PHENOBARBITAL-INDUCED SYNTHESIS OF THE OXIDATIVE DEMETHYLATING ENZYMES OF RAT LIVER MICROSOMES.

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Rat liver microsomes catalyze the TPNH-dependent oxidative demethylation of various drugs, e.g. aminopyrine (La Du et al., 1955). TPNH-cytochrome c reductase (Horecker, 1950) and the hemoprotein, known as the CO-binding pigment (Klingenberg, 1958; Omura and Sato, 1963), are probably involved in liver-microsomal drug-hydroxylating reactions (Orrenius et al., 1964; Orrenius and Ernster, 1964). Administration of phenobarbital or related compounds to rats causes an increased rate of drug-hydroxylation (Kato et al., 1962; Remmer and Merker, 1963). Studies of the phenobarbital-induced increase of the microsomal aminopyrine-demethylating activity are the subject of this paper.

The methods used were as described earlier (Ernster et al., 1962; Dallner, 1963; Orrenius et al., 1964), except the composition of the incubation system used for measurement of the oxidative demethylation activity, which contained microsomes, 5 mM aminopyrine, 0.05 M tris-buffer, pH 7.5, 50 mM nicotinamide, 5 mM $MgCl_2$, 0.5 mM TPN, and a TPNH-generating system consisting of 5 mM DL-isocitrate, 0.01 mM $MnCl_2$ and isocitric dehydrogenase enough to reduce 0.32 μmoles TPN per min., in a final volume of 2 ml.

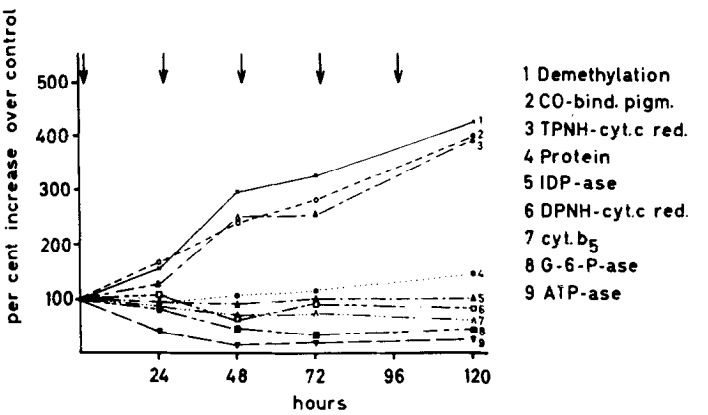

Fig. 1. Effect of phenobarbital treatment on certain microsomal enzymes.
Phenobarbital injections are marked with arrows.

Rats of both sexes were injected i.p. with 100 mg of phenobarbital per kg body-weight once every 24 hours. After five phenobarbital injections there was a fourfold increase over the controls in the aminopyrine-demethylating and TPNH--cytochrome c reductase activities, as well as in the content of CO-binding pigment, all as compared on the protein basis; the total liver-microsomal protein of the drug-treated animals was ca. 1.5 x that of the controls. The levels of another microsomal flavoenzyme, DPNH-cytochrome c reductase, and another hemoprotein, cytochrome b_5, were slightly diminished, as were also the microsomal glucose-6-phosphatase and nucleoside di- and triphosphatase activities (Fig. 1).

Fig. 2 shows that after the fifth and last phenobarbital injection there was a decrease in the rate of aminopyrine--demethylation and after another four days the activity was about the same as in the control-group. The amount of CO-binding pigment declined somewhat more rapidly than the TPNH-cytochrome

c reductase activity. The latter followed closely the amino-pyrine-demethylating activity, indicating that, under the prevailing conditions, the TPNH-cytochrome c reductase was the rate-limiting component of the oxidative demethylation system.

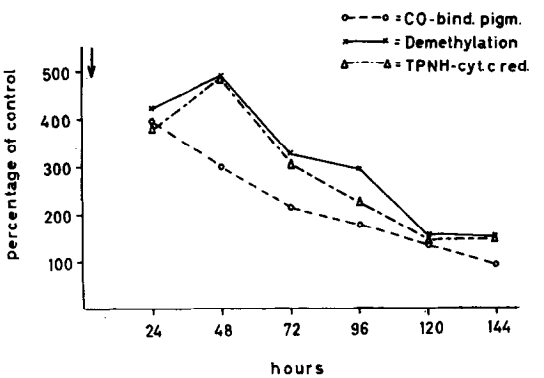

Fig. 2. Decrease in TPNH-cytochrome c reductase and oxidative demethylation activities and amount of CO-binding pigment after completed phenobarbital treatment.

The arrow marks fifth and last phenobarbital injection.

Data in Table I illustrate the effect of phenobarbital treatment upon the total microsomal fraction and subfractions. The smooth vesicle fraction exhibited the greatest increase in the content of protein and CO-binding pigment as well as in the activities of TPNH-cytochrome c reductase and amino-pyrine demethylation.

Simultaneous administration of 8 μg/100 g body-weight of actinomycin D abolished initially the phenobarbital-stimulated increase in TPNH-cytochrome c reductase, CO-binding pigment and oxidative demethylation activity (Fig. 3); a complete inhibition required that the dose of actinomycin D was raised with 4 μg/100 g body-weight for the second and each of the following injections.

Table I. Effect of phenobarbital treatment on TPNH-cytochrome c reductase and oxidative demethylation activities and content of CO-binding pigment of total microsomes and microsomal subfractions.

No. of treatm.	Fractions	Protein mg/g liver	RNA / Protein mg/mg	Phospholip. / Protein mg/mg	TPNH-cyt.c red.act. μmoles TPNH ox./min/mg protein	CO-bind. pigm. $E_{450-500}$ mμ/mg protein	Aminopyrine demeth.act. mμmoles formaldehyde/min/mg protein
	Total microsomes	27.6	0.23	0.28	0.028	0.018	2.36
0	Rough vesicles	16.3	0.34		0.033	0.018	2.84
	Smooth vesicles	9.1	0.05		0.027	0.014	2.80
	Total microsomes	21.8	0.25	0.41	0.035	0.030	3.66
1	Rough vesicles	13.4	0.36		0.032	0.027	3.90
	Smooth vesicles	8.7	0.05		0.031	0.023	3.40
	Total microsomes	31.2	0.23	0.43	0.071	0.052	7.60
3	Rough vesicles	16.2	0.32		0.043	0.037	6.35
	Smooth vesicles	14.0	0.08		0.114	0.068	10.40
	Total microsomes	38.0	0.22	0.45	0.110	0.071	10.00
5	Rough vesicles	19.1	0.34		0.060	0.051	8.60
	Smooth vesicles	20.1	0.08		0.159	0.085	10.80

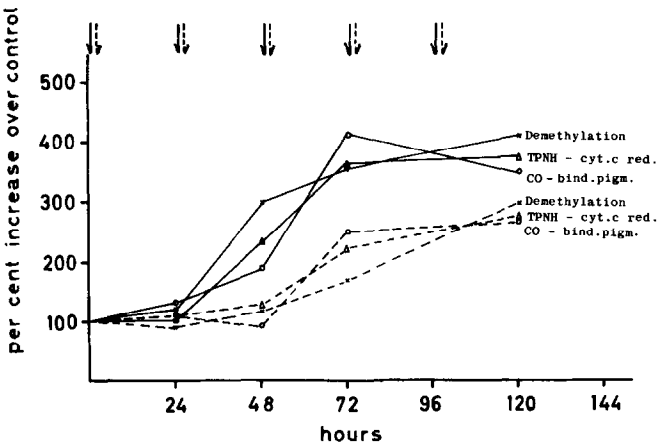

Fig. 3. Initial abolition of the phenobarbital-induced increase in oxidative demethylation, TPNH--cytochrome c reductase and CO-binding pigment, by simultaneous administration of actinomycin D.
Solid arrows mark injections with phenobarbital; dashed arrows mark injections with phenobarbital + actinomycin D.

These results add strong support to the concept that TPNH-cytochrome c reductase and the CO-binding pigment are components of the oxidative demethylation system. The results also suggest that the substrate-induced activation of this system takes place at the level of messenger-RNA synthesis.

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