ABO INCOMPATIBILITY AND HAEMOLYTIC DISEASE OF THE NEWBORN

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Since the work of Boorman, Dodd and Trinick (1949), incompatibility in the ABO system has been recognized with increasing frequency as a cause of haemolytic disease. Robinson, Phillips and Prystowsky (1951), Shumway, Miller and Young (1955), Davidsohn (1956) and others have written on this condition. And yet haemolytic disease due to ABO incompatibility is much less widely known than that due to Rhesus incompatibility. The failure widely to recognize ABO disease may be due to several reasons. Chiefly it is due to the fact that it is usually not a serious condition. In its severe degree it is uncommon. In its common form it is so slight as scarcely to be recognized. So the disease is held to be rare. Belief that a disease is rare leads to a lessened vigilance in its detection, and it is remarkable how once a disease is found to be not uncommon how quickly it becomes commonplace. Then again, that elegant test, the direct antiglobulin test of Coombs, which so readily detects haemolytic disease in blood group incompatibilities other than ABO, is usually negative by the more usual techniques. By the special technique of Rosenfield (1955), however, it may be shown to be positive. With heterospecific pregnancies with A or B incompatibility, antibodies are by definition present in the blood of the mother, although where disease is present they are believed to be of a different nature from the 'natural' antibody. In Rhesus disease the presence of antibody with Rhesus incompatibility indicates disease. It is not necessarily so in ABO disease. Moreover, no immune antibody may be detectable ante-natally in ABO disease, although it may appear after delivery. Unlike Rhesus disease, ABO disease cannot be anticipated by ante-natal testing with any certainty. Finally, it may be that the term 'physiological jaundice' makes for a too easy road to diagnostic apathy, and one that is trod too often.

It is stated by Davidsohn (1956) that ABO haemolytic disease appears in 1 in 3,000 random pregnancies and by Gunson (1956) that it accounts for 15% of all cases of haemolytic disease of the newborn. Certainly it is generally believed to be less common than disease due to Rhesus incompatibility. However, Rosenfield (1955) found laboratory evidence of abnormality attributable to ABO incompatibility in 38 cord blood specimens of 1,480 random samples.

The present small survey purports to show that clinically recognizable ABO haemolytic disease is a common condition and that it may quite often need close watching and active treatment.

Survey and Findings

This survey, conducted in a hospital which serves a population of about 60,000 of mixed European descent, deals with the period August 1, 1956, to July 19, 1957. During this time there were 1,000 live births. It is the universal practice in this locality for deliveries to be in hospital. The survey therefore covers 1,000 random live births.

During the period under review all babies judged to show the slightest jaundice within the first 24 hours after birth were reported. It was found that minimal jaundice was difficult to detect in artificial light, and it is more than likely that some escaped investigation if they became jaundiced but slightly at night toward the end of the 24-hour limit set by the terms of the survey. Nevertheless, in 1,000 live births, 21 babies were found to be recognizably jaundiced within 24 hours. Six were found to have haemolytic disease due to antibody anti-D and one to be due to anti-E, and these are excluded from the series. Fourteen babies with jaundice believed not due to Rhesus disease were investigated. It should be emphasized that no baby who became jaundiced within 24 hours is excluded from the investigation apart from the seven in whom D or E incompatibility was found. Nothing was known about the blood groups of mother or baby until early jaundice had prompted investigation.

Table 1 relates to the parity of the mother, the birth weight in pounds and ounces and the time of onset of the jaundice in hours after birth. It will be noted that in three instances first babies were involved and that one came within the World Health Organization definition of prematurity. Cases 2 and 3 were dizygotic twins of the same blood group, A. There was no difference in sex incidence. The time of onset of the jaundice varied between four and 23 hours with a mean of 14 hours. The smaller babies did not become jaundiced earlier than those of larger size.

Table 2 refers to the initial bilirubin estimation and to the maximal bilirubin estimation. Bilirubin estimations were not done at night if the jaundice did not seem to be deepening rapidly, nor were they repeated if the initial level was low and the baby not becoming more icteric.

It will be seen that we were able just to detect jaundice at a level of 4 mg. % but it was our experience that it did not become easily visible until a level of about 6 mg. % was reached. In three cases the maximal bilirubin reached the figure of 20 mg. % or over, and in these babies exchange transfusions were done. In one case, Case 6, three exchanges were required to keep the bilirubin at a safe level. The average time for the onset of jaundice in these three cases was 14 hours, which does not differ from the series as a whole.

Table 3 shows the first haemoglobin level determined after the jaundice was first reported. In no case was the haemoglobin below 15 g. %, and in the majority it was considerably higher. The most severely jaundiced did not have lower haemoglobin levels than the remainder. In twelve instances it is recorded that splenic enlargement was specifically sought. In two only was it found.

Table 4 shows the presence or absence of spherocytes in a stained smear and the osmotic fragility of the red cells in venous blood expressed as the percentage concentration of sodium chloride in which haemolysis is first apparent.

In eight instances spherocytosis is recorded as being present. It is remembered that it was also present in two further cases, Cases 2 and 3, but there is no written record of this. All cases showing spherocytosis showed haemolysis in concentration of 0·60% saline, or greater, but those showing the most obvious spherocytosis were not those with the greatest fragility. Reticulocyte counts were done in only nine of the 14 babies. In only three was the count above the normal of 4·35% (Washburn, 1941) for this age group.

Table 5 shows the reaction on a slide of the whole blood of the baby when mixed with complement inactivated normal AB serum compared with the same serum with the addition of bovine albumin (Witebsky, 1954). Thirteen of the 14 babies had blood which agglutinated with normal AB serum within 90 seconds. One baby showed no reaction where reaction might have been expected. In five there was agglutination also in the AB serum with bovine albumin.

Experience with this test has shown that it is most difficult to interpret with confidence. The agglutination may be very fine and scarcely visible. It is easily confused with rouleaux formation. The time limit of 90 seconds must be strictly enforced or false positive results will be found. Incorrect readings in our hands have been too frequent to make the test really valuable.

The direct antiglobulin test of Coombs was performed in the 14 babies in the series. In only one, Case 4, was macroscopic agglutination seen with a test carried out as described by Zuelzer and Cohen (1957).

Table 6 shows the blood groups of the babies compared with those of their mothers. It will be seen that in every one of the 14 cases of babies becoming jaundiced within the first 24 hours an incompatibility, O-A or O-B, was found. In 12 A was the incompatible, in two it was B. No attempt at subgrouping into A_1 or A_2 was made.

Table 7 shows the ABO phenotype distribution in 50 mother-child pairs randomly selected in our area. For clarity, those where incompatibility of A or B exists are marked with an asterisk. It is seen that such incompatibility was present in 10 births out of 50. If this finding is compared with Table 6 a marked and highly significant difference is obvious. That every mother in the series was group O and every baby either group A or B can scarcely be due to chance.

It might be argued that 50 random mother-child combinations is too small a number to rule out a peculiar bias in blood group distribution, but the percentage of compatible and incompatible pairs in that small number is almost identical with the number which would be expected on the basis of the ABO gene frequencies. This calculation was made and resulted in the expectation of 81% compatible mother-child pairs, assuming that mating is random and unrelated to blood groups.

Table 8 shows the maximal bilirubin level in relation to the D factor of Rhesus of the babies and their mothers. It is seen that of 14 babies jaundiced in the first 24 hours no less than seven were d/d: in common parlance, they were Rhesus negative. Using tables (Mainland, 1948) giving the confidence limit for small samples, it can be shown that this

TABLE 1
MATERNAL PARITY, BIRTH WEIGHT AND TIME OF ONSET OF JAUNDICE

Case No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Parity of mother ..	1	2	2	1	2	2	3	3	1	2	2	4	9	2
Birth weight (lb. oz.)..	7·2	6·8	4·15	8·10	6·6	6·10	8·5	6·14	6·4	6·5	8·9	8·14	6·13	6·4
Onset of jaundice (hr. P.N.) ..	4	18	22	6	20	23	7	11	12	8	18	10	9	20

TABLE 2
INITIAL AND MAXIMAL BILIRUBIN ESTIMATIONS

Case No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Initial bilirubin (mg.%)	6·5	8·0	5·0	8·6	11·9	13	7·0	7·5	9·2	7·0	11·3	6·4	4·0	8·6
Maximal bilirubin (mg. %)	6·5	9·0	5·0	8·6	14·6	25·0*	7·0	22·0*	10·3	20·0*	14·0	6·4	16·2	8·6

* Exchange transfusion.

TABLE 3
INITIAL HAEMOGLOBIN LEVELS

Case No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Haemoglobin (g. %) ..	19·5	18·0	18·5	20·0	17·0	20·0*	18·5	17·0*	16·0	16·5*	16·5	19·5	15·0	19·0

* Exchange transfusion.

TABLE 4
SPHEROCYTOSIS AND OSMOTIC FRAGILITY IN BABIES

Case No.	1	2	3	4	5	6*	7	8*	9	10*	11	12	13	14
Spherocytosis ..	—	?	?	++	+	+	+	—	+	—	—	+++	+	+
Osmotic fragility (% sodium chloride)	0·64	0·64	0·60	0·60	0·80	—	0·80	0·52	0·84	0·56	0·56	0·60	0·60	—

* Exchange transfusion.

TABLE 5
WITEBSKY TEST REACTIONS

Case No.	1	2	3	4	5	6*	7	8*	9	10*	11	12	13	14
Reaction in AB serum	+	+++	+	+	+++	+	+	+	0	+++	+	+	+++	+
Reaction in AB serum + albumin ..	0	+	0	+	0	+	+	0	0	0	+	0	0	0

* Exchange transfusion.

TABLE 6
BLOOD GROUPS OF MOTHERS AND BABIES

Case No.	1	2	3	4	5	6*	7	8*	9	10*	11	12	13	14
ABO:														
Baby	A	A	A	A	B	A	A	B	A	A	A	A	A	A
Mother	O	O	O	O	O	O	O	O	O	O	O	O	O	O
Rhesus:														
Baby	D	D	D	D	d/d	d/d	d/d	d/d	d/d	d/d	D	d/d	D	D
Mother	d/d	D	D	D	D	d/d	D	d/d	D	D	D	d/d	D	D

* Exchange transfusions.

TABLE 7
ABO PHENOTYPE DISTRIBUTION IN 50 RANDOM MOTHER-CHILD PAIRS

ABO phenotype	O-O	O-A	O-B	A-O	A-A	B-O	B-A	B-B	AB-O	AB-B	AB-AB
No. of pairs	11	6*	2*	6	18	1	2*	1	1	1	1

* Incompatibility of A or B.

TABLE 8
RHESUS D FACTOR IN MOTHER AND BABIES AND MAXIMAL BILIRUBIN LEVEL

Case No.	1	2	3	4	5	6*	7	8*	9	10*	11	12	13	14
Rhesus:														
Baby	D	D	D	D	d/d	d/d	d/d	d/d	d/d	d/d	D	d/d	D	D
Mother	d/d	D	D	D	D	d/d	D	d/d	D	D	D	d/d	D	D
Bilirubin, maximal	6·5	9	5	8·6	14·6	25	7	22	10·3	20	14	6·4	16·2	8·6

* Exchange transfusion.

TABLE 9
BLOOD GROUP OF BABY AND ANTIBODY LEVEL IN MATERNAL BLOOD

Case No.	Baby	Mother		Nature of Antibody
	ABO	Anti-A	Anti-B	
1	A	—	—	—
2	A	320	40	Both resistant to neutralization. Anti-A eluted from baby's cells.
3	A	320	40	Both resistant to neutralization. Anti-A eluted from baby's cells.
4	A	40	20	Both weakly immune. Findings not too conclusive.
5	B	40	320	Anti-B is strongly immune. Anti-A weakly so.
6	A	—	—	—
7	A	640	80	Only anti-A is of immune variety.
8	B	640	80	Both antibodies strongly immune.
9	A	640	80	Both antibodies strongly immune.
10	A	640	160	Both antibodies of immune variety.
11	A	—	—	—
12	A	1,000	320	Both of immune variety. Blood taken 6 weeks after delivery.
13	A	160	40	Both antibodies of immune variety.
14	A	320	20	Anti-A is strongly immune. Blood taken 6 weeks after delivery.

observed distribution of Rhesus negative babies with jaundice and AB incompatibility is a departure from the expected in a population such as ours at a low level of significance (significant at 5% level, but scarcely so at 1% level). It will also be noted that the three babies requiring transfusion were Rhesus negative (d/d), and that the average maximal bilirubin value in this series was higher for the Rhesus negative (d/d) babies than for the D positive infants. The mean difference was 5·3 mg. %, the standard error of difference 3·20. In this small series the difference between the maximal bilirubin levels of the D positives and the Rhesus negatives (d/d) was not significant.

Table 9 illustrates the level of antibodies found in the blood of the mother. Except where indicated in the table blood samples were taken two to four days after delivery. The antibody titrations were most kindly carried out at the Ortho Research Foundation, Raritan, New Jersey, under the direction of Dr. Phillip Levine, whose comments are given in brief.

Antibody studies were carried out on the blood of the mothers of 11 of the 14 babies in the survey. It will be seen that a high titre of immune antibody is present against the factor carried by the baby with a frequency which seems far too high for it to be the result of mere chance or of immunization

unrelated to the pregnancy. However, in Case 4 the antibody levels were not very convincing, while in Case 8, where the baby was group B, immune anti-A was present in a titre of 1:640 and immune anti-B in a titre of 1:80 only.

Discussion

Investigations on the cases presented here are in some instances incomplete, but the following facts clearly emerge. The slightly lower birth weights of those requiring treatment was not a significant difference. There was no difference in the average time of onset of the jaundice in those in whom the jaundice later became severe. Late onset did not mean mild disorder. The Coombs test was positive in one baby, but this was one with a low level of bilirubin throughout. The initial haemoglobin level was no lower in those threatened with kernikterus than in the others, nor was splenic enlargement a feature of severe disease. Spherocytosis was present or absent in mild and in severe cases alike, as was an increased osmotic fragility of the red cells. Agglutination of the baby's cells in AB serum was found in the three severely affected babies, but it was also found in most of the others, including that with the lowest bilirubin. A reticulocyte count of 11% was found in one of those with marked icterus, but a similar count was found also in one of the mildest of the series. Immune anti-A was found in a titre of 1:640 in one of the babies with severe jaundice, but it was present in the same titre in a baby whose bilirubin never rose above 7 mg. %. Incompatibility of group B was present in a severe case, but was present also in a case of only moderate severity.

There was thus, in this series, no difference between the cases with severe jaundice and those with mild jaundice apart from the degree of jaundice itself.

The 14 cases where ABO incompatibility existed appeared to represent a uniform picture of the same disease manifesting itself in greater or less severity. There was no way of forecasting which would become an anxiety and which would cause no alarm. All need close watching.

The true incidence of ABO disorder is difficult to determine. Rosenfield (1955) found laboratory evidence in 38 specimens of 1,480 cord blood samples collected at random. It appeared that the disorder was present in the babies of 11% of mothers who lacked A or B when this was possessed by the foetus. The high degree of significance in the present small series for the frequency of incompatibility compared with that expected suggests that these cases, selected purely because they were clinically jaundiced, were indeed examples of ABO haemolytic disease. If this be accepted, we found ABO haemolytic disease to be clinically recognizable in one in every 71 births and likely to be present in 7% of mother-child pairs where A or B incompatibility exists.

An arbitrary limit of 24 hours was set for the onset of jaundice coming within this review. When one sees that of the 14 cases believed to have ABO haemolytic disease, four first showed jaundice at 20 hours or over, one immediately suspects that many cases of jaundice with onset after 24 hours may in fact be examples of haemolytic disease. This we have found to be so. It is clear, however, that the disorder does not always appear, in form detectable by known methods, every time that incompatibility exists. Why this curious selection occurs is at present unknown. It is likely that a combination of circumstances must exist before the disorder is manifest. It appears (Zuelzer and Cohen, 1957) that babies of subgroup A_2 escape altogether and that full maturity of the antigenic potential of A_1 is not developed in the newborn. It may be that in some babies the immune antibody does not cross the placental barrier or is neutralized in so doing by the A or B substances in the placental tissue. It may be that there is an antigenic fraction associated with, but not identical with the A and B antigens, which is present in some but not in others. Unger and Wiener (1954) have demonstrated a factor C in the ABO system, and to this factor they attribute ABO haemolytic disease. If the group O mother of a baby of group A had, herself, a mother who was of group A and a secretor one could see that she, while herself a foetus, could develop tolerance to the A antigen from being subjected to A in intrauterine life. Were this to happen one could imagine that such a mother would not herself have a baby with ABO haemolytic disease for, to her, the A factor would no longer be antigenic. Probably there is no single explanation of why the disorder does not always appear when conditions seem to be fulfilled for its appearance.

In this survey a discrepancy at a low level of significance is noted between the incidence of Rhesus negativity in mother-child incompatible babies affected with ABO haemolytic disease and what would be expected from the random distribution. If this difference be real it is difficult to explain. Until a larger series proves or disproves the significance of this observation it would be idle to speculate further.

Some would discount the levels of antibody titrations presented in Table 9, saying rightly that many normal people who have never received inoculation with human blood or tissue products

may have a high level of immune anti-A or anti-B. Nevertheless, taken in conjunction with other evidence, it does not seem that the titres found here can really be disregarded.

It is a matter for regret that we did not use the test for free homologous antibody using adult cells of the same group as the baby. At the time this survey started the value of this test (Zuelzer and Cohen, 1956) was not realized. We are now employing this test using the A or B cells of the baby's father for, if his baby is affected, he will himself possess cells upon which free antibody in the baby's serum will react; that reaction being detected by the indirect Coombs test.

Summary

From 1,000 unselected live births all babies showing jaundice in the first 24 hours were investigated. Twenty-one such babies were found. Seven were diagnosed as being examples of Rhesus haemolytic disease and these are excluded from this series. Fourteen were considered to have ABO haemolytic disease, and for this evidence is presented.

ABO haemolytic disease can occur in clinically recognizable degree as often as once in every 71 births and may arise in 7% of AB incompatible mother-child pairs. It is thus about three times more common than haemolytic disease due to Rhesus incompatibility.

There is always a reason for jaundice within 24 hours of birth. The diagnosis of 'physiological jaundice' should not be invoked to explain such a happening.

My thanks are profoundly due to many: to the nursery nurses for their unfailing enthusiasm and vigilance, to the doctors who permitted me to see their patients, to the laboratory technicians for their most painstaking work at all hours and to the staff of the medical records department. I am also most grateful to the Ortho Research Foundation and to Dr. Phillip Levine for the reports on the antibody titrations and to Dr. Carol Buck of the University of Western Ontario for the statistical examinations.

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