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Microsome-specific stimulation by phenobarbital of amino acid incorporation *in vivo*

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THE administration of phenobarbital to rats produces a marked increase in the activity of a number of liver microsomal enzyme systems.[1, 2] Many of these require NADP and oxygen and metabolize foreign compounds by N-demethylation, O-demethylation, and ring hydroxylation. The question arises as to the mechanism of this effect; i.e. is the increased enzyme activity due to the activation of pre-existing enzymes or to enzyme synthesis? Earlier studies demonstrated an increase in amino acid incorporation *in vitro* in microsomes from phenobarbital-treated rats[3] and an increase in the amount of microsomal protein in rats chronically treated with phenobarbital.[4] Others found that the administration of ethionine or puromycin inhibits the phenobarbital-induced increases in enzyme activity.[5, 6]

More recently, Remmer and Merker[7] have shown by electron microscopy that chronic administration of phenobarbital causes a proliferation of endoplasmic reticulum of rat liver. Thus, the major enzyme changes and the alterations in fine structure induced by phenobarbital are localized in the microsomes.

In this investigation we studied the effect of phenobarbital on the incorporation *in vivo* of L-leucine-^{14}C in the various subcellular fractions of rat liver.

Table 1 shows the effect of phenobarbital treatment on total liver weight and on liver microsomal protein. Although these results were obtained under somewhat different experimental conditions, they represent a confirmation of the work of Conney *et al.*,[4] who showed an increase in net microsomal protein after phenobarbital treatment. We found that 40 hr after phenobarbital treatment there was an increase of 17% in liver weight and 35% in total microsomal protein.

TABLE 1. EFFECT OF PHENOBARBITAL TREATMENT ON RAT LIVER WEIGHT AND MICROSOMAL PROTEIN CONTENT*

	Controls	PB-Treated	Difference (%)	P
Body weight (g)	146 ± 3	147 ± 5	+ 1	>0·05
Liver weight (g)	4·91 ± 0·5	5·74 ± 0·5	+17	<0·01
Microsomal protein (mg/g liver)	28·9 ± 2·1	37·8 ± 2·7	+35	<0·001
Total microsomal protein (mg)	142·9 ± 6·3	222·6 ± 9·8	+57	<0·001

The rats were treated with phenobarbital and microsomes isolated as described in Table 2.
* Protein was determined gravimetrically.[8]

Table 2 shows the effect of phenobarbital on the labeling *in vivo* of protein of various liver subcellular fractions. These results indicate that the predominant site of the phenobarbital-induced increase in protein synthesis is the microsomes. Thus, in two experiments there was an increase of 23% and 34% in the specific activity of microsomes from phenobarbital-treated rats. When one takes into account both the increase in the specific activity of the microsomes and the increase in net microsomal protein, the total incorporation of leucine-^{14}C after phenobarbital treatment is increased by 93% and 110%. When the microsomes were fractionated further into ribosomes and deoxycholate-soluble material, the latter composed largely of endoplasmic reticulum, we observed a similar phenobarbital-induced increase in the labeling of these subfractions. Thus, in two experiments the proteins from phenobarbital ribosomes were 24% and 35% more radioactive than comparable control proteins. The proteins from phenobarbital deoxycholate-soluble material were 21% and 38% more radioactive than similarly prepared proteins from control rats.

TABLE 2. THE EFFECTS OF PHENOBARBITAL ON THE INCORPORATION *IN VIVO* OF L-LEUCINE-^{14}C IN SUBCELLULAR FRACTIONS OF RAT LIVER

Groups of 6 female rats of the Sprague-Dawley strain and weighing about 180 g were injected i.p. with 80 mg phenobarbital/kg at 42 and 18 hr prior to decapitation. The rats were fasted from food during the entire 42-hr interval. In experiment I each rat was injected i.p. with 10 μc L-leucine-^{14}C/kg (S.A. 5·67 mc/mmole) 20 min prior to death. In experiment II each rat was given 100 μc L-leucine-^{14}C/kg (S.A. 5·52 mc/mmole) 45 min before sacrifice. The livers were removed and homogenized in five volumes of 0·25 M sucrose. The nuclear fraction and cell debris were sedimented at 700 *g* for 10 min. The nuclear fraction was further purified by centrifugation through high molarity sucrose according to the procedure of Sporn and Dingman.[9] The mitochondria were sedimented at 10,000 *g* for 10 min, and the microsomes and supernatant fractions were isolated by centrifugation at 100,000 *g* for 1 hr. The microsomes were further fractionated by the procedure of Korner,[10] with deoxycholate to solubilize the endoplasmic reticulum. The proteins from each fraction were precipitated with trichloroacetic acid and washed, plated, and the radioactivity measured by a procedure previously described.[8] Each value represents the average and standard deviation.

	Exp. I				Exp. II			
	Specific activity (cpm/mg protein)							
Fraction	Control	PB	%	P	Control	PB	%	P
Nuclei	69 ± 8	71 ± 4	+ 3	>0·05	382 ± 17	445 ± 39	+16	<0·05
Mitochondria	121 ± 14	115 ± 11	− 5	>0·05	397 ± 19	428 ± 25	+ 8	>0·05
Microsomes	216 ± 21	265 ± 10	+23	<0·001	1,159 ± 69	1,551 ± 134	+34	<0·001
Ribosomes	84 ± 6	104 ± 6	+24	<0·001	490 ± 48	662 ± 108	+35	<0·001
DOC-soluble microsomes	239 ± 36	290 ± 12	+21	<0·01	1,042 ± 95	1,436 ± 221	+38	<0·001
Supernatant	55 ± 4	57 ± 7	+ 3	>0·05	279 ± 23	267 ± 19	− 4	>0·05

In contrast to the phenobarbital stimulation of microsomal leucine incorporation, there was either a small or no effect on the labeling of proteins of highly purified nuclei or of mitochondria. This indicates that the increased incorporation observed in the microsomes is not due to a phenobarbital-induced decrease in the endogenous pool size of leucine-^{12}C which would result in a relatively higher specific activity of the leucine-^{14}C precursor and hence show an apparent but not an actual increased rate of leucine-^{14}C incorporation. Table 2 also shows that the specific activity of supernatant protein from the control and phenobarbital-treated rats was identical. Since the supernatant proteins are thought to be synthesized in the microsomes, our results suggest that the increased protein-synthesizing activity of the microsomes due to phenobarbital treatment is restricted to the synthesis of those proteins which become part of the endoplasmic reticulum and does not involve an increase in the synthesis of proteins that are transferred to the soluble fraction. The radioactive proteins precipitated with the ribosomes represent to a large extent proteins that are being synthesized and subsequently transferred to other fractions.

Current concepts of protein synthesis suggest that the proteins synthesized in the microsomes reflect the nature of the messenger RNA molecules directing their synthesis. Our results are consistent with the hypothesis that phenobarbital affects the production of specific messenger RNA molecules which direct the synthesis of endoplasmic reticulum and the enzymes associated with this fraction. In other studies, methylcholanthrene, an inducer of microsomal enzymes, was shown to stimulate the production of nuclear messenger RNA as well as increase microsome amino acid incorporation *in vitro*.[11–14] The latter was due to both an increased messenger RNA content and an increased sensitivity of microsomes to added messenger RNA. This may represent part of the mechanism of phenobarbital action.

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