short duration and does not lead to a lethal outcome. Meanwhile the late effect of injection of heterologous autoantibodies is independent of the class to which they belong. This effect has a marked individual character even for inbred mice, and it is manifested several weeks after injection of antibodies without an immunodepressant. It can be tentatively suggested that the late effect of injection of heterologous autoantibodies is connected with triggering of an autoimmune process involving targets other than NAR of the myoneural junction. Animals in which this effect was exhibited often died. The time course of the action of neostigmine in this case was different from that of its action in the first phase. This also indicated that in the second phase of action of the preparation of heterologous autoantibodies in the passive transfer model, targets other than NAR of the myoneural junction are involved.

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HUMAN CHORIONIC PREALBUMIN: IDENTIFICATION, PHYSICOCHEMICAL PROPERTIES AND ITS DETECTION IN BLOOD SERUM IN TROPHOBLASTIC DISEASES

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Investigations of hormones produced by the human placenta began in the 1930s-1950s after the discovery of chorionic gonadotropin [2] and of placental lactogen [4]. However, a new stage in the study of placental proteins began with the discovery of trophoblastic γ-globulin [1, 5], which was followed by the discovery of a whole group of "specific" placental proteins, possessing neither hormonal nor enzymic activity [3].

This paper gives information about human chorionic prealbumin (CPA), some of its physicochemical properties, and the results of its immunodiffusion analysis in patients' tissues and blood sera.

EXPERIMENTAL METHOD

Antiserum to CPA was prepared by immunizing rabbits with semipurified preparations of CPA isolated from extracts of the chorion obtained during therapeutic abortion at the 8th-12th week of pregnancy. The semipurified preparation was obtained by freezing and thawing chorionic extracts 3 times, with the addition of 1% Triton X-100, followed by dialysis against 0.15 M sodium chloride in 20 mM Tris-HCl, pH 8.0, and by salting out the fraction obtained between 40 and 70% saturation with ammonium sulfate. The fraction thus obtained was dialyzed against 20 mM Tris-HCl buffer, and subjected to adsorption chromatography on columns containing DEAE-52

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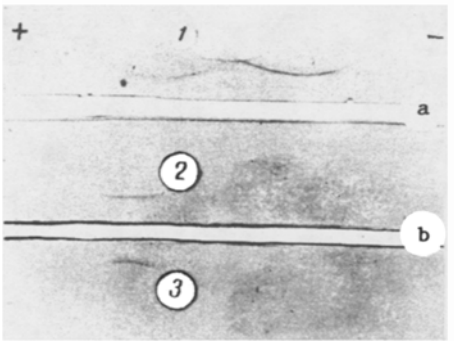

Fig. 1

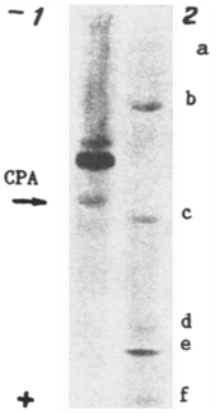

Fig. 2

Fig. 1. Immunoelectrophoresis of CPA in agar gel. 1) Donor's blood serum in dilution of 1:16; 2, 3) semipurified preparation of CPA. a) Polyvalent antiserum to donors' blood serum; b) monospecific antiserum to CPA. 1.5% "Difco" agar, electrode buffer 30 mM Tris-Veronal, pH 8.6, 300 V, 6 mA, glass plates 6 × 9 cm, duration of electrophoresis 60 min.

Fig. 2. Disc electrophoresis (in 10% SDS-PAG) of CPA preparation after chromatography and subsequent gel-filtration on Sephadex G-200. 1) CPA preparation; 2) set of markers for SDS-PAG electrophoresis ("Pharmacia"): a) phosphorylase B (94 kD), b) BSA (67 kD), c) ovalbumin (43 kD), d) carbonic anhydrase (30 kD), e) soy trypsin inhibitor (20 kD), f) α-lactalbumin (12 kD).

cellulose in the same buffer. Elution was carried out with 2 M NaCl and 20 mM Tris-HCl, pH 8.0.

The CPA preparations thus obtained were studied by gel-filtration on Sephadex G-200 and disc electrophoresis with the addition of sodium dodecylsulfate (SDS). The precipitation arc of CPA after electrophoresis in agar gel was stained to reveal the ability of CPA to take up dyes, which are bound either by the carbohydrate or by the lipid components.

The CPA concentration in the blood serum or tissue extracts of the placenta and various organs of fetuses and adult individuals was determined by the use of a standard test system, consisting of monospecific antiserum to CPA and the semipurified CPA preparation. The sensitivity of the test system (curvature of the precipitation arc) was about 1 mg/liter. Patients' blood sera were obtained from different clinics of the N. I. Pirogov Second Moscow Medical Institute and the Oncologic Scientific Center, Academy of Medical Sciences of the USSR, in the period 1976-1986. The collection of blood sera was preserved and kept in the frozen state at –20°C.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that during immunoelectrophoresis in agar gel CPA migrates in the prealbumin zone. Staining for carbohydrates (the PAS reaction) of the precipitation arc of CPA was weakly positive. On gel filtration on Sephadex G-200 the molecular weight of CPA was about 100 kD, and by SDS-electrophoresis, its molecular weight was about 45 kD (Fig. 2). This suggests that CPA consists of two subunits with equal molecular weight.

Data on the content of CPA in various placental tissues at different times of pregnancy are given in Table 1. The highest CPA concentration was observed in the early chorion (Fig. 3), whereas in the terminal placenta the CPA content in the chorion was about 5 times less. No CPA was discovered in the amniotic membrane or umbilical cord. Trace amounts of CPA could be found in the decidual membrane. Evidently CPA is synthesized in the chorion starting from the early stage of pregnancy. However, only direct placental tissue culture experiments can reveal the site of CPA synthesis.

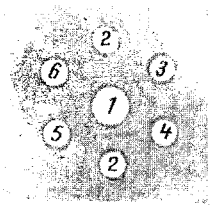

Fig. 3. Immunodiffusion analysis of CPA in 1.5% "Difco" agar. 1) Monospecific antiserum to CPA; 2) CPA preparation; 3) extract of 12-week chorion; 4) umbilical cord extract; 5) blood serum from patient with chorionepithelioma of the uterus; 6) blood serum from healthy blood donor.

TABLE 1. CPA Concentration in Various Placental Tissues

Tissue	Amount of tissue, g	Tissue extract, ml	CPA concentration	
			titer	mg/liter
Chorion 12 weeks	2	1.5	1-128	128
Chorion 40 weeks	2	1.5	1-4	4
Decidua 40 weeks	2	0.5	n/r	1
Amnion 40 weeks	2	0.5	absent	absent
Umbilical cord	2	0.3	absent	absent

Note: n/r indicates no reaction.

TABLE 2. Immunodiffusion Determination of CPA in Blood Serum from Cancer Patients

Diagnosis	Number of individual samples of patients' blood sera	Result of analysis	
		positive	negative
Chorionepithelioma of uterus			
Before treatment	9	3	6
After treatment	17	1	16
Lung tumor	39	0	39
Ovarian tumor	5	0	5
Tumor of the testis	16	0	16
Tumor of the bladder	15	0	15
Tumor of the genital organs	6	0	6
Hydatidiform mole, after treatment	21	2	19
Total	128	6	122

Legend. Concentration of CPA in cases with positive reaction was 1-4 mg/liter.

Immunodiffusion analysis revealed no CPA in fetal or adult (autopsy) tissues. No CPA likewise could be found in human seminal plasma, by contrast with chorionic gonadotropin and trophoblastic β-glycoprotein.

Table 2 gives the results of immunodiffusion analysis of blood sera from cancer patients. In chorionepithelioma before treatment, CPA can be found in the blood serum of about one-third of patients, but after treatment it is found extremely rarely. So far as other forms of tumor are concerned, in no case was CPA found. Possibly if the sensitivity of immunochemical analysis can be increased CPA will be discovered in a higher percentage of cases of chorionepithelioma and trophoblastic diseases.

The test system for determination of CPA was compared by immunodiffusion analysis with known placental proteins, including chorionic gonadotropin, placental lactogen, trophoblastic β-glycoprotein, placenta-specific α_1-microglobulin (PP-12), α_2-microglobulin (PP-14), lactoglobulin, and prostatic β-globulin. No cross reactions of CPA with the above-mentioned proteins were discovered. In our opinion CPA is one of the "specific" proteins of the chorion. The biological role and physiological function of CPA require special investigation. In the future it is hoped to develop a highly sensitive method of quantitative determination of CPA in biological fluids and to undertake trials of a test for CPA under normal conditions and in pathology of pregnancy, including trophoblastic diseases.

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