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The Effects of Nonadecafluoro-*n*-decanoic Acid on Serum Retinol and Hepatic Retinyl Palmitate Hydrolase Activity in Male Sprague-Dawley Rats

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ABSTRACT: The effects of nonadecafluoro-*n*-decanoic acid (NDFDA) on serum retinol levels and hepatic retinyl palmitate hydrolase (RPH) activity were investigated in male Sprague-Dawley rats given a single intraperitoneal (IP) dose of 0, 50, or 100 mg/kg NDFDA and sacrificed at two, eight, or 11 days. Treated animals exhibited depressed serum retinol levels, lymphoid involution, and failure to gain weight in proportion to the dose. Hepatic RPH activities were depressed in both treatment groups at all time points and correlated with serum retinol levels. Hepatic retinol levels were also depressed by Day 11. Extraction of hepatic homogenates with acetone removed NDFDA and increased RPH activities twofold and threefold for the low- and high-dose groups, respectively. Analysis of partially purified RPH showed both NDFDA and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to be noncompetitive inhibitors: K_I = 450 and 750 μM, respectively. We conclude that NDFDA causes a decrease in the mobilization of vitamin A from the liver by noncompetitive inhibition of RPH.

KEY WORDS: Nonadecafluoro-*n*-decanoic acid, NDFDA, TCDD, retinol, retinyl palmitate hydrolase

INTRODUCTION

Nonadecafluoro-*n*-decanoic acid ($CF_3(CF_2)_8COOH$), also known as perfluorodecanoic acid, is a member of a class of perfluorinated alkanoic acids

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which have been utilized in a wide variety of research and industrial applications (1, 2). Recently, it has been shown that certain of these chemicals evoke a toxic response at relatively high doses in rats (1). The symptoms of toxicity caused by NDFDA include anorexia, cachexia, lymphoid involution, a delayed onset of death that cannot be shortened by higher doses, and failure to gain weight at a normal rate or weight loss. These symptoms are similar to those described for animals suffering from a vitamin A deficiency (3) and also to those caused by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (also called dioxin) and similar compounds (4, 5), which have also been shown to affect vitamin A levels in treated animals (6–12).

An attractive hypothesis, then, is that the toxicity of NDFDA and compounds causing symptoms of vitamin A deficiency may be associated with their ability to perturb some aspect of vitamin A metabolism directly. This could account for some of the common symptoms caused by structurally dissimilar chemicals and could also help explain the similarities between NDFDA and dioxin-type toxicosis and vitamin A deficiency.

Vitamin A has been shown to be absolutely required for the maintenance of several physiological functions, including growth, reproduction, immune response, and vision (13). The consequences of a severe vitamin A deficiency in mammals are quite pronounced and may eventually be fatal (14, 15).

Vitamin A metabolism appears to be a tightly regulated process, as evidenced by the narrow range within which animals maintain their serum retinol levels (16). Mobilization of vitamin A from liver stores occurs with the hydrolysis of retinyl esters by retinyl palmitate hydrolase to yield retinol (R), which is then bound to the specific serum retinol transport protein, retinol-binding protein (RBP). The R-RBP complex exits the liver and is transported in the serum as part of a ternary complex with prealbumin (transthyretin) (17, 18).

Preliminary experiments in our laboratory showed that a single IP dose of NDFDA rapidly caused a depression in levels of serum retinol not observed in pair–fed control animals and other symptoms of vitamin A deficiency in rats (19). This finding is in contrast to the slightly elevated or normal levels of serum retinol observed immediately following doses of TCDD or similar compounds (10, 11). We suspected that NDFDA caused the decline in serum retinol by acting as an inhibitor of RPH, thereby limiting the amount of retinol available for complexation with RBP and, ultimately, serum transport. That this enzyme is subject to inhibition has been shown by Napoli and Beck (20), who demonstrated the noncompetitive inhibition of RPH by α-tocopherol and phylloquinone at physiologically relevant levels. We have therefore examined the effect of a single IP dose of NDFDA on serum and hepatic retinol levels, as well as hepatic RPH activity, in male rats. We have also investigated the ability of NDFDA and TCDD to inhibit the activity of partially purified RPH isolated from control rat liver.

MATERIALS AND METHODS

Chemicals

Retinol, phenyl-agarose, NDFDA, and retinyl palmitate were obtained from Sigma Chemical Co, St Louis. Lichrosorb SI-60 was from Merck. Boron triflouride/methanol was from Pierce Chemical Co, Rockford, Ill.

Animal Treatment and Sacrifice

Male Sprague-Dawley rats, weighing from 120 to 140 g, were obtained from Charles River Laboratory (Portage, Mich) and acclimated for one week on hardwood bedding with access to a standard diet (Wayne Rodent-Blox) and distilled water *ad libitum*. The animals were given a single IP dose of NDFDA at 0, 50, or 100 mg/kg in peanut oil (10 ml/kg). Three animals per dose were sacrificed at two, eight, and 11 days after dosing by carbon dioxide (CO_2) anesthesia and exsanguination via open chest cardiac puncture of the right ventricle. Serum was prepared by allowing the blood to clot for one hour at 4 °C, followed by centrifugation at 2,000 *g* for ten minutes. Serum was aspirated and stored at –20 °C. Liver and thymus were excised, rinsed in cold 1.15% potassium chloride/water (KCl/H_2O), weighed, immediately frozen on dry ice, and stored at –20 °C. Liver homogenates were prepared by homogenization of liver in 10 vol of 50 mM 3-(*N*-morpholino)-propane-sulfonic acid, 0.02% sodium azide, pH 7.2, containing 2% (w/v) sodium cholate (MOPS-cholate) in a glass-Teflon homogenizer. Acetone-dried preparations of the liver homogenates were made by mixing the homogenate with 17 vol reagent-grade acetone. The mixture was centrifuged at 2,000 *g* for five minutes and the supernatant decanted and discarded. The extraction and centrifugation step was repeated two additional times, and the resultant pellets were solubilized in 1.0 ml MOPS-cholate buffer by sonication. The mixture was centrifuged at 2,000 *g* for five minutes, and the supernatant was stored at 4 °C until use.

Retinol Levels

Serum and hepatic levels of retinol were determined by a modification of the method of Dennison and Kirk (21). A 200 μl aliquot of serum or liver homogenate was mixed thoroughly in a centrifuge tube containing 1 ml distilled H_20, 1 ml sodium chloride (NaCl)–saturated H_2O, and 1 ml ethanol containing 0.1% (w/v) sodium ascorbate. Retinol was extracted by the addition of 0.5 ml UV-grade hexane and thoroughly mixing. The samples were briefly centrifuged at 1,000 *g* to facilitate phase separation and the supernatants assayed for retinol by high performance liquid chromatography (HPLC) using a fluorescence detector (Shimadzu RS-530-S, λ_{ex} = 330 nm, λ_{em} = 470 nm) and a 4 x 250 mm column packed with Lichrosorb SI-60. Isocratic elution was performed using 25% hexane/chloroform at 2

ml/min. Retinol was eluted by this system at a retention time of 5.5 minutes with a detection limit of 500 pg. Retinol standards were prepared in ethanol and verified by $\epsilon^{1\%}_{324nm}$ = 1,835 (22). High performance liquid chromatography standards of 1.0 and 0.1 ng/μl were prepared by dilution of the stock standard with hexane. All standards were stored at –20 °C and remained stable for at least six months.

Analysis of RPH Activity

A modification of the method described by Prystowsky et al (23) was used to assay for RPH activity. A 200 μl aliquot of the hepatic homogenate or the acetone-dried homogenate preparation described above was added to 1.78 ml MOPS-cholate and 0.20 μmol retinyl palmitate in ethanol and incubated for 20 minutes at 37 °C. The reaction was terminated by the addition of 1 ml ethanol (0.1% sodium ascorbate w/v) and 1 ml NaCl-saturated H_2O. Retinol was extracted and quantitated by HPLC as described above. Samples were corrected for the amount of endogenous retinol present in unincubated samples. Protein concentration of the homogenate was determined by a bicinchoninic acid microassay (24).

Partial Purification of RPH

Retinyl palmitate hydrolase was partially purified from the livers of control rats by a modification of the procedure described by Prystowsky et al (23). An acetone-dried powder was prepared by thorough homogenization of livers (98 g) in 500 ml acetone in a Waring blender. The homogenate was centrifuged at 10,000 *g* for 15 minutes, and the supernatant was discarded. Residual acetone was removed on a rotary evaporator at < 35 °C. The acetone-dried powder was solubilized in 800 ml MOPS-cholate buffer and material precipitating between 33% and 70% saturation of ammonium sulfate (19% to 47% w/v) collected by centrifugation. The pellet was resolubilized in 200 ml of 50 mM MOPS containing 19% ammonium sulfate (w/v) and dialyzed extensively against the same buffer. An 80-ml aliquot of the dialyzed extract was loaded and washed with 1.0 of 50 mM MOPS, 19% ammonium sulfate (w/v) on a 5 x 20 cm column of phenyl-agarose previously equilibrated with the same buffer and further washed with 1.0 l of 50 mM MOPS. Retinyl palmitate hydrolase activity was eluted with 1.0 l of a 0% to 2% gradient of sodium cholate in 50 mM MOPS. Fractions were assayed for protein content and RPH activity by the methods described above. Fractions containing RPH activity were pooled and used for the kinetic experiments described below.

In Vitro Inhibition of RPH by NDFDA and TCDD

The effects of NDFDA and TCDD on the kinetics of the hydrolysis of retinyl palmitate by RPH were determined using the standard RPH incu-

bation described above, modified as follows: substrate (retinyl palmitate) was provided at 0, 2.5, 5.0, 10, 20, and 40 μM. For all assays, 50 μl of partially purified RPH solution (6.9 mg/ml protein) was used. The effects of NDFDA and TCDD on the activity of RPH were studied in separate assays, using in each case a concentration of 500 μM of the compound being tested. All assays were performed in triplicate, the experiments repeated in the absence of NDFDA and TCDD, and the results expressed as a mean ± standard deviation (S.D.). Kinetic constants K_m, V_{max}, and K_I for NDFDA and TCDD were calculated from Lineweaver-Burke (double-reciprocal) plots of the data.

In Vitro Inhibition of RPH by Hepatic Homogenates

A 0.5 ml aliquot of the hepatic homogenate from each of the Day 11 samples was incubated with 0.5 ml of a partially purified RPH solution in 50 mM MOPS and 0.20 μmol retinyl palmitate for 20 minutes at 37 °C. Retinol formed was extracted and quantitated by HPLC as described above. Samples were corrected for the amount of retinol present in corresponding incubations containing no enzyme.

Analysis of Hepatic NDFDA Levels

A 0.2 ml aliquot of the liver homogenate (described above) was transferred to a centrifuge tube containing 1 ml NaCl-saturated H_2O and 1 ml ethanol (containing 0.1% sodium ascorbate w/v) and mixed thoroughly. This mixture was extracted three times with 1 ml of hexane, and the extracts were

Table 1. ***The Effect of a Single IP Dose of NDFDA on Hepatic and Thymic Body Weight Ratios in Male Sprague-Dawley Rats***

	Days Following Treatment			
Group	0	2	8	11
Control				
Liver weight/body weight[a]	5.19 ± 0.32	5.20 ± 0.21	5.48 ± 0.48	4.57 ± 0.24
Thymus weight/body weight[b]	3.69 ± 0.51	2.80 ± 0.18	2.49 ± 0.14	2.17 ± 0.33
50 mg/kg				
Liver weight/body weight[a]	———	5.91[c] ± 0.18	7.09[d] ± 0.40	6.72[e] ± 0.28
Thymus weight/body weight[b]	———	3.34 ± 0.34	2.35 ± 0.08	2.17 ± 0.20
100 mg/kg				
Liver weight/body weight[a]	———	5.40 ± 0.08	5.98 ± 0.73	6.87[d] ± 0.71
Thymus weight/body weight[b]	———	2.76 ± 0.23	1.20[d] ± 0.66	0.85[d] ± 0.48

[a] x 10^2
[b] x 10^3
[c] Significantly different from control values; $p < 0.10$
[d] Significantly different from control values; $p < 0.05$
[e] Significantly different from control values; $p < 0.01$

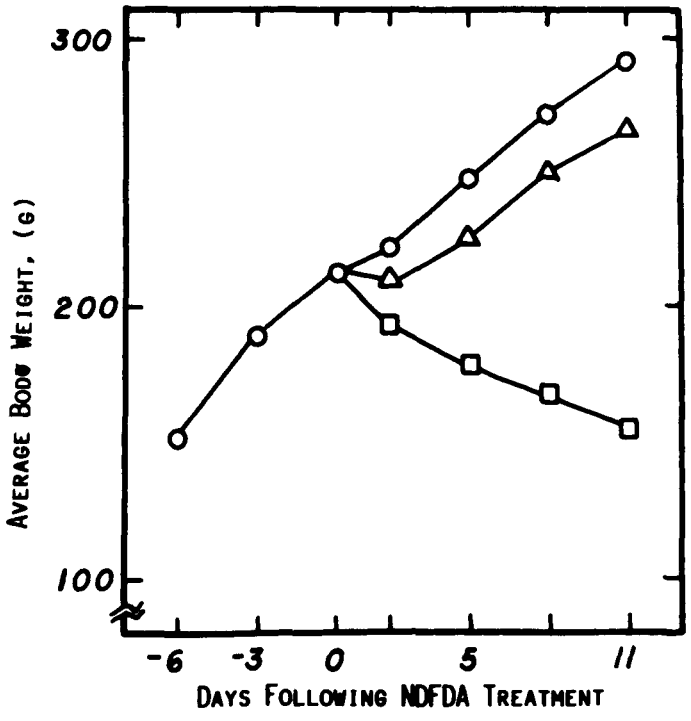

Figure 1. Body-weight change in control and NDFDA-treated rats. Values are means for groups of n = 3 treated with 0 (○), 50 (△), or 100 (□) mg/kg.

combined and evaporated to dryness at < 35 °C. A 2.0 ml volume of 14% boron trifluoride/methanol was added and the sample heated at 60 °C for 90 minutes. The sample was allowed to cool, diluted with 2 ml NaCl-saturated H_2O, and extracted with 1.0 ml hexane. The extract was assayed for NDFDA methyl esters (Me-NDFDA) using a gas chromatograph equipped with an electron-capture detector and a 6 ft x 4 mm column packed with 3% OV-101 on 80/100 Gas Chrom Q at 80 °C with nitrogen (N_2) at 15 ml/min. Standards were prepared by a quantitative derivatization and extraction of 200 mg of NDFDA. Standard purity was evaluated by gas liquid chromatography/ mass spectrometry (GC/MS) and found to be > 98% Me-NDFDA by total ion current.

RESULTS

Body and Organ Weights

A rapid and pronounced dose-related effect was observed on the body weight of the NDFDA-treated animals, with the high-dose group showing a progressive decline in weight (Figure 1). However, while those in the

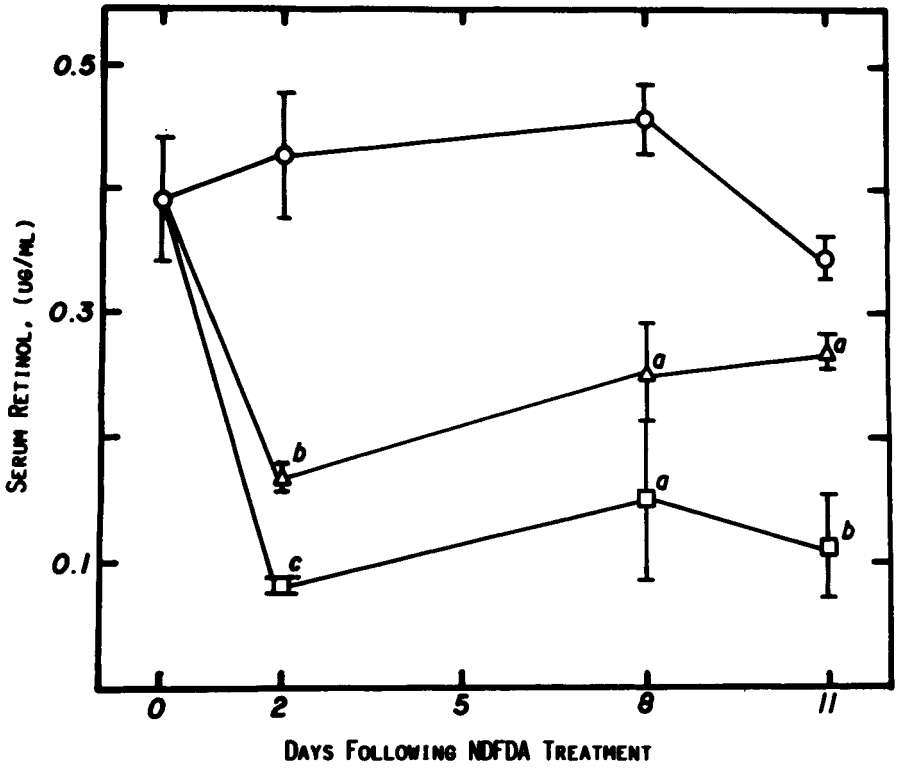

Figure 2. Serum retinol levels in control and NDFDA-treated rats. Values are means ± S.E. for groups of n = 3 treated with 0 (○), 50 (△), or 100 (□) mg/kg. Significantly different from control value: a = $p < 0.025$, b = $p < 0.010$, c = $p < 0.005$.

low-dose group initially lost weight, they seemed to recover to a near normal growth rate from Day 5 through Day 11. An increase in the liver wt/body wt ratio and lymphoid involution, as measured by a decline in the thymus wt/body wt ratio, were observed in treated animals, as shown in Table 1.

Serum and Hepatic Retinol

A significant effect of NDFDA on serum retinol levels was observed (Figure 2). By two days posttreatment, the average serum retinol concentration in the high-dose–treated group was decreased to only 18% of that of the control animals. The low-dose–treated group was similarly affected, at 39% of the control level. While the low-dose–treated animals showed some recovery by Day 11 (to 78% of control values), the high-dose group was unable to increase serum retinol levels significantly. Hepatic retinol levels did not correlate with serum values and were only significantly depressed relative to control-animal values at Day 11, as shown in Figure 3.

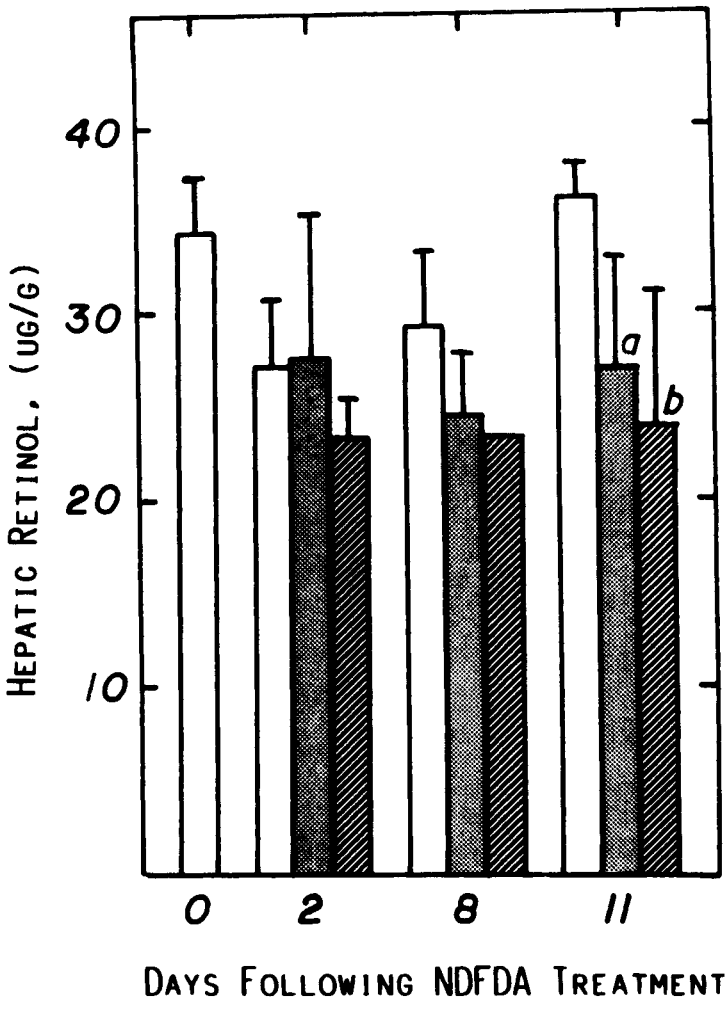

Figure 3. Hepatic retinol levels in control and NDFDA-treated rats. Values are means + S.E. for groups of n = 3 treated with 0 (□), 50 (▒), or 100 (▨) mg/kg. Significantly different from control value: a = $p < 0.10$, b = $p < 0.05$.

Hepatic RPH Activity

A pronounced depression in RPH activity in the hepatic homogenates from both NDFDA-treated groups was noted at Day 2, and activities remained low with respect to control values throughout the study (Figure 4). As with other parameters, the low-dose–treated animals seemed to recover somewhat during the course of the study when activity was considered as a percentage of control activity. Retinyl palmitate hydrolase activity in homogenates was positively correlated with serum retinol levels ($r = 0.86$), as shown in Figure 5. The RPH activity in acetone-extracted hepatic homog-

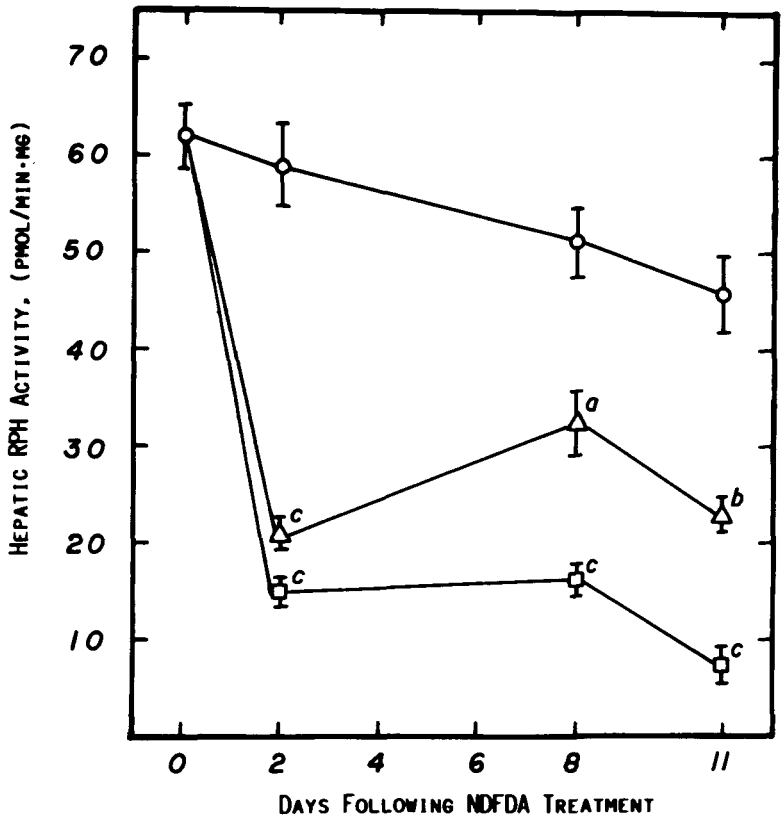

Figure 4. Hepatic retinyl palmitate hydrolase activity in NDFDA-treated rats. Values are means ± S.E. for groups of n = 3 treated with 0 (○), 50 (△), or 100 (□) mg/kg. Significantly different from control value: a = $p < 0.025$, b = $p < 0.010$, c = $p < 0.005$.

enates prepared from treated animals was significantly greater than the RPH activity in the original homogenate, reaching a ratio of 4.5:1 in the Day 11 high-dose group (Figure 6). In contrast, the RPH activity in acetone-extracted hepatic homogenates prepared from control animals averaged only 1.35 times that of the activity of the corresponding homogenate for all time points.

In Vitro Inhibition of RPH by NDFDA and TCDD

In vitro analysis of the ability of NDFDA and TCDD to inhibit the activity of partially purified RPH at a concentration of 500 μM showed that each compound was able to cause significant inhibition of the enzyme (Figure 7). Analysis of the data by a double-reciprocal plot suggested that both compounds act as noncompetitive inhibitors, with K_I = 450 μM for NDFDA and 750 μM for TCDD.

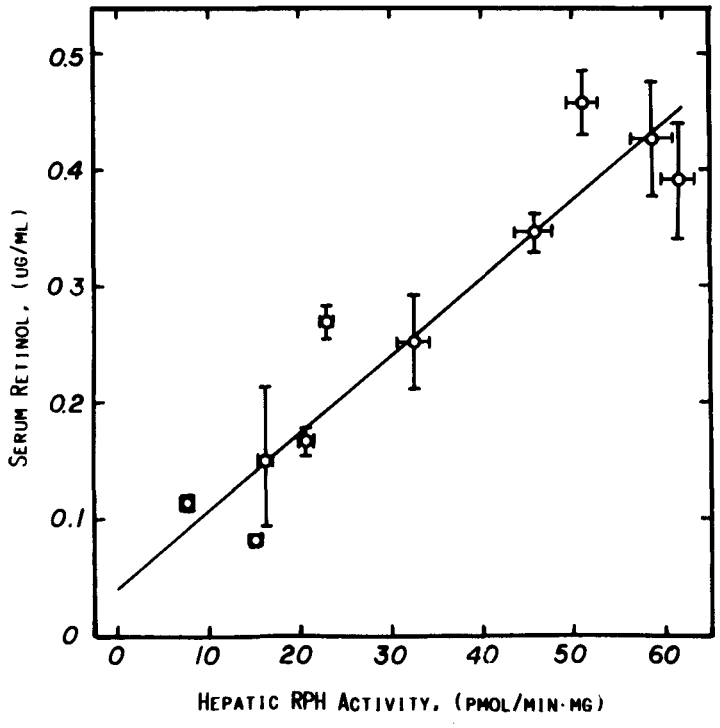

Figure 5. Correlation of serum retinol levels with hepatic RPH activity in NDFDA-treated and control rats. Values are means ± S.E. for groups of n = 3.

In Vitro Inhibition of RPH by Hepatic Homogenates

Partially purified RPH activity was significantly inhibited by an aliquot of the hepatic homogenates from NDFDA-treated animals. The incubations containing homogenate derived from the low- and high-dose–treated animals averaged 40.2% and 17.5%, respectively, of the activity of incubations containing homogenate derived from control animals.

Table 2. *Hepatic NDFDA Levels Following a Single IP Dose*[a]

	Days Following Treatment			
Treatment Group	0	2	8	11
Control	ND[b]	ND[b]	ND[b]	ND[b]
NDFDA 50 mg/kg	———	124 ± 21	105 ± 41	69 ± 9
NDFDA 100 mg/kg	———	265 ± 50	116 ± 40	127 ± 34

[a]Mean PPM NDFDA for groups of n = 3 ± S.D.
[b]Not detectable.

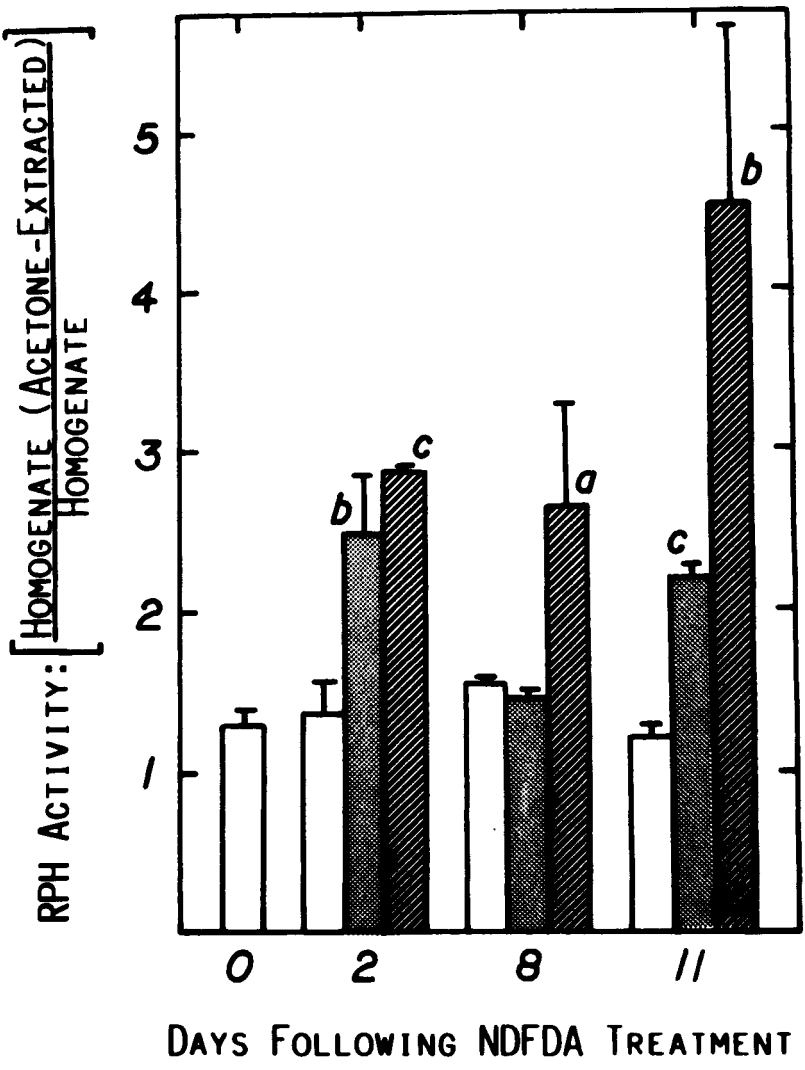

Figure 6. Increase in RPH activity in acetone-extracted hepatic homogenates from NDFDA-treated rats. Values are means + S.E. of the ratio of activity of the acetone-extracted homogenate to the original homogenate activity for groups of n = 3 treated with 0 (□), 50 (▒), or 100 (▨) mg/kg. Significantly different from control value: a = $p < 0.10$, b = $p < 0.05$, c = $p < 0.01$.

Hepatic NDFDA Levels

Hepatic NDFDA concentration was inversely correlated with serum retinol levels (Day 2: r = –0.66, Day 8: r = –0.93, Day 11: r = –0.92) and positively correlated with liver RPH activity (Day 2: r = 0.64, Day 8: r = 0.58, Day 11: r = 0.85). In the treated animals NDFDA levels were found to decline throughout the study, as shown in Table 2.

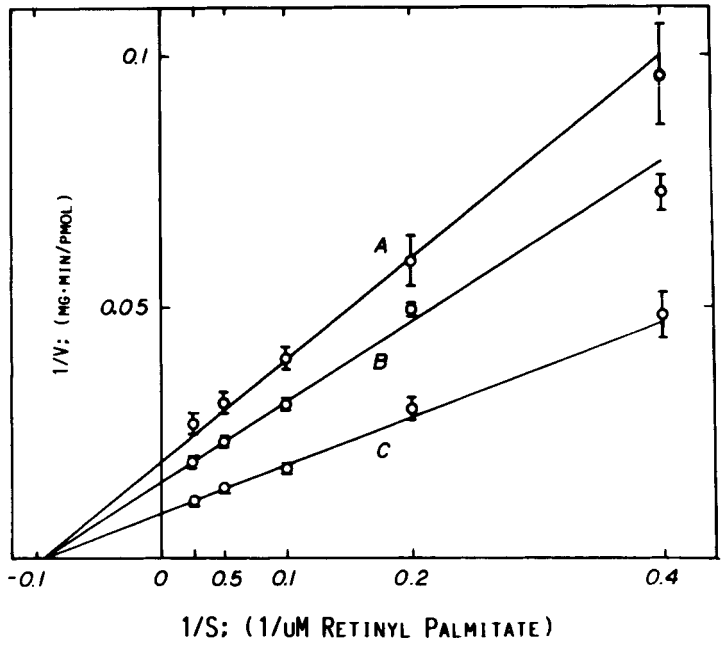

Figure 7. Inhibition of RPH activity by NDFDA and TCDD. Values are mean ± S.E. of 1/RPH activity (1/pmol/min-mg) versus 1/substrate concentration (1/[μM retinyl palmitate]). Incubation containing (A) 500 μM NDFDA, (B) 500 μM TCDD, (C) control.

DISCUSSION

Treatment of male Sprague-Dawley rats with NDFDA caused a rapid onset of symptoms of toxicity that included anorexia, cachexia, and loss of weight. In addition, thymic involution was quite pronounced in the 100 mg/kg NDFDA-dose group, indicating depressed immunologic capability. These symptoms are also observed in animals treated with toxic levels of TCDD and similar compounds (6, 7). However, in marked contrast to TCDD toxicity, serum retinol levels in the treated animals were severely and rapidly depressed to levels significantly below normal. The same pattern of symptoms, including depressed serum retinol levels, is exhibited by animals suffering from a severe vitamin A deficiency (3). This suggested that NDFDA may be toxic either by interfering directly with some important metabolic function of vitamin A or by affecting the availability of retinol to its sites of utilization. Many of the "toxic" responses that might be attributed to NDFDA, then, would actually be consequences of a vitamin A deficiency caused by immediately reduced retinol availability.

The marked effect of NDFDA on serum retinol levels is important to the consideration of the mechanism of toxicity, since these levels are normally

maintained within a fairly narrow range. Lowered serum retinol availability could cause the expression of symptoms of vitamin A deficiency in many target tissues. The rapid depression in serum retinol levels observed following NDFDA administration suggested that the compound may cause this effect via one of three mechanisms: (a) by causing a rapid depletion of hepatic retinyl ester stores, (b) by affecting the formation or secretion of the R-RBP complex by the liver, or (c) by causing a lesion in the process of hepatic retinyl ester hydrolysis.

Because the onset of symptoms characteristic of a vitamin A deficiency, including severely depressed serum retinol levels, occured so rapidly following NDFDA administration, it seemed highly unlikely that depletion of hepatic retinyl ester stores could be a contributory mechanism. The amount of stored retinyl esters in the liver is generally well in excess of metabolic needs, even in young, growing animals, and it continues to increase with age. This reflects the fact that dietary vitamin A availability is routinely in excess of daily requirements, resulting in the relatively large quantity of stored retinyl esters (16). Therefore, the process of causing symptoms of a vitamin A deficiency in rats by complete elimination of dietary availability of retinoids may take many weeks (25). In contrast to the rapid effect caused by NDFDA treatment, depressed serum retinol levels in the case of a severe vitamin A deficiency are symptomatic only in the final stages, when the hepatic reserves of retinyl esters are nearly exhausted (16). Treatment of animals with TCDD or similarly acting compounds seems either to have no effect on serum retinol levels or to cause an initial moderate rise in those levels followed by an eventual decline as hepatic retinyl ester stores are depleted (5, 12, 26).

Retinol appears to be mobilized from the liver only as a component of the R-RBP complex. Formation of the complex is dependent upon the availability of retinol and, therefore, on the hydrolysis of retinyl esters (27). It had been previously reported that apo-RBP is normally present in excess in the liver (28); therefore, because of the extremely short period between the NDFDA dose and the onset of lowered serum retinol levels, it seemed unlikely that an interference with RBP synthesis could produce the results described above. If NDFDA did interfere with the synthesis or levels of apo-RBP, the binding of retinol to apo-RBP, or the secretion of the R-RBP complex, a build-up in hepatic retinol levels in proportion to the NDFDA dose would be predicted. However, while hepatic retinol levels were not significantly depressed by the NDFDA dose until Day 11, there was no evidence of an accumulation of retinol in the liver. Further, the contribution to the measurement of endogenous retinol from other sources in the cell beyond that resulting directly from the hydrolysis of retinyl palmitate may have obscured any pronounced change.

The pronounced depression of RPH activity in hepatic homogenates observed in the NDFDA-treated rats suggested that inhibition of this enzyme was the most likely mechanistic basis for the depressed serum

retinol levels. This was supported by the strong correlation observed between RPH activity as measured in the hepatic homogenate and the serum retinol levels in the corresponding treatment group. Previous workers have been unable to demonstrate a strong correlation between these parameters (23), although the possibility was noted by Goodman and Blaner (17). We believe our data supports a cause and effect relationship between RPH activity and the mobilization of retinol into serum.

A comparison of RPH activity in liver homogenates with that in acetone-extracted liver homogenates showed that NDFDA was able to inhibit liver RPH activity directly in vivo. The extraction of liver homogenates with acetone removed NDFDA present in the homogenate (data not shown) and resulted in a pronounced increase in RPH activity. Enzymatic activities of the acetone-extracted homogenates were always slightly higher than for the corresponding homogenate, but the amount of increase remained constant for samples from control animals. However, a significantly greater increase in activity occurred in samples from the NDFDA-treated animals. The percentage increase in activity was in proportion to dose and time following exposure to NDFDA, suggesting a specific interaction between the chemical and the enzyme. These results strongly indicate that NDFDA acts as an inhibitor of RPH in vivo.

It has been recently suggested by George and Anderson (29) that NDFDA is probably esterified to a more polar species. Since the derivatization technique utilized in the detection of NDFDA would probably also form Me-NDFDA from many of the esters of the compound that could be formed in vivo, we did not attempt to differentiate or characterize any metabolite of NDFDA that may have been formed by the treated animals. Because the in vivo inhibition of RPH activity was maintained for some time, despite the tendency for recovery within the low-dose–treated group, we suggest that metabolism of NDFDA yields species also able to inhibit the enzyme. Further, the data showing that the hepatic homogenate from the treated animals was able significantly to inhibit a partially purified RPH preparation in a dose-dependent manner compared to control values suggest that NDFDA and/or its metabolites maintained their ability to inhibit RPH throughout the course of the experiment.

Partially purified RPH was used to determine the capacity of NDFDA and TCDD to inhibit the enzyme in vitro. Assessment of inhibition of RPH activity was performed with a concentration of NDFDA in the reaction mixture (500 μM) that approximated NDFDA levels in the livers of rats treated with a dose of 100 mg/kg (recognizing that considerations of molar concentrations may not necessarily apply when membrane or highly hydrophobic enzymes such as RPH are being considered). In separate ways, TCDD was incorporated at the same concentration, although with a lethal dose-50 (LD-50) 1,000 times lower than that of NDFDA (50 μg/kg vs 41 mg/kg, respectively, in rats [1, 26]), levels of TCDD in the livers of treated animals would not be expected to approach such a high concentration.

Under the particular incubation conditions used, NDFDA and TCDD caused 60% and 42% inhibition of RPH activity, respectively. Analysis of the data showed both compounds to act as noncompetitive inhibitors, with K_I = 450 and 750 μM, respectively. Thus, the data suggested that at concentrations of NDFDA present in the livers of animals exhibiting toxic symptoms (ie, depressed serum retinol, anorexia, etc) there was also a significant amount of inhibition of RPH activity both in liver homogenates and in partially purified RPH. Further, the data strongly suggested that while TCDD was capable of causing inhibition of RPH, the concentrations necessary were so far in excess of a lethal dose that inhibition of RPH should probably not be associated with the toxic mechanism of the compound.

It is noteworthy that both phylloquinone and α-tocopherol appear to act as noncompetitive inhibitors of the enzyme. As pointed out by Napoli and Beck (20), if inhibition of RPH is to affect vitamin A metabolism, a noncompetitive mechanism is mandated because the substrate concentration (retinyl esters) is routinely maintained at high levels and would necessitate a correspondingly high concentration of inhibitor to compete effectively with substrate and control the enzyme activity by a competitive mechanism.

As noted above, compounds causing dioxin-type toxicity have been reported to cause (eventually) a decline in serum retinol levels in treated animals (6, 8–10). However, such a rapid depression in serum retinol levels as was caused by NDFDA has not yet been described for a xenobiotic agent. Retinyl palmitate hydrolase has not been identified as a regulatory enzyme for the control of serum vitamin A levels. The strong correlation between RPH activity in hepatic homogenates and serum retinol, while not conclusive evidence of a regulatory role for the enzyme, certainly suggests that this potential exists and clearly demonstrates the rapidity and extent to which an inhibition of the enzyme results in an effect on serum retinol levels. Therefore, NDFDA may prove to be a useful tool for investigations on the mobilization of retinol from the liver, as well as for evaluations of the consequences of depressed serum retinol levels.

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FOOTNOTE

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