THE EFFECT OF PHENOBARBITAL ON THE SYNTHESIS OF MICROSOMAL PHOSPHOLIPID IN FEMALE AND MALE RATS

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Treatment of animals with phenobarbital leads to an increase in the activity of enzymes associated with the hepatic microsomes and to an increase in hepatic microsomal protein and phospholipid. Conney and Gilman (1963) showed that puromycin and actinomycin prevented the increase in enzyme activity induced by phenobarbital and thus suggested that the barbiturate acted by increasing protein synthesis. This has recently been confirmed by the work of Kato et al. (1965a,b) who have found that pretreatment of rats with phenobarbital increased the incorporation (both in vitro and in vivo) of C^{14}-labeled amino acids into liver microsomes.

Orrenius et al. (1965) reported that 3-24 hours after a single injection of phenobarbital there is a two to sixfold increase in the incorporation of P^{32} into the microsomal phospholipids of fasted male rats. In contrast to their findings the results of the present study indicate that the increase in phospholipid after phenobarbital treatment is not necessarily associated with an increase in synthesis. Instead, it appears to be caused mainly by inhibition of catabolism of the phospholipid.

MATERIALS AND METHODS

Eight to ten-week-old Sprague Dawley rats (female 160-180 g, male 300-380 g) from the NIH colony received either 80 mg/kg daily i.p. of phenobarbital or an equivalent volume of saline. Animals were fasted for the entire experiment beginning 24 hours before the first dose of phenobarbital.

Each of five experimental and control animals was given 0.2-0.25 mc of P^{32} via the caudal vein at various times after the first dose of phenobarbital and killed by decapitation one hr later.

The livers were removed, chilled, and homogenized in 3 volumes of KCl (144 mM)-tris (20 mM) buffer pH 7.4. Each homogenate was centrifuged at 9000 x g for 10 min. Twelve ml of the supernatant was centrifuged at 144,000 x g for 1 hr. The pellet was resuspended in the KCl-tris buffer and centrifuged at 144,000 x g for 1 hr. The microsomal pellet was resuspended in 4 ml of the KCl-tris buffer. The phospholipids were extracted from 0.25 ml of the microsomal suspension with methanol-chloroform-water (10:5:4) by a modification of the method of Bleigh and Dyer (1959). Phospholipid phosphorus in the organic extract was determined by the molybdate-ascorbic acid method of Chen et al. (1956). Four-tenths ml of 9000 x g supernatant was precipitated with 10 ml of 5% TCA. The inorganic phosphorus in the aqueous phase was determined by the method of Chen et al. (1956). The radioactivity was determined by liquid scintillation counting in a Tri Carb 4000. The phospholipid extract (2 ml) was dissolved in 15 ml of 4% BBOT (2,5-bis-[5'-tert. butyl-benzoxazolyl (2')]-thiophene) in toluene. Aqueous samples (0.4 ml) were dissolved in a mixture of 15 ml of the toluene scintillator and 8 ml of absolute ethanol.

The phospholipids of microsomes from animals receiving 5 mc of P^{32} 72 hrs after the first dose of phenobarbital were separated into fractions by thin layer chromatography according to the method of Parker and Peterson (1965). The spots were identified by exposure to iodine vapor, the silica gel scraped into counting vials, and the radioactivity determined by liquid scintillation counting.

The fractional turnover rate was taken as the ratio of specific activity of the phospholipid phosphorus to the specific activity of the inorganic phosphorus. The rate of synthesis was calculated by multiplying the fractional turnover rate by the amount of microsomal phospholipid phosphorus in the

liver. Control and experimental values were compared at each time interval by the Student t-test, and a $P < 0.05$ was taken as significant.

RESULTS

The fractional turnover rate in the female rats was depressed at all times (16-32%) (fig. 1).

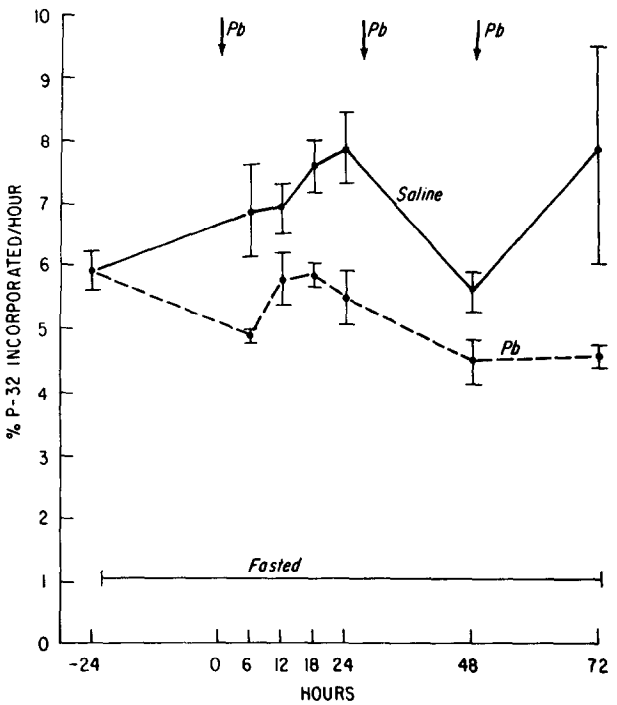

Fig. 1. Effect of phenobarbital (80 mg/kg daily, i.p.) on the fractional turnover of P^{32} into hepatic microsomal phospholipids of fasted female rats.

These results would indicate that phospholipid synthesis is depressed at all times, but if the rate of incorporation of phosphorus into total hepatic microsomal phospholipid is compared, then no significant difference in total synthesis occurred at any time (fig. 2). Despite the failure of phenobarbital to increase the rate of synthesis, there was a significant increase in the total hepatic microsomal phospholipid phosphorus (fig. 3).

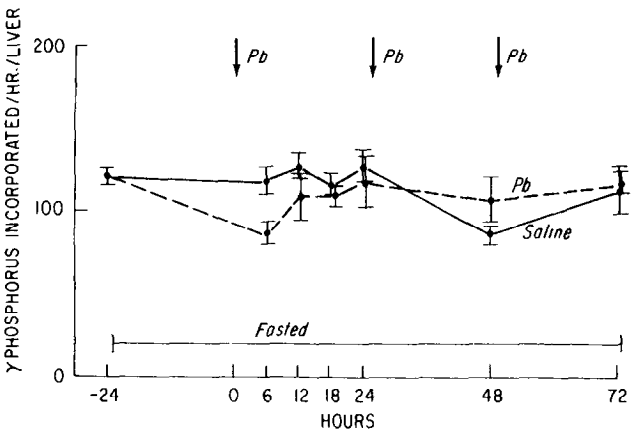

Fig. 2. Effect of phenobarbital (Pb) (80 mg/kg daily, i.p.) on rate of synthesis of hepatic microsomal phospholipids of fasted female rats.

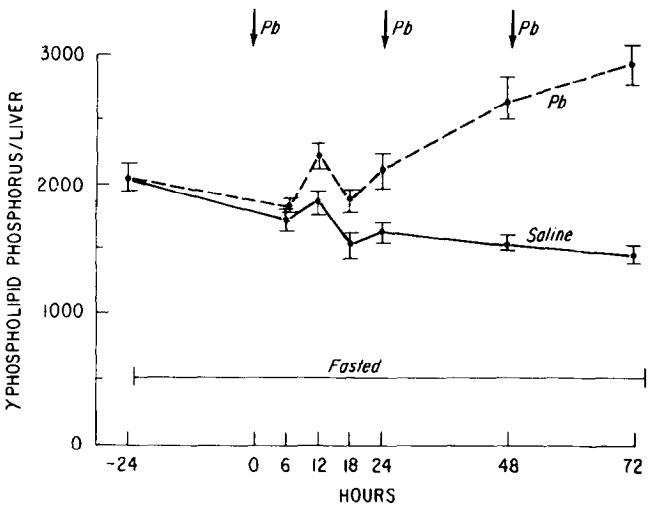

Fig. 3. Effect of phenobarbital (Pb) (80 mg/kg daily, i.p.) on total hepatic microsomal phospholipid of fasted female rats.

Similarly, in the male rats the fractional turnover rate was significantly depressed at 48 (29%) and 72 hrs (31%), though it was significantly increased at 18 hrs (37%). The rate of phospholipid synthesis was not significantly different except at 18 hrs (+41%). Yet there was a significant increase in total hepatic microsomal phospholipid phosphorus.

In female rats the distribution of the label among the various phospholipid fraction was unchanged by phenobarbital treatment. However, in the male rats phenobarbital decreased the incorporation of the P^{32} label into the lecithin fraction and increased (51.8%) the incorporation of P^{32} into phosphotidylethanolamine (46.5%).

DISCUSSION

The work of Conney and Gilman (1963), Kato et al. (1965a,b), and Orrenius et al. (1965) led to the belief that phenobarbital increases in the enzymatic activity, protein, and phospholipid of hepatic microsomes solely by enhancing the synthesis of these constituents. The data from the present study do not support the view that phenobarbital increases phospholipids by stimulating their synthesis. In both male and female rats significant increases in the total phospholipid were not observed until at least 24 hrs after the initial dose of phenobarbital, at which time the synthesis was not significantly enhanced by the treatment. Thus, phenobarbital may increase components of the endoplasmic reticulum in two ways: 1) By stimulating the synthesis of protein and 2) by inhibiting the catabolism of phospholipid.

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