first series was significantly greater (twice as many) and LDH activity was almost 25% higher than in the second group, where these indicators of the degree of destruction of neurons were comparable with the control values.

The results as a whole thus indicate that free-radical reactions (most probably lipid peroxidation) play an important role in neuronal destruction during hypoxia and subsequent reoxygenation; these reactions, moreover, are involved in the injury process mainly during the reoxygenation stage.

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COMPARATIVE ELECTROPHORETIC CHARACTERISTICS OF ALKALINE PHOSPHATASE ISOZYMES IN MATERNAL BLOOD PLASMA AND EXTRACTS OF CHORION FRONDOSUM AT DIFFERENT STAGES OF PREGNANCY

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In recent years the number of enzymic methods used in clinical practice for the early detection of pathological changes has increased considerably [1]. One of the most important conditions in this connection is the use of methods of investigation of enzymes or their molecular forms in blood, urine, saliva, and seminal fluid which correlates most closely with tissue enzymes, for this not only determines the interpretation of clinical data, but also makes it possible to estimate the quality and efficacy of treatment and the prognosis for development of pathological processes. One of the best ways of tackling this problem is through a parallel study of the activity of an enzyme in

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blood and in a tissue extract obtained by biopsy or surgical operation. Another convenient model is to study correlation of the corresponding isozymes in maternal blood and in placental extracts obtained immediately after abortion or spontaneous delivery. Determination of alkaline phosphatase (AlP) activity is most commonly used in obstetric practice in order to assess the state of the fetoplacental complex [2, 4]. Determination of the time course of blood enzyme activity and the relations between thermolabile and thermostable fractions throughout pregnancy is of great diagnostic value in relation to somatic, obstetric, and placental pathology. It must be pointed out, however, that at least four forms of AlP are present in the plasma: intestinal, skeletal, hepatic, and placental. According to data in the literature [2] activity of thermostable AlP (TAlP) in the blood of pregnant women correlates with that in the placenta. However, we showed previously that relationships of this kind are observed only during the normal development of pregnancy, whereas in the presence of many pathological processes blood TAlP activity does not always coincide with that of the placenta [5].

The aim of this investigation was to study TAlP isozymes in plasma and placental extracts of women at different stages of pregnancy, assuming that AlP is a heterogeneous enzyme, whose isozymes differ in their electrophoretic mobility [7].

EXPERIMENTAL METHOD

Venous blood and placentas of women at the following stages of pregnancy were studied: 6-12 weeks (7 cases), 24-27 weeks (4 cases), 28-40 weeks (6 cases). Heparinized blood (5-7 U/ml) was centrifuged at 3000 rpm for 5 min at room temperature. The chorionic villi of the placentas were used as material from which to prepare Triton extracts: 1 g tissue, washed in 0.85% NaCl, was homogenized in 2 ml of 0.05 M Tris-HCl buffer, pH 7.2, with the addition of one drop of Triton, and extracted in the cold for 18 h, after which the sample was centrifuged for 20 min at 6000 rpm. The plasma and supernatant were kept at −12°C. The specimens thus prepared were analyzed by vertical electrophoresis (Eph) in plates measuring 225 × 130 × 2 mm at room temperature. The upper separating gel was: T = 3.85% C = 2.60%, riboflavin 5 μg/ml, and the lower separating gel T = 10.395%, C = 2.59%, ammonium persulfate 0.175 mg/ml. The working buffer was: 0.07 M Tris-HCl, pH 7.5, N,N,N′,N′-tetramethylenediamine 0.3 μl/ml, and the electrode buffer: 0.08 M Tris, titrated with boric acid to pH 7.0. A lower gel 9 cm high and an upper gel 2.5 cm high were formed. To the upper electrode buffer solution was added 1.5 ml of 0.001% aqueous solution of bromphenol blue to 1000 ml of solution. The thawed out specimens of plasma and extract were mixed with an equal volume of 0.5 M sucrose in fivefold diluted working buffer and of the solution of plasma and chorionic extract thus prepared 50 μl obtained at 6-12 weeks was added to the gel, 20 μl at 24-27 weeks, and 10 μl of solution at 38-40 weeks.

Eph was carried out under the following conditions: 12 mA for the first hour and 25 mA subsequently until the band of bromphenol blue had moved 7 cm away from the beginning of the lower separating gel. After Eph, activity of AlP was determined by the azo-coupling method [3]. The gel was incubated in a mixture of: naphthol-AS-TR phosphate 3.7 mM, dimethylformamide 5 vol. %, Fast Blue RR 1 mg/ml, 0.1 mM $MgCl_2$ in 0.1 M Tris-HCl buffer, pH 9.0, for 30 min at 37°C in darkness on a bath with a shaker, after which it was washed with distilled water, covered with a fresh solution of the mixture, and allowed to stand overnight in a refrigerator. To detect thermostable forms of the enzyme, before incubation the gels were preheated in the corresponding buffer at 65°C for 15 min. After staining the gels were thoroughly washed with distilled water. The gels were fixed in ethyl alcohol–distilled water–glacial acetic acid mixture in the ratio of 5:10.5:1.5 [7]. The stained gels were subjected to photometry on a DM-1 densitometer. The mobility of the proteins was calculated relative to bromphenol blue. The distance traveled by the proteins and dye was measured from the beginning of the lower gel. The following reagents were used: Tris-(hydroxymethyl)-aminomethane was from "Biokhimreaktiv" (USSR), glycine, N,N,N′,N′-tetramethylenediamine, and riboflavin were from "Reanal" (Hungary), ammonium persulfate was from "Serva" (Switzerland), Triton X-305 from "Ferak" (Germany), hydrochloric acid from "Riap," naphthol-AS-TR phosphate, Fast Green RR salt from "Chemapol" (Czechoslovakia), and dimethylformamide from "Reakhim" (USSR).

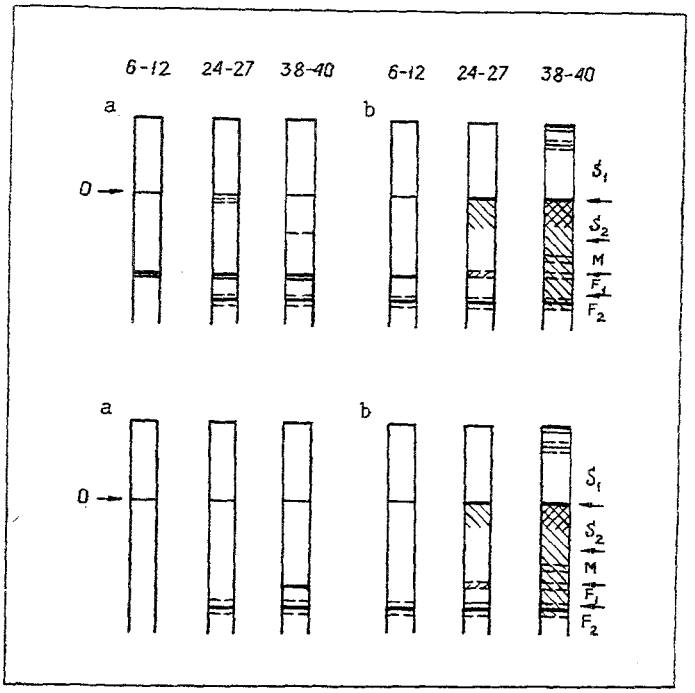

Fig. 1. Electrophoretic fractionation of alkaline phosphomonoesterases of human blood plasma (a) and extracts of chorion frondosum (B) at different times of pregnancy. a and b) total, a′ and b′) thermostable forms of AlP, O) boundary separating upper and lower gels, S_1 (upper gel) and S_2 (R_f = 0-0.120) – "slow" fractions, M (R_f = 0.120-0.200) – "medium" fractions, F_1 (R_f = 0.200-0.260) – "fast" fractions, F_2 (R_f = 0.260) – "fastest" fractions.

EXPERIMENTAL RESULTS

The experimental results showed that chorionic extracts in the first months of pregnancy (6-12 weeks) contained one "fast" (F_1, see Fig. 1b) thermolabile and one or three more labile (F_2, see Fig. 1b) thermostable fractions, which appeared after the 7th week of intrauterine development of the fetus. At 24-27 weeks a narrow bright band appeared on the boundary between the upper and lower gels, below which there was a more or less wide, less deeply stained zone in the region of "slow" fractions (S_2, see Fig. 1b). In two cases narrower and almost invisible bands were observed in the region of the "fast" forms, evidence of the appearance of new AlP isozymes. Under the conditions of the electrophoretic system used, in the early stages of pregnancy (6-12 weeks) only two thermolabile AlP isozymes could be seen in the maternal blood in the region of "fast" fractions (F_1, see Fig. 1a). The absence of thermostable and thermolabile forms of tissue of chorionic AlP in the first three months of pregnancy will be noted. At 24-27 weeks, besides the two thermosensitive forms, one or three "fastest" thermostable fractions also appeared, and were completely identical in Eph characteristics to the placental extract. In one case two additional slow isozymes (S_2, Fig. 1a), but disappearing after heating and absent in placental extract, were recorded.

At birth diffuse staining of the lower gel was observed in extracts of placental tissue, often with the appearance of 1-3 narrow bands with unequal mobility (S_2, M, and F_1 – see Fig. 1b and b′). By contrast with the previous stage of development, the chorion of the full-term placenta was characterized by the presence of 3-5 additional bands in the start region. It must be emphasized that under these conditions of Eph all the AlP fractions detected in the second half of pregnancy were resistant to a high temperature, and indeed, sometimes additional bands appeared after heating in the M or F_1 region. The existence of temperature activation of AlP was described previously by Tsirel'nikov and co-workers [6]. At birth, the electrophoretic picture of the blood plasma was distinguished by the appearance of an AlP resistant to a high temperature in the F_1 region (F_1, see Fig. 1a and a′), and with mobility identical with that of the hepatic isozyme, and not detected in placental extract. In one case, at an early stage, two

additional AlP bands were found in a woman receiving hormone therapy in the region of the "fastest" fractions in the placenta. In another case, mentally defective, an increase in mobility of the wide band in the S_2 region was noted. Absence of AlP activity in the chorion also was found in spontaneous abortion. Thus the comparative study of electrophoretically separated molecular forms of AlP in the blood plasma and placenta (chorion frondosum) of women at different stages of pregnancy showed that only the "fastest" (F_2) placental forms of the enzyme appear in the maternal blood in the second trimester of pregnancy. We succeeded for the first time in separating the thermolabile fraction of AlP from thermostable fractions by Eph in tissues of the chorion frondosum at the early stages of pregnancy. Close correlation was found between activity of placental AlP and its definite polymorphism, on the one hand, and the clinical state of women with a complicated course of pregnancy, on the other hand.

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