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IMMUNOCHEMICAL IDENTIFICATION AND PHYSICOCHEMICAL CHARACTERISTICS OF SPECIFIC PROTEINS OF SEMINAL PLASMA

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Investigation of the antigens of seminal plasma is particularly important in order to characterize the functional state of the reproductive system and of the blood–testis barrier and also to evaluate any possible immunological causes of sterility. Seminal plasma contains normal blood serum proteins (albumin, immunoglobulins) [11, 12] and also certain unidentified antigens [7, 8]. The present writers found placental α_2-microglobulin in sperm [3]. It is suggested [9, 10] that the study of sperm proteins and their exposure to various factors is the most specific method of verification of male fertility.

The aim of the present investigation was to seek specific proteins in human seminal plasma and to study them.

EXPERIMENTAL METHOD

To obtain antisera rabbits were immunized with pooled healthy human seminal plasma. The antisera were exhausted with dry blood plasma obtained from donors. The schemes of immunization, and methods of preparation of the tissue extracts and of determination of the physicochemical parameters of the proteins were described by the writers previously [2, 5]. Antigens were determined semiquantitatively by the double immunodiffusion method with a standard test system [6]. Immunoelectrophoresis was carried out in 1% agar gel, from Serva (West Germany).

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TABLE 1. Physicochemical Characteristics of α_1-GT and α_2-MGF

Properties	α_1-GT	α_2-MGF
Molecular weight, daltons	60,000 ± 7,000	25,000 ± 2,000
Relative electrophoretic mobility	0.92 ± 0.11	0.65 ± 0.01
Staining for lipoproteins	Negative	Negative
Staining for glycoproteins	Positive	Positive
Binding with conA-sepharose	Does not bind	Does not bind
Changes in electrophoretic mobility under the influence of neuraminidase	Reduced	Unchanged
Resistance to trypsin	Destroyed	Destroyed
Salting out with ammonium sulfate, % saturation	35–55	25–69
Precipitation with 0.5% rivanol	Not precipitated	Precipitated
Precipitation with 2% TCA	" "	"
Precipitation with 0.6 M sulfosalicylic acid	" "	"
Resistance to the temperature factor	Thermostable	Thermolabile

EXPERIMENTAL RESULTS

As a result of immunization of rabbits with healthy human seminal plasma two types of antisera were obtained, each of which, after neutralization of antibodies against normal blood serum antigens, continued to reveal one antigenic component in each case in seminal plasma. One such antigen, with the electrophoretic mobility of α_1-globulins (α_1-G) and a molecular weight of 60,000 ± 7000 daltons, was found to be thermostable. On heating to 100°C for 1 h there was no appreciable change in the immunological and physicochemical properties of this antigen, and it was accordingly described as thermostable α_1-G (α_1-GT). α_1-GT was found to be immunochemically not identical with placental α_1-microglobulin, placental lactogen, α_2-globulin of the "pregnancy zone," chorionic gonadotropin, α-fetoprotein, or C-reactive protein.

The second antigen, with the electrophoretic mobility of α_2-globulins and a molecular weight of 25,000 ± 2000 daltons, was found to be completely immunochemically identical with placental α_2-microglobulin (α_2-MG). Considering that placental α_2-MG was found not only in the placenta, but also in the endometrium in the secretory phase of the menstrual cycle, and also in sperm [3], and bearing in mind its role in reproductive function [4], this protein was called α_2-MG of fertility (α_2-MGF).

The results of determination of the physicochemical properties of α_1-GT and α_2-MGF are given in Table 1. α_1-GT is an antigen of glycoprotein nature, the carbohydrate component of which contains sialic acids in its composition. This is shown by positive staining of the α_1-GT precipitation arc for glycoproteins and the reduction in the anodal electrophoretic mobility of this antigen after treatment with neuraminidase. α_1-GT does not bind with concanavalin A-sepharose, it is resistant to precipitation by 0.6 M sulfosalicylic acid solution, 0.4% rivanol solution, and 2% TCA solution, and is not precipitated by ammonium sulfate at 35-55% saturation. As our investigations showed, α_2-MGF present in seminal plasma is similar in its physicochemical properties to α_2-MGF isolated from the placenta [2].

Standard monospecific test systems were obtained for α_1-GT and α_2-MGF and used for their immunochemical identification in tissues and biological fluids. As Table 2 shows, these two antigens both possess high specificity. α_2-MGF was found by immunodiffusion to be present in men only in sperm and the seminal vesicles, which are evidently the place where it is produced. In women α_2-MGF is present in the endometrium in the secretory phase of the menstrual cycle, and in menstrual blood and the placenta.

α_1-GT was found in sperm within a wide range of concentrations (the titers ranged from 1:4 to 1:64), but it was not found in blood serum or in the tissues of most adult human organs. However, a detailed study of the organ-specificity of α_1-GT demonstrated its presence in the saliva of men (in titers of 1:4 to 1:32). The

TABLE 2. Results of Immunochemical Determination of α_1-GT and α_2-MGF in Biological Fluids and Tissues

Test material	α_1-GT			α_2-MGF		
	No. of samples tested	No. of positive tests	Titer	No. of samples tested	No. of positive tests	Titer
Blood serum	29	0	–	38	–	–
Lung	11	0	–	11	0	–
Heart	9	0	–	9	0	–
Liver	17	0	–	17	0	–
Kidney	17	0	–	17	0	–
Adrenals	5	0	–	5	0	–
Spleen	8	0	–	8	0	–
Thymus	3	0	–	3	0	–
Mucosa of small intestine	8	0	–	8	0	–
Brain	5	0	–	5	0	–
Thyroid gland	1	0	–	1	0	–
Pituitary	1	0	–	1	0	–
Ovaries	6	0	–	6	0	–
Endometrium						
in phase of secretion	17	0	–	17	17	1:2–1:16
in phase of proliferation	8	0	–	8	–	–
Menstrual blood	17	0	–	17	17	1:2–1:16
Prostate	7	0	–	7	0	–
Epididymis	4	0	–	4	0	–
Seminal vesicles	4	0	–	4	4	1:1–1:4
Testes	7	5	±–1:1	7	0	–
Seminal plasma	17	17	1:4–1:64	17	17	1:2–1:16
Saliva	11	10	1:4–1:32	11	0	–

presence of this antigen, incidentally, also was found in tissues of the testes and parotid gland, but in extremely low concentrations (titers 1:1). This suggests that α_1-GT is produced by these glands and is actively secreted by them into the saliva and sperm. No α_1-GT could be found in the blood serum not only of healthy donors, but also in any of 50 samples of blood serum taken from patients with inflammatory diseases and malignant tumors in different situations. The diagnostic role of α_1-GT and α_2-MGF in different forms of sterility is a matter for special study.

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