Immunoglobulins in Umbilical Cord Plasma

III: Haemolytic Disease of Newborn and Respiratory Distress Syndrome

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In a study of the serum proteins in respiratory distress syndrome of the newborn (RDS), Hardie, Heese, and Kench (1965) found that the concentration of electrophoretically separated γ -globulin was significantly reduced below the levels normally found in umbilical cord blood, and fell even further in the first few days of life. Later, Hardie and Kench (1967) demonstrated reduced concentrations of γ -globulin also in the serum of mothers whose infants developed RDS. Low levels of γ -globulin were demonstrable in the mother's plasma throughout pregnancy from about the 16th–20th week of gestation, and rose to normal about 6 weeks after delivery. One suggestion of the authors was that RDS might have an immunological basis, and they showed similar low concentrations of γ -globulin in the umbilical cord sera of 3 infants with haemolytic disease of the newborn.

Sternberg, Dagenais-Perusse, and Dreyfuss (1956) had previously shown that the ratio of fetal to maternal γ -globulin concentration was lower in infants with haemolytic disease than in normal infants. Similarly, Nejedlá (1967) found that the titres of various antibodies acquired transplacentally as well as the γ -globulin concentration were low in infants with haemolytic disease before exchange transfusion, and that those infants who had exchange transfusions showed falling concentrations of γ -globulin and impaired 7S-antibody production for some months after birth.

These previous investigations suggest that the total plasma γ -globulin concentration may be abnormally low in haemolytic disease of the newborn and in RDS, and that in the former the immune response may be impaired for some months if replacement transfusion is carried out.

An investigation is reported here in which the concentrations of the individual plasma immuno-

globulins were measured by a specific immunological technique in infants having either one of the diseases.

Material and Methods

Selection of cases. Maternal and umbilical cord blood specimens were collected from a random group of 16 cases of haemolytic disease of the newborn, and umbilical venous blood only from a further 26 cases. In 39 of the 42 cases the incompatibility was due to the Rhesus D antigen, in one to Rhesus E, and in two to ABO factors. A high titre of antibody (1/40 or over) was recorded during the pregnancy in 28 of the 40 rhesus cases. In 36 instances the infant was treated by replacement transfusion shortly after birth.

The 15 cases of RDS studied were removed from a series of infants who were apparently healthy when born and from whom umbilical venous blood was collected for a study of normal values (Thom, McKay, and Gray, 1967b). RDS was defined for the purposes of this study in the clinical terms described by Troelstra *et al.* (1964) and on the basis of characteristic *x*-ray appearances. All the infants had symptoms of moderate or severe degree. Maternal blood was collected at time of delivery in 10 instances. Three of the infants were over 2500 g. birthweight, and the remainder under this weight, but in the 12 infants, including 2 of the 3 over 2500 g., whose gestational age was known accurately, it was 37 weeks or less.

The statistical analysis for both haemolytic disease and RDS was carried out on a birthweight rather than a gestational age classification of the infants into premature and mature groups, since this allowed inclusion of more cases. The results are compared with those previously found in healthy infants (Thom *et al.*, 1967b).

Quantitation of proteins. Individual plasma proteins were quantitated by single radial diffusion in agar as described previously (Thom, McKay, and Gray, 1967a). In the case of IgA, preliminary screening by double diffusion in agar was carried out.

Absorption of anti-Rh antibodies. Plasma specimens at delivery from 3 women immunized against the

D antigen, and the umbilical cord plasma specimens of their children who were all CDe/cde, were tested before and after absorption with equal volumes of pooled $R_1 R_1$ and $R_2 r$ cells. Two of the maternal plasmas contained high titre incomplete anti-Rh, and the cord plasmas of their children contained this antibody in somewhat lower titre. The total IgG concentrations in these mothers (1803 and 1578 mg./100 ml.) were also higher than the concentrations in the cord specimens (1294 and 984 mg./100 ml., respectively), as would be expected since both were of low birthweight (2268 g. and 2424 g.). Nearly all antibody was removed from these maternal plasmas by two absorptions, and from the cord plasmas by one absorption. The maternal plasma of the third case contained low titre incomplete anti-Rh and the cord plasma of her child gave only a weakly positive reaction in the indirect antiglobulin test. This infant weighed 3459 g. and was born at 40 weeks' gestation, so that the maternal and umbilical cord total IgG concentrations were closer than in the other two pairs (maternal concentration = 1489 mg./100 ml.; infant concentration = 1343 mg./100 ml.). All antibody was removed from both sera by one absorption.

Results in Haemolytic Disease of the Newborn

Quantitation of proteins in maternal plasma.

In 16 specimens of plasma collected at time of delivery from mothers with anti-Rh antibody titres ranging from 1 in 1 to 1 in 60 (9 being 1/40 or higher), the mean concentration of IgG from the log value was 1352 mg./100 ml. with a ± 2 SD range from 890 to 2053 mg. This concentration is not significantly different from the mean level of 1309 mg./100 ml. previously found by us in 60 healthy mothers ($t = 0.511$, $0.7 > p > 0.6$).

Quantitation of proteins in umbilical cord plasma.

The concentrations of albumin, IgG, and IgM in the umbilical plasma of 42 affected infants practically all fell within the ± 2 range found in healthy infants (Fig. a, b, c). Although, in the case of IgG, and IgM, more values fell below the normal mean than above it, no significant difference was found in the mature by weight infants between the mean concentrations of corresponding plasma proteins in healthy infants and those with haemolytic disease (Table I). The concentrations of albumin and IgG in the 42 specimens were signi-

FIG.—Albumin (a), IgG (b), and IgM (c) concentrations in umbilical venous plasma in 15 infants with respiratory distress syndrome $\circ$, and 42 infants with haemolytic disease $\bullet$. Regression of IgG concentration on birthweight with 95% confidence limits for healthy premature infants and mean concentration and 95% confidence limits for healthy mature infants are illustrated.

TABLE I

Comparison of Concentrations of Albumin, IgG, and IgM in Umbilical Cord Plasma of 36 Mature by Birthweight Infants with Haemolytic Disease of Newborn and 44 Healthy Mature by Birthweight Infants

	Haemolytic Disease	Normal	‘t’	
No. of cases	36	44		
Mean birthweight (g.)	3124	3185	0.673	0.6 > p > 0.5
Mean log [plasma albumin concentration (% concentration in reference plasma)]	1.915	1.907	0.491	0.7 > p > 0.6
Mean log [plasma IgG concentration (mg./100 ml.)]	3.179	3.195	0.588	0.6 > p > 0.5
Mean log [plasma IgM concentration (mg./100 ml.)]	0.993	1.024	0.742	0.5 > p > 0.4

ificantly correlated ($r = 0.671$; $p < 0.001$), as was found previously in healthy infants.

In 40 of the infants IgA was not detected in the umbilical cord plasma by double diffusion in agar studies, indicating that if IgA were present its concentration was below 2.6 mg./100 ml. One infant had a concentration of 10 mg./100 ml. and one 46 mg./100 ml. The latter infant also had a slightly raised level of IgM of 35 mg./100 ml.

Relation between maternal and infant IgG concentrations. No significant correlation was found between maternal and cord plasma IgG concentrations in all 16 paired specimens ($r = 0.185$, $p > 0.1$) or in the 13 mature by weight infant-mother pairs ($r = 0.309$, $p > 0.1$).

Absorption of anti-Rh antibodies. The concentrations of albumin and IgG were estimated before and after absorption of anti-Rh antibody from 3 maternal and 3 umbilical specimens as described above. The albumin values were used as an index of dilution of the specimens occurring during absorption, and the IgG concentrations after

absorption were corrected using this factor. As shown in Table II, the alteration in IgG concentration following absorption ranged from -8% to +12% of the original concentration.

Results in Respiratory Distress Syndrome

Quantitation of proteins in maternal plasma.

The mean concentration of IgG in the venous plasma at time of delivery of 10 mothers whose infants developed RDS was (from logs) 1335 mg./100 ml. with a ± 2 SD range from 704 to 2531 mg./100 ml. Since the gestation period of all these mothers was 37 weeks or less, these results were compared with the findings in a group of 22 mothers of similar gestation period who produced healthy infants (mean IgG concentration from logs = 1236 mg./100 ml. ± 2 SD range from 771 to 1982 mg./100 ml.). There was no significant difference between the results in the two groups ($t = 0.747$, $0.5 > p > 0.4$).

Comparison of the albumin concentrations in the two groups of mothers also showed no significant difference (mean RDS concentration (from logs) =

TABLE II

Alteration in Plasma IgG Concentrations Following Absorption of Anti-Rh Antibodies

Specimen	Titre of Incomplete Anti-Rh (D) Antibody	IgG concentration				
		Before Absorption (A) (% reference plasma conc.)	After Absorption (B) (% reference plasma conc.)	(A) Corrected for Dilution (= C) (% reference plasma conc.)	Alteration Due to Absorption of Antibody (C)-(A) (% reference plasma conc.)	% Change Due to Absorption of Antibody $\left(\frac{C-A}{A} \times 100\right)$
I Maternal	1/60	104.5	74.5	68.9	+5.6	+5.3
Umbilical	1/40	67.5	51.0	45.3	+5.7	+8.4
II Maternal	1/60	63.0	41.5	46.5	-5.0	-7.9
Umbilical	1/20	52.5	39.5	38.4	+1.1	+2.1
III Maternal	1/1	110.5	79.5	74.9	+4.6	+4.2
Umbilical	Trace	97.5	80.0	68.7	+11.3	+11.6

61% of concentration in standard reference plasma, ± 2 SD range from 40 to 92%; mean normal concentration = 64%, ± 2 SD range from 47 to 86%; $t = 0.681$, $0.6 > p > 0.5$).

Quantitation of proteins in umbilical cord plasma. As illustrated in Fig. a, b, and c, the concentrations of albumin, IgG, and IgM in 15 affected infants all lay within the ± 2 SD range for healthy infants apart from one high albumin value.

IgA was not detected in any of the specimens.

Discussion

Even in a high titre maternal serum Boursnell, Coombs, and Rizk (1953) found only small amounts of anti-D antibody (5.4 mg./100 ml.), and on the infant's red cells Hughes-Jones, Hughes, and Walker (1967) estimated that 0.4–18 μ g. of anti-D was present per ml. of cells, which is less than 3 mg. for the total circulating red cell mass of an average healthy full-term infant. The total amount of IgG absorbed on the red cells in haemolytic disease of the newborn at any one time is therefore very small compared to the total IgG complement of the adult or of the full-term healthy infant. Even allowing for rapid haemopoiesis in the affected infant, which may increase the utilization of antibody, and even though it is possible that anti-Rh antibody is preferentially transferred to the fetus, the amount of IgG utilized is unlikely to lower the fetal γ -globulin concentration to the levels found by Hardie *et al.* (1965) in 3 infants with haemolytic disease of the newborn.

We were unable to demonstrate any reduction in the concentration of IgG in 3 specimens of maternal plasma and 3 of umbilical cord plasma following absorption of anti-Rh antibody. The antibody titres in these sera were probably lower than that in the serum studied by Boursnell *et al.* (1953) where the total anti-Rh antibody would constitute as little as 0.3–0.5% of the normal maternal IgG concentration. It must be remembered also that the error of the single radial diffusion in agar method of quantitation applies here to the estimation of IgG and also of albumin used to correct for dilution occurring during the absorption. In this laboratory the coefficient of variation for the method of estimation is 6.6% for albumin and 5.0% for IgG.

In the present series the plasma concentration of IgG in mothers whose infants were affected by haemolytic disease was not reduced below the levels found in normal mothers, and in the affected infants, though the greater proportion of the IgG concentrations in umbilical cord plasma fell below the normal mean, the results in the two groups were

not significantly different. These findings differ from results of Nejedlá (1967) who found low γ -globulin levels in haemolytic disease infants, Sternberg *et al.* (1956) who found the infant IgG concentrations low relative to the maternal concentration, and Hardie *et al.* (1965) who reported low concentrations in 3 affected infants. In these 3 series γ -globulin was estimated by paper electrophoresis, so that the results are not strictly comparable with the present series where individual immunoglobulins were quantitated by single radial diffusion in agar. It is of interest that Hardie and Kench (1967) found a large discrepancy between their estimations of γ -globulin concentration on paper electrophoresis and of separate immunoglobulins by specific immunological methods.

In normal healthy infants close correlation exists between the concentrations of IgG in maternal venous and umbilical venous plasma at delivery (Thom *et al.*, 1967b). No significant correlation was found between the corresponding concentrations for the series of haemolytic disease cases studied. The reason for this finding is not clear. It may have been due to changes in the maternal plasma proteins or to a general alteration in the concentrations of fetal plasma proteins as by haemodilution or haemoconcentration, since the close correlation normally found between IgG and albumin concentrations in umbilical cord plasma was still evident in the affected infants.

The raised IgA concentrations found in 2 specimens of umbilical cord plasma are unexplained by the haemolytic disease process, and scrutiny of the maternal case notes revealed no history of infections or other abnormalities during the pregnancy. It is possible, however, that raised concentrations of IgA or IgM in umbilical cord plasma may arise from subclinical infection of the fetus at some stage of pregnancy.

In infants with RDS, Hardie *et al.* (1965) found low concentrations of γ -globulin, and suggested that one possible explanation was the operation of an immunological reaction with utilization of γ -globulin in the infant. However, any single antibody is unlikely to exceed 10% of the total γ -globulin and, as shown above for anti-Rh antibodies, even a high titre of antibodies may comprise a much lower percentage. In the present series of infants with RDS, though more of the IgG concentrations in umbilical cord plasma were below the normal mean than above, they all fell within the 2 standard deviation range of normal values. Also, contrary to the findings of Hardie and Kench (1967), the IgG concentrations in the mothers of these infants were within normal limits for mothers of

healthy infants of similar gestation. The results in the present series neither support nor contradict the suggestion of Markarian, Jackson, and Bannon (1966) that the disease process begins *in utero*.

In 1960, Cooke suggested that RDS was associated with low concentrations of total plasma proteins in umbilical cord blood, and that administration of albumin was beneficial. Fraillon and Kitchen (1962) also found that the serum protein level was related to the development of respiratory distress, but no more closely than was the birthweight of the infant, and the total plasma protein concentration of infants normally rises steadily with increasing birthweight. The findings of Markarian *et al.* (1966) were similar, except that they showed some reduction in total protein concentration in the umbilical cord blood of affected infants apart from that arising from birthweight effects, though the latter were more important. Serum albumin concentration was also reduced in the smaller affected infants in the series of Hardie *et al.* (1965). In the present series, where albumin concentrations were considered in relation to birthweight, no difference was found between distressed and healthy infants.

Summary

The concentrations of IgG, IgA, IgM, and albumin were estimated in the umbilical cord plasma of 42 cases of haemolytic disease of the newborn and 15 infants who developed respiratory distress syndrome.

No differences of statistical significance were found from the concentrations present in healthy infants.

No difference was found in the concentration of IgG in samples of maternal and umbilical cord plasma from cases of haemolytic disease of the newborn before and after absorption of anti-Rh antibodies.

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