Phenobarbital-Induced Fatty Livers in the Rat[1] (37425)

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(Introduced by H. Baker)

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One general response of the liver to injury is the diffuse accumulation of fat within hepatic cells. In the past 50 yr, many diverse agents have been reported which produce this change in liver tissue. Although phenobarbital has not been considered as one of these agents, several workers have reported that phenobarbital administration produced enhanced hepatic microsomal phospholipid levels (1–4), no doubt resulting from the known increase of smooth membranes of the endoplasmic reticulum produced in the liver by this drug.

Observations by Salvador *et al.* (5) have shown that sex differences occurred in lipid metabolism in response to phenobarbital administration to normal mice. These workers reported that serum levels of cholesterol, triglyceride and phospholipid were lowered in response to the drug in male and female mice and that the liver concentrations of triglyceride were increased twofold in the female mice.

Koff *et al.* (6) have reported that the pretreatment of animals with phenobarbital protected against fatty livers produced by a single dose of ethanol. Aside from this observation, the data of these authors shows that phenobarbital alone did not produce an increase in hepatic triglycerides. Ariyoshi and Remmer (7) have shown that phenobarbital does not protect against fatty livers produced by chronic feeding of alcohol or choline-free diets to animals and unlike the report of Koff *et al.* (6) demonstrated that phenobarbital *per se* promotes the increase of hepatic triglycerides in control animals.

In reporting their results, Koff *et al.* (6) and Ariyoshi and Remmer (7) did not publish the composition of the diets fed to their animals. Our data demonstrate that dietary components are important in studying the effects of phenobarbital on lipid metabolism and this may account for some of the differences in results reported by the workers mentioned above. This study confirms the observations of others (2, 7) that phenobarbital produced fatty infiltration of the livers in normal animals fed regular laboratory diet and in addition demonstrates that this drug magnifies the effect of a choline deficiency. This effect was influenced by dietary levels of choline and fat.

Materials and Methods. Male, Sprague–Dawley rats weighing approximately 250 g were used in each of the experiments. All experimental animals were pair fed with an animal of equal age and size. Those animals receiving phenobarbital were injected intraperitoneally with 50 mg/kg sodium phenobarbital every 12 hr for 5 days, the experimental period used in each study. Following each experiment, the animals were killed and the livers removed and frozen in air-tight plastic containers at −50° until analyzed. Lipids were extracted from the liver tissue by homogenization with chloroform–methanol 2:1 (v/v) according to Folch *et al.* (8). Total cholesterol was measured in the lipid extracts by the method of Zak (9).

Using another aliquot of the lipid extract, triglycerides and phospholipids were separated from one another using silicic acid columns as described by Sardesai and Manning (10).

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TABLE I. Fat, Protein and Choline Composition of Diets Fed Rats in Phenobarbital Study.

	Diets	Fat content (%)	Protein content (%)	Choline dihydrogen citrate content (%)
A	Purina rat chow	6	23	0.4
B	French choline-control diet	35	12	0.7
C	French choline-deficient diet	35	12	0.0
D	French choline-supplemented diet	35	12	2.1
E	French choline-supplemented diet	35	12	4.2
F	French low-fat diet	15	12	0.7
G	French high-protein diet	35	24	0.7

Liver triglyceride levels were measured using the method of these same authors (10) and phospholipid levels were determined by the method of Bartlett (11) for phosphorous analysis.

Experiment 1. Eight control and eight experimental animals were pair fed on Purina rat chow (Table I, Diet A) and water *ad libitum*. The experimental animals were injected with phenobarbital according to the stated regimen and the control animals received sham injections of physiological saline.

Experiment 2. Twelve rats were maintained on the French (12) control diet and twelve rats on the French choline-deficient diet (Diets B and C, Table I). Each of these groups was subdivided into groups of six animals. The experimental animals received phenobarbital injections throughout the 5-day period and the control animals received sham injections of physiological saline at each injection period. During the period of the experiment, the animals receiving the phenobarbital were pair fed with an animal of their respective control group, and all animals received water *ad libitum*.

Experiment 3. From results obtained in Expts 1 and 2 it was apparent that choline, fat and protein content of the diet may play a role in influencing liver triglycerides, phospholipids and total cholesterol during phenobarbital administration. In view of this a third experiment was conducted in which the control French diet was modified to determine whether dietary fat was responsible for elevated liver lipids during phenobarbital administration and if elevated dietary choline or protein could prevent this fat accumulation. The control experiments were conducted in rats fed the French control (Table I, Diet B). Increased dietary choline was given to two separate groups of rats by adding two different levels of choline dihydrogen citrate to Diet B to form Diets D and E (Table I). Diet F with lowered fat content was fed to a fourth group of rats. In formulating Diet F, the saturated fat content of the French control diet was lowered to reduce the total fat content from 35 to 15%. This fat was replaced isocalorically with sucrose. A fifth group of rats was fed a modified French control diet (Diet G) which had its protein content (casein and soya protein) doubled at the isocaloric expense of sucrose. In this experiment it was not possible to pair-feed all the experimental groups to the control animals but record of food consumption showed approximately an equal daily intake of all animals in all groups. All animals in the 5 different groups of this experiment received phenobarbital injections by the regimen described above.

The fat, protein and choline contents of all diets used in this study have been summarized in Table I for purposes of clarity.

In studies involving the development of fatty livers, it has been shown by Barak (13), Thomson *et al.* (14), and Mendenhall (15) that a good means of circumventing the problem of the reference unit is to list analytical values obtained on the basis of the total liver. Consequently, comparative values shown in this study are expressed as μmoles/total liver. The Student t test was used throughout this study to determine the significance of differences between the means of groups being compared.

Results. The effects of phenobarbital ad-

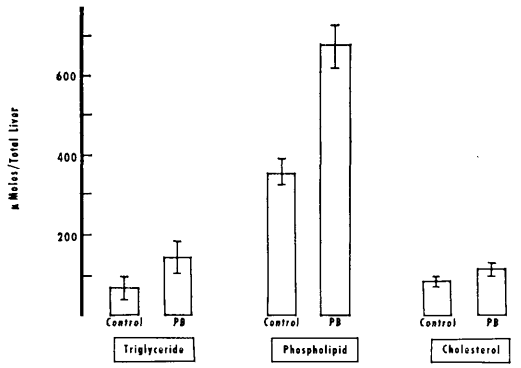

FIG. 1. Effect of phenobarbital on hepatic triglyceride, total cholesterol and phospholipid in rats fed commercial rat chow (Means ± SD). p values between control and phenobarbital data were all < 0.001.

ministration on liver lipids under normal dietary conditions (Diet A) are shown in Fig. 1. Under these conditions the triglycerides and phospholipids were approximately doubled and the cholesterol was increased significantly.

When animals were maintained on a high-fat choline diet (Diet B) the administration of phenobarbital produced a 7-fold increase in triglycerides, but as with the commercial rat chow, a doubling of the phospholipids and a significant increase in total cholesterol (Fig. 2).

That the French choline-deficient diet (Diet C) is effective in producing fatty in-

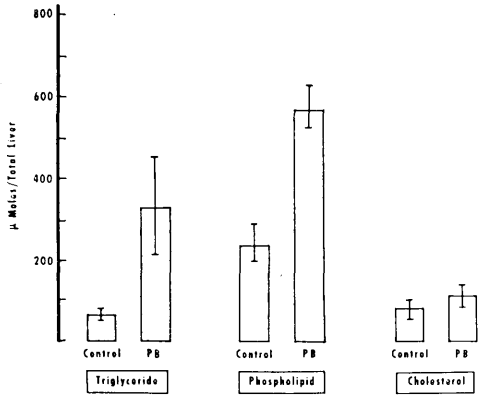

FIG. 2. Effect of phenobarbital on hepatic triglyceride, total cholesterol and phospholipid in rats fed the French choline diet (Means ± SD). p values between control and phenobarbital data were all < 0.001.

filtration of the liver in 5 days is shown in Fig. 3. The triglycerides were increased 25-fold in the liver but the phospholipids remained constant and the cholesterol showed some increase.

Despite the fact that extremely high levels of hepatic triglycerides are produced with the French choline-deficient diet (Diet C), in 5 days, this family of lipids was found to double when animals were given phenobarbital along with this diet. Again with this regimen, the phospholipids were shown to in-

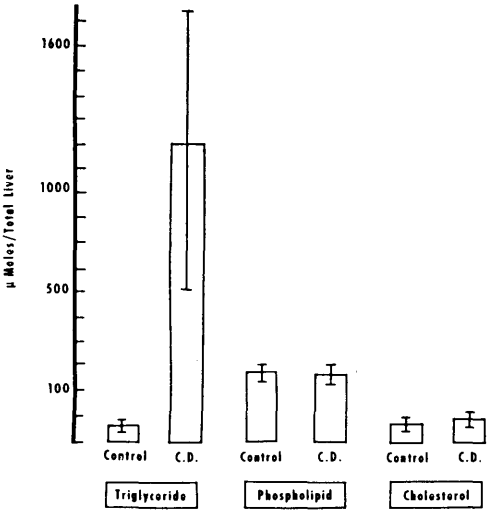

FIG. 3. Effect of choline deficiency on hepatic triglycerides, total cholesterol and phospholipid levels (Means ± SD). p values between control and choline deficiency data were: (a) < 0.001 for triglycerides. (b) N.S. (not significant) for phospholipid. (c) < 0.002 for cholesterol.

crease 2-fold and the total cholesterol increased significantly (Fig. 4).

Even though choline deficiency enhanced the effect of phenobarbital in this study, Table II demonstrates that supplementing the French diet 3-fold and 6-fold with choline only partially prevented the phenobarbital-induced accumulation of hepatic triglycerides and cholesterol. The partial protective effect of high levels of choline in the diet apparently was not mediated through phospholipid utilization because this family of lipids remained at the same levels as seen in the other experiments with phenobarbital. Table II also demonstrates that reducing levels of saturated

TABLE II. Preventive Ability of Dietary Factors Against Accumulation of Phenobarbital-Induced Liver Lipids.

Type of diet with phenobarbital	No. of animals	Mean liver triglycerides ± SD (μmoles/total liver)	p	Mean liver phospholipids ± SD (μmoles/total liver)	p	Mean liver cholesterol ± SD (μmoles/total liver)	p
Control diet							
B + PB	6	330 ± 122		579 ± 45		113 ± 19	
Choline supplement							
D Diet + PB[a]	8	213 ± 59	<0.05	611 ± 71	NS	92 ± 10	<0.05
E Diet + PB[b]	5	188 ± 32	<0.05	619 ± 44	NS	110 ± 11	NS
Low fat							
Diet F + PB	6	311 ± 104	NS	591 ± 63	NS	124 ± 25	NS
High protein							
Diet G + PB	6	297 ± 38	NS	674 ± 77	<0.05	122 ± 12	NS

[a] Choline dihydrogen citrate added to regular French Control Diet (11) to give level of 2.1% by weight.
[b] Choline dihydrogen citrate added to regular French Control Diet (11) to give level of 4.2% by weight.

FIG. 4. Effect of phenobarbital on hepatic triglycerides, total cholesterol and phospholipid in rats fed the French choline deficient diet (Means ± SD). p values between control and phenobarbital data were: (a) < 0.002 for triglycerides. (b) < 0.001 for phospholipid. (c) < 0.001 for cholesterol.

fat or increasing levels of protein in the diet did not have much influence in changing the effect of phenobarbital on liver fats.

Discussion. These data indicate that the hepatic levels of three general families of lipids (triglycerides, phospholipids, and cholesterol) are affected by phenobarbital administration in the rat. Triglycerides appeared to be more influenced by dietary levels of choline and fat than phospholipids and cholesterol. A unique observation in this study is that phenobarbital produces approximately the same increases in phospholipid and total cholesterol regardless of fat or choline levels in the diet. This occurs even in a choline deficiency, which poses a question as to the source of choline necessary for the maintenance of the elevated phospholipid. The observations of Holtzman and Gillette (1) that phenobarbital reduces the catabolism of microsomal phospholipid may partially explain the maintenance of elevated hepatic phospholipid levels in these studies. The raised phospholipid levels despite choline deprivation suggests that the integrity of endoplasmic reticulum membranes may have a high priority.

Nothing in these data indicate the mechanism of action of phenobarbital in producing hepatic triglyceride accumulation. As shown in Table II, reducing the dietary fat from

35 to 15% did not lower the phenobarbital effect. Further reduction in dietary fat to the 6% level, as present in the Purina rat chow (Diet A), did lower hepatic lipid accumulation. However, absolute comparison with the French diet is tenuous because of wide differences in the constituents of the two diets.

Although protein has been shown to be a factor in the transport of liver lipids, these data show that increasing protein from 12 to 24% in the French control diet containing 35% fat did not protect against the phenobarbital induced hepatic steatosis.

One of the natural compounds which must be considered in studies relating to fatty livers is a key lipotrope, choline. Our data suggest that the mechanism of action of phenobarbital in inducing hepatic triglyceride accumulation may be partially mediated through the metabolic process which renders choline lipotropic. Phenobarbital produced high levels of hepatic lipids when administered to animals fed a high fat diet containing essential amounts of choline (Table II). This measure was partially prevented when the diet was supplemented with excess amounts of choline. With the same diet but with the complete lack of choline (Fig. 4) phenobarbital produced extremely high levels of hepatic triglyceride which were not explained by the additive effects of phenobarbital and choline deficiency.

It is difficult to speculate where phenobarbital has its effect on lipid metabolism. Its possible effect on choline metabolism is also perplexing because the lipotropic nature of choline is not understood. Work in this laboratory (16) has shown that the major uptake of choline by the isolated perfused liver is regulated by the oxidation of choline in the liver. Preliminary studies in this laboratory have shown that acute or chronic administration of phenobarbital inhibits choline uptake by the perfused liver. It is feasible that if phenobarbital inhibits hepatic choline oxidase this would make more choline available for the expansion of the phospholipid pool noted with the use of this drug. Normally adequate amounts of choline in the diet may not be sufficient to fulfill the increased demands for choline imparted by phenobarbital administration. Only in the case of excessive amounts of choline in the diet would choline offer some lipotropic protection against infiltration of liver with triglycerides. Lack of complete protection with high levels of dietary choline would indicate that other metabolic mechanisms are operative.

Summary. Administration of phenobarbital to rats fed a standard laboratory diet or 6 different synthetic diets produced elevated levels of triglyceride, phospholipid and total cholesterol in the livers of these animals. By varying the choline, fat, and protein constituents in these diets it appears that choline and fat content are factors influencing the amount of triglyceride accumulating in livers as a result of phenobarbital administration. These dietary factors, however, did not appear to regulate the manner in which phenobarbital elevated phospholipid and total cholesterol levels.

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