PHA than in the control, there was a correspondingly unchanged and increased titer of antistaphylococcal antibodies. Injection of mineral oil, leading to inhibition of inflammation, meanwhile, had no effect on the humoral immune response. Despite this difference in the severity of inflammation of the lungs, all the immunomodulators used led to activation of the cellular immune response, as shown by the results of the skin tests (compared with the control). The different intensities of the cellular response depending on the substances injected likewise did not correspond to differences in the course of the inflammatory process. No correlation was found between the character of the immune response and activation of proliferative processes leading to connective tissue formation, which can be regarded as an indicator of transition from acute to chronic inflammation [4]. As was noted, the most marked manifestations of organization with connective tissue formation were observed in animals receiving cyclophosphamide and PHA: The former responded to inhibition of the humoral response, the latter to its activation. Compared with the control, heparin caused maximal, and mineral oil caused minimal activation of the cellular immune response, whereas in this case there was no significant difference in the intensity of proliferation compared with the control.

Besides existing views on the importance of immunodeficiencies and immunopathological reactions in the development of inflammatory processes, the results of this investigation thus provide a basis for the view that the course of inflammation may be independent of the state of immunity.

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IMMUNOMORPHOLOGICAL INVESTIGATION OF INDUCTION OF CYTOCHROME P-450PB IN THE EMBRYONIC AND PREGNANT FEMALE RAT LIVER IN RESPONSE TO PHENOBARBITAL

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The microsomal monooxygenase system plays an important role in the detoxication of foreign lipophilic compounds entering the body. In the intermediate stages of metabolism, highly reactive compounds may be formed, capable of combining with biological macromolecules and, consequently, giving rise to mutagenic, carcinogenic, toxic, teratogenic, and other effects. Since embryonic tissues are sensitive to factors of this kind, the state of this enzyme system and, in particular, of its terminal component (cytochrome P-450) both in embryos and in pregnant females is interesting. A quite considerable number of investigations has already been conducted on the cytochrome P-450 content in the embryonic liver and its induction [4-6, 9]. It has been shown that the cytochrome P-450 content in the rat embryonic liver is low, and that there is no response to administration of cytochrome P-450 inducers.

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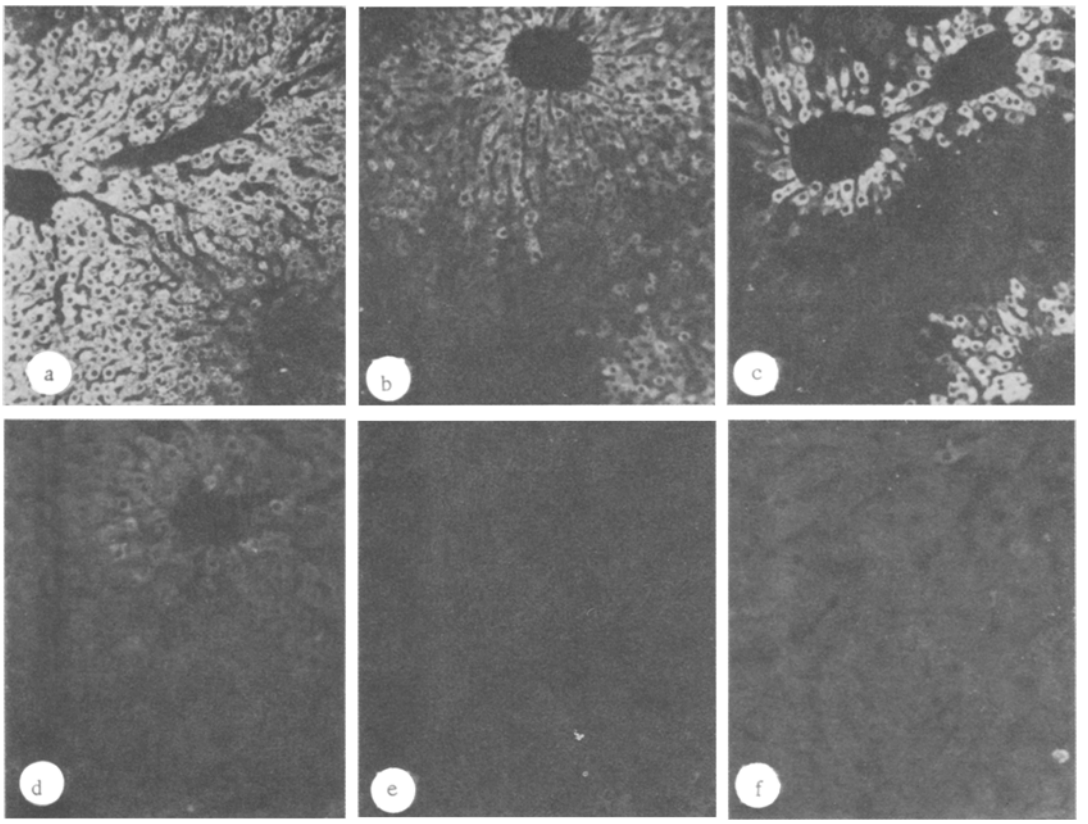

Fig. 1. Localization of cytochrome P-450PB in rat liver (indirect immunofluroescence). a) Male after injection of PB; b) female after injection of PB; c) pregnant female after injection of PB; d) intact male; e) intact female; f) intact pregnant female. Magnification: a, b, c, e) 200×; d, f) 300×.

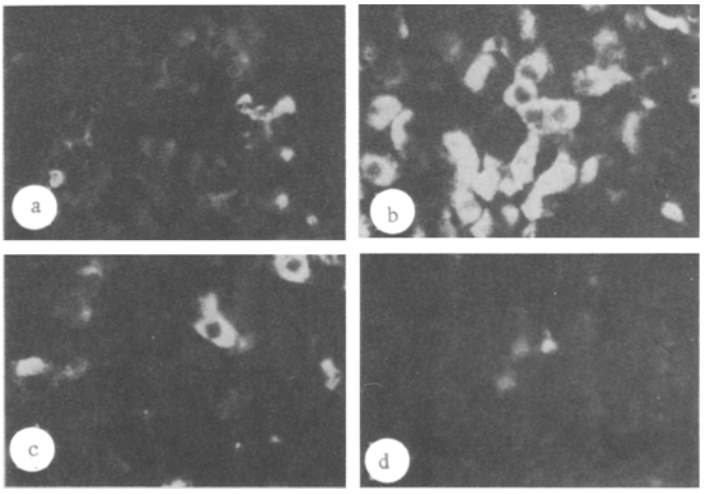

Fig. 2. Localization of cytochrome P-450PB in embryonic rat liver (indirect immunofluorescence). a) Absence of staining in hepatocytes after transplacental action of PB; b) staining in groups of cells after transplacental action of PB; c) staining of single cells after transplacental action of PB; d) absence of staining in liver of control embryos. 400×.

TABLE 1. Change in Cytochrome P-450 Concentration in Liver of Pregnant and Nonpregnant Females after Injection of Phenobarbital (M ± m)

Group No.	Experimental conditions	No. of animals	P-450, nmoles/mg protein
1	Intact rats	4	0,49±0,05
2	Pregnant rats	7	0,36±0,03
3	Intact rats + PB	4	0,75±0,04
4	Pregnant rats + PB	7	0,57±0,06

Immediately after birth of the animal the cytochrome P-450 content in the liver began to increase and the liver became able to respond to injection of inducers of cytochrome P-450 by its synthesis. All these data were obtained by spectral methods and by methods based on metabolism of various substrates in embryonic liver homogenates. The immunomorphological method which we have used is capable of detecting induction of cytochrome P-450 even if it takes place only in single cells of the embryonic liver.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats aged 3-4 months, bred at the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, weighing 160-200 g and on pregnant Wistar rats weighing 350 g. Altogether 25 animals were investigated: males and females, and pregnant females. Phenobarbital (PB, from Serva, West Germany) was injected intraperitoneally into the animals in a single dose of 150 mg/kg body weight in Hanks' solution (30 mg/ml). The rats were killed 24 h after receiving the injection of PB and the liver was perfused with 0.15 M KCl solution. Pieces of liver measuring 3 × 4 mm were taken for fixation. The liver of 17.5-18.5-day embryos (without perfusion) also was taken from the pregnant females. The material was fixed and processed histologically by the method in [2] with slight modifications [3]. Sections were stained by the indirect immunofluorescence method, using rabbit antibodies against cytochrome P-450 obtained previously [3]. Goat antibodies against rabbit γ-globulins, labeled with fluorescein isothiocyanate (from Daco, Denmark) were used as second antibodies.

Isolation of the microsomal fraction of the liver after perfusion with 0.15 M KCl was done by the standard method [1]. Differential spectra of CO-bound cytochrome P-450 were recorded by the method in [8] on an OW-3 spectrophotometer (Aminco Chance, USA). The protein concentration was determined by Lowry's method [7] using bovine albumin as the standard.

EXPERIMENTAL RESULTS

The dose of PB used (150 mg/kg) was the highest dose tolerated by our animals. In males this dose induced cytochrome P-450PB in nearly all cells of the hepatic lobule except one or two rows of hepatocytes by the portal veins (Fig. 1a). After injection of PB into females in the same dose, cytochrome P-450PB was induced in about half of the cells of the hepatic lobule equivalent to 10-20 rows of cells around the central veins (Fig. 1b). In pregnant females, after injection of PB induction of cytochrome P-450 was observed in about one-quarter of the area of the hepatic lobule, equivalent to 2-6 rows of cells near the central veins (Fig. 1c). In both males and females gradients of distribution of cytochrome P-450PB were clearly visible. In these cases cells located around the central veins were stained more intensely, and toward the periphery of the lobule the density of staining diminished. There was no gradient of cytochrome P-450 distribution in the pregnant animals. The intensity of staining in this case was the same in all cells stained and corresponded to the intensity of staining of hepatocytes adjacent to the central veins in males. Staining for cytochrome P-450PB was virtually absent in the liver of animals not receiving PB (Fig. 1d-f).

According to the immunomorphological data, induction of cytochrome P-450PB was thus strongest of all in the male liver, almost 50% weaker in nonpregnant females, and weaker still in pregnant females. To determine whether ability to induce cytochrome P-450 is really reduced during pregnancy, its concentration in the liver of pregnant and nonpregnant rats, both treated and not treated with PB, was measured by spectral methods (Table 1). The results showed that the cytochrome P-450 level was lower in pregnant females than in the control (group 1). This difference is statistically significant ($P < 0.05$). After injection

of PB the cytochrome P-450 concentration was increased in both nonpregnant (group 3) and pregnant (group 4) females; the degree of induction (i.e., the ratio of the original level of the enzyme to that found after injection of the inducer) was approximately the same (157 and 148% respectively).

Together with immunomorphological data on the nonstandard (polar) distribution of cytochrome P-450PB in the liver of the pregnant animals, these results indicate that only a quite limited zone of the hepatocytes near the central veins is capable of participating in the response to injection of the inducer, and they do so with maximal strength. Evidently all the other hepatocytes of the hepatic lobule perform certain other functions at this time and are unable to participate in the response to the inducer. This may be connected with hormonal factors arising during pregnancy and (or) with the functional load on the liver.

We were in fact unable to detect induction of cytochrome P-450PB by an immunomorphological method in the liver of embryos aged 17-18 days (Fig. 2a). In only one of seven cases was positive staining for cytochrome P-450PB observed in small groups (Fig. 2b) and individual cells (Fig. 2c) of the embryonic liver. The basic criterion in this case was absence of staining of the cell nucleus although the cytoplasm was stained. In control sections of uninduced embryos no such cells could be found (Fig. 2d). These results are in good agreement with those obtained by biochemical methods [4-6] on resistance of the embryonic liver to PB. Weak induction of cytochrome P-450, incidentally, also was observed in these investigations in rare cases [9].

We may conclude by stating that the principal load associated with detoxication of foreign compounds in the mother–embryo system falls on the mother, for microsomal oxygenases of the fetal liver are virtually nonfunctioning. Meanwhile the maternal microsomal monooxygenases are also unable to respond fully to the presence of xenobiotics in the body. Caution must therefore be observed when substances of this kind (phenobarbital, for instance) are used during pregnancy.

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