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EFFECT OF ANTITUBERCULOSIS AGENTS ON CYTOCHROME P450 ISOFORM COMPOSITION IN RAT LIVER MICROSOMES

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The cytochrome P450-hydroxylase system of endoplasmic reticulum membrane of liver cells plays an important role in the metabolism of nonpolar exogenous and endogenous compounds [1]. The final electron acceptor in the oxidation chain, namely cytochrome P450, can exist as several isoforms. Under the influence of phenobarbital, a subfraction cytochrome $P450_B$, with subunit molecular weight of 52,000 daltons, is induced in the rat liver, whereas by the action of 3-methylcholanthrene, subfraction cytochrome $P450_C$, with subunit molecular weight of 56,000 daltons is induced [9]. Many substances inducing cytochrome P450 in the liver are now known. Between 20 and 30 different isoforms of this hemoprotein have now been identified

TABLE 1. Effect of Antituberculosis Drugs, Phenobarbital, and 3-Methylcholanthrene on Cytochrome P450 Content in Rat Liver Microsomes (M ± m)

Preparation	Cytochrome P450, nmoles/mg protein
Phenobarbital sodium	2,8±0,2
3-Methylcholanthrene	1,5±0,1
Isoniazid	1,1±0,05
Phthivazid	1,2±0,06
PAS	0,9±0,1
Streptomycin	1,1±0,2
	1,0±0,1

Legend. Mean results of four experiments shown. Microsome preparation from livers of five animals.

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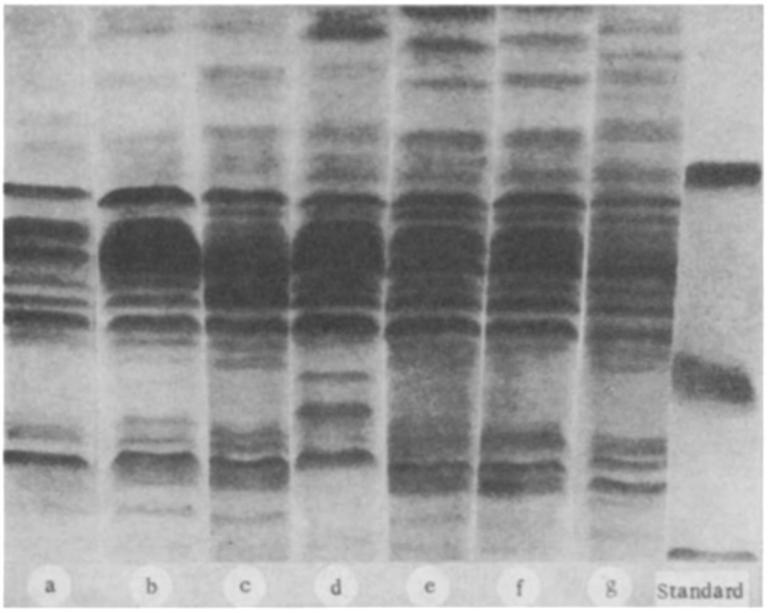

Fig. 1. Results of electrophoretic fractionation of membrane proteins of microsomes isolated from rat liver: a) control, b) phenobarbital, c) 3-methylcholanthrene, d) isoniazid, e) phthivazid, f) PAS, g) streptomycin. The following standards for molecular weight were used (from top to bottom): ovalbumin (43,000 daltons), albumin (67,000 daltons), phosphorylase B (94,000 daltons) — standard.

in rat and rabbit liver microsomes [9]. The question whether this number of isoforms is final or whether there is an infinite number of them, and what is the stimulus which induces its own specific isoform of cytochrome P450 cannot yet be answered.

It was accordingly decided to study the effect of antituberculosis drugs on the cytochrome P450 isoform composition in liver microsomes in an attempt to elucidate the mechanism of development of drug tolerance in the treatment of tuberculosis.

EXPERIMENTAL METHOD

Noninbred male rats weighing 120-150 g, kept on the standard animal house diet, were used.

The microsome fraction from rat liver was obtained by differential centrifugation as described previously [2]. The isolation medium for microsomes contained 0.15 M KCl, 0.5 mM EDTA, and 5 mM phosphate buffer, pH 7.4. The resulting microsome fraction was suspended in 100 mM phosphate buffer, pH 7.4, containing 0.5 mM EDTA.

To induce cytochrome P450, phenobarbital sodium (80 mg/kg), 3-methylcholanthrene dissolved in olive oil (40 mg/kg), isoniazid (200 mg/kg), phthivazid (400 mg/kg, suspension in 1% starch), PAS (1500 mg/kg), and streptomycin sulfate (80 mg/kg, or 80,000 Units/kg) were injected into the animals intraperitoneally at intervals of 24 h for 3 days. The rats were decapitated 48 h after the last injection.

Cytochrome P450 in preparations of rat liver microsomes was assayed spectrophotometrically [11] in a "Unicam SP-800" spectrophotometer. The protein concentration was determined by Lowry's method [8] in the presence of 0.1% sodium deoxycholate.

The electrophoretic study of the polypeptide composition of the liver cell microsome membranes was carried out by the method in [7], using an acrylamide concentration grid from 5 to 15%. The conditions of fractionation were application of a steady current of 50 mA to a plate measuring 180 × 200 × 2.7 mm in a chamber of type GE-2/4 (Pharmacia Fine Chemicals), with water cooling system. Proteins (from Sigma, USA) with the following subunit molecular weights were used as standards: α-lactalbumin, 14,400 daltons, trypsin inhibitor 20,000 daltons, ovalbumin 43,000 daltons, bovine serum albumin 67,000 daltons, phosphorylase B 94,000 daltons. After electrophoresis the gels were fixed in a mixture of 70% isopropanol and 10%

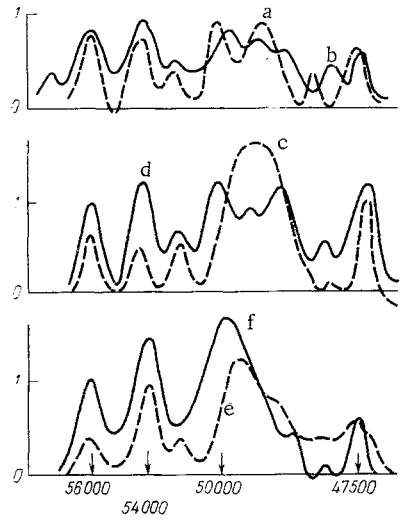

Fig. 2. Densitograms of electrophoretic fractionation of microsome preparations illustrated in Fig. 1. a) Phthivazid; b) control and experiment with streptomycin; c) phenobarbital; d) PAS; e) isoniazid; f) 3-methylcholanthrene. Abscissa, molecular weights (in daltons); ordinate, optical density 560 nm (in relative units).

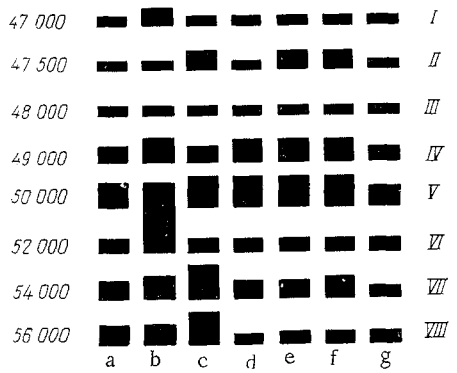

Fig. 3. Diagram showing changes in cytochrome P450 isoform composition in rat liver microsomes. a) Control, b) phenobarbital, c) 3-methylcholanthrene, d) isoniazid, e) phthivazid, f) PAS, g) streptomycin. Isoforms of cytochrome P450 with the following subunit molecular weight (daltons): I) 47,500, II) 47,500, III) 48,000, IV) 49,000, V) 50,000, VI) 52,000, VII) 54,000, VIII) 56,000.

acetic acid and stained with 0.05% Coomassie brilliant blue R-250 in a system of isopropanol—acetic acid—water (25:10:65). The gels were washed in 10% acetic acid until the background was completely decolorized. The gels were scanned on a "Gilford" spectrophotometer at 560 nm.

EXPERIMENTAL RESULTS

The inducing action of the antituberculosis drugs and also of phenobarbital and 3-methylcholanthrene on cytochrome P450 of rat liver microsomes was assessed on the basis of their effect on the specific concentration of this enzyme. As Table 1 shows, phenobarbital increased the cytochrome P450 content 2.8 times and the 3-methylcholanthrene content 1.5 times

compared with the control. Meanwhile the antituberculosis drugs studied did not give this effect, in agreement with data in the literature [13].

Electrophoretic analysis of liver microsome membranes from rats receiving antituberculosis drugs showed qualitative changes in cytochrome P450 isoform composition (Figs. 1 and 2) in cases of animals receiving isoniazid, phthivazid, and PAS. Injection of streptomycin did not affect the isoform composition of cytochrome P450. It will be clear from Fig. 2 that isoniazid induced cytochrome P450 isoforms with a subunit molecular weight in the region of 49,000-50,000 daltons. These fractions were induced to the greatest degree by phenobarbital (Fig. 2) [5, 6, 14, 16]. Phthivazid induced an increase in the hemoprotein subfractions with subunit molecular weight in the region of 50,000, 49,000, and 47,500 daltons. PAS induced an increase in isoforms with subunit molecular weights in the regions of 54,000, 50,000 49,000, and 47,500 daltons. Subfractions of cytochrome P450 with subunit molecular weights in the regions of 56,000, 54,000, 50,000, and 47,500 daltons were induced by 3-methylcholanthrene [2, 4, 10, 15]. The changes observed are shown schematically in Fig. 3.

The investigation thus showed that some antituberculosis drugs (isoniazid, phthivazid, and PAS), while causing practically no increase in the specific content of cytochrome P450 in rat liver microsomes, can induce qualitative changes in the isoform spectrum of that enzyme, increasing the contribution of some subfractions. The fact that changes occurred in the isoform composition of cytochrome P450 in rat liver microsomes under the influence of antituberculosis drugs may perhaps lie at the basis of the development of drug tolerance during the treatment of tuberculosis.

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