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# IMMUNOHISTOCHEMICAL LOCALIZATION OF CYTOCHROME P-450


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Synthesis and production of steroid hormones are observed in many organs and tissues: the adrenal cortex, ovaries, testes, and placenta [6, 7]. These synthetic processes are varied and quite complex and they include a complete cascade of reactions of hydroxylation of the side chains of the steroid molecules. However, the limiting stage in these conversions is oxidation of cholesterol into pregnenolone. This process is catalyzed by cytochrome P-450 [1].

The study of the morphological and functional organization of enzyme systems of cytochromes of the adrenals, ovaries, and testes has been pursued quite actively. For reasons which will be understood, most attention has been paid to hormone synthesis in the adrenals. The immunochemical characteristics and structural localization of this cytochrome have been investigated. It has been shown that cytochrome P-450 is located on the inner cristae of the mitochondria of the adrenal cortex [9]. A study of the distribution of the mixed monooxygenase system in the ovaries has shown that cytochrome P-450 is located in ripe follicles [8]. In the testes P-450 is located in the Leydig's cells [3].

Much less attention from this standpoint has been paid to the placenta. It has been shown that in man and animals oxidation of cholesterol into pregnenolone takes place in the mitochondrial fraction of the placenta [4]. As yet, however, there has been virtually no research into the structural localization of cytochrome P-450 in the placenta. The aim of this investigation was accordingly to demonstrate cytochrome P-450 (forms C, D, and B) in sections of the human placenta with the aid of specific antibodies.

## EXPERIMENTAL METHOD

The placenta was taken immediately after normal birth (12 deliveries) into 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The placenta was fixed for 4 h, and then taken through a series of alcohols and xylol and embedded in paraffin wax. Sections (4 $\mu$ thick) of the placenta were mounted on a slide, dewaxed in xylol (for 10 min twice), rehydrated in alcohols, and carried through to phosphate buffer. Specific antibodies against cytochrome P-450 of the C, D, and B


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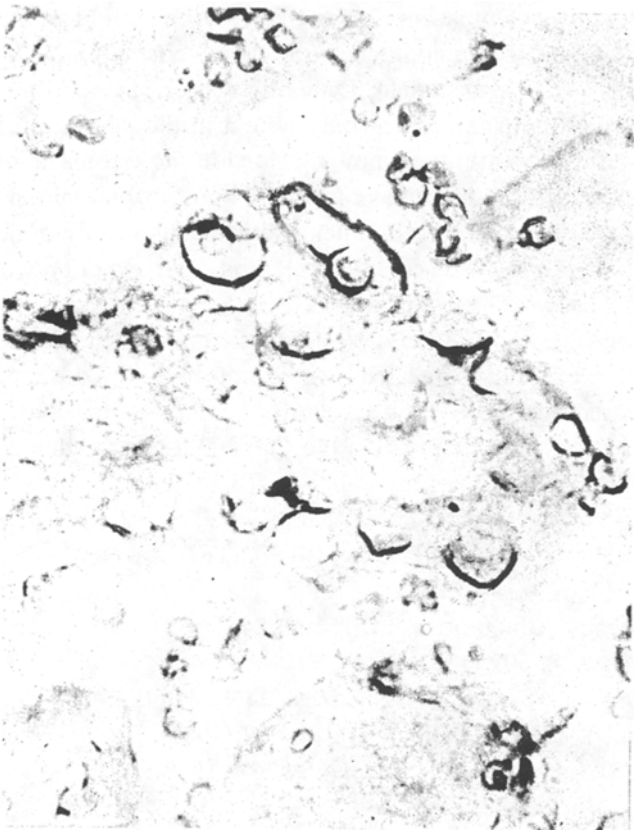

Fig. 1. Immunohistochemical localization of cytochrome P-450 (C, D) in human puerperal placenta.

types were obtained from the Department of Cell Physiology and Pathology, Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. Colloidal gold particles were prepared by Franz's method. For this purpose, to 100.0 ml of a 0.001% aqueous solution of chloroauric acid was added 2.5 ml of a 1% solution of trisodium citrate and the mixture was boiled for 10 min. The optimal quantity of antirabbit antibodies (determined by Zsigmondy's reaction) was mixed with the gold sol, the pH of which had previously been adjusted to 8.0 with an aqueous solution of $K_2CO_3$; the whole complex with antibodies was centrifuged (25,000*g*, 40 min) and the residue was resuspended in 1.0 ml of phosphate buffer, containing polyethylene-glycol (20 kD) in a concentration of 0.2 mg/ml. Sections of the placenta were incubated for 30 min in a 1.0% solution of bovine serum albumin, after which they were incubated without rinsing for 1 h at 37°C in a humid chamber with rabbit antibodies against cytochrome P-450 C, D, and B separately, in a dilution of 1:100. After incubation the samples were carefully washed to remove excess of primary antibodies for 30 min with phosphate buffer, and incubated with antirabbit antibodies, conjugated with colloidal gold particles, in a humid chamber at 37°C for 2 h. After rinsing, the sections were dehydrated and mounted in balsam without counterstaining. The control consisted of sections not incubated with specific antibodies and also sections treated only with secondary antibodies. For routine morphology, sections were stained with hematoxylin and eosin.

## EXPERIMENTAL RESULTS

On treatment of the sections of polyclonal antibodies against cytochrome P-450, C and D, in all cases the cytoplasm of endothelial cells stained in the sections (Fig. 1, in the section collodial gold appears red). It must be pointed out, moreover, that the endothelial cells which were stained were mainly those of vessels of capillary type located at the edges of the villus, namely epithelioid lamellae of the membrane. Antibodies did not bind with cells of the trophoblast, syncytiotrophoblast, and connective tissue of the sections. Incubation of placental sections with antibodies against cytochrome P-450B gave negative staining in all cases.

The cytochrome P-450 present in the placenta plays a key role in steroid hormone synthesis and in metabolism of various xenobiotics [2]. However, whereas this immunochemical characteristic has been studied in fair detail, the structural localization of the enzyme remains virtually unstudied. Only one article has been published in recent years. Its authors attempted to demonstrate the distribution of the cytochrome system in the placentas of women smokers [5]. According to their data cytochrome P-450 (of methylcholanthrene type) is located in the cytoplasm of cells of the cytotrophoblast of the placental microvilli in women smokers. In the placentas of women nonsmokers, minimal staining of the cytoplasm of cells of the cytotrophoblast of the large villi was observed When these results are examined it must be pointed out that the authors used the peroxidase-antiperoxidase (PAP-complex) method. Our experience of working with the PAP complex shows that it is very unreliable when working with sections of the placenta, because the latter possesses very strong endogenous peroxidase activity, which is very difficult to suppress, and which, moreover, is very quickly restored. The method with colloidal gold, which we used, is free from these defects and, consequently, it is a more reliable way of assessing the distribution of the mixed monooxygenase system in the human placenta.

Our results thus showed that cytochrome P-450 of the C and D forms are detectable in the human placenta and are located in the cytoplasm of the endothelial cells of the villi.

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