Some Properties of Human Fetal Ceruloplasmin

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Ceruloplasmin was isolated from the blood of women during childbirth and from cord blood. By its sensitivity to chelating agents, affinity for concanavalin A, resistance to specific degradation, and specific copper content cord blood ceruloplasmin differs from adult ceruloplasmin and is similar to breast milk ceruloplasmin. It is assumed that the similarity between fetal and breast milk ceruloplasmin reflects the involvement of the latter in copper metabolism in newborns.

Key Words: *fetal ceruloplasmin; copper metabolism during ontogeny*

Ceruloplasmin (CP, EC 1.16.31.1), a copper-containing plasma glycoprotein in vertebrates, is a polyfunctional protein. Being a ferrooxidase, CP catalyses reduction of Fe(II) to Fe(III) and participates in conversion of apotransferrin to transferrin. CP stimulates angiogenesis, inhibits erythrocyte lysis induced by heavy metals, and exhibits weak superoxide dismutase [6] and thiol-dependent peroxidase activities [7]. Moreover, CP belongs to the family of acute phase proteins [6]. However, the most important function of CP is copper ion transport through cell-cell channels [10]. It was shown that tissue-specific CP is synthesized in the mammary gland and provides newborns with copper ions [2,3]. Milk CP than appears in the circulation and participates in copper turnover as long as fetal copper metabolism persists [1]. We have early demonstrated that milk and blood CP are different even in the same individual [4]. These differences probably reflect the role of milk CP for embryonic copper metabolism, which persists in humans during the first year of life. However, embryonic copper metabolism probably depends on fetal CP. These data will provide new insight into copper metabolism and substantiate scientific recommendations for maintenance of copper homeostasis in newborns, since deficiency or excess of copper ions in the body in combination with copper-sensitive genotype can cause severe microelementosis [5,11]. In the present study we compared CP from newborn cord blood and maternal serum (maximum genotypic similarity).

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MATERIALS AND METHODS

Blood serum, breast milk, and colostrum from healthy women and cord blood from healthy newborns were examined. CP was isolated by ion-exchange chromatography on columns with diethylaminoethyl Sephadex (pH 7.0) in 0.05 M sodium acetate buffer. Fractions were eluted with a linear 0.05-0.3 M NaCl gradient and then CP-containing fractions (immunoelectrophoresis data) were concentrated and further purified by precipitation with chrolophorm-ethanol (1:9) mixture. Polyacrylamide gel electrophoresis (PAGE) in a discontinuous buffer system (pH steps) and under denaturating conditions in the presence of SDS was performed [8]. Quantitative rocket and 2D-immunoelectrophoresis were carried out as described previously [9]. The concentration of copper ions was measured by atomic absorption spectrometry with Zeeman background correction on a 4100ZL Perkin-Elmer spectrometer. Serum content of CP was assessed by the total oxidase activity [13], protein concentration was determined by the method of Lowry.

Milk (lactation day 20) and colostrum (day 3) were analyzed. To this end, 200-μl aliquots of the serum, milk, and colostrum were centrifuged for 20 min at 13,000 rpm, and 0.1 mg dry Chelex 100 was added to each sample. After overnight binding of copper ions (4°C and constant shaking), the samples were centri-

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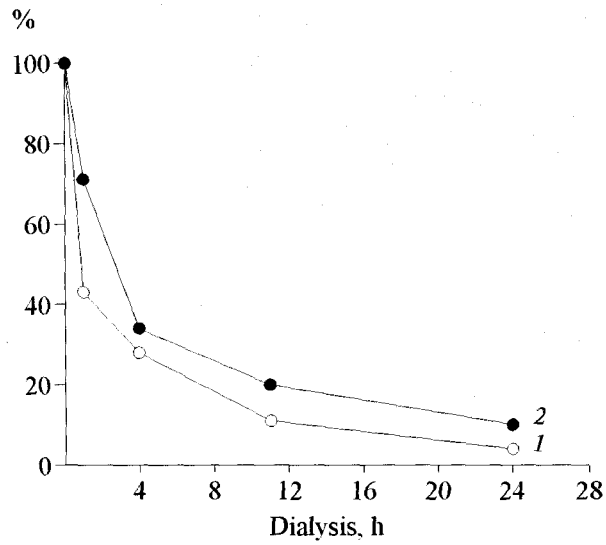

Fig. 1. Dynamics of oxidase activity in fetal (*1*) and adult (*2*) sera during dialysis in the presence of 100 mM EDTA. Initial oxidase activity is taken as 100%. The data are the means of 10 measurements.

fuged at 13,000 rpm and the content of copper ions was measured in the supernatant. The concentration was calculated basing on immunological and enzymological data on serum content of CP. Commercial preparation of human CP isolated by the method of M. M. Shavlovskii (L. Pasteur Institute of Epidemiology and Microbiology) was used as the standard.

RESULTS

Experimental data on oxidase activity and quantitative immunoelectrophoresis showed that the content of CP in women after delivery was 94.6±18.1 mg/100 ml, i.e. 3-fold surpassed the normal value for adults (35.5± 3.5 mg/100 ml). These findings confirm previous reports on a 2-5-fold increase in the content of serum CP during pregnancy. The content of CP in cord blood varied from 7.0 to 20.8 mg/100 ml (average 12.5±4.4 mg/100 ml), which also agree with the data on low concentration of CP in newborns. In both cases, the data of rocket immunoelectrophoresis and oxidase assay coincided. Serum oxidase activity gradually decreased after dialysis against 500-fold volume of 50 mM sodium-acetate buffer (pH 5.5) containing 100 mM EDTA (Fig. 1). After 24-h dialysis, oxidase activity in adult and fetal serum decreased 11- and 25-fold, respectively (8.6±0.37 and 0.51±0.18 mg/100 ml). Blood serum contained only one protein whose oxidase activity remained unchanged after overnight dialysis against sodium-acetate buffer (pH 5.5) [2]. Hence, similarly to breast milk CP [4], fetal CP more readily losses copper ions in the presence of EDTA than CP in adult plasma. Incubation of cord and adult sera with Chelex 100 (removal of chelate-sensitive copper ions) showed

TABLE 1. Effect of Chelex 100 on CP Synthesized at Different Periods of Ontogeny or in Different Tissues (n=10)

Sample	Copper atoms per CP molecule	
	without treatment	after treatment
Serum		
mother blood	5.2	4.3
cord blood	6.8	4.4
Colostrum, day 3	6.9	5.0
Breast milk, day 20	5.8	4.2

that adult CP losses approximately 1 copper atom per 1 CP molecule, while fetal CP under these conditions losses 2 copper atoms per 1 CP molecule (Table 1). CP of cord blood binds more copper ions than adult CP. These findings agree with the data of Japanese investigators on elevated copper/CP ratio in colts during the first week of life [12].

In contrast to adult CP, electrophoretically pure CP from cord blood (Fig. 2, *a*) contained no fast-mo-

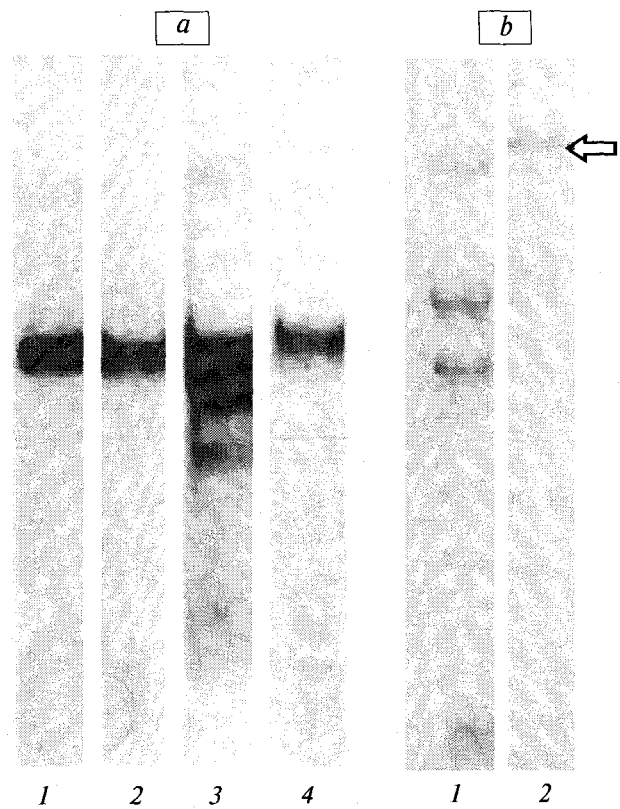

Fig. 2. Electrophoresis of ceruloplasmin (CP) preparations isolated from adult and cord blood. *a*) electrophoresis in 7.5% polyacrylamide gel of adult (*1,3*) and fetal (*2,4*) CP. *1* and *2*) staining with o-dianisidine (CP-specific chromogen); *3* and *4*) immunoblotting. *b*) electrophoresis in 7.5% polyacrylamide gel with SDS. *1*) adult CP, *2*) cord blood CP. Arrow indicates the position of full-length CP from rat blood (132 kD).

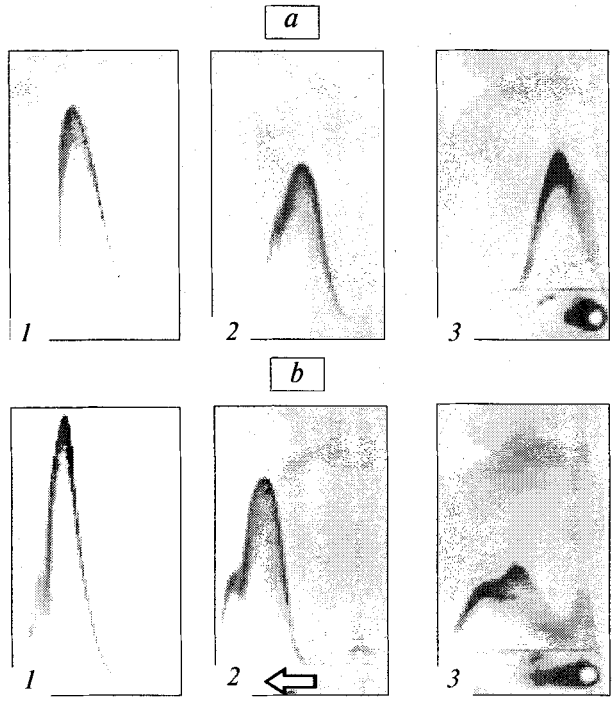

Fig. 3. Two-dimensional immunoelectrophoresis of CP from adult serum (*a*) and cord blood (*b*). Native CP (*1*), CP treated with Chelex 100 (*2*), or concanavalin A excess (*3*). Each sample contains 200 μg CP. Arrow indicates electrophoresis direction.

ving fraction, which possesses no oxidase activity and can be identified by blotting as apo-CP.

Adult and fetal CP isolated simultaneously and stored under the same conditions differ by their sensitivity to spontaneous proteolytic degradation (Fig. 2, *b*). By these two characteristics CP from cord blood is similar to breast milk CP [7].

Cord blood and adult CP were incubated overnight (4°C, constant shaking) with concanavalin A and analyzed by two-dimensional immunoelectrophoresis. Adult CP interacts with concanavalin A as a homogenous preparation (Fig. 3), while fetal preparation contains much CP with does not interact with concanavalin A, and only a minor fraction binds lectin (Fig. 3). Two-dimensional immunoelectrophoresis demonstrated molecular microheterogeneity of cord blood CP and confirmed its sensitivity to Chelex 100 (Fig. 3).

Our findings suggest that adult and cord blood CP differ by packing of copper ion and sensitivity to spontaneous proteolytic degradation. Moreover, fetal CP contains no apo-CP and is characterized by marked heterogeneity. Considerable fraction of fetal CP does no interact with concanavalin A, which probably suggest that these molecules can bind asialoglycoprotein receptors on hepatocytes. Hence, fetal CP is similar to breast milk CP by a number of properties [4]. This similarity probably reflects their functional analogy in embryonic copper metabolism.

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