EXPERIMENTAL BIOLOGY

ON THE PROBLEM OF THE IMMUNOBIOLOGICAL RELATIONSHIPS BETWEEN THE HUMAN MOTHER AND FETUS

COMMUNICATION II. DETERMINATION OF ANTIGEN GROUPS IN AMNIOTIC FLUID

L. S. Volkova

From the Laboratory of the Biology of Antigens (Director: Professor P. N. Kosyakov) of the Institute of Experimental Biology of the Academy of Medical Sciences of the USSR (Director: Professor I. N. Maisky)

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In the study of the immunobiological relationships between the mother and fetus, the amniotic fluid interested us as the medium in which the fetus develops during its entire intrauterine life.

Data which exist in the literature indicating that the exchange of certain substances can occur between mother and fetus through the amniotic fluid also [4, 6, 7, 8, 12], served us as an additional basis for the study of this fluid.

Work touching the question of the grouping of embryonic fluids, unfortunately, is rare, while the results of investigations are contradictory.

A number of investigators [11, 14, 15] established the presence of grouped antigenic substances identical with the hemagglutinins of the fetal blood in the amniotic fluid. However, according to the data of these authors, grouped antigens were not found in all cases in the amniotic fluid, which led the indicated investigators to the conclusion that two types of women exist: "secretors," whose embryonic fluids always contain grouped antigens, and "nonsecretors," who do not have these substances in the fluids.

Ito (Ito, 1938) investigated 65 samples of amniotic fluid and established that in 3.1% of the cases grouped substances were not found in the fluids; the author explains the coincidence with the blood group of the neonates by the pollution of the fluids by the epithelial peelings and vernix caseosa which, in Ito's opinion, have group specificity typical of the child.

Thus, the problem of the grouping of the amniotic fluid has not been solved and requires further study.

EXPERIMENTAL METHODS

Into each of two rows of agglutination tubes (5 tubes in each row) were poured 2 drops of the amniotic fluid being tested, which had been diluted 10 times with physiological solution beforehand; 2 drops of standard α serum were added to the first tube of the first row, the tube was shaken and left at room temperature for 15–30 minutes; then two drops of the contents of this tube were transferred to the second (of the same row). The mixture was shaken and after 15 minutes of standing two drops of the contents of the second tube were transferred into the third, out of the third (also after 15 minutes and the same volume) into the fourth and fifth. Out of the fifth (last) tube of the first row, the two excess drops of liquid were removed after 15 minutes. In the same way the dilution of the standard β serum by amniotic fluid was carried out in the second row of tubes. As a result the following dilutions of standard α- and β-sera by the tested fluid were obtained: 1:2, 1:4, 1:8, 1:16 and 1:32. Then into each of the tubes of the first row was added one drop of a 3% suspension of the group A

test-erythrocytes, and into each of the tubes of the second row a drop of group B erythrocytes (in the same concentration). The tubes with the fluid being tested were shaken 3-4 times carefully after the standard erythrocytes had been added and centrifuged for a minute (at 1.5-2 thousand RPM) and the test results read.

Simultaneously a control experiment was set up for the determination of the agglutinability of the standard sera and the agglutination properties of the test erythrocytes.

In all, we investigated 197 samples of amniotic fluid and the same number of blood samples of the corresponding mothers and neonates.

The results of the investigation of the groups amniotic fluid are shown in Table 1.

TABLE 1

Amniotic Fluid Groups

Object of investigation	Number of samples investigated	A-B-antigens not found	Antigens found		
			A	B	AB
Amniotic fluid	197	39	88	45	25
Blood of neonate	197	39	88	45	25
Blood of mother	197	56	74	39	28

It is evident from Table 1 that out of 197 samples of amniotic fluid tested, neither A nor B antigens were found in 39 samples. In all these cases the blood of the newborn was also free from group antigens, while the blood of the mother belonged to different groups.

In the remaining 158 samples of amniotic fluid the presence of A-, B- or AB-antigens was established, and here also complete correspondence between the grouping of the fluid and the blood grouping of the newborn was found. Thus, in 88 cases, when A-antigens were contained in the blood of the child, the amniotic fluid agglutinated the α-agglutinins in the standard sera and consequently, also contained A-group substances. In these cases, the blood of the mothers belonged to various groups. In 45 samples of amniotic fluid the presence of B-antigens was established; in these cases all the children (45) belonged to Group B (Group III). In 25 samples of amniotic fluid, as in the blood of the newborn, were found AB-substances; in these cases the blood of the mothers belonged to different groups.

Thus, the data we obtained indicated that the amniotic fluid of man is differentiated into groups and contains antigens which are identical with the blood antigens of the fetus (newborn).

In no case were hemagglutinins found in the amniotic fluid which were lacking in the blood of the newborn, or vice versa.

Our data, thus, confirm the hypothesis, expressed by P. N. Kosyakov and Z. L. Rovnova [3] and proved by them on another object (saliva), regarding the fallacy of the hypothesis of Schiff and Sasaki [13] regarding the "secretors" and "non-secretors." If this hypothesis were correct, we would not have been able to find some of the group antigens which were present in the blood of the newborn in 20% of the tested samples of amniotic fluid. In our experiments, on the contrary, complete correspondence between the grouping of the amniotic fluid and the groups found in the blood of the newborn was found.

The discrepancy between the results of our experiments and the data of other investigators [9, 10, 11, 14, 16], who were not always able to find hemagglutinins in the amniotic fluid, is explained by the difference in the methods used to study this fluid.

Through our use of the method of consecutive dilution of the standard sera by the same sample of tested fluid, the most complete recovery of antibodies occured. This circumstance, as well as the use of amniotic fluid in comparatively low dilutions—1:10, 1:2 or undiluted—permits the recovery of even small amounts of the group substances present in the amniotic fluid.

In determining the quantitative content of the group substances in the amniotic fluid, it was established that it is considerably greater than the amount of hemagglutinins present in the blood serum of the newborn and noticeably greater than in the serum of the mothers (Table 2).

TABLE 2

Comparative Titer of A- and B-group Antigens in Amniotic Fluid and in the Sera of Mothers and Newborn

Object of investigation	Antigen group	Number of samples tested	Titer of group antigens			
			from 1:10 to 1:40	from 1:80 to 1:160	from 1:320 to 1:640	from 1:1280 and higher to 1:10240
Amniotic fluid	A	85	9	8	32	36
	B	40	2	7	13	18
Mother's retroplacental blood serum	A	74	17	19	27	11
	B	37	6	16	11	4
Newborn's cord	A	21	15	6	—	—
Blood serum	B	21	11	9	1	—

It is apparent from Table 2 that the greatest number of samples of amniotic fluid contained group A, B antigens in a titer of from 1:1280 to 1:10,240: A in 36 cases out of 85, B in 18 out of 40 tested fluids; the group antigens found in the retroplacental blood sera of the mothers and in the cord blood sera of the newborn are present in low titers in the majority of cases: titers of 1:80–1:640 in the mothers' blood, titers to 1:40 in the newborn's blood.

The quantitative differences among the group antigen contents of the amniotic fluid and the blood sera of the newborn and mothers indicates first of all that the group antigens of the embryonic fluids do not originate from the blood of the mother or the fetus.

A basis exists for the statement that the group antigens of the amniotic fluid, like the fluid itself, form as a result of the secretory activity of the amnion, whose glandular structure has been proved conclusively [1, 2, 5].

The presence of a large amount of group antigens in the amniotic fluid, which are identical with the blood antigens of the child, can play a significant role in the immunobiological relationships between the mother and fetus. The group substances in the amniotic fluid can, when there is serological incompatibility between the mother and child, agglutinate the corresponding isoantibodies of the mother's blood and protect the child's system from possible injury by this means.

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