FAILURE TO DETECT Rh-SUBSTANCE IN LIQUOR AMNII

BY

WILLIAM WALKER AND BERYL M. BAILEY

From the Children's Department, Royal Victoria Infirmary, Newcastle-on-Tyne

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Under certain circumstances it is desirable to predict the Rh group of a foetus before delivery. As predictions based on the genotypes of the parents are uncertain it would be useful to be able to tell from an examination of amniotic fluid whether or not a foetus was Rh positive, and our purpose was therefore to determine whether Rh substance could be detected in liquor amnii by means of a simple technique.

Material

Fifty specimens of liquor amnii were obtained from women where artificial rupture of the membranes by means of a Drewe-Smythe catheter was performed for some independent reason. Care was taken to avoid contamination with blood, and only specimens free from contamination were tested. In addition a specimen of cord blood was obtained from the baby at birth.

Methods

The ABO and Rh groups were determined on the cord blood.

The liquor amnii was tested for the presence of A and B substance. It was heated in a boiling water-bath for 10 minutes, and filtered to remove solid particles. One volume of liquor amnii was added to 1 vol. of anti-A and to 1 vol. of anti-B grouping sera and allowed to stand at room temperature for 15 minutes. One volume of a 3% suspension of the appropriate red cells, A₂ or B, was added and any agglutination was recorded after a further 15 minutes. The absence of agglutination was taken to mean that soluble group substance was present.

The liquor amnii was then tested for the presence of Rh substance. Except where indicated the same antiserum, a pure saline anti-D, was used, because Rh substance in low concentration is best detected by means of saline antibody (Witebsky, personal communication, 1955). For all tests a freshly prepared 3% red cell suspension of O R₁ R₂ cells was used. Witebsky (1948) increased the concentration of liquor 10-fold by means of dialysis, but we varied the proportions of liquor and antiserum by setting up a titration of doubling dilutions of antiserum using the test liquor as diluent. Thereby, in the first tube was undiluted serum and in the tenth a mixture of one part of serum to 511 parts of liquor. Two

controls were set up alongside; the first a titration of the antiserum in saline, and the second a titration in liquor from an Rh-negative foetus. After standing at 37° C. for two hours, an equal volume of red cell suspension was added and agglutination read microscopically after a further period of two hours at 37° C. The degree of agglutination was recorded in accordance with the recommendations of the M.R.C. Subcommittee on blood grouping (1943).

Before testing, the specimens of liquor were heated in a boiling water-bath for 10 minutes and filtered. They were stored at -20° C. for periods varying between one and 14 days before testing. Ten specimens of liquor were titrated at a time and the appropriate controls were set up with each batch.

TABLE I
A AND B SUBSTANCE

ABO Group	Soluble Group Specific Substance		
	Present	Absent	Total
A	15	2	17
B	5	2	7
AB	1 (both)	1 (both)	2
O	—	—	24

Results

A and B Substance.—In the majority of cases the results were clear-cut, although the degree of inhibition was less with some specimens of liquor than with others. There was no apparent relationship between the Rh group and the ability to "secrete" A or B substance (Table I).

Rh Substance.—The controls gave essentially the same results throughout the period of these experiments and no titration in liquor differed significantly from the controls or its fellows, although of course all titrations were not identical. For simplicity in presentation we have given arbitrary scores to the different degrees of agglutination in individual tubes; thus an aggregate score was arrived at for each titration (Table II).

The small number of specimens of Rh-negative liquors precludes any formal statistical comparison between the results in Rh-positive and Rh-negative

TABLE II
AGGLUTINATION SCORE*

Titration	No.	Range	Average
Controls { In saline	5	21-26	23.4
" Rh-negative liquor ..	5	20-30	24.6
Test { Liquor from Rh-negative foetus	6	14-26	20.5
liquor { " " " positive "	44	14-27	18.6

* For these scores the first tube containing undiluted serum has not been included.

cases. However, it is obvious that the range in both groups is sufficient to prevent a confident prediction of the Rh group of any individual case. It would seem from these findings that Rh substance if present in liquor amnii is there inconsistently and in small quantities.

Because these findings conflict with those of Witebsky and his co-workers (Witebsky and Mohn, 1945; Witebsky, 1948) we considered whether certain other factors might have been responsible for our failure to detect Rh substance. We therefore conducted a number of experiments designed to test the effect of these variables. For these experiments we used one volume of antiserum in seven of liquor, because the antiserum gave clear macroscopic agglutination at this dilution.

We tested 20 specimens of liquor amnii from Rh-positive cases under the following conditions:

Conditions for Neutralization (Liquor and Serum Only)	Conditions of Agglutination (After Addition of Rh-positive Cells)
* 2 hours at 4° C.	2 hours at room temperature
2 " " 4° C.	2 " " 37° C.
2 " " room temperature ..	2 " " room temperature
2 " " " " " " " " " " " "	2 " " 37° C.
2 " " 37° C. " " " " " "	2 " " 37° C.
24 " " 4° C. " " " " " "	1 hour at room temperature
24 " " 4° C. " " " " " "	2 hours " 37° C.

* The conditions employed by Witebsky.

In 127 of the 140 tests, definite macroscopic agglutination was evident while microscopic agglutination was present in the other 13. Of the 12 controls, 10 showed macroscopic and two only microscopic agglutination.

Different Antisera.—Witebsky and Mohn (1945) had found that some antisera were more suitable than others for demonstrating Rh substance in liquor. Therefore we carried out tests with five different saline anti-D sera together with saline and Rh-negative liquor controls. The liquor used for this experiment was from a group A Rh-positive baby, known to secrete A substance. The neutral-

ization was carried out for two hours at room temperature, 37° C., and 4° C., after which the cells were added and incubated for two hours. There was no difference in the degree of agglutination of the test specimens or controls.

Twenty specimens of liquor from Rh-positive babies, 10 of which were known secretors of A or B substance, were titrated with two albumin anti-D sera, using the test red cells suspended in albumin. The results were similar in all cases and did not differ from the albumin or Rh-negative liquor controls.

Inactivation.—In case inactivation by boiling might destroy Rh substance, 10 samples of liquor from Rh-positive ABO secretors were tested before and after boiling, but no differences were observed.

Conditions of Storage.—The same 10 samples of liquor were tested after storage at -20° C. for various periods up to 14 days. The results in all cases were essentially the same, nor did the activity of A and B soluble substance seem to diminish.

Discussion

We were unable to diminish appreciably the titre or avidity of Rh antisera by absorption with liquor amnii obtained from babies of different ABO and Rh groups. Although the degree of agglutination was somewhat less after neutralization with Rh-positive than with Rh-negative liquor this was not consistent nor definite enough to be used as a means of identifying the Rh group of the foetus before delivery. These findings are at variance with those of Witebsky, who found that liquor amnii from 80% of Rh-positive babies contained soluble Rh substance. He regarded the presence of this substance as able to protect the foetus from the action of Rh antibodies, but it is unlikely that this hypothesis is true, because it is generally accepted that in all cases where a mother has antibodies an Rh-positive baby will have haemolytic disease, if one accepts a positive Coombs test on cord blood as defining the limits of this condition. Moreover, Crawford, Cutbush, and Mollison (1953) found that A "secretors" are not protected from ABO—haemolytic disease. In our series liquor was obtained from three immunized Rh-negative mothers. All babies were Coombs positive, two were severely affected, while the other was mildly affected and required no treatment. The titration results in these specimens of liquor were not different from one another nor from the group as a whole.

Using the methods described we were unable to detect Rh substance in liquor amnii in sufficient quantity to allow prediction of the Rh group of the

foetus. For this, one must continue to rely on the father's genotype as determined by tests on his blood and that of his parents or children.

Summary

Fifty specimens of liquor amnii were tested for blood group activity in relation to ABO and Rh substances. Cord blood from the corresponding babies was tested for ABO and Rh group.

Rh substance was not detected in any specimen of liquor.

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