A SURVEY OF RECENT DEVELOPMENTS IN BLOOD TRANSFUSION

PART II

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3. Blood Grouping and Compatibility Tests; The Study of Blood Group Antigens and their Practical Importance, especially the Rh Factor

Considerable advances have been made in blood grouping and serological technique. Blood grouping may, on occasion, have to be done by the clinician. Unfortunately, many transfusion fatalities have resulted from blood grouping errors made by inexperienced workers. Nothing is more lamentable than the way these tests have been light-heartedly undertaken by inexperienced workers, with little realization of the hazards involved. In ABO grouping tests, highly potent sera with good avidity are essential. For absolute accuracy, tests must be carried out on both cells and serum. In mass grouping tests when some hundreds of blood samples are ABO grouped in a day, a useful additional control is the checking of all cells tested with a potent group O serum (high titre alpha and beta) because even the best technician may, on rare occasions, inadvertently omit to put the anti-A and anti-B sera up with some cells under test. Care must be taken to distinguish group AB, especially A_2B and A_3B from group B blood. Control A, B and O cells must be used in the tests. Controls in saline of the various cells under test should be put up. Clean, fresh blood samples are essential and clotted blood is the ideal (about 3 cc.). A detailed discussion of technique is out of place here, but any clinician who is likely to have to perform ABO blood grouping or compatibility tests should first undergo instruction. The only technique upon which, virtually, absolute reliance can be placed is grouping in tubes. Recording of results must always be checked. Blood grouping on tiles or glass slides, especially on cells only, is not a reliable technique. Compatibility tests by a slide technique are quite unreliable.

In order to ensure even bleeding of donor panels, and in the interests of safety, only blood which is homologous with that of the recipient in the ABO (and Rh) system should be transfused.

The transfusion, for instance, of group O blood containing potent anti-A or anti-B isoagglutinins or both, to recipients of groups AB, A or B, may be dangerous since sufficient destruction of the recipient's cells may occur to cause a disastrous reaction. Likewise, neither group A blood with high titre anti-B, nor group B blood with high titre anti-A should be given to AB recipients. The sera of all A, B and O donors should be titrated for high titre anti-A or anti-B isoagglutinins. The blood of those donors whose sera contain potent anti-A or anti-B isoagglutinins should not be issued to blood banks, but should be used for grouping sera. If a high titre serum because of zoning or haemolysis is not suitable for grouping serum, the donor blood can be used for strictly homologous transfusion, e.g., A to A, etc.

Although compatibility tests have been greatly improved in recent years, no absolutely reliable technique has, as yet, been devised. Certainly, compatibility tests in which a saline suspension of donor's cells are mixed with recipient's serum cannot be completely relied upon. On the other hand, much greater reliance can be placed upon compatibility tests carried out in albumin, or, alternatively, using the Coombs' test. This is the most reliable compatibility test yet devised. The albumin technique may fail sometimes because of zoning of the recipient's serum which should, therefore, be diluted once with AB Rh-negative serum. Very rarely, cross-matching of blood may prove an exceedingly difficult procedure and such cases are really research problems and should be dealt with by highly trained laboratory workers. Nevertheless, cases occur in which these techniques fail. I have encountered such cases, but must point out that the Rh factor was involved, and had Rh tests been done before transfusion, disastrous reactions would not have occurred. The patients were Rh-negative and were given Rh+ blood, sometimes persistently, despite febrile reactions. Compatibility tests, using saline suspensions of donor cells mixed with

equal volumes of recipients' sera, failed because of the presence of Rh blocking antibody in the recipient's sera. Blocking antibody sensitizes cells but does not agglutinate them. Other patients were Rh-negative mothers who had been pregnant with Rh+ fetuses. Transfusions of these mothers with Rh+ blood resulted in haemolytic reactions and a few days after transfusion Rh antibodies in the recipients' sera were readily detected by tests in albumin, or by the Coombs' test. Pre-transfusion samples of serum of these mothers were available and compatibility tests, in saline, albumin, or by the Coombs' technique, revealed no incompatibility with the donor bloods or random Rh+ bloods. However, the transfusion of Rh+ blood very rapidly evoked the Rh antibody. All traces of Rh antibodies, as can be detected by present tests, may disappear from a recipient's serum, yet a single injection or transfusion of blood containing the offending Rh antigen, perhaps many years later, will rapidly evoke the Rh antibody and destruction of the transfused blood will then quickly occur, perhaps with disastrous result. This is the anamnestic reaction. On this account Rh-negative females of childbearing or pre-childbearing age (even if infants) must never be transfused or injected with Rh+ blood, for if sensitization to the Rh factor occurs, and years later pregnancy occurs with an Rh+ foetus, the Rh agglutinins may be evoked and the foetus harmed. These facts, therefore, should be borne in mind by all concerned with transfusions. The rule of thumb is a simple one, namely, *never give an Rh-negative recipient, or a recipient whose Rh group is not known, any blood other than Rh-negative blood.* If the latter is not available, tide the patient over the emergency on plasma transfusion. Some 15 per cent. of European whites are Rh-negative. Since Rh-negative blood is scarce, *it is essential that all prospective recipients of transfusion should be ABO grouped and Rh typed, so that blood homologous in the ABO and Rh systems can be transfused.* Out of every 100 donors bled, only five or six will be O Rh-negative. There are as many A Rh-negative as O Rh-negative persons. Since O Rh-negative blood is such a valuable and scarce commodity, it is quite unjustifiable to transfuse it needlessly to recipients of groups AB, A or B, who should receive blood of the same ABO group as that to which they belong. *Very seldom will difficulty arise with compatibility tests if care is taken to select blood of the same ABO and Rh group as that of the recipient.*

The discovery of the Rh factor (Landsteiner and Wiener, 1940) is the greatest advance made since the discovery of the ABO groups. Its discovery has stimulated search for other antigen-antibody systems with the result that such systems

as those of Lewis, Kell, and Lutheran, which are of clinical importance have been discovered and, doubtless, others exist.

The literature on the Rh factor is now vast, but some of the developments of importance to clinician and laboratory worker may be mentioned here. The fallibility of cross-matching tests in saline in the presence of Rh blocking antibody has been mentioned. Blocking antibodies are readily detected by tests in albumin media (see Mollison *et al.*, 1948). Agglutination occurs roughly in two stages: (1) the cells acquire agglutinins; (2) the cells then agglutinate. When cells acquire blocking antibody, they become sensitized, but agglutination, i.e. the second stage, does not occur in saline. Coombs *et al.* (1945) devised a test for detection of sensitization of red cells. The iso-antibodies occur in the globulin fraction of serum. By injecting human serum into rabbits, a rabbit anti-human-globulin serum can be produced. Sensitized red cells, i.e. cells which have absorbed Rh blocking antibody, can be made to agglutinate by mixing them with suitably prepared rabbit anti-human-globulin serum. This test, known shortly as the Coombs' test, is a very great advance for it is an extremely sensitive instrument for detecting sensitization of red cells. It is commonly used in testing the red cells of a new-born infant to find out whether it is afflicted with haemolytic disease. The Coombs' test is not specific for the Rh system. For instance, in the acquired form of haemolytic icterus (acholuric jaundice) the test is positive directly on the patient's washed red cells (Loutit and Mollison, 1946), and distinguishes between the congenital and acquired forms of the disease. It seems that in acquired acholuric jaundice, the patient's red cells absorb an immune antibody from his or her own plasma. The Coombs' test may be of great use as a form of compatibility test, but, as indicated above, it may, on rare occasions, fail.

Space does not permit of a detailed consideration of the Rh antigens. In the Fisher classification there are six Rh antigens, namely, C, D, E, c, d, and e, and there are subgroups of some of these. The Rh-positive antigens are C, D and E. They are positive in the sense that they are the most prone to provoke formation of immune iso-antibodies if injected into persons whose blood lacks these antigens. The antigens c, d and e very rarely provoke formation of immune iso-antibodies. The allelomorphs of C, D and E are c, d and e. The common or standard Rh antigen is D, which is present in the blood of 85 per cent. of European whites who are defined as Rh+(D+). Rh-negative blood is defined as that lacking the Rh+ antigens C, D or E. Partially Rh+ blood lacks the antigen D, but con-

tains C or E, and such persons, from the point of view of receiving standard Rh+(D+) blood, must be regarded as Rh-negative. Further, Rh-negative females, and females whose blood contains C or E, but lacks D, may become immunized to the Rh factor D through pregnancy with a D+ foetus, or in consequence of injection or transfusion of D+ blood. *It is now known that 50 per cent. of D-negative persons, male or female, may become Rhesus-immunized by the injection or transfusion of D+ blood.* Immunization of D-negative mothers to the antigen D in pregnancy seems to occur in only about 5 per cent. of cases. Immunization to C is less common, and to E very uncommon. Rh+ persons, e.g. CDe/CDe, whose blood lacks the antigen c may become immunized to the Rh antigen c, for instance, through transfusion with Rh-negative blood, but this is very rare. Anti-d and anti-e are extremely rare.

Blood which is partially Rh+, i.e. contains the antigens C or E, but lacks D, must not be given to Rh-negative persons (cde/cde) lest immunization to C or E occur. The importance of the Rh antigens is that once a person becomes sensitized to a particular Rh antigen, whether by transfusion or pregnancy, such sensitization will persist for life. Therefore, no person, especially a female, should be transfused with Rh+(D+) blood unless he or she is Rh+(D+). The risk of immunization with the other Rh antigens is not so great. No baby, especially a female, should ever be given an injection of blood, either intramuscularly or intravenously, from a random blood donor, least of all its father, lest the donor be Rh+ and the baby Rh-negative and Rh-immunization occur. If an injection of blood is to be given an infant, its Rh group should be ascertained and blood homologous in the Rh system procured. It should be borne in mind that Rh-negative babies (D-negative) can result from matings of Rh+ heterozygous (Dd) persons. The severest forms of haemolytic disease are seen in those infants whose mothers have been immunized to the Rh factor by transfusions or injections of Rh+ blood, perhaps years previously. To transfuse or inject Rh+ blood deliberately into Rh-negative persons, or persons whose Rh group is not known, when facilities for Rh tests are available, is thoroughly bad practice, especially in the case of females of pre-childbearing or childbearing age. An Rh-negative female (D-negative) who became Rh-immunized in consequence of transfusion or injection of Rh+(D+) blood, and who in consequence could not produce normal, or even living, Rh+(D+) infants, could, no doubt, sue for damages on the grounds of negligence.

When a D-negative person not yet immunized to the Rh factor receives a transfusion of

Rh+(D+) blood, the chance of Rh-immunization of the recipient occurring is roughly 1 in 2. If Rh-immunization does not occur, the donor blood will survive *in vivo* normally. However, should the recipient become Rh-immunized, the donor's red cells will only survive about 50 days in the recipient's circulation and, a few days after their total elimination, Rh antibodies, the titre of which will rise for a few days, may be detected in the recipient's serum. This slow, but nevertheless abnormal, destruction of the donor red cells is termed inapparent haemolysis. The evidence of abnormal destruction of the transfused red cells is their diminished survival time *in vivo*. Of course, a subsequent transfusion of D+ blood will have very different effect for the donor red cells will be abruptly destroyed with, perhaps, disastrous effects.

Antenatal tests on pregnant women should be done as a routine to find out their Rh group and whether they are Rhesus-immunized. Such Rh antenatal testing is now done on a colossal scale mainly by the Regional Transfusion Laboratories. It ensures that mothers in need of transfusion will get blood of the correct group (ABO and Rh) and that infants affected with haemolytic disease will receive Rh-negative blood transfusion, if it is necessary.

The agglutinins anti-M, -N, -S,* and anti-P are not of importance as a cause of haemolytic transfusion reactions or haemolytic disease of the newborn. Very rarely, anti-A or anti-B may cause haemolytic disease of the foetus. The subgroups of A are not of clinical importance.

4. Diagnosis and Treatment of Haemolytic Transfusion Reactions, including Incompatible Transfusion

(a) *Diagnosis of Haemolytic Reactions*

A haemolytic reaction is, strictly speaking, one in which destruction of red cells, either of donor or recipient, occurs in the recipient's circulation. However, it is convenient to include here transfusions of haemolyzed blood, e.g. blood which has been frozen, overheated or grossly infected, or which is long time-expired and has, in consequence, become grossly haemolyzed though still sterile. A haemolytic reaction may be attributable to transfusion of red cells incompatible with the recipient's serum, e.g. in the ABO or Rh system. On the other hand, the transfused red cells may suffer no harm, but the donor's isoagglutinins may destroy the recipient's red cells. The re-

* At the time of going to press a report (Cutbush and Mollison, *Lancet*, 1949, 2, 102) of a haemolytic reaction apparently due to anti-S has appeared. An anomalous feature of this case, however, is the fact that the patient subsequently also destroyed S-negative blood.

infusion of a patient's own blood, e.g. from the peritoneal cavity, as in ruptured spleen or ruptured ectopic gestation, may result in a fatal haemolytic reaction if the blood has been present in the peritoneal cavity for two or three days. The investigation of a transfusion reaction should establish whether or not the transfused red cells have been destroyed, whether the transfused blood was haemolyzed when transfused, or whether destruction of the recipient's own red cells occurred. The cause of the reaction should be established.

It is now possible, especially with the aid of differential agglutination, to state with some precision whether destruction of the transfused red cells or of those of the recipient occurred. Of course simple inquiry may determine the cause of a reaction, e.g. transfusion of blood which has been frozen and therefore haemolyzed. Having established that blood was normal when transfused, had been properly preserved with glucose, and was not time-expired (i.e. aged 21 days or more), it is then necessary to establish whether incompatible blood was transfused. The blood groups (ABO and Rh) of donor and recipient should be re-determined. The ideal is a sample of blood from the bottle used and pre- and post-transfusion samples of the recipient's blood. In all transfusions, pre-transfusion samples of blood of donor and recipient should be kept for a couple of days in cold storage. Further, as a routine, the bottle used in transfusion should be conserved in cold storage with its contained remnant of blood for 24 hours lest investigations prove necessary. Cross-matching tests should be repeated and tests in albumin may be necessary or Coombs' test may have to be applied. When incompatible blood in the ABO system has been given, it may be possible to find agglutinates of donor red cells in the recipient's blood for one to seven days after transfusion. The fact that incompatible blood has been transfused may have to be inferred from laboratory investigations when samples of the donor blood are not available. This is quite readily done by studying the *in vivo* survival of the donor cells and by studying the serum of the recipient for changes in isoagglutinin titre. For instance, Rh antibodies may appear in the serum of an Rh-negative recipient transfused with Rh+ blood. Again, if A blood has been given to a B recipient, there will be an initial absorption of the recipient's anti-A isoagglutinins by the transfused red cells. Therefore, for a day or two after transfusion, the anti-A titre will be low or no anti-A isoagglutinin may be detectable. In the latter event a cross-match test will be fallacious for the donor cells will seem to be compatible. However, if at intervals after transfusion the recipient's serum be titrated, the titre of the anti-A agglutinins will rise steadily

until about the twentieth day, whereafter it will decline gradually to its normal pre-transfusion figure. The same changes will occur when Rh+ blood is transfused to a Rhesus-immunized Rh-negative recipient. In a haemolytic reaction, e.g. incompatible transfusion, there may be clinical evidence of blood destruction such as haemoglobinuria and jaundice, but these do not necessarily always occur. The colour of the urine may be deep orange and pigmented casts may be present. When haemoglobinuria occurs the urine, if alkaline, will be red due to oxyhaemoglobin, but if acid or neutral its colour will be brown or dark red like port wine, or even black, the dark colour being due to methaemoglobin. Glycosuria may occur. Masses of haemoglobin debris may be present and occasional red cells may be seen.

Differential agglutination may establish normal *in vivo* survival of the transfused red cells, and if there is evidence of haemolysis, e.g. hyperbilirubinaemia or jaundice shortly after transfusion, the donor's isoagglutinins should be titrated to determine whether destruction of the recipient's red cells has occurred. In certain disease processes, e.g. nocturnal haemoglobinuria, transfusion may precipitate severe destruction of the recipient's own red cells, though the donor red cells survive normally (Dacie and Firth, 1943).

It must be emphasized here that the *proper time to investigate a transfusion reaction is at the time of its occurrence, not some days later*. The time of maximal blood destruction or elimination, either of effete red cells, of incompatible blood, or of haemolyzed blood, is within one to five hours of transfusion, though elimination of course commences during transfusion. Therefore such phenomena as haemoglobinaemia, hyperbilirubinaemia, and agglutinates of donor red cells must be sought for an hour or two after transfusion. Intravascular destruction of blood is to be suspected when jaundice or haemoglobinuria complicate transfusion. All febrile transfusion reactions, especially chill or rigor, should be investigated as a routine procedure. A slight febrile reaction may be the sole evidence of an incompatible transfusion (Drummond, 1944). However, the great majority of chills and rigors are due to causes other than destruction or elimination of transfused blood or, alternatively, destruction of the recipient's erythrocytes. When homologous stored blood, because of faulty preservation or mishandling, or because it is effete, causes a haemolytic reaction, little difficulty will occur in establishing the diagnosis, especially as there will be no changes in the isoagglutinin titre of the recipient. The occurrence and degree of haemoglobinaemia and hyperbilirubinaemia will depend upon the quantity of blood destroyed in the re-

recipient's circulation and the rate of its destruction, also upon the quantity of haemolyzed blood transfused and the rate of transfusion. For example, the very slow transfusion and destruction of, say, 100 cc. of transfused blood may result in only slight hyperbilirubinaemia and no haemoglobinuria, but if the same quantity is rapidly transfused and abruptly destroyed, both haemoglobinuria and marked hyperbilirubinaemia may occur within an hour or so of transfusion. As a haemolytic reaction may be due to transfusion of haemolyzed blood, a sample of blood from the bottle used should be centrifuged and the supernatant fluid examined for haemolysis. The transfusion of glucose-citrated blood properly stored for 21 days is not followed by haemoglobinaemia or methaemalbuminaemia, though transient hyperbilirubinaemia will occur since about 20 per cent. of the red cells transfused will be effete (see Mollison, 1943a). Hamilton-Fairley (1940) could not find methaemalbumin spectroscopically after experimental intravenous injections in man of 14 to 25.4 gm. of haemoglobin though Schumm's test was positive within four to ten hours. On the other hand, methaemalbumin was demonstrated in the plasma of recipients transfused with amounts of incompatible blood containing the equivalent of 45 to 90 gm. of haemoglobin. When intravascular haemolysis is suspected the plasma should be examined spectroscopically for methaemalbumin.

The following procedures should be taken in the investigation of a transfusion reaction:—(1) If fever, chill, rigor or haemoglobinuria occur, stop the transfusion at once, re-cap the bottle and conserve remnant of transfusion fluid in cold storage. (2) One hour after transfusion has ceased take for investigation 20 cc. of recipient's blood with a dry sterile, or freshly-boiled syringe. It is useful to have a similar blood sample taken a few hours later. *Never use a syringe sterilized in spirit or antiseptic since haemolysis will result.* Put the bulk of the blood into a dry container (preferably a sterile screw-cap bottle) and 2 cc. into a dry oxalated tube. If pre-transfusion samples of recipient's and donor's bloods are available these should be referred for investigation. (3) Examine for haemoglobinuria and casts all urine voided during transfusion and for 48 hours afterwards. If haemoglobinuria occurs after transfusion, but no reaction was noted during transfusion, take immediately 20 cc. of recipient's blood and a catheter or clean sample of urine. Conserve abnormally coloured urine for investigation. A sample of the donor blood, preferably from the bottle, should be referred for investigation. (4) Do blood culture on recipient if there is persistent rigor, or a series of rigors; if possible,

take blood culture during rigor. Also take blood from bottle for culture. (5) When jaundice is noted within a few hours of transfusion and no other symptoms have been noted, take at once 20 cc. of the recipient's blood, a catheter or clean sample of urine and conserve remnant (if any) of transfusion fluid. All samples mentioned above should be referred without delay for investigation. Any pre-transfusion samples of blood of the recipient or donor which may be available should, of course, also be referred for investigations. (6) Chart the fluid intake and output for 14 days when a reaction complicates transfusion.

(b) *Treatment of Haemolytic Reactions*

The treatment of haemolytic reactions has recently been given renewed study and there is now a better understanding of the principles involved. When there has been gross destruction of transfused blood or, alternatively, much haemolyzed blood has been transfused, renal excretion of some of the haemoglobin may occur and this may result in damage to the renal tubules and, in consequence, their function may be impaired. Suppression of urine may occur and the patient may pass into, and die of, uraemia. Usually, however, regeneration of the damaged cells of the renal tubules will occur in a few days. With regeneration of the damaged tubules, function will be restored though some time may elapse before renal function is full. Treatment should be conservative. Alkalinization of the urine before transfusion may prevent precipitation of haemoglobin in the renal tubules, but will not remove casts of pigment precipitate already present. Intravenous infusion of sodium-citrate immediately a haemolytic reaction occurs may be of some benefit. The salt-and-water metabolism of the patient requires most careful attention. About half of the total fluid loss of the body is in the urine and, accordingly, when there is urinary suppression, the water intake must be restricted (Black and Stanbury, 1948; Muirhead *et al.*, 1948). The fluid intake should be restricted to sufficient to balance loss in sweat and urine, and should be about 1½ litres per diem when urinary suppression is complete. A patient with fever will need more fluid, and loss of fluids as in exudates, vomiting, or diarrhoea, should be corrected. Salt intake, too, should be restricted especially when no urine is being voided because the skin is the only other channel for excretion of salt. The patient should therefore be on a salt-free diet when the kidneys fail to pass urine.

Black and Stanbury (1948) recommend that the diet contain 30 gm. of protein per diem. Incidentally, anaemia and oligoemia, when present, should be corrected by compatible transfusion, since a good circulation of blood through the

kidneys is obviously desirable. Such heroic treatment as decapsulation of the kidneys is to be avoided, being as likely to kill as cure. No attempt should be made to "force" the kidneys into action in the oliguric period as, for instance, by excessive fluid intake (Muirhead *et al.*, 1948). Considerable harm may be done by giving the patient excessive fluids during the phase of urinary suppression. The forcing of fluids during the phase of urinary suppression may, in fact, result in generalized oedema and, in short, water poisoning. During the phase of suppression of urine the patient may manifest symptoms of uraemia; the blood urea may or may not rise to a high level. Recovery is heralded by the onset of diuresis which Muirhead *et al.* (1948) term the 'salt-losing diuresis.' During this phase the water lost must be replaced lest dehydration occur. The loss of salt in the urine, too, must be corrected and in the salt-losing diuresis phase the body needs may amount to 20 to 40 gm. salt and 5,000 to 10,000 cc. water daily (Muirhead *et al.*, 1948). This phase will last about five days and, thereafter, the patient can be allowed to regulate his or her own fluid intake and output. Complete recovery of renal function may not occur for several weeks.

There remain for consideration those cases of severe urinary suppression in which recovery is despaired of, despite correct treatment. In these cases it may be worth trying blood, peritoneal or intestinal dialysis for removing urea and retained products from the recipient's circulation. For a discussion of these procedures see Black and Stanbury (1948) and Joekes (1949).

5. The Study of Disease with the Aid of Transfusion

Knowledge of the normal *in vivo* survival of the transfused erythrocyte has been used successfully in the study of certain disease processes. In haemolytic disease of the foetus, of which the commonest form is that due to Rhesus-immunization of the mother, the red cells of the foetus undergo destruction by immune iso-antibodies transmitted from the mother to the foetus during pregnancy. Successful treatment of the infant must therefore involve transfusion of blood which lacks the antigen, present in the infant's blood, which stimulated the production of the harmful immune iso-antibodies in its mother. Nearly always the mother is Rh-negative (D-negative) and the affected infant Rh+(D+). When this is the case, the affected infant must not be transfused with D+ blood because such blood will be rapidly destroyed by the harmful Rh antibodies submitted from the mother. Mollison (1943b) has shown that Rh+(D+) blood will be completely eliminated from such an infant's circulation

within ten days. On the other hand, Rh-negative blood has a normal *in vivo* survival in the affected infant, though in occasional cases some initial destruction of transfused Rh-negative blood may occur. The affected infant will be kept alive by the transfused Rh-negative blood if given in adequate amount, but the destruction (or elimination) of the infant's own red cells proceeds so that in some cases no red cells of the infant will be detected in its circulating blood and only the Rh-negative red cells of the donor will be present. The infant will then appear to be Rh-negative. However, after two or three weeks, differential agglutination studies will reveal that the infant's red cells have re-appeared in its circulating blood, indicating that the haemolytic process is coming to an end. As the infant gets older the harmful, immune iso-antibodies transmitted from the mother are entirely eliminated and transfused Rh+(D+) blood will then have a normal *in vivo* survival. In haemolytic disease of the foetus, differential agglutination studies have thus clearly demonstrated that the fundamental pathology, long suspected of being a haemolytic mechanism, is destruction (haemolysis) of the infant's red cells by harmful immune iso-antibodies transmitted from the mother. The red cells of the infant, incidentally, being sensitized by the maternal immune iso-antibodies, will give a positive Coombs' test (Coombs *et al.*, 1946), and this very sensitive test is extremely useful and very accurate in diagnosing the disease. It is much the most reliable test yet devised.

The congenital and acquired forms of haemolytic icterus (acholuric jaundice) have been studied with the aid of transfusion and differential agglutination (Dacie and Mollison, 1943; Loutit and Mollison, 1946; Mollison, 1947). The following facts have been established by Loutit and Mollison (1946):—(1) In the congenital or familial form of acholuric jaundice the *in vivo* survival of transfused red cells is normal, but the red cells of the patients, when transfused to normal recipients, are eliminated unduly rapidly. (2) In the acquired form of acholuric jaundice, transfused red cells are destroyed abnormally rapidly, whereas the red cells of patients affected with acquired acholuric jaundice survive normally in normal recipients. Further, the Coombs' test is positive on the washed red cells of patients suffering from the acquired form of acholuric jaundice, but in the congenital or familial form the Coombs' test on the patient's cells is negative. Even after splenectomy the Coombs' test has been found to be positive in the acquired form of the disease. These facts are important and indicate that 'there are two different conditions having a different aetiology within the syndrome of

acholuric jaundice' (Loutit and Mollison, 1946). It would seem that in the acquired form of the disease the patient has an immune antibody which destroys not only the patient's red cells, but those of transfused blood. In some cases of acquired acholuric jaundice studied by Mollison (1947), the effect of splenectomy was beneficial in that the rate of elimination of transfused red cells became less than it was before splenectomy. The fact that in the congenital or familial form of the disease the Coombs' test on the patient's red cells is negative would seem to indicate that no antigen-antibody system exists which causes haemolysis of the patient's red cells. The congenital or familial form of acholuric jaundice is, therefore, apparently due to an inherent (hereditary) defect of the erythron and, as noted, the red cells of patients affected with the congenital or familial form of acholuric jaundice do not have a normal *in vivo* survival in the circulation of normal recipients. This suggests that the red cells in the congenital or familial form of the disease are unduly susceptible to normal destructive processes. Normal erythrocytes retain their normal fragility in the circulation of patients suffering from congenital or familial haemolytic anaemia (Mollison, 1947) which suggests that, in congenital or familial haemolytic anaemia, the basic abnormality lies in the erythrocytes. It is clear that the congenital and acquired forms of acholuric jaundice can be distinguished with the aid of transfusion and differential agglutination studies, and by the Coombs' test.

The red cells of a patient with nocturnal haemoglobinuria, when transfused to normal subjects, were eliminated more rapidly than normal, i.e. even in a normal environment they underwent destruction more rapidly than normal (Dacie and Mollison, 1949). On the other hand, the red cells of normal persons have a normal *in vivo* survival in patients suffering from nocturnal haemoglobinuria (Dacie and Firth, 1943; Mollison, 1947). Therefore, in this disease, as in familial haemolytic anaemia, abnormal destruction of transfused normal erythrocytes does not, apparently, occur. These observations make it clear that there must be some fundamental abnormality of the patient's red cells in nocturnal haemoglobinuria since they become haemolyzed in human serum *in vivo* and, as is known, *in vitro* as well. There is no evidence that the patient's red cells are harmed by some antibody elaborated by the patient since transfused normal red cells have

a normal *in vivo* survival in the recipient's circulation. Mollison (1947) found that in the acute haemolytic anaemia associated with chronic malaria ('black water fever') transfused red cells were rapidly eliminated during the phase of haemoglobinuria, but that thereafter the elimination was within the limits of normality.

All these observations are of interest and indicate that blood transfusion and its allied tests can profitably be used in research in certain diseases, especially haemolytic processes. Transfusion, a powerful instrument in the hands of the clinician, may likewise become a useful instrument in the hands of the research worker.

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