IMMUNOHISTOCHEMICAL LOCALIZATION OF CYTOCHROME P-450 IN RAT LIVER DURING PHENOBARBITAL INDUCTION

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The enzyme system of the microsomal oxygenases, the terminal region of which is cytochrome P-450, plays an important role in the activation of chemical carcinogens and in metabolism of drugs and steroids. It has now become evident that cytochrome P-450 has many different forms, and that administration of various substances (inducers) can give rise to the appearance, intensification, or, conversely, disappearance of particular forms of the enzyme. Meanwhile we know that the cell population of the liver, an organ in which this enzyme system is most strongly represented, is heterogeneous. The distribution of different forms of cytochrome P-450 among cells of the hepatic lobule has not been studied until very recently. This problem can be investigated now that immune sera have been obtained against various forms of cytochrome P-450 [2].

In this investigation the dynamics of changes taking place in the distribution and concentration of cytochrome P-450 among cells of the hepatic lobule was studied during induction by phenobarbital (PB).

EXPERIMENTAL METHOD

Cytochrome P-450PB (i.e., cytochrome P-450 from rats induced with PB; P-450MCh — from rats induced with 3-methylcholanthrene; P-450U — from uninduced rats) was isolated by the method in [6-8] from the liver of male Wistar rats weighing 120-150 g, which were given daily intraperitoneal injections of PB in a concentration of 75 mg/kg body weight for 4 days. The relative content of cytochrome P-450 in the resulting preparation was 17 nmoles/mg protein. Protein was determined by Lowry's method in the modification in [5]. Rabbits were immunized in the popliteal lymph nodes. The resulting antiserum was exhausted with microsomes of normal rats under control of the double diffusion in gel test. The precipitate obtained by exhaustion was discarded and the serum was passed through a sorbent with P-450PB. The sorbent was prepared on CNBr-Sepharose by the method in [3]. Cytochrome P-450PB, obtained from the liver of rats treated previously with PB, as mentioned above, was used as the antigen. The preparation was used after the second stage of purification according to the method in [7].

By means of the antibodies obtained as described above, the distribution of cytochrome P-450PB at different stages of induction, due to a single injection of PB in a dose of 75 mg/kg, was investigated by the unlabeled antibody immunoperoxidase method (PAP). The animals were killed 2, 6, 12, 18, 24, and 48 h after injection of PB. Pieces of liver of control rats and rats treated with PB were fixed at 4°C in a mixture of acetone—formalin—30 mM phosphate buffer, pH 7.0 (9:5:6), washed, and embedded in paraffin wax [1]. Serial paraffin sections 3 μm thick, after inhibition of endogenous peroxidase in methanol with 0.3% H_2O_2, were treated with rabbit antibodies against cytochrome P-450PB and tested by the PAP method with hog antiserum against rabbit IgG and a complex of horseradish peroxidase and rabbit antibodies against peroxidase (from DAKO, Denmark). Peroxidase was revealed by the standard method [4].

Control sections were treated with a preparation of antibodies against cytochrome P-450PB, neutralized with purified P-450PB.

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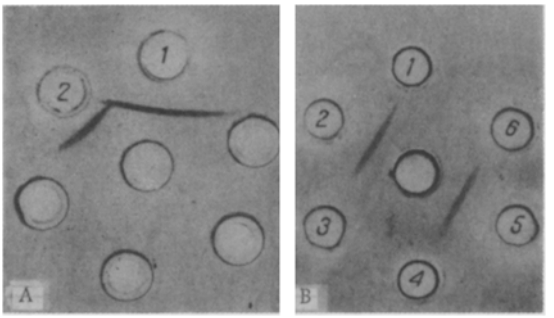

Fig. 1. Immunodiffusion in gel test in 0.9% agarose [10]. A) Antiserum against P-450PB (native) in center: 1) cytochrome P-450PB (2.0 nmoles per well), 2) cytochrome P-450U (0.7 nmole per well); B) monospecific antibodies against cytochrome P-450PB in center; 2,5) cytochrome P-450PB (2.0 nmoles per well), 3,6) cytochrome P-450U (0.7 nmole per well), 1, 4) physiological saline.

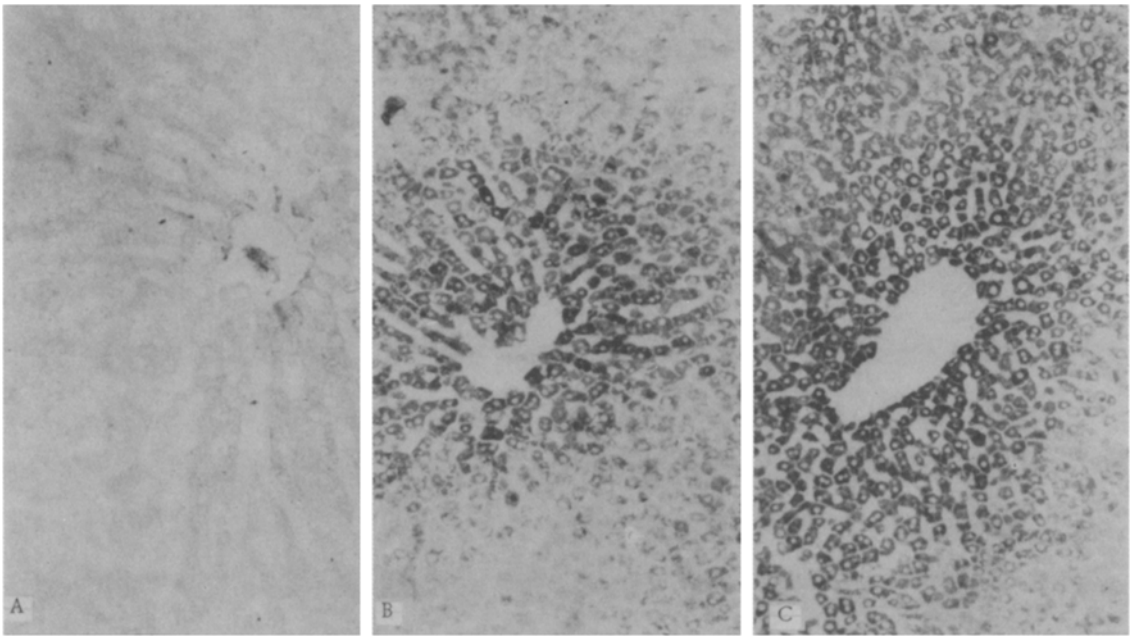

Fig. 2. Immunohistochemical localization of cytochrome P-450PB in rat liver (magnification 200 ×). A)Section through liver of intact rat, B) section through rat liver 12 h after injection of PB, C) the same, 24 h after injection of PB.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1A that the serum against cytochrome P-450PB was heterogeneous. The presence of partial nonidentity (a spur) between P-450PB and P-450U made exhaustion possible, and this was made use of in this investigation. Similar nonidentity between cytochromes P-450PB and P-450MCh was described by Thomas et al. [10].

Serum against P-450PB, exhausted by microsomes of intact liver, behaved as nonspecific in the gell diffusion test (Fig. 1B). Because of exhaustion, the resulting serum detected only forms of the enzyme which appeared during induction by PB.

The insoluble dimerization product of reduced 3,3-diaminobenzidine, chelated with OsO_4, formed at the site of the anti-P-450PB + PAP, when examined under the light microscope, had the appearance of black granules.

In sections through the liver of intact rats anti-P-450PB + PAP stained only the first row of cells around the central veins weakly (Fig. 2A). The same picture was observed 2 h after injection of PB. The intensity of staining increased 6 h after injection of PB. A positive reaction was now given by two or three rows of cells around the central veins. In sections through the liver of rats killed 12 h after injection of the inducer, the zone of

intensively stained cells extended to five or six rows around the central veins (Fig. 2B). The intensity of the immunoperoxidase reaction of the liver sections after 18 h was higher than after 12 h: seven or eight rows of cells now stained. After 24 h, eight to 10 rows of cells stained intensively and the intensity of the reaction was higher than after 18 h (Fig. 2C). The intensity of staining and the localization of the stained cells were the same after 48 h as after 24 h. The pattern of staining of the liver section of a rat receiving PB in a dose of 75 mg/kg daily for 4 days before sacrifice was similar to the picture obtained 24 h after a single injection of PB.

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