BILIRUBIN VALUES OF CORD BLOOD IN HETERO-SPECIFIC PREGNANCY

BY

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Following the discovery of the Rhesus factor by Landsteiner and Wiener (1940) and the demonstration by Levine, Burnham, Katzin, and Vogel (1941) that haemolytic disease of the newborn generally is the result of mother–child Rh incompatibility several reports of erythroblastosis foetalis attributable to sensitization of the mother by the A or B foetal antigens have been published, notably those of Boorman, Dodd, and Trinick (1949) and Mollison and Cutbush (1949a). In view of the hyperbilirubinaemia in cord blood which may be attained in cases of Rh sensitization, and the demonstration that erythroblastosis foetalis may occasionally result from mother–child ABO incompatibility, it was decided to examine the bilirubin level of cord blood to try to determine whether the ABO and Rh blood group systems exert any influence on this level in heterospecific pregnancies.

Material

The ABO and Rh blood groups of each mother delivered in this hospital, and of each live-born infant, were determined, together with the cord blood bilirubin value, whenever sufficient serum was available. A direct Coombs test was also performed on each cord blood sample. While there was no selection of cases the sample contains a high percentage of Rh-negative subjects (18%), as Rh-negative mothers tend to be sent to hospital for supervision during pregnancy.

Methods

ABO and Rh blood groups were determined by the tube technique as described in the M.R.C. War Memorandum No. 9 (1943) and Mollison, Mourtant and Race (1948). Bilirubin values were estimated by the method of Rappaport and Eichhorn (1943) as modified by Gray and Whidborne (1946). In some instances of haemolytic anaemia six hours may be necessary for full colour development by this method; all tests were allowed to stand six hours in the dark. The methyl red standard was used and taken to be equivalent to 3.6 mg. bilirubin per 100 ml. serum.

Results

Mean Bilirubin Value.—The mean serum bilirubin value of 1,459 cord blood samples was 1.27 mg./100 ml., the distribution of values being shown in Fig. 1. This mean value is lower than the 1.69 mg. per 100 ml. serum reported by Davidson, Merritt, and Weech (1941) using an acetone protein precipitation method (94 samples), and the 1.5 mg. per 100 ml. plasma given by Mollison and Cutbush (1951) for 100 samples.

The red cells of eight infants gave a positive direct Coombs test and they, together with 98 families in which a full blood group examination of either the mother or infant was not made for any reason, have been excluded from the following analysis.

ABO Heterospecific Pregnancies.—The mean serum bilirubin values, the standard errors, and deviations of the means in ABO homospecific and heterospecific pregnancies.
were determined and are shown in Table I. The difference in the means is statistically significant (t = 3.74, n = 1,352, P < 0.001).

<table>
<thead>
<tr>
<th>Type of Pregnancy</th>
<th>No. of Observations</th>
<th>Mean Bilirubin Value (mg.%</th>
<th>Standard Error</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO homospecific</td>
<td>1,078</td>
<td>1.248</td>
<td>0.013</td>
<td>0.451</td>
</tr>
<tr>
<td>ABO heterospecific</td>
<td>275</td>
<td>1.365</td>
<td>0.034</td>
<td>0.564</td>
</tr>
</tbody>
</table>

* A heterospecific pregnancy is one in which the foetus has an A and/or B antigen not possessed by the mother.

The raised mean serum bilirubin values in the heterospecific group of pregnancies (Table I) may be accounted for in one or both of two ways: either there is a general higher trend for bilirubin values in heterospecific pregnancies throughout the entire sample, or a small number of high readings has raised the mean bilirubin value for this group.

Examination of the distribution of the bilirubin values of ABO homo- and hetero-specific pregnancies (Fig. 2) shows that the proportion of heterospecific to homospecific pregnancies is much higher in the group of readings above 2 mg. bilirubin per 100 ml. than in the group below this level. The actual figures are 35:71 (0.49:1.00) and 240:1,007 (0.24:1.00) respectively (Table II). In addition, while the mean bilirubin value of those hetero-specific pregnancies in the group of readings below 2 mg. bilirubin per 100 ml. is very similar to that of the homospecific pregnancies in this range, the mean bilirubin value for heterospecific pregnancies is distinctly raised above that for homospecific pregnancies in the group of readings over 2 mg. bilirubin per 100 ml. (Table II).

**TABLE II**

<table>
<thead>
<tr>
<th>Type of Pregnancy</th>
<th>Up to and Including 2 mg. Bilirubin/100 ml. Serum</th>
<th>Over 2 mg. Bilirubin/100 ml. Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Observations</td>
<td>Mean Bilirubin Value</td>
</tr>
<tr>
<td>ABO homospecific</td>
<td>1,007</td>
<td>1.178</td>
</tr>
<tr>
<td>ABO heterospecific</td>
<td>240</td>
<td>1.210</td>
</tr>
</tbody>
</table>

It would appear therefore that the raised mean bilirubin value for all heterospecific pregnancies as compared with that for all homospecific pregnancies is largely accounted for by the increased proportion of readings over 2 mg. bilirubin per 100 ml. serum in the heterospecific group. In addition this group accounts for almost all of the readings over 3 mg. bilirubin per 100 ml. serum.

**ABO and Rh Heterospecific Pregnancies.**—Four ABO and Rh combinations are possible in a pregn-

**TABLE III**

<table>
<thead>
<tr>
<th>Type of Pregnancy</th>
<th>No. of Observations</th>
<th>Mean Bilirubin Value (mg.%</th>
<th>Standard Error</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>965</td>
<td>1.253</td>
<td>0.014</td>
<td>0.445</td>
</tr>
<tr>
<td>ABO homospecific</td>
<td>Rh homospecific</td>
<td>246</td>
<td>1.363</td>
<td>0.036</td>
</tr>
<tr>
<td>Group II</td>
<td>113</td>
<td>1.211</td>
<td>0.048</td>
<td>0.508</td>
</tr>
<tr>
<td>ABO homospecific</td>
<td>Rh heterospecific</td>
<td>29</td>
<td>1.350</td>
<td>0.095</td>
</tr>
</tbody>
</table>

* * An Rh-negative mother with an Rh-positive child has been considered as an Rh-heterospecific pregnancy, and any other combination as homospecific.**

From Table III it is again seen that in ABO heterospecific pregnancies the mean
BILIRUBIN VALUES OF CORD BLOOD

bilirubin value is higher than in ABO homospecific pregnancies, and also that this difference is unrelated to Rh compatibility or incompatibility. Six intra-group comparisons may be made (Table IV). Comparison between groups I and II and groups II and III show differences between the means which are statistically significant. These differences are due to ABO incompatibility and are independent of Rh incompatibility. From this it might have been expected that comparison between groups I and IV would have yielded a higher value for t. However, group IV contains only 29 observations and may be too small for accurate comparison.

Table IV

Comparison of the Bilirubin Values in Each Type of Pregnancy

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Degrees of Freedom</th>
<th>Values for t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups I and II</td>
<td>1,209</td>
<td>3.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Groups I and III</td>
<td>1,076</td>
<td>0.95</td>
<td>&lt;0.30</td>
</tr>
<tr>
<td>Groups II and IV</td>
<td>273</td>
<td>2.43</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Groups III and IV</td>
<td>140</td>
<td>1.30</td>
<td>&lt;0.20</td>
</tr>
</tbody>
</table>

Discussion

Recent investigations of the ability of the infant to excrete bilirubin and bromsulphalein have led to general acceptance of the view that hyperbilirubinaemia in the infant is largely due to functional immaturity of the foetal liver (Findlay, Higgins, and Stanier, 1947; Weech, 1947; Fashena, 1948; Mollison and Cutbush, 1949b; Yudkin, Gellis, and Lappen, 1949; Pickles, 1949; Mollison, 1951; Mollison et al., 1952; Obrinsky, Denley, and Brauer, 1952).

The present work has shown that in ABO heterospecific pregnancies there is a slight but significant increase in the mean cord-blood serum bilirubin value above that for homospecific pregnancies, and that this difference is unaffected by the Rh status of the pregnancy, provided that cases of maternal Rh iso-immunization are excluded. Further, this difference is largely accounted for by an increased proportion of higher readings over 2 mg. bilirubin per 100 ml. serum in the heterospecific group. The most probable explanation of this is that a few of these higher readings in the heterospecific sample are from pregnancies in which mild AB iso-immunization of the mother had occurred although clinically no instance of erythroblastosis foetalis from such a cause was observed. One may speculate that in such rare cases immune anti-A or anti-B agglutinins may have developed in the mothers, and, were they transferred to the foetus over a prolonged period, it might reasonably be expected that some increase in the rate of blood destruction above normal would occur and thus produce a quantity of bilirubin too great to be excreted by a liver lacking in functional reserve.

In the 3,070 consecutive deliveries from which this sample is taken, each case of unequivocal haemolytic disease of the newborn was shown to be due to Rh iso-immunization of the mother. No instance was found to satisfy the criteria of Pickles (1949) for the diagnosis of erythroblastosis due to maternal AB iso-immunization. Few of the recorded cases of haemolytic disease of the newborn alleged to be due to anti-A or anti-B do indeed satisfy these criteria, but this may not be surprising in view of the findings of Mollison (1951; Mollison et al., 1952) that in such cases, even when severe, the Coombs test may be only very weakly positive, or even negative.

It is unfortunate that owing to the distance separating the various units from which this material was drawn the incidence of physiological jaundice was not recorded in the present work, but Davidson et al. (1941), while confirming that physiological jaundice does not necessarily depend on the degree of bilirubinaemia, showed that a direct relationship does exist between the bilirubin value at birth and the peak bilirubin value attained during the first few days of life. Halbrecht (1944) introduced the term icterus praecox to describe jaundice which occurs in infants within 24 hours of birth and in which there is an excellent prognosis, but he did not mention the Rh status of these infants. From examination of cord blood bilirubin values he believed this condition, but not physiological jaundice, to be due to the action of anti-A or anti-B agglutinins in ABO heterospecific pregnancies. On the other hand Fortunato and Rondinini (1950) recorded the incidence of neonatal jaundice in 200 infants and found a very much higher percentage in ABO heterospecific pregnancies than in homospecific pregnancies; they did not distinguish between physiological jaundice and icterus praecox.

Clearly, if AB iso-immunization in heterospecific pregnancies is responsible for an increase in the serum bilirubin value of infants it will inevitably bring some up to the level at which they become classified as "physiological jaundice of the newborn," but it is abundantly plain that ABO heterospecific pregnancy is not a prerequisite for physiological jaundice.
Summary

The mean serum bilirubin value of 1,459 cord blood samples was 1.27 mg. per 100 ml.

The ABO and Rh groups of 1,353 mothers and their infants were determined together with the estimation of the serum bilirubin value of each cord blood sample.

The mean serum bilirubin value in ABO heterospecific pregnancies was found to be 1.36 mg. per 100 ml. and significantly higher than the value of 1.24 mg. per 100 ml. in homospecific pregnancies. This difference was unrelated to the Rh status of the pregnancy. It is suggested that this difference is accounted for by a greater proportion of higher readings over 2 mg. bilirubin per 100 ml. serum in the ABO heterospecific group, some of which are possibly due to mother–child ABO iso-immunization.

I am indebted to Professor D. F. Cappell for his advice and criticism, to Dr. A. C. Spence for affording me facilities in order to perform this work, and to Mr. D. M. Stern for access to the clinical material, to the medical and nursing staff of his department for their co-operation, and to Dr. M. Lubran for his advice on the statistical analysis of the data.

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Medical Research Council War Memorandum, No.9 (1943). London.
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