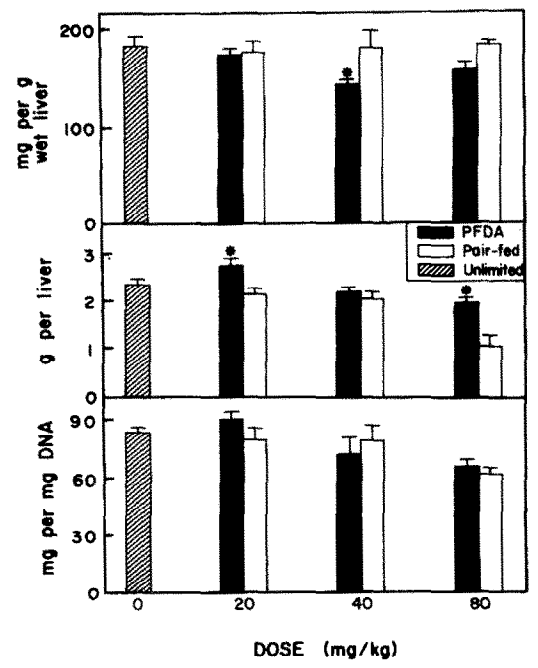

Fig. 3. Hepatic protein in PFDA-treated rats. Other conditions were as in Fig. 2. An asterisk indicates a significant difference between a PFDA-treated group and its respective pair-fed set at a given dose ($P < 0.05$).

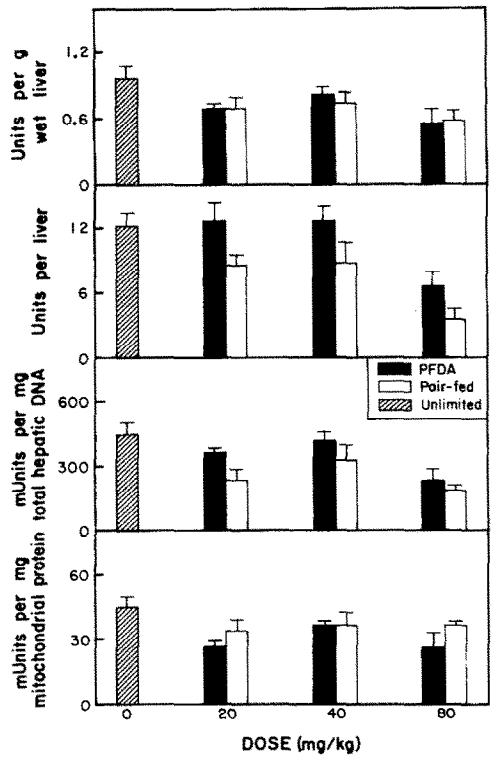

Fig. 4. Succinate dehydrogenase activity in the liver of PFDA-treated rats. Other conditions were as in Fig. 2.

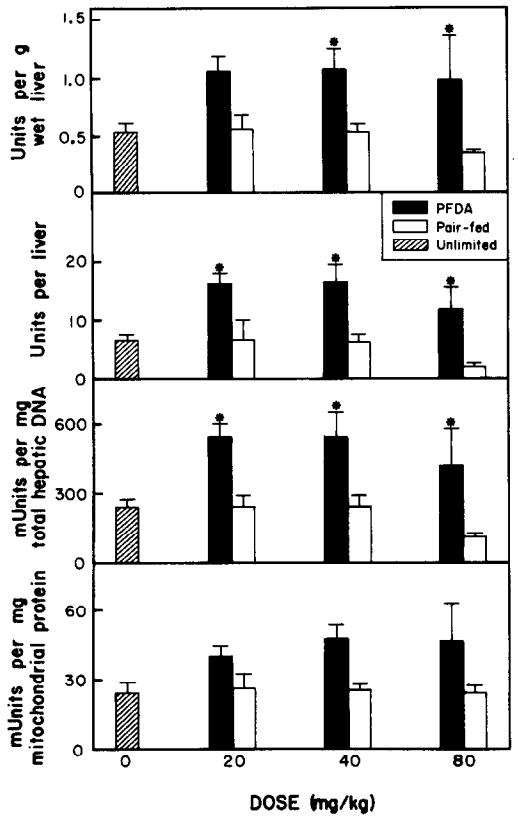

Fig. 5. L-Glycerol-3-phosphate dehydrogenase activity in the liver of PFDA-treated rats. Other conditions were as in Fig. 2. An asterisk indicates a significant difference between a PFDA-treated group and its respective pair-fed set at a given dose ($P < 0.05$).

liver was depressed significantly ($P < 0.05$) at the 80 mg/kg dose level in the PFDA-treated rats and their pair-fed counterparts in comparison to the unlimited-fed rats. A depression in succinate dehydrogenase activity per mg DNA across doses for both the PFDA-treated rats and their pair-fed counterparts (Fig. 4) was also indicated by one-way ANOVA. Irrespective of the manner in which succinate dehydrogenase activity was expressed, the activity of the enzyme in PFDA-treated and pair-fed rats at the same dose level was similar.

Activity of mitochondrial L-glycerol-3-phosphate dehydrogenase per g wet liver was increased following PFDA treatment (Fig. 5). When the increase in hepatic mass associated with PFDA treatment (Table 1) is taken into account by expressing enzyme activity per total liver or per cell (mg DNA), L-glycerol-3-phosphate dehydrogenase activity was significantly higher in PFDA-treated rats than in pair-fed animals at all dose levels (Fig. 5). In PFDA-treated rats, the trend towards an increase in activity of L-glycerol-3-phosphate dehydrogenase per mg mitochondrial protein was significant ($P < 0.05$) in comparison to vehicle-treated animals, either with unlimited access to feed or pair-fed, by one-way and two-way ANOVA, respectively (Fig. 5).

Supernatant enzyme activity. Lactate dehydrogenase activity in the high-speed supernatant fraction (100,000 $g \times 30$ min) of liver homogenate (Fig. 1) was increased by PFDA treatment relative to their respective pair-fed counterparts or those with unlimited access to feed (Fig. 6). Hepatic lactate dehydrogenase activity tended to be diminished in pair-fed animals with decreased feed intake at the higher doses of PFDA. This decrease was significant ($P < 0.05$) at the 80 mg/kg dosing level relative to the unlimited-fed group whether lactate dehydrogenase activity was expressed per g wet liver, total liver, or mg DNA (Fig. 6).

Cytosolic malic enzyme activity in the liver was increased markedly by PFDA treatment (Fig. 7). Whether expressed per g wet liver, total liver, mg DNA or mg supernatant protein, activity of malic enzyme in PFDA-treated rats was significantly greater than that of the unlimited-fed group or pair-fed groups at all dose levels. However, the increase in activity declined at 80 mg/kg PFDA, the highest dose examined. Activity per mg supernatant protein in PFDA-treated rats at 20 or 40 mg/kg was 10–20 times greater than that of the respective pair-fed groups.

Glucose-6-phosphate dehydrogenase activity in the high-speed supernatant of liver homogenate was increased in rats treated with 20 and 40 mg/kg PFDA, but was not affected by treatment with 80 mg/kg, in comparison to vehicle-treated rats with unlimited access to feed (Fig. 8). The increased activity of this enzyme following PFDA treatment

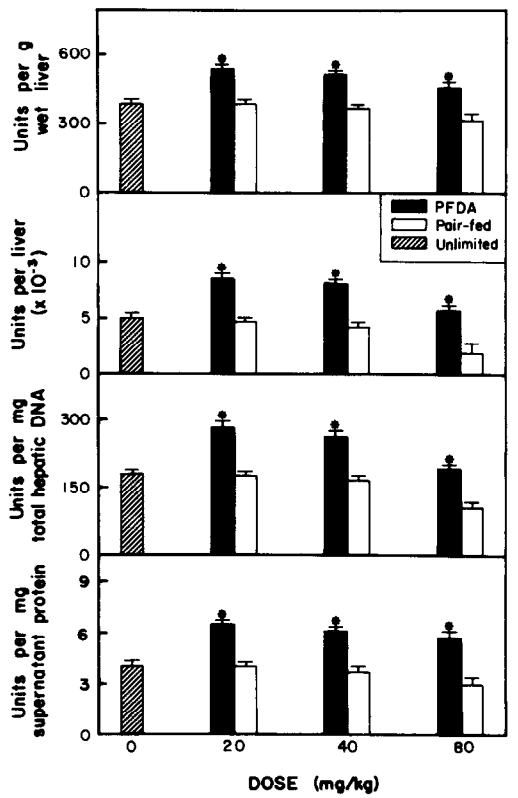

Fig. 6. Lactate dehydrogenase activity in the liver of PFDA-treated rats. Other conditions were as in Fig. 2. An asterisk indicates a significant difference between a PFDA-treated group and its respective pair-fed set.

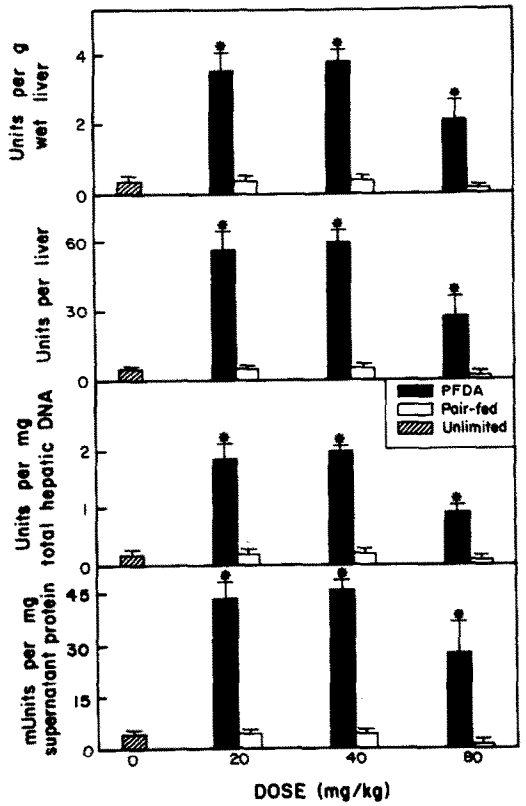

Fig. 7. Malic enzyme activity in the liver of PFDA-treated rats. Other conditions were as in Fig. 2. An asterisk indicates a significant difference between a PFDA-treated group and its respective pair-fed set at a given dose ($P < 0.05$).

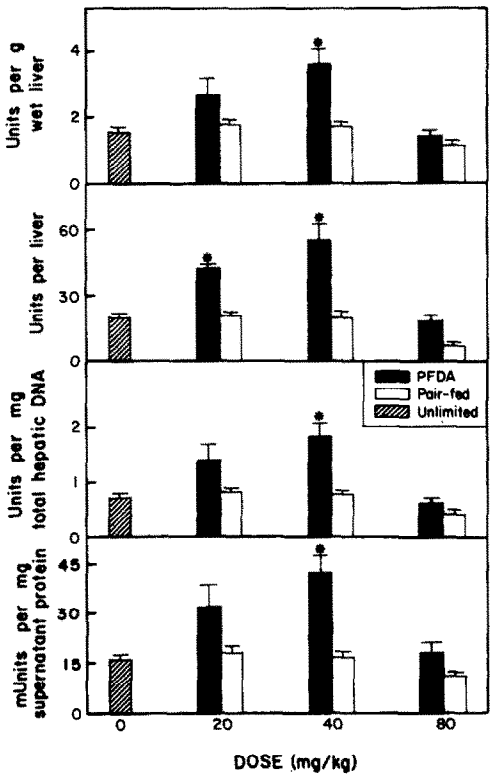

Fig. 8. Glucose-6-phosphate dehydrogenase activity in the liver of PFDA-treated rats. Other conditions were as in Fig. 2. An asterisk indicates a significant difference between a PFDA-treated group and its respective pair-fed set at a given dose ($P < 0.05$).

was also significant in comparison to pair-fed rats at the 20 and 40 mg/kg dose level when expressed per total liver, but only at 40 mg/kg of PFDA when expressed per g wet liver, mg DNA or mg supernatant protein. Irrespective of the manner in which it was expressed, activity of glucose-6-phosphate dehydrogenase in rats pair-fed to PFDA-treated counterparts at the 20 and 40 mg/kg dosing levels was similar to that of the unlimited-fed animals. Yet enzyme activity in the pair-fed rats at the 80 mg/kg level was depressed (Fig. 8).

Activity of 6-phosphogluconate dehydrogenase was decreased in a dose-related manner by PFDA when expressed per g wet liver (Fig. 9). This decrease was significant at 40 and 80 mg/kg of PFDA in comparison to both respective pair-fed groups and the unlimited-fed animals. Whether the activity of 6-phosphogluconate dehydrogenase was expressed per total liver, mg DNA or mg supernatant protein, the level of activity observed in PFDA-treated animals was similar at 20 and 40 mg/kg, but was depressed at 80 mg/kg in comparison to the group with unlimited access to feed (Fig. 9). Hepatic 6-phosphogluconate dehydrogenase activity in the pair-fed treatment groups followed a pattern similar to that found in PFDA-treated animals, in that activity in rats at the 20 and 40 mg/kg dosing levels was similar to that of the unlimited-fed group. Also its activity at the 80 mg/kg dosing level in both PFDA-treated rats and their pair-fed counterparts was less than that in the unlimited-fed rats regardless of whether activity was expressed per g wet liver, total liver, mg hepatic DNA, or mg supernatant protein (Fig. 9).

DISCUSSION

Expression of the effects of thyroid hormones in target tissues and control of their concentration in plasma are both under homeostatic influence. Toxicants that perturb the circulating level of thyroid hormones and/or cause severe feed deprivation can potentially upset this equilibrium. Under such conditions, the concentration of thyroid hormones at the cellular level provides a more accurate index of functional thyroid status than that obtained from the circulating level of thyroid hormones [15]. However, direct analysis of thyroid-responsive tissue functions provides the ultimate proof of physiological thyroid status. The circulating concentration of T_4 has been reported to be decreased following sublethal or lethal doses of PFDA while that of T_3 was also decreased relative to *ad libitum*-fed vehicle-treated rats [3]. In addition, PFDA treatment results in a severe, long-lasting hypophagic response [2]. These observations affirm the need for an examination of thyroid hormone function at the tissue level in PFDA-treated rats.

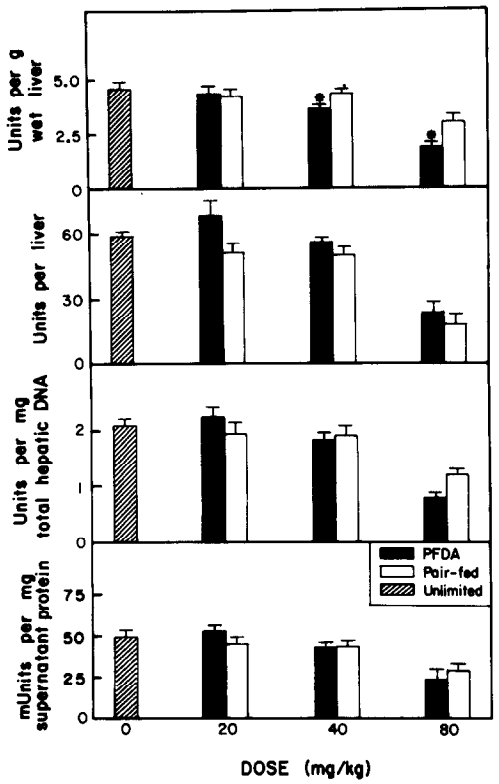

Fig. 9. 6-Phosphogluconate dehydrogenase activity in rats treated with PFDA. Other conditions were as in Fig. 2. An asterisk indicates a significant difference between a PFDA-treated group and its respective pair-fed set at a given dose ($P < 0.05$).

Assessment of an interaction of PFDA treatment upon hepatic indices of thyroid status in rats is complicated by other toxic effects of the compound. Administration of PFDA to the rat results in a loss of body weight associated with a hypophagic response [2–3]. In the present study, the use of rats pair-fed to their PFDA-treated counterparts permitted an evaluation of hepatic alterations that are secondary to PFDA-induced hypophagia rather than a primary effect of PFDA on thyroid status. PFDA treatment resulted in hepatomegaly, whereas liver mass was diminished in their pair-fed counterparts. PFDA-induced hepatomegaly was found to be due to hypertrophy, as the content of DNA per total liver was similar in PFDA-treated rats and vehicle-treated rats allowed unlimited access to feed. Reduction in liver mass in rats pair-fed to those receiving the highest dose of PFDA examined (80 mg/kg) was associated with a decrease in cell number, as evidenced by a decrease in hepatic DNA content per total liver. Likewise, a significant reduction in the protein content per total liver in rats pair-fed to those treated with 80 mg/kg PFDA was also attributable to the decrease in liver mass. However, when the change in cellularity was also taken into account, the amount of protein per cell (mg DNA) was similar in the livers of PFDA-treated rats and their pair-fed counterparts at the 80 mg/kg dose level.

An evaluation of alterations in the activities of hepatic enzymes that are secondary to PFDA-induced hypophagia, rather a primary effect of PFDA on thyroid status, was also made in the present study. Hepatic activities of succinate dehydrogenase, a mitochondrial marker enzyme, and 6-phosphogluconate dehydrogenase, a cytosolic enzyme of the hexose monophosphate shunt, diminished modestly with increasing dose levels of PFDA. In that the activities of both enzymes were also reduced in rats pair-fed to the PFDA-treated counterparts, the diminished activities of both succinate dehydrogenase and 6-phosphogluconate dehydrogenase could be attributed to the secondary influence of PFDA-induced hypophagia.

Even though a modest reduction of the hepatic activity of 6-phosphogluconate dehydrogenase occurred in PFDA-treated rats, the activity of glucose-6-phosphate dehydrogenase, also a cytosolic enzyme of the hexose monophosphate shunt, was maximally increased approximately 1.5-fold in livers of rats treated with 40 mg/kg PFDA. However, increasing the dose of PFDA to 80 mg/kg resulted in a decrease in the hepatic activity of glucose-6-phosphate dehydrogenase to a value that was not different from that of rats pair-fed to this high dose of PFDA. Thus, the severe hypophagic effect of PFDA at 80 mg/kg and its resulting consequences on the nutritional and hormonal milieu would seem responsible for obliterating the increase in activity of glucose-6-phosphate dehydrogenase in liver that followed the administration of lower doses of PFDA (20 and 40 mg/kg).

The activity of lactate dehydrogenase, a cytosolic marker enzyme, was increased by PFDA treatment. In contrast to the increase in hepatic activity of glucose-6-phosphate dehydrogenase that was absent at the highest dose of PFDA examined (80 mg/kg), the increases, approximately 1.5-fold, in the activity of lactate dehydrogenase expressed per mg liver supernatant protein were similar at all the doses examined. Likewise, L-glycerol-3-phosphate dehydrogenase, a liver mitochondrial enzyme sensitive to thyroid status [4–7], exhibited a modest augmentation in activity (2-fold at maximum) in PFDA-treated rats. This increase in hepatic L-glycerol-3-phosphate activity was also present in rats treated with 80 mg/kg PFDA.

Hepatic activity of cytosolic malic enzyme has been used extensively as a measure of thyroid hormone effect by a number of investigators [4–7]. The activity of cytosolic malic enzyme, expressed per mg supernatant protein, was increased markedly in PFDA-treated rats. At doses of 20 and 40 mg/kg PFDA, there was an approximately 10-fold augmentation in hepatic malic enzyme activity. As with glucose-6-phosphate dehydrogenase, the drastic reduction in feed intake at 80 mg/kg PFDA and its consequences on the homeostatic mechanism interacted with the induction of malic enzyme activity. Even though an increase in the activity of malic enzyme was found in the livers of rats treated with 80 mg/kg PFDA, it was blunted when compared to that found in rats administered lower doses of PFDA. In addition to the regulatory influences of nutritional and hormonal status [5–7, 16–18], hepatic activity of

malic enzyme is increased following the treatment of rats with halothane [19], clofibrate [20, 21], phenobarbital [19, 22], 3-methylcholanthrene [23] and polychlorinated biphenyls [24]. The mechanism of the striking elevation in hepatic activity of malic enzyme following the administration of xenobiotic compounds is still obscure. Recently, it has been suggested that an increase in NADPH consumption through augmentation of detoxification processes induces the synthesis of malic enzyme [25]. In support of their contention was the finding that the administration of 1,3-bis (chloroethyl-1-nitrosourea, a glutathione reductase inhibitor, together with *t*-butyl hydroperoxide, abolished induction of liver malic enzyme by the latter compound.

Depletion of the hepatic NADPH supply for the reductive step of monooxidation could be especially crucial if PFDA were to act like perfluoro-*n*-hexane, which uncouples the electron transport process from monooxygenation of substrate [26, 27]. Due to its lipophilic properties, perfluoro-*n*-hexane can form a cytochrome P-450 substrate complex and stimulate electron flow to cytochrome P-450, leading to oxygen activation. However, because of the stability of the carbon–fluorine bonds, the active oxygen cannot be incorporated into the perfluoro-*n*-hexane molecule, resulting in further utilization of NADPH to reduce the activated oxygen to water. Although PFDA has yet to be demonstrated to induce mixed-function oxidation of fatty acids in rats or to act as a "dead-end inhibitor" of monooxygenation, these possibilities cannot be excluded, considering the structural similarity between PFDA and perfluoro-*n*-hexane.

In closing, a potential effect of PFDA treatment on the functional thyroid state of rats precipitated the present investigation. A severe depression in serum T_4 along with hypothermia and bradycardia in PFDA-treated rats led Langley and Pilcher [3] to the suggestion that these effects might be reflective of a hypothyroid state that is secondary to an alteration in the circulating level of thyroid hormones. However, the pattern of activity of thyroid-responsive hepatic enzymes in the present study is not compatible with a functional shift toward a lessened thyroid state in rats 1 week after PFDA administration.

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