INDUCTION OF CYTOCHROMES P-450 and b_5 IN HEPATOCYTE SUBPOPULATIONS BY PHENOBARBITAL

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The microsomal mono-oxygenase system of the liver plays an important role in metabolism of lipophilic compounds of endogenous (steroids) and exogenous (xenobiotics) origin. During metabolism of the latter, highly reactive intermediates may be formed, capable of inducing mutagenic, carcinogenic, and toxic effects. We know that these effects are not manifested in all hepatocytes, and that they depend on their localization in the lobules [12]. Selective damage to hepatocytes by hepatotoxins, such as CCl_4, can be explained by the uneven distribution of cytochrome P-450 among these cells [7]. The use of immunohistochemical methods has shown that cytochrome P-450 is located mainly in centrilobular hepatocytes [1, 10]. On injection of phenobarbital (PB) into animals, induction of cytochrome P-450 spreads from the center to the periphery of the hepatic lobule [1]. The promising approach to the study of this phenomenon is biochemical analysis of isolated hepatocyte subpopulations. Data on the distribution of cytochrome P-450 in hepatocyte subpopulations, currently available, are contradictory [6, 11, 13]. The problem of correlation between hepatocyte subpopulations isolated by centrifugation in a density gradient and periportal and centrilobular zones of the lobule likewise remains unsolved [5, 14].

Accordingly, in the investigation described below the distribution of cytochromes P-450 and b_5 between hepatocyte subpopulations with different buoyant densities was studied in intact animals in rats induced with PB.

METHODS

Female Wistar rats weighing 200-250 g were used in the experiments. PB (from Serva, West Germany), made up in 0.9% NaCl solution, was injected intraperitoneally into the animals in a dose of 80 mg/kg body weight daily for 3 days. The animals were decapitated 24 h after the last injection. Hepatocytes were isolated by the method in [4] with some modifications [2, 3]. The liver was reperfused in vitro for 20-30 min with Krebs–Ringer solution (pH 7.4, 37°C), containing 0.03% of collagenase (type I, from Sigma, USA). The isolated hepatocytes were suspended in 10% Ficoll (from Pharmacia, Sweden), their concentration was adjusted to 13 x 10^6 cells in 1 ml, and 8 ml of the suspension was layered above the density gradient. A stepwise gradient was prepared in a test tube (29•103 mm, using Ficoll solutions with the following densities: 1.126, 1.073, 1.054, and 1.044 $g \cdot cm^{-3}$. The suspensions were centrifuged in the J-13 bucket rotor of the J2-21 centrifuge (Beckman, USA) for 40 min at 11,000 rpm [3]. The resulting cell fractions were suspended in cold Henks' solution and centrifuged for 2 min at 50 g. The cells were counted in a Goryaev's counting chamber. The viability of the cells was estimated by the trypan blue test [4]. The concentrations of cytochromes P-450 and b_5 were determined in cell homogenates by the method in [9] in the modification in [8], using a Hitachi 200-20 spectrophotometer (Japan).

RESULTS

The yield of isolated hepatocytes was $(36 \pm 4) \cdot 10^6$ cells per gram of tissue. More than 85% of the cells excluded trypan blue. The original hepatocyte suspension contained 0.32 ±

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TABLE 1. Effect of PB on Distribution of Hepatocytes among Fractions in Ficoll Density Gradient (M ± m, n = 8)

Fraction	Density, $g \cdot cm^{-3}$	Number of cells, %	
		control	experiment
F-1	1,044	22,5±1,8	32,7±4,0
F-2	1,044±1,054	23,4±1,1	23,3±3,0
F-3	1,054—1,073	17,4±1,7	12,2±3,3
F-4	1,073—1,126	23,3±2,8	19,7±2,5
F5 (bottom)	1,126	13,2±2,8	12,0±4,6

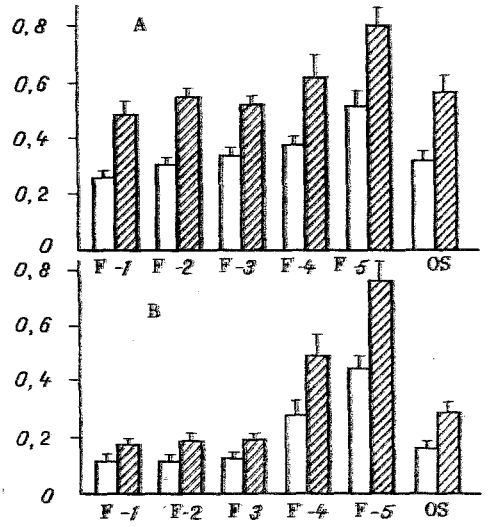

Fig. 1. Induction of cytochromes P-450 (A) and b_5 (B) in suspensions of hepatocytes (F1-F5) and in original suspension (OS) of hepatocytes following administration of PB to rats. Ordinate, concentration of cytochromes (in nmoles/10^6 cells). Unshaded columns, control; shaded, experiment.

0.02 nmole of cytochrome P-450 and 0.16 ± 0.02 nmole of cytochrome b_5 per 10^6 cells. On isodensity centrifugation the original hepatocyte suspension separated into four fractions (F1-F4) plus the cell residue at the bottom of the tube (F5; Table 1). About 85% of the hepatocytes had buoyant densities of between 1.044 and 1.126 $g \cdot cm^{-3}$. The yield of cells after centrifugation in a Ficoll density gradient was 70 ± 5%. A similar distribution of hepatocytes under analogous conditions was obtained previously [15]. During fractionation in metrizamide the hepatocytes separated into three fractions with densities of 1.10, 1.12, and 1.17 $g \cdot cm^{-3}$ [6], whereas in a Percoll gradient most of the cells were distributed in densities from 1.09 to 1.10 $g \cdot cm^{-3}$ [13]. The buoyant density of the cells evidently depended substantially on the properties of the medium used to prepare the gradient. The conditions of their isolation and fractionation may also affect the buoyant density of hepatocytes.

Analysis of the fractions revealed substantial heterogeneity of the hepatocytes with respect to concentrations of P-450 and b_5. The concentration of cytochrome P-450 in the hepatocytes rose gradually with an increase in their buoyant density. "Heavy" hepatocytes (F4 and F5) contained 1.5-2 times more ($P < 0.01$) cytochrome P-450 than "light" hepatocytes (F1) (Fig. 1). The concentration of cytochrome b_5 also was higher in the "heavy" cells. However, unlike cytochrome P-450, cytochrome b_5 was distributed uniformly in fractions F1, F2, and F3. Differences between the "light" and "heavy" fractions also were more marked in the latter case (Fig. 1). The results are in agreement with those of immunohistochemical investigations [1, 10], reflecting the uneven distribution of components of the mono-oxygenase system

throughout the hepatic lobule. It has been shown by immunohistochemical methods that cytochromes P-450 and b_5 are localized mainly in the centrilobular zones of the lobule [10]. Consequently, centrilobular hepatocytes are distributed in a Ficoll density gradient in the "heavy" fractions, whereas periportal hepatocytes are distributed in "light" fractions. Previously, the writers reached the same conclusion from data on the distribution of key enzymes of glycolysis and glyconeogenesis between hepatocyte subpopulations [3]. With this approach it is possible to examine the problem why periportal hepatocytes are the "lightest" cells. However, not all investigators share this view [6, 13 14].

On administration of PB to the animals the concentrations of cytochrome P-450 and b_5 increased 1.8 times to 0.52 ± 0.05 and 0.28 ± 0.04 nmole/10^6 cells respectively ($P < 0.01$). Similar changes for these enzymes have been observed in liver microsomes [7]. Induction of cytochrome P-450 was observed in all fractions of hepatocytes; moreover, it was most marked in the case of fractions F1 and F2. Conversely, maximal induction of cytochrome b_5 was discovered in fractions F4 and F5 (Fig. 1). After injection of PB, incidentally, the number of cells in fraction F1 increased (Table 1), probably due to a decrease decrease in the buoyant density of the most inducible (centrilobular) hepatocytes [14]. The possibility cannot be ruled out that the increase in the concentration of cytochromes P-450 and b_5 in the "light" fraction was due to some extent to a redistribution of the cells in the gradient.

The results are thus evidence of a difference in the character of distribution of cytochromes P-450 and b_5 in the hepatocyte subpopulations. The highest concentration of these enzymes in both intact and PB-treated animals was found in the "heavy" (centrilobular) hepatocytes. These cells are evidently the most active in metabolism of PB and other xenobiotics.

Differences in relations between sinusoidal (Kupffer, endothelial) cells and hepatocytes in the different zones of the lobule are probably one factor which determines functional specialization of hepatocytes in general, and in connection with biotransformation of xenobiotics in particular.

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