COMPLEX FORMATION BETWEEN GONALDOTROPINS AND γ-GLOBULIN FRACTION OF PLACENTAL BLOOD SERUM

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By means of the Ascheim – Zondek test on sexually immature female rats, the γ-globulin isolated from placental blood serum by various methods is shown to possess gonadotropic (follicle-stimulating) activity. It is shown that complex formation can take place between γ-globulin and chorionic gonadotropin in vitro.

Serum from placental blood and from blood collected at therapeutic abortion operations is the principal source of commercial production of albumin and γ-globulin. Both these products contain total gonadotropins [2, 3], which may play a definite role in the pharmacodynamics of these substances [5].

Considering the extensive use of γ-globulin in clinical and experimental practice and the absence of information on its linkage with hormones, an investigation was carried out to determine the presence of gonadotropins and the stability of their bonds with protein in a product isolated from the same raw material, but by the methods of gel-filtration and ion-exchange chromatography, which are milder than alcoholic precipitation, and also to investigate the ability of γ-globulin to bind chorionic gonadotropin in vitro.

EXPERIMENTAL METHOD

Measles γ-globulin from the Moscow Research Institute of Epidemiology and Microbiology and from the I. I. Mechnikov Ufa Research Institute of Vaccines and Sera, obtained by alcoholic fractionation [6] of serum obtained during abortion operations and from placental blood, was used in the investigation.

γ-Globulin was isolated from placental blood serum on columns with DEAE-Sephadex A-50 by the method of Sela et al. [10], with DEAE-cellulose by the method of Fahey et al. [7], and with Sephadex gel G-100 by the method of Lourent et al. [9]. The purity of the isolated γ-globulin was verified by electrophoresis in agar by Grabar's method [8]. Artificial complex formation between γ-globulin and chorionic gonadotropin (Choriogonin, Richter, Hungary) was carried out by incubation of a mixture of the protein and hormone for 24 at 37°C and for 12 h at 4°. The dose of hormone was 35 i.u./100 mg γ-globulin. Separation of γ-globulin from the unfixed hormone was achieved by chromatography of the mixture on a column with Sephadex G-200 as described by Nezlin and Kul'pina [1].

Biological tests of the γ-globulin for total gonadotropins were carried out by the Ascheim – Zondek method [11] on infantile female albino rats aged one month and weighing 40-45 g, noting macroscopic changes in the ovaries and uterus and also the weight of these organs.

The numerical data were analyzed by the Fisher – Student method. Differences between the experiment and control were taken as significant when $P \leq 0.05$.

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TABLE 1. Ascheim – Zondek Reaction in Infantile Female Albino Rats Receiving Placental Blood Serum or Its γ-Globulin Fraction Parenterally

Group	Number of animals	Substance injected and method of preparation	total dose per animal (in mg protein)	Reaction		
				type of reaction of gonads in Ascheim – Zondek test	mean weight (in mg)	
					of ovaries	of uterus
					(M ± m)	
Control	14	–	–	–	15.6 ± 0.9	26.1 ± 1.8
Exptl.	10	Placental blood serum	~90	1-2-3	21.6 ± 2.4 P < 0.05	72.0 ± 12.0 P < 0.01
"	15	γ-Globulin, alcoholic fractionation	90	1-2-3	20.2 ± 0.02 P < 0.02	40.0 ± 1.4 P < 0.01
"	6	γ-Globulin, DEAE-Sephadex A-50 column	90	Weak, 1	17.1 ± 0.5 P > 0.1	30.0 ± 1.7 P > 0.1
"	6	γ-Globulin, DEAE-cellulose column	90	Marked, 1	20.0 ± 1.1 P < 0.05	42.1 ± 1.2 P < 0.01
"	6	γ-Globulin (Sephadex G-100 gel column)	90	I	18.2 ± 1.8 0.1 > P > 0.05	35.0 ± 1.6 P < 0.02
"	4	γ-Globulin incubated with choriogonin, gel-filtration (Sephadex G-200)	30	Marked, 1-2-3	24.3 ± 2.2 P < 0.05	63.2 ± 8.1 P < 0.01

EXPERIMENTAL RESULTS

The results in Table 1 show that γ-globulin isolated from placental blood serum by alcohol precipitation in the cold possesses total gonadotropic (follicle-stimulating and luteinizing) activity, including all three Ascheim – Zondek types of reaction of the gonads in rats, and giving rise to a statistically significant increase in weight of the ovaries and uterus.

It will be noted that whereas native placental blood serum also induced all three types of reaction of the gonads in the experimental animals, its γ-globulin fraction, isolated on columns, induced only growth of the follicles in the ovaries and swelling of the uterine cornua (type 1 reaction). Fractionation on columns under the conditions used evidently does not abolish the follicle-stimulating activity of this fraction, but abolishes its luteinizing activity. In the same way, in previous investigations [4] the luteinizing activity was also removed from commercial preparations of γ-globulin by column chromatography. It can be concluded that follicle-stimulating activity is firmly bound with the γ-globulin fraction, and the bond could not be broken by ion-exchange chromatography or by molecular filtration.

Inequality of the weight of the uterus and ovaries of the rats after receiving identical doses of γ-globulin isolated from placental blood serum by the four different methods indicates that the γ-globulin fraction obtained on a DEAE-cellulose column possessed the highest activity. Next followed γ-globulin obtained by alcoholic fractionation, followed by that isolated by filtration through Sephadex G-100 gel, and last, that obtained on a column with DEAE-Sephadex A-50.

It was also found that γ-globulin can bind some choriogonin in vitro. Under these circumstances the reaction of the rats' gonads to parenterally injected artificial complex of γ-globulin with choriogonin included all three Ascheim – Zondek types, while the weight of the uterus and ovaries for a dose of protein three times smaller was considerably higher than in all the preceeding experiments (Table 1).

γ-Globulin obtained from placental blood serum by methods of alcoholic fractionation, and ion-exchange and distributive chromatography on columns, thus possess an irremovable follicle-stimulating activity. The γ-globulin fraction placental blood serum can evidently form a complex with gonadotropins not only in vivo, but also in vitro, and this must be taken into consideration in experimental work and also when assessing the therapeutic properties of preparations of placental γ-globulin.

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