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ABO — Rh Interaction

A study of five cases *

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SUMMARY

Five families with cases of erythroblastosis foetalis have been studied for the ABO, Rh, MN, and Kell-Cellano blood group systems. In all of them, the father and mother were found to be compatibly mated, and so were the children with their mother, in all systems, except Rh.

Most children were of CDe/cde type, and all mothers cde/cde. The influence of other systems (MN and Kell-Cellano) on Rh incompatibility remains to be investigated. They might play a role in counteracting the effect of Rh incompatibility.

The antibody titre in the mother's blood has clearly appeared to decline gradually, with an increasing time gap between successive pregnancies.

Whether the possibility of having a normal full term pregnancy, resulting in the birth of a healthy child, increases, if the time gap between two successive pregnancies is larger, remains to be investigated.

Introduction

The haemolytic disease of the newborn is determined by a blood group incompatibility between mother and child; mostly, Rh or ABO incompatibility. There is considerable evidence that the most serious cases of haemolytic disease of the newborn are due to Rh factor, especially Rh factor D incompatibility.

In a case of haemolytic disease due to Rh factor D incompatibility, the foetus would derive its gene for factor $Rh^+(D^+)$ from its father; i.e., both father and foetus would be Rh (D) positive. The mother in such cases would be Rh negative, and to her the factor would be antigenic. The transfer of any foetal cell into the mother's circulation might result in sensitization, i.e., antibodies will be produced, and the passage of sufficient quantities of them across the placenta into the foetal circulation would bring about a premature destruction of foetal red cells, which are Rh (D) positive.

In 1943, Levine observed that cases of haemolytic disease of the newborn are more frequent in ABO compatible than ABO incompatible cases. This can be explained as follows:

If the erythrocytes (red cells) entering maternal circulation from the foetus are of a compatible ABO group, they are likely to be accepted and to have a normal survival time. Such cells will accumulate, and will be much more likely to cause

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sensitization than will the erythrocytes of an incompatible ABO group, which would be promptly eliminated. An ABO incompatible pregnancy may be defined as a pregnancy in which the foetus has factors A or B and the mother a so-called " naturally occurring " agglutinin against one of these factors. Antibodies produced by the mother pass across the placenta, enter the foetal circulation and bring about the destruction of foetal red cells.

The protective effect of ABO incompatibility in Rh incompatibility between mother and foetus, in respect of pregnancy induced isoimmunization, has been recognized since the publication of Levine's paper in 1943. This has been confirmed by other workers (Levine, 1948; Glass, 1949; Malone, 1949; Lucia et al, 1950*a*, 1950*b*, 1950*c*; Brendemoen, 1952; Race, 1952; Nevanlinna, 1953; Mollison, 1954; Potter, 1954; Heisto, 1955; Nevanlinna and Vaino, 1956; Stern et al, 1956; Allen and Diamond, 1958; Clarke et al, 1958; Levine, 1958; Cohen and Glass, 1959; Cohen, 1960; Anderson et al, 1961; Kirk, 1961; Mourant, 1961; Thomson, 1961; Reepmaker et al, 1962; Sundal, 1962; Goldsmith, 1963; Levine et al, 1963; Donohue and Wake, 1964; Newcombe, 1964; Race, 1965).

Stern et al (1956) introduced Rh positive blood to Rh negative male volunteers and showed that antibodies were produced more often in cases where the recipient was of a compatible rather than incompatible ABO blood group.

Cohen (1960), using two sets of Rh negative mothers, Rh sensitized and Rh non-sensitized, proved that ABO incompatibility does give protection against Rh incompatibility.

In the present report, five cases have been studied. Beside blood groups, the mother's serum was subjected to titration for estimating the amount of antibodies present at the time of investigation.

Method

Whole blood specimens of the father, mother and all alive children were obtained in dry sterile test tubes, numbered serially by finger-prick method. Case histories were obtained from the hospital record. Birth ranks of all children (living or dead) were derived from the parents. Information concerning any previous obstetric difficulties, history of any previous blood transfusion, whether the children suffered from any disease and the clinical history of the child after birth was also obtained from the parents.

After collecting blood samples, the tubes were sealed by means of rubber bungs and were immediately put into a beaker inside a thermos containing ice to prevent any possible haemolysis and contamination. They were immediately taken to the Serological Laboratory of the University of Delhi. Maternal blood serum was separated by centrifugation to determine the titre of the antibodies, if present. After separation of the serum, the clotted blood specimens were suspended in normal saline (0.85%) and washed three times by centrifugation at 1000 r.p.m. The washed cells were resuspended in normal saline to make a 2% cell-suspension.

Blood specimens were then subjected to the grouping tests for the ABO, MN, Rh and Kell-Cellano blood group systems. All $D^-(Rh^-)$ samples were further tested for D^u.

The serum was titrated in saline just like anti-A, anti-B and anti-H with one variation, i.e., instead of the tile, precipitin tubes were used for the titration. To the diluted serum, one drop of freshly prepared O, Rh^+ cell suspension was added to each of the tubes. The tubes were then gently shaken for about five minutes to ensure through mixing of the cell suspension and the serum. The tubes were then kept in the incubator for 1 ½ hr at 37°C. After incubation, the tubes were centrifuged. The cell-serum mixture was then examined under a powerful microscope for possible agglutination reaction. In case no reaction was observed, the specimen was centrifuged once more. The supernatant fluid was pipetted out. The cells were then washed three times in normal saline solution. After the last wash, the supernatant saline was pipetted out completely. The button of cells was then injected to a drop of Coomb's anti-human globulin serum on a flat white opal glass tile. The cells were mixed with Coomb's reagent by means of a small glass stirer. The tile was gently shaken, and after about 10 minutes the results were declared. Indirect Coomb's test is performed to observe the presence of any incomplete antibody capable of coating cells, which would otherwise escape attention. The titre of the serum is the reciprocal of the greatest dilution of the serum at which agglutination reaction was observed.

Case Histories

Case No. 1 (Family 1)

First child: female.

The baby developed jaundice after birth. The condition of the baby was such that she could not be given exchange transfusion and died on 11.12.'62.

Diagnosis: Rh-incompatibility.

Second child: male, born on 18.12.'63.

Two expert doctors were consulted from the third month of pregnancy at every six weeks' interval. No complications; the baby was normal at birth and is in good health now.

The mother did not receive any blood transfusion.

Case No. 2 (Family 2)

First child: male, 5 years. Normal full term delivery.

Second child: male, was born at home on 10.8.'62 at 9 p.m., developed jaundice and was admitted to the hospital.

Expired on 3.9.'62.

Third child: female, born on 7.9.'63. Jaundiced at birth. The baby was saved by exchange transfusion and is in good health now. The mother did not receive any blood transfusion.

Case No. 3 (Family 3)

First child: normal full term baby (female) died after birth, 1958.

Cause of death: unknown.

Second child: male, born in 1962 at home. The baby developed jaundice and was taken to the hospital. Since there was no improvement, he was taken by the father to another hospital. Exchange transfusion was given and the baby was saved. The child, however, died at about the age of 1 year.

The mother did not receive any blood transfusion.
Third child: female, born on 22.8.'64.
Jaundiced at birth.
Diagnosis: Rh-incompatibility.
The child was given exchange transfusion, improved, and is now in good health.

Case No. 4 (Family 4)

Female child, born on 8.1.'64.
Diagnosis: Icterus Gravis Neonatorum.
Jaundice after three days (10.1.'64).
Serum bilirubin – 16.4 mg% on 10.1.'64.
Serum bilirubin – 6.62 mg% on 13.1.'64.
Previous pregnancy: one full term normal delivery; male child, alive, aged 2.
Exchange transfusion was not given.
Husband: O, Rh.
Mother: O, Rh^-.
The mother did not receive any blood transfusion.

Case No. 5 (Family 5)

First child: female, born on 17.4.'51, normal full term pregnancy.
Second child: female, born on 18.12.'54, full term pregnancy, mild jaudice after birth.
Mother did not have any blood transfusion.
Third child: female, born on 20.2.'62.
Severe jaundice at birth.
Diagnosis: Icterus Gravis Neonatorum.
The child improved, and is now in good health.
The investigation of these cases was done during November, 1964 to April, 1965.

Results

The results of the tests for different blood group systems of all surviving members of the family are given in Tab. I. It will be observed that, in all cases presented, the parents were compatibly mated for the ABO and other blood group systems, except for Rh, and that the children also had ABO, MN and Kell-Cellano blood groups compatible with their mother. This is in conformity with the findings of the other workers. It is observed that all mothers were of the genotype ccddee (cde/cde) for the Rh system. Whether cde/cde mothers are more susceptible to Rh isoimmunization, cannot be said as yet, due to the small number of cases examined.

The serum of the mothers examined for the presence of Rh antibodies, showed in one case a titre of 8 192, while in two others 32 and 64 respectively. No antibodies could be detected in the serum of the other two mothers. It is probable that the Rh titre increases with each pregnancy, if the time gap between one pregnancy and the other is too small. The amount of antibodies declines gradually as the time gap becomes larger.

Tab. I. Results of the blood grouping tests

		Titre of the Rh antibodies in serum
Family 1		
Father	O, MM, CcDEe, kk	
Mother	AB, MM, ccddee, kk	—
Child	B, MM, ccDdEe, kk	
Family 2		
Father	O, MM, CCDee, kk	
Mother	B, MM, ccddee, kk	—
Child — 1	O, MM, CcDdee, kk	
Child — 2	B, MM, CcDdee, kk	
Family 3		
Father	A, MM, CCDee, kk	
Mother	A, MM, ccddee, kk	8,192
Child	A, MM, CcDdee, kk	
Family 4		
Father	O, MM, CcDee, Kk	
Mother	O, MN, ccddee, Kk	64
Child — 1	O, MN, CcDdee, Kk	
Child — 2	O, MM, CcDdee, Kk	
Family 5		
Father	A, MN, CcDee, kk	
Mother	A, MN, ccddee, kk	
Child — 1	A, MN, CcDdee, kk	32
Child — 1	O, MN, CcDdee, kk	
Child — 3	A, MN, CcDdee, kk	

Discussion

Investigations by different workers have established that ABO incompatibility does give protection against Rh incompatibility. This protection, according to Donohue and Wake (1964) is not absolute. Anderson et al (1961) reported two families in which both infants were ABO incompatible with the mother and the second infant had erythroblastosis. Donohue and Wake (1964) also reported some such cases, where the infants were ABO incompatible with the mother. They concluded: "... It is likely that passage of foetal cells into the maternal circulation, either during pregnancy or at delivery, is a relatively common occurrence. There must, however, be wide variation in the susceptibility of the mothers to isoimmunization". In the five cases studied in the present report, the husband and wife are found to be compatibly mated

for all blood systems (ABO, MN, and Kell-Cellano) except for Rh, and so were the children with their mother.

Murrary (1957) provided very strong evidence that cDE/cde children are more prone to be affected by haemolytic disease of the newborn than are CDe/cde children and that their disease is more severe. A study of 320 Western Australian families with haemolytic disease did not show the same disturbance (Kirk et al, 1959). In the five families studied in Delhi, most children were of the CDe/cde type. Since the number of families studied is too small, it is not possible to make any definite conclusion in this respect.

Stern and Berger (1960) found that injections of Rh positive ABO compatible cells fail to stimulate anti-D, if they have previously been sensitized with anti-D. Finn et al (1961 and 1962) have remarked a very promising series of experiments directed at a possible method of preventing Rh haemolytic disease. They have observed that injected Rh positive blood survives less well in the Rh negativee male volunteers if the injection is followed by another of anti-D. Clarke et al (1963) concluded: "We are hopeful, however, that the technique will prevent most cases of Rh immunization and thus in time help to eliminate Rh haemolytic disease of the newborn ". Davidson (1966) also expressed a similar view and concluded: "... It is possible that prevention of sensitization to the Rh factor which had been tried unsuccessfully many times in the past, may finally become a reality... ". Studies by Woodrow et al (1965) and Clarke et al (1966) indicate that protection against Rh immunization is possible. Small doses of anti-D gamma-globulin from hyperimmunized volunteers seems to be effective. Another possibility which seems probable in preventing haemolytic disease of the newborn is to increase the time gap between two pregnancies.

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References

Allen F. H., Diamond L. K. (1958). Erythroblastosis Foetalis. Little, Brown and Co., Boston and Toronto.

Anderson J., Bentzon M. W., Larsen S. O. (1961). ABO incompatibility and Rh immunization in pregnancy. Vox Sang., **6:** 3-20.

Brendemoen O. J. (1952). Some factors influencing Rh immunization during pregnancy. Acta Path. Microbiol. Scand., **31:** 579-583. (cf. Race and Sanger, 1962).

Clarke C. A., Finn R., McConnell R. B., Sheppard P. M. (1958). The protection afforded by ABO incompatibility against erythroblastosis due to Rhesus anti-D. International Archives of Allergy and Applied Immunology, **13:** 5-6, Proc. of the British Association of Allergists.

— Donohue W. T. A., McConnell R. B., Woodrow J. C., Finn R., Krevans J. R., Kulke W., Lehane D., Sheppard P. M. (1963). Further experimental studies on the prevention of Rh haemolytic disease. Brit. Med. J., **1:** 979-984.

— Finn R., Lehane D., McConnell R. B., Sheppard P. M., Woodrow J. C. (1966). Doses of anti-D gamma-globulin in prevention of Rh-haemolytic disease of the newborn. Brit. Med. J., **1:** 5481 (213-214) (cf. Excerpta Medica, Vol. 19; Section 4, p. 1546, 1966).

Cohen B. H., Glass B. (1959). The relation of ABO and Rh blood groups to differential reproduction. J. Med. Educ., **34:** 414-417.

— (1960). ABO-Rh interaction in an Rh-incompatibly mated population. Amer. J. Hum. Genet., **12:** 180-209.

— (1962). ABO incompatibility and Rh immunization. Amer. J. Hum. Genet., **14:** 433.

Davidsohn I. (1966). Recent advances in blood group incompatibility of the newborn. Illinois Med. J., **129:** 39-42.

Donohue W. L., Wake E. J. (1964). Effect of ABO incompatibility on pregnancy induced Rh immunization. Canad. Med. Ass. J., **90:** 1-5.

Glass B. (1949). The relation of Rh incompatibility to abortion. Amer. J. Obstet. Gynec., **57:** 323-332.

Goldsmith K. L. G. (1963). Blood Transfusion Serology. In: Clinical Aspects of Immunology. Ed. by Gell and Coombs, **7:** 162-193.

Finn R., Clarke C. A., Donohoe W. T. A., McConnell R. B., Sheppard P. M., Lehane D., Kulke W. (1961). Experimental studies on the prevention of Rh-haemolytic disease. Brit. Med. J., **1:** 1486-1490.

— — McConnell R. B., Woodrow J. C., Kulke W., Lehane D., Sheppard P. M. (1962). Isoimmunization in the rabbit with antibody-coated erythrocytes. Nature (London), **193:** 990-992.

Heisto H. (1955). The relation of the ABO blood groups to Rh immunization in pregnancy with special reference to the A_1 A_2 sub-groups. Acta Path. Microbiol. Scand., **36:** 257-262 (cf. Race and Sanger, 1962).

Kirk R. L., Vos G. H., Kelsall G. A. (1959). Rh genotype of the father and severity of haemolytic disease of the newborn. Brit. J. Haemat., **5:** 158-168.

— (1961). Blood group interaction and the world distribution of the ABO gene p^2 and the gene r (cde). Amer. J. Hum. Genet., **13:** 224-232.

Levine P. (1943). Serological factors as possible causes of spontaneous abortions. J. Hered., **34:** 71-80.

— (1948). A Survey of the Significance of the Rh Factor. In: The Rh Factor in the Clinic and in the Laboratory. Ed. by Joseph M. Hill and William Dameshek. Grune and Stratton, New York.

— (1958). The influence of the ABO system on Rh haemolytic disease. Hum. Biol., **30:** 14-28.

— Vogel P., Rosenfield R. E. (1953). Haemolytic disease of the newborn. Advances Pediat., **6:** 97-156.

Lucia S. P., Hunt M. L. (1950*a*). The significance of ABO compatibility and its relationship to the intensity of Rh immunization. Blood, **5:** 767-772.

— — (1950*b*). Hemolytic disease of the newborn. I. An analysis of a sample of an obstetric population. J. Lab. Clin. Med., **35:** 24-27.

— — (1950*c*). Hemolytic disease of the newborn. II. An analysis of maternal antipartum factors in a sample of sensitized Rh negative women in relation to the clinical condition of the child. J. Lab. Clin. Med., **35:** 28-34.

Malone R. H. (1949). ABO incompatibility between husband, wife and its relation to Rh immunization. Brit. J. Soc. Med., **4:** 228-229.

Mollison P. L. (1954). Clinical consideration. Part 2. In: The Rh Blood Groups and their Clinical Effects. Medical Research Council Memor. No. 27, London. Her Majesty's Stationery Office, pp. 21-46.

Mourant A. E. (1961). Blood group incompatibilities in genetical counselling. Proc. 2nd. Int. Congr. Hum. Genet., Rome, **3:** 1974-1981.

Murray S. (1957). The effect of Rh genotype on severity in haemolytic disease of the newborn. Brit. J. Haemat., **3:** 143-152 (cf. Race and Sanger, 1962).

Nevanlinna H. R. (1953). Factors affecting maternal Rh immunization. Ann. Med. Exp. Finn., Suppl. 2, **31:** 60 (cf. Race and Sanger, 1962).

— Vaino T. (1956). The influence of mother-child ABO-incompatibility on Rh immunization. Vox Sang., **1:** 26-36.

Newcombe B. H. (1964). Risk of fetal death to mothers of different ABO and Rh blood types. Amer. J. Hum. Genet.

Potter E. L. (1954). Erythroblastosis foetalis. Report of the 7th M and R Pediatric Research Conference, 78 (cf. Allen and Diamond, 1958).

RACE R. R. (1952). The Rh Groups. Part I. In: The Rh Blood Groups and their Clinical Effects. Medical Research Council Memor. No. 27, London. Her Majesty's Stationery Office.
— (1965). Contributions of blood groups to genetics. Proc. Roy Soc. (Biol.). **163:** 151-168.
— SANGER R. (1962). Blood Groups in Man. 4th Ed. Blackwell Scientific Publ., Oxford.
REEPMAKER J., NIJENHUIS, L. E., VAN LOGHEM J. J. (1962). The inhibiting effect of ABO incompatibility on Rh immunization in pregnancy: a statistical analysis of 1742 families. Amer. J. Hum. Genet., **14:** 185-198.
STERN K., DAVINSOHN I., MASITIS L. (1956). Experimental studies on Rh immunization. Amer. J. Clin. Path., **26:** 835-843.
— BERGER M. (1960). Experimental isosensitization to hemoantigens in man. Abstract. 13th AABB Program, 39 (cf. Race and Sanger, 1962).
SUNDAL A. (1962). Erythroblastosis foetalis: a survey of 491 consecutive cases of Rh immunization in pregnancy. I. Stillbirths due to erythroblastosis foetalis. Acta Paediat. (Uppsala), Suppl. 135, **51:** 203-208. (Excerpta Medica, **16:** 84, 1963).
THOMSON E. B. (1961). A Short Text Book of Haematology. Pitman Medical Publ. Co. Ltd., London, pp. 46-56.
WIENER A. S., NAPPI R., GORDON E. B. (1953). Studies in Rh sensitization. VI. ABO blood groups and Rh subtypes in sensitized and non-sensitized Rh negative pregnant women. Blood, **8:** 1024-1028.
WOODROW J. C., CLARKE C. A., DONOHOE W. T. A., FINN R., MCCONNELL R. B., SHEPPARD P. M., LEHANE D., RUSSEL S. H., KULKE W., DURKIN C. M. (1965). Prevention of Rh-haemolytic disease: a third report. Brit. Med. J., **1:** 279-283.
VAUGHAM V. C., ALLEN F. H. Jr., DIAMOND L. K. (1950). Erythroblastosis fetalis. IV. Further observations on Kernicterus. Pediatrics, **6:** 706-716 (cf. Allen and Diamond, 1958).

RIASSUNTO

Sono state studiate 5 famiglie con casi di eritroblastosi fetale, in relazione ai gruppi sanguigni ABO, Rh, MN e Kell-Cellano. Tutti i genitori sono risultati compatibili tra di loro, e così pure i figli nei confronti delle madri, tranne che per il fattore Rh.

La maggior parte dei figli erano del tipo CDe/cde, e tutte le madri cde/cde. L'influenza di altri sistemi (MN e Kell-Cellano) sull'incompatibilità Rh è ancora da valutare. Questi potrebbero avere un ruolo nel neutralizzare l'effetto dell'incompatibilità Rh.

La titolazione anticorpale nel sangue materno ha chiaramente presentato un graduale declino, aumentando l'intervallo di tempo tra le successive gravidanze. Resta ancora da vedere se la possibilità di avere una gravidanza normale a termine, con la nascita di un figlio sano, aumenta quando l'intervallo di tempo tra le successive gravidanze è più ampio.

RÉSUMÉ

Cinq familles avec des cas d'érythroblastose fétale ont été étudiées en relation aux groupes sanguins ABO, Rh, MN et Kell-Cellano. Tous les parents sont résultés compatibles entre eux, et aussi les fils en relation aux mères, sauf que pour le facteur Rh.

La majorité des fils étaient du type CDe/cde, et toutes les mères cde/cde. L'influence d'autres systèmes (MN et Kell-Cellano) sur l'incompatibilité Rh doit encore être évaluée. Ces systèmes pourraient jouer un rôle dans la neutralisation de l'effet d'incompatibilité Rh.

Le titrage anticorpal dans le sang maternel a présenté une diminution graduelle, en relation à l'augmentation de l'intervalle de temps entre les grossesses successives. Il reste à voir si la possibilité d'avoir une grossesse normale à terme, avec naissance d'un fils sain, augmente avec l'augmentation de l'intervalle de temps entre les grossesses successives.

ZUSAMMENFASSUNG

Bei 5 Familien mit Fällen von Fetal-Erythroblastose wurden die Blutgruppen ABO, Rh, MN und Kell-Cellano untersucht. Mit Ausnahme für den Rh-Faktor bestand keine Unverträglichkeit weder zwischen irgendeinem der Elternpaare noch jemals zwischen Mutter und Kind.

Die meisten Kinder gehörten dem Typ CDe/cde und alle Mütter dem Typ cde/cde an. Der Einfluss anderer Systeme (MN und Kell-Cellano) auf die Rh-Unverträglichkeit ist noch nicht festgestellt, doch wäre es möglich, dass gerade sie die Unverträglichkeitswirkung des Rh neutralisieren.

Bei Anstieg des Zwischenraums zwischen den weiteren Schwangerschaften war deutlich ein allmähliches Absinken des Antikörper-Titers im mütterlichen Blut festzustellen. Es wäre noch zu untersuchen ob bei grösserem Zwischenraum zwischen den folgenden Schwangerschaften die Möglichkeit besteht, eine vollausgetragene Schwangerschaft und Geburt eines normalen Kindes zu erreichen.

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