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The Incidence and Severity of Jaundice in the Newborn in Salisbury, Southern Rhodesia

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Introduction

It became apparent during 1959 and 1960 that the numbers of newborn babies requiring transfusion to prevent kernicterus in Salisbury, S. Rhodesia, were far above those reported elsewhere.

Mollison (1956) states: " . . . Haemolytic disease of the newborn due to ABO incompatibility has a minimum incidence of one in 150 births; the word minimum is used because the estimate is based on the proportion of infants who develop jaundice. It may be taken as certain that other infants have a mild haemolytic process due to ABO incompatibility, but never display jaundice. However, only one in 2,000 or less of all newborn infants have a sufficiently severe degree of haemolytic disease of the newborn due to ABO incompatibility to require treatment."

Weiner, Wexler and Hurst (1949) say that when the haemolytic disease is due to ABO incompatibility the infant as a rule is not seriously affected.

Valentine (1958) states " . . . that haemolytic disease can occur in clinically recognisable degree as often as once in every 71 births," and describes a series of 14 cases where three were sufficiently affected to require exchange transfusion.

Cunningham (1959), however, from a series of investigations on babies suffering from "physiological jaundice," did not support this view. He also showed that from 1,000 babies investigated, none at full time with normal weight required transfusion and only one (weight 2¾ lb.) required transfusion from physiological jaundice.

In view of the former findings it was decided to correlate results obtained in the laboratory for the years 1959 and 1960.

(1) To establish whether there is an increased incidence of haemolytic disease of the newborn due to immune anti-A or B.
(2) To show whether there is an increased severity of the disease in this area.
(3) To emphasise the need for adequate ante-natal serological investigation of all women, irrespective of Rh group or number of pregnancies.
(4) To provide a scheme of investigation for all jaundiced babies so that the antibody involved, if any, may be identified quickly and compatible blood provided in the shortest possible time.

Materials and Methods

Throughout the period under discussion the sources of grouping sera and the methods used in all tests remained constant.

Groups A, B and O sera were supplied by the South African Institute for Medical Research, Johannesburg. The various Rhesus grouping sera and the Coombs reagent were obtained from the Certified Blood Donor Service Inc., Jamaica 35, New York. The 20 per cent. bovine albumin solution used in titrations, compatibility tests for exchange transfusions and in ante-natal tests was obtained, sterile, in 20 ml. quantities, from Armour Laboratories (Armour & Co. Ltd.), Hampden Park, Eastbourne, England. Merck, Sharpe & Dohme (Division of Merck & Co. Inc.), Philadelphia, P.A., supplied the blood group specific substance A and B for neutralisation tests used in ante-natal and cord blood investigations. The Salisbury and District Blood Transfusion Service donated regular samples of blood from European donors who had all been genotyped as far as possible. This provided a satisfactory panel of blood group antigens for ante-natal antibody screen tests and in investigations involving the identification of an unknown antibody.

The estimation of cord blood haemoglobin was made on an M.R.C. wedge photometer which was kept constantly checked.

The bilirubin estimation employed was that of Malloy and Evelyn (1937), Watson (1946) and Gray (1947), readings being made on a Hilger biochemical absorbtionometer.

(a) *Ante-Natal Tests*

All investigations over the whole of the two-year period were made essentially on women of European descent. Each patient was grouped for ABO and Rhesus (D) by the methods on

pages 140 and 161 of Dunsford and Bowley's *Techniques in Blood Grouping*, 1955. Those that were found to be Rhesus (D) negative were further tested for the antigen Du (Dunsford and Bowley's Technique No. 55).

All specimens of serum whether from Rhesus negative or Rhesus positive patients, were then screened for the presence of haemolysins against A and B cells (Dunsford and Bowley's Technique No. 45 iii). Other blood group antibodies were tested for in saline at 37° C., in 20 per cent. bovine albumin solution at 37° C., and by indirect Coombs test (Dunsford and Bowley's Technique No. 65), against a panel of cells of known groups. Tests in saline at room temperature were not made except in special circumstances where the antibody detected appeared to have an optimum degree of agglutination at a temperature lower than 37° C.

In cases where the haemolysin test was positive the husband's blood was obtained and the blood group determined. When this proved to be of a group unrelated to the wife's haemolysin her serum was tested directly against her husband's cells in saline at 37° C., 20 per cent. albumin solution at 37° C. and by indirect Coombs test to exclude all abnormal antibodies. When, however, the group did correspond to the haemolysin, the wife's serum was subjected to a thermal optimum test against her husband's cells and a neutralisation test, using blood group specific substance A and B (Dunsford and Bowley's Technique No. 45). If either of these were positive her doctor was advised accordingly and cord blood was requested as soon as possible after delivery.

(b) *Cord Blood Tests*

Haemoglobin.—It was found that in infants affected with haemolytic disease of the newborn due to immune anti-A and anti-B, the cord haemoglobin levels were almost always above the average range (normal average range is 15-18 gm. per cent.).

Bilirubin.—The normal range of bilirubin in cord serum is accepted as 0-2.8 mg. per cent. Where the antibody was immune anti-A or anti-B, bilirubin tests were performed daily, and exchange transfusion was given only when the level neared or exceeded 20 mg. per cent.

Group and Rhesus, Dunsford and Bowley's Techniques Nos. 38 and 50.

Direct Coombs Test.—Cells from the cord were washed three to four times in normal physiological saline and a Coombs test done. The Coombs serum was diluted serially from 1/1 to 1/256 in order that an optimum dilution might be routinely present. This was often found helpful in cases of immune anti-A and anti-B sensitisation where the agglutination tended to be stronger in the 1/2 to 1/8 range of dilutions.

Mother's Serum Against Baby's Cells.—When the baby's cord cells gave a negative direct Coombs test the mother's serum was tested directly against them in saline at room temperature and at 37° C., in 20 per cent. bovine albumin solution at 37° C. and by indirect Coombs test as a final check for antibodies. An avid immune anti-A or anti-B in the mother's serum rapidly haemolysed the baby's cells (if appropriate group), in saline at 37° C. in under 30 minutes, and complete or partial haemolysis was taken as an indication of the degree of avidity of the haemolysin.

(c) *Exchange Transfusion*

Fresh compatible donor blood was concentrated just before use by centrifuging, and approximately 100 c.c. plasma removed. It is realised that the use of washed cells might have been preferable, particularly in the case of infants affected by immune anti-A or anti-B, but at present it is not possible to effect this in Salisbury. In ABO affected cases Group O blood was used, with the Rhesus group corresponding to that of the infant and especially selected for low titre non-immune anti-A or anti-B. Where the jaundice appeared to be due to physiological causes, blood of the infant's own group was chosen. Bilirubins were estimated on specimens taken from the cord both at the commencement and at the termination of the exchange transfusion, but all later tests were made on serum obtained from blood taken from heel puncture.

Discussion

The statistics quoted were from full-term infants, all of normal average weight (6-9 lb.), so that prematurity is not a factor that influenced the increased severity of these cases.

It is apparent that the numbers of babies requiring transfusion for jaundice other than that due to Rh antibodies (Table I) are far in excess of the numbers reported by Mollison, Weiner or Valentine, although the ratio of babies affected with haemolytic disease of the newborn due to immune anti-A or anti-B to the total births compares favourably with Valentine and Mollison's figures (Table I).

The incidence and severity of "physiological jaundice" is greater than that reported by

Cunningham. The disease is more severe in Salisbury (S.R.) than is encountered elsewhere.

The infants transfused in the ABO and physiological groups had bilirubins of between 18 and 28 mg. per cent. before exchange transfusion was done. All had been watched clinically from birth and regular bilirubins estimated until it was apparent that the increasing bilirubinaemia was approaching the danger zone for the development of kernicterus.

It is interesting to note that the number of infants with severe hyperbilirubinaemia requiring blood transfusions, caused by ABO haemolytic disease and "physiological jaundice" (Table II), is greater than among those infants requiring blood transfusion from haemolytic disease of the newborn due to Rh antibodies (Table II).

Using Coombs reagent in optimum dilution, it has been found that 44 out of 44 cord bloods were Coombs positive in cases of haemolytic disease of the newborn due to immune anti-A or anti-B. With the system of investigation as illustrated in the representative case shown, there

Table I

	Number of Live Births	RH Babies	ABO Babies	Physiological Jaundice
	5,425	39	44	27
Ratio to live births		1: 139	1: 123	1: 201
Normal expected ratio		1: 200	1: 75	Nil full time (Cunningham)
Requiring transfusion		37	26	19
Ratio to live births requiring transfusion		1: 147	1: 208	1: 285
Normal expected ratio to live births requiring transfusion		1: 300 (Mollison)	1: 2,000 (Mollison)	Nil full time (Cunningham)

Table I shows the number of live births in Salisbury, Southern Rhodesia, during the period January, 1959, to December, 1960.

The number and proportion of infants requiring transfusion is compared with those expected from previously accepted statistics (Mollison; Cunningham).

Table II

Type of Jaundice	Total Numbers	Number Needing One Exchange Transfusion	Per Cent.	Number Needing Two or More Exchange Transfusions	Per Cent.	Total Per Cent. Needing Transfusion
RH	39	25	64.1	12	30.7	94.8
ABO	44	20	45.4	6	13.6	59.0
Phys.	27	14	51.8	5	18.5	70.3

Table II shows the percentage of each group requiring exchange transfusion.

Table III

Total Number of Jaundiced Infants	Total Number Transfused	Transfused for Rh Antibodies	Transfused for ABO and Physiological
110	82	37	45

Table II

Maximum Total Bilirubin	ABO	Physiological
16-18 mg. per cent.	2	—
18-20 mg. per cent.	6	1
20-22 mg. per cent.	6	6
22-24 mg. per cent.	5	5
24-26 mg. per cent.	4	3
26-28 mg. per cent.	3	4
	26	19

Case History

Mrs. S—

Antenatal: Group "O" Rh positive (D).

Rh antibodies: Nil. (Panel of known cells: Saline 37° C., 20 per cent. albumin 37° C., Trypsin, indirect Coombs test techniques.)

Haemolysin test: Positive against A cells.
Husband's group: A Rh positive (D).
Thermal optimum test against husband's cells—

	4°	22°	37°
Titre	64	128	512

Immune anti-A.

Cord blood: Haemoglobin = 145 per cent. = 26.46 gm. per cent.

Direct Coombs test (Coombs serum in serial dilution): Positive 1/2 to 1/16.

Group "A" Rh positive (D).

Bilirubin: Total, 3.1 mg. per cent.; indirect, 2.5 m. per cent.; direct, 0.6 mg. per cent.

	Saline R.T.	20 per cent. Albumin 37° C.	Haemolysin 37° C.
Mother's serum x baby's cells	+	+	++

Mother's serum neutralised with A and B specific substance—

	Saline 4°	Albumin 37° C.	Indirect Coombs	Haemolysin
x baby's cells	—	—	+*	—
x O panel	—	—	—	—
x A cells	—	—	+	—
x B cells	—	—	—	—
x husband's cells	—	—	+	—

Baby's bilirubin (mg. per cent.)—

	Second Day	Third Day	Fourth Day
Total	8.2	15.8	20.1
Direct	0.8	1.2	1.0
Indirect	7.4	14.6	19.1
	Post Transfusion	Fifth Day	Sixth Day
Exchange transfusion given	9.4	14.6	11.0
	0.6	0.9	0.6
	8.8	13.7	10.4

* If the baby's cells are positive by direct Coombs test, this reading will be positive in any case and may be left out of the series.

should be no difficulty in conclusively demonstrating the presence of active immune anti-A or anti-B.

From the statistics given in Figs. 1, 2, 3 and 4 there is some doubt as to whether the ABO-affected infants requiring transfusion may not in fact have superimposed "physiological jaundice" as well.

The reasons for the higher incidence of severity of ABO haemolytic disease of the newborn and of "physiological jaundice" in Salisbury may be accounted for in at least two ways.

(1) *"Physiological Jaundice"*

The average haemoglobin estimation in this group ranges between 18.07 gm. per cent. and 22 gm. per cent.

The possibility of dehydration was considered during the course of investigation, as on these babies it was noted on many occasions that the bilirubin concentration fluctuated in unison with the clinically assessed degree of hydration or dehydration.

The regime adopted at the maternity hospital was found to be:

From Birth—

3-5 lb.: No fluids for 48 hours.
5 lb. +: No fluids for 24 hours.
Then 2 oz. (approximately) of total fluids per lb. body weight is given in 24 hours.

This is less than the amount given in temperate climates (15-19 oz. in 24 hours). Also the nursery is mainly glass enclosed and average temperature throughout the year is 78°-80° F. It is suggested that the fluid intake of these babies is insufficient, especially as the humidity in Salisbury (Meteorological Office) is sometimes as low as 10 per cent. and the average summer temperature range 70°-90° + and in winter mean temperature of 56.5°.

(2) *Immune Anti-A and Anti-B.*

The majority of the adult European population in S. Rhodesia receive inoculations such as T.A.B., yellow fever and smallpox vaccination on repeated occasions. This may stimulate the production of all types of antibodies, including the ABO antibodies. From Table I it may be noted that the ratio of Rh-affected infants (1:139 of all pregnancies) is high and the percentage (94.8 per cent.) requiring exchange transfusion is greater than encountered elsewhere. Whether this may be explained by the increased heterologous antibody production due to inoculations is a matter for conjecture.

As stated earlier, the possibility of superimposed "physiological jaundice" due to dehydration must always be considered, although for the purpose of exchange transfusion it is advisable to treat the individual case as being ABO-affected.

Conclusions

(1) It has been shown that the jaundice among full-term infants of normal birth weights in Salisbury (S.R.) is far greater than that reported by Mollison, Weiner and Cunningham in other countries.

(2) Although the incidence of ABO-affected babies is less than that reported by Valentine, the proportion of infants with sufficient jaundice requiring exchange transfusion is far higher.

(3) Hypotheses have been put forward in an attempt to explain this high incidence and severity of jaundice.

(4) It could not be proved that the severity of jaundice in the ABO-affected infants was entirely due to immune anti-A or anti-B, or whether "physiological jaundice" of dehydration origin was a contributory factor.

(5) (a) To conclude, we should like to emphasise and confirm the findings of Valentine: that infants should be closely watched for haemolytic disease of the newborn due to immune anti-A or anti-B and not simply dismissed as cases of "physiological jaundice."

(b) We would go further and say that in Central Africa all European women, whether Rhesus positive or Rhesus negative, should be checked ante-natally for antibodies and haemolysins, and if the presence of immune anti-A or anti-B is found and the husband is of significant group, the cord blood of the baby should be investigated at birth.

(c) That dehydration should be seriously considered as a cause of the increased bilirubinaemia in all groups of jaundiced babies reported in this paper.

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