The purpose of the duplicate test is to avoid the occurrence of an artefact due to traumatisation of the red blood cells, particularly when separating the clot from the wall of the tube. Naturally, care must be taken that the needles and syringes used for the test are perfectly dry.

If there is no change in the appearance of the patient's plasma, and the need for blood is urgent, one can then proceed with the transfusion of larger amounts of blood from the same donor. When time permits, however, it is preferable to inject another test dose of 50 cc. of the donor's blood and to draw a third sample of blood after an additional hour. In this way more reliable results can be obtained and the reaction in positive cases is more striking. In positive reactions, one hour after the test injection of 50 cc. of blood, the patient may have a severe chill and rise in temperature, but the clinical symptoms may be quite mild, and occasionally they may be entirely absent. More reliance is to be placed on the appearance of the patient's plasma, which will show a distinct rise in the icteric index as compared with the pre-transfusion sample. The donor's blood used for the biological test should be fresh; if only bank blood is available, no sample more than three days old should be used. The reason for this is that blood stored for periods longer than 7 to 10 days, even though apparently intact when transfused, often breaks down rapidly in the patient's circulation. In this way a positive biological reaction might be obtained even with serologically compatible blood.

SUMMARY

The practical points in this short review may be summarised as follows. Before a transfusion is given the direct and indirect blood matching tests should be carried out using an exact technique. Particular care should be taken in direct matching recipients who have had previous transfusion, or who are or have recently been pregnant. Where there is any doubt as to the presence of atypical agglutinins in the recipient's serum, a biological test should be a routine preliminary to transfusion.

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THE APPLICATION OF PRESENT KNOWLEDGE ABOUT THE RH FACTOR

By P. L. MOLLISON, M.B., M.R.C.P.

A. A BRIEF OUTLINE OF KNOWN FACTS ABOUT RH AGGLUTINOGEN

The facts about the Rh agglutino-gen are at first sight easy to understand. 85 per cent of human beings (whites) are Rh positive (that is their erythrocytes contain the Rh agglutino-gen); 15 per cent are Rh negative. This newly recognised grouping is independent of the four main blood groups and differs from the latter system in one very important respect, namely, in that persons whose erythrocytes lack the Rh agglutino-gen do not normally have the corresponding anti-Rh agglutinin in their serum. It will be recalled that, by contrast, in the four main blood groups lack of the A or B agglutinogens from the erythrocytes is always accompanied by the presence of the corresponding anti-A or anti-B agglutinins in the serum.

Rh negative persons can, however, form anti-Rh agglutinins under either of two circumstances, and in this fact lies the importance of the Rh agglutino-gen. These circumstances are—

- (i) (in persons of either sex) after the transfusion of Rh positive blood;
- (ii) (in women) during pregnancy, when the foetus is Rh positive (the Rh agglutino-gen having been inherited from the father).

The consequences of the formation of anti-Rh agglutinins are also apparently clearly defined:—

- (i) Rh negative persons whose serum has come to contain anti-Rh agglutinins as a result of one of the circumstances defined above, will suffer a haemolytic reaction if they subsequently receive a transfusion of Rh positive blood.
- (ii) When an Rh negative woman forms anti-Rh agglutinins during pregnancy, in response to stimulation by the Rh agglutinin passed across the placenta from an Rh positive foetus, the anti-Rh agglutinins so formed pass back across the placenta into the foetal circulation and cause the destruction of foetal erythrocytes. This in turn gives rise to the condition known as erythroblastosis foetalis or, better, haemolytic disease of the foetus.

This process of the formation of immune agglutinins by a member of a given species against some antigen lacking from its own tissues, but present in the tissues of another member of the same species is known as *iso-immunisation*. Unfortunately, the simple outline, presented above, of the way in which persons become sensitised to the Rh agglutinin and of the consequences of this sensitisation, provides only a rough account of the role played by iso-immunisation in clinical medicine. Many modifications and additions must be made before a true picture can be presented. The modifications reside chiefly in the irregularity with which Rh negative persons respond to the stimulus of the Rh agglutinin. The additions are mainly due to two facts. Firstly, the "Rh agglutinin" is not a single agglutinin, but a group of related agglutinogens; the erythrocytes of an "Rh positive" person may contain only one or some of these agglutinogens, and such a person can still form "anti-Rh" agglutinins against other varieties of Rh agglutinogens. Secondly, red cell agglutinogens other than Rh are also antigenic in man.

Irregularity of Response to the Rh Agglutinin

1. Response of Rh negative recipients to transfusions of Rh positive blood.

Wiener and Peters (1940) published the first report of haemolytic transfusion reactions caused by the interaction of anti-Rh agglutinins in the recipient's serum and Rh agglutinin in the donor's erythrocytes. Wiener (1941) later reported ten similar cases. He pointed out that sometimes the destruction of Rh positive erythrocytes by anti-Rh agglutinins might occur so slowly that no obvious signs of haemolysis were produced. The only evidence of such "inapparent haemolysis" might be a failure of the recipient's haemoglobin value to increase after transfusion, or rather to remain increased after transfusion. Conclusive evidence of this destruction could be produced by carrying out differential agglutination tests and demonstrating that the donor's erythrocytes had been eliminated. A good example of inapparent haemolysis is provided by the following case (described by Boorman, Dodd and Mollison, 1942).

CASE 1.—A man, aged twenty-five, had had symptoms of ulcerative colitis for eight years. In November 1940 he had a relapse and received two transfusions, totalling 2,500 c.c. of group O blood. The response to these transfusions was satisfactory. In April 1942 he was readmitted to hospital for further treatment and received a transfusion of 1,000 c.c. of group O blood. Forty-eight hours after this transfusion it was noted that his haemoglobin value had scarcely been raised, and that his serum had become more yellow. By the fifth day after transfusion it could be demonstrated that only a trace of the donor blood remained in the circulation, and that the recipient had produced anti-Rh agglutinins. By the seventh day, no trace of the donor blood remained. It was demonstrated that the donor blood was Rh positive, and that the recipient was Rh negative. Apart from the failure of the patient's haemoglobin value to increase after transfusion, there were no signs from the clinical point of view that a haemolytic reaction had occurred.

From cases published by Wiener (1941) and by Vogel, Rosenthal and Levine (1942), it is evident that if the "inapparent haemolysis" produced by the development of anti-Rh agglutinins is not detected and further transfusions of Rh positive blood are given, the reactions are likely to become far more severe and be in every way comparable to those of ordinary "wrong group" transfusions. It is also evident, however, that in many instances repeated transfusions of Rh positive blood must be given over a period of many months, or even years, before the recipient develops anti-Rh agglutinins capable of causing a serious haemolytic reaction.

By contrast, Dacie and Mollison (1943) reported a case in which a patient (Rh negative),

who had never previously received a transfusion and whose only child was Rh negative, developed anti-Rh agglutinins after her first transfusion, which was of Rh positive blood. In this case the response was very slow and was only detected by estimating the survival rate of the transfused erythrocytes. The latter were completely eliminated within 59 days of transfusion instead of the usual 100 days or more.

On the whole, the impression obtained from the evidence so far available is that Rh is a poor antigen for man, that is to say, that several stimuli are usually necessary before there is a good antibody production. This impression is increased by observations which indicate that Rh negative women only irregularly become sensitised to the Rh agglutigen as a result of becoming pregnant with an Rh positive foetus.

2. Response of Rh negative women during pregnancy to the presence in utero of an Rh positive foetus.

Landsteiner and Wiener (1940) have shown that when the father is Rh positive and the mother Rh negative, the Rh group of the infant will depend upon whether the father is homozygous (*RhRh*) or heterozygous (*Rhrh*) for the Rh factor. If the former, all the children will be Rh positive, but if the latter, half the children will be Rh positive and half Rh negative. In this paper the symbol *Rh* is used for any Rh positive gene, irrespective of subgroup.

It has been estimated that the combination of an Rh negative mother and an Rh positive foetus occurs in approximately 1 in 10 of all pregnancies. On the other hand, the incidence of haemolytic disease of the foetus has been estimated at only 1 in 400 births (Javert, 1942). Evidently, therefore, only once in 40 times does the potentially dangerous combination actually lead to morbidity. One of the main reasons for this is almost certainly the fact that many Rh negative women require the stimulus of more than one pregnancy before they respond by producing anti-Rh agglutinins.

In testing different families in which the birth of one or more infants affected with haemolytic disease has occurred, it is found that in only a small proportion of cases is the first child affected, and quite often the birth of several normal Rh positive children occurs before the mother becomes sensitised and starts giving birth to affected children. The following case provides a good example of this:

CASE 2.—The seventh infant of Mrs. M. developed severe jaundice shortly after birth, and was found to be affected with icterus gravis neonatorum. Its red cells were Rh positive, whereas those of its mother were Rh negative, and her serum contained anti-Rh agglutinins. Five of the six previous children were seen and tested: all were Rh positive and all had been healthy since birth.

Such women who only respond to the Rh agglutigen after repeated stimuli may be compared with the persons referred to above who only develop anti-Rh agglutinins after the repeated transfusion of Rh positive blood.

Although the presence of anti-Rh agglutinins in a mother's serum at the time of delivery is usually associated with haemolytic disease of the foetus, it is not necessarily so. Either the foetus may be Rh negative (the anti-Rh agglutinins having been formed in a previous pregnancy) or the foetus, although Rh positive, may be unaffected.

The following cases illustrate these points:

CASE 3.—Mrs. H., was first seen during her fourth pregnancy. Of her three previous children, the first had been healthy from birth and was found to be Rh positive. The second had died within a few days of birth from "icterus gravis neonatorum." The third infant was normal and was Rh negative. Mrs. H. was Rh negative, and (at the sixth month of her fourth pregnancy) her serum contained anti-Rh agglutinins. The question arose as to whether the presence of anti-Rh agglutinins indicated that the foetus *in utero* was Rh positive or whether the agglutinins had simply persisted since the second pregnancy. When the patient was delivered it became clear that the latter explanation was correct for the foetus was Rh negative and healthy.

CASE 4.—Mrs. J. was first seen during her fourth pregnancy. Her first three infants had been normal. Her blood group was A Rh negative, and her serum contained very weak anti-Rh agglutinins. At the time of delivery the presence of weak anti-Rh agglutinins was confirmed. The infant appeared healthy at birth, and although Rh positive it maintained a normal blood picture.

It must be added that no close correlation is found between the strength of anti-Rh agglutinins in the mother's serum and the severity of haemolytic disease in the infant. Sometimes only weak anti-Rh agglutinins are found, although the infant is affected with hydrops foetalis and is born dead.

3. Haemolytic reactions of varying severity after sensitisation to the Rh Agglutinin during pregnancy.

Haemolytic transfusion reactions due to the sensitisation of a woman to the Rh agglutinin during pregnancy are liable to be more severe than reactions caused by the development of anti-Rh agglutinins after repeated transfusion. This is partly because in the latter case some warning is usually given by the increasingly unfavourable response to transfusion so that further transfusions of Rh positive blood are withheld, whereas in the case of a woman immunised by pregnancy there is only the warning of the diseased foetus, and if the latter is still *in utero* there may be no warning at all. The following case serves to illustrate this latter point.

CASE 5 (from Mollison, 1943a).—Mrs. X. First two pregnancies normal. The third pregnancy ended in a miscarriage. The fourth pregnancy resulted in the birth of an apparently normal child, which later became very anaemic. The fifth pregnancy proceeded normally, but bleeding, due to placenta praevia, developed when the patient went into labour. A transfusion was given, and was followed by a rigor. A very pale dead infant was delivered. On the following day the patient became jaundiced and passed very little urine. Investigations carried out five days after transfusion showed that the patient was group A Rh negative, and that her serum contained anti-Rh agglutinins. Her husband was found to be group B Rh positive, and the donor group O Rh positive. No group O blood was found to be surviving in the circulation. Ten days after the first transfusion, group O Rh negative blood was transfused with a satisfactory result: tests showed that these erythrocytes survived normally in the recipient's circulation. Although at the time of transfusion it was not known that the foetus *in utero* was diseased, enquiry would have shown that the previous infant manifested the typical picture of congenital anaemia of the newborn.

Fatalities caused by the transfusion of Rh positive blood to women immunised by pregnancy have been described by Wiener (1941), Burnham (1941), Boorman, Dodd and Mollison (1942), and others. On the other hand, the reactions may be extremely mild as in the following case:

CASE 6.—This patient has already been referred to above (case 4): as has been stated, her serum contained weak anti-Rh agglutinins. After her delivery she bled profusely, due to a retained placenta and required massive transfusion. Only a small quantity of Rh negative blood was available, and some 4,000 c.c. of Rh positive blood was given during a period of several hours whilst the patient continued to bleed. Eventually the placenta was delivered and the patient recovered rapidly. During the following week a rapid elimination of the donor erythrocytes was observed, although this process was not complete until 12 days after transfusion (this is approximately nine times the normal rate of elimination of transfused erythrocytes). From the clinical point of view the only sign of this destruction was the rapidly increasing pallor during the week following transfusion.

Iso-immunisation to sub-groups of Rh and to Agglutinogens other than Rh

It is generally agreed that not all mothers of infants affected with haemolytic disease are Rh negative (Levine *et al*) (1941); Wiener (1942); Boorman *et al* (1942); Race *et al* (1943). At first it appeared that when a mother was found to be Rh positive it could be assumed that she could not have become immunised to the Rh agglutinin. However, it has since become apparent that many sub-groups of Rh exist, and that a woman whose erythrocytes contain one of the Rh agglutinogens, and who is therefore classified as Rh positive, may form "anti-Rh agglutinins" against another type of Rh agglutinin.

Wiener (1943) describes two main sub-groups of Rh, namely, Rh₁ and Rh₂. Most human anti-Rh sera react with both sub-groups Rh₁ and Rh₂, but occasional human anti-Rh sera (anti-Rh₁) only react with Rh₁ bloods (that is, approximately 70 per cent of the 85 per cent total of Rh positive bloods). Wiener has also described an anti-Rh₂ agglutinin reacting with approximately 35 per cent of bloods. Boorman, Dodd and Mollison (1943) obtained a serum which

was almost certainly identical with Wiener's anti-Rh₂, from an Rh positive woman whose infant was affected with haemolytic disease of the newborn. From another Rh positive woman, who also had given birth to an infant affected with haemolytic disease of the newborn, Race and Taylor (1943) obtained a serum containing agglutinins of yet a different kind. This serum reacted with all Rh negative bloods and with a large proportion of Rh positive bloods. The relationship of the many different sub-groups of Rh has been discussed by Wiener (1943), and by Race, Taylor, Boorman and Dodd (1943).

The question must also be considered whether the immunisation of a woman to the A or B agglutinogens during pregnancy is ever responsible for causing haemolytic disease of the foetus. Dienst (1905) first showed that immune responses to the A and B agglutinogens might occur in recently delivered women. Boorman, Dodd and Mollison (1943) have observed that in at least half the cases in which a woman's serum contains anti-A or anti-B agglutinins, and her infant's erythrocytes contain the corresponding A or B agglutinogens, an immune response follows delivery.

Since incompatibility of the mother's serum with the infant's erythrocytes, due to the presence of anti-A or anti-B agglutinins in the former, occurs in approximately 1 in 5 of all pregnancies, it seems at first sight that this incompatibility is unlikely to be related to disease in the foetus. However, in two families out of a series of a 100 in which haemolytic disease of the foetus occurred, Boorman, Dodd and Mollison (1943) consider that the destruction of the foetal erythrocytes was caused by immune anti-A or anti-B agglutinins (anti-Rh agglutinins were thought to be responsible in the remaining 98 cases). Their reasons for this conclusion are, firstly, that in these two cases no atypical agglutinins, other than immune anti-A or anti-B agglutinins, could be demonstrated in the mother's serum, whereas in the cases in which the mother was Rh negative anti-Rh agglutinins were demonstrated in 93 out of 97 cases, and secondly, the immune anti-A or anti-B agglutinins in these two cases were exceptionally potent.

Tests for Rh Agglutinogens and Anti-Rh Agglutinins

The fact that anti-Rh agglutinins in human sera passed unnoticed for so many years is undoubtedly largely due to the poor reactions which these agglutinins give *in vitro*. It must be emphasised that when a potent human anti-Rh serum is mixed on a slide with a suspension of Rh positive erythrocytes and observed for a period of, say, 15 minutes, no reaction of any kind develops. The mixture of serum and cell suspension must be left in a test-tube for approximately two hours before being examined. Furthermore, the tube must be kept at 37° C. during this period, since almost all anti-Rh sera are more active at this temperature than at room temperature. When the tube is examined at the end of the period of incubation, all the red cells will have settled to the bottom of the tube; a portion of this sediment must be gently withdrawn with a Pasteur pipette, gently transferred to a glass slide, and then be examined for the presence of agglutinates under a microscope. Due to gentle handling of the sediment, free cells sometimes form "drifts" and "nests," appearances which may be mistaken for agglutination until experience has been acquired.

Although emphasis has been placed upon the weakness of some of the reactions and the care that must be taken to avoid breaking up the agglutinates, the perspective must be restored by pointing out that some anti-Rh sera are encountered which give really strong reactions *in vitro*.

Although the use of the technique described above will almost always serve to detect any incompatibility between the donor's erythrocytes and the recipient's serum, due to the presence of anti-Rh agglutinins in the latter, more extensive tests are necessary for the proper identification of anti-Rh agglutinins and of Rh positive and Rh negative erythrocytes, and in the present state of knowledge there can be no question that this branch of serology is better left to specialists. In explanation of this contention it will be sufficient to repeat that "Rh" is no more than a convenient term for a group of antigens whose analysis is still by no means complete, and that, as mentioned above, a person may be "Rh positive" with one anti-Rh serum, and yet may form "anti-Rh" agglutinins against another sub-division of the Rh group of agglutinogens.

B. THE APPLICATION OF KNOWN FACTS ABOUT THE RH AGGLUTINOGEN**Blood Transfusion****1. Prevention of sensitisation of Rh negative recipients.**

Evidently the sensitisation of Rh negative persons by Rh positive transfusions could be avoided by testing the Rh group of all recipients and then finding Rh negative donors for the Rh negative recipients. However, as far as males are concerned, this is hardly necessary unless it is anticipated that the recipient is likely to require a series of transfusions. In the latter case, the recipient should certainly be tested whenever possible; obvious examples are patients with aplastic anaemia or haemophilia. In the case of battle casualties with septic wounds, for instance, who may require many transfusions, experience so far gained suggests that repeated transfusions over a period of, say, three weeks are most unlikely to lead to trouble, whereas a few transfusions spread over a period of many months may well do so. Exceptions to this rule seem most likely to be provided by patients with some variety of haemolytic anaemia, who appear to be particularly liable to form immune iso-agglutinins.

The case of female recipients is very different from that of males. Whereas with males, once the recipient has become sensitised to the Rh agglutinin, Rh negative blood can be chosen if a further transfusion is required, in the case of women, subsequent pregnancies have to be considered. By transfusing an Rh negative woman with Rh positive blood one may sensitise her to the Rh agglutinin so that if she later becomes pregnant with an Rh positive foetus the chances of her producing anti-Rh agglutinins are increased and she may give birth to a dead child instead of a live one. That this is not mere hypothesis is illustrated by cases reported by Diamond (1942). Unfortunately, until facilities for making Rh tests have been increased, this risk will often have to be taken.

2. Clinical diagnosis of sensitised recipients.

The person who has become sensitised to the Rh agglutinin as a result of repeated transfusions can often be detected before a serious haemolytic transfusion reaction develops. Characteristically the reactions become steadily more severe as antibody production increases. At first there is "inapparent haemolysis"—the haemoglobin rises after transfusion, but falls again within a few days. In these cases serial estimations of the serum bilirubin concentration are likely to show a temporary increase. After further transfusions the reactions become more severe, and jaundice may be noted. Usually by this time suspicion has been aroused, and serological investigation will reveal the true state of affairs. If the warning is ignored and still further transfusions are given, without reference to Rh tests, a fatal haemolytic reaction may follow.

Women who have become sensitised to the Rh agglutinin as a result of pregnancy must be detected in a different way. Here the warning must be obtained from the previous obstetric history or from the state of the foetus. It has now been made abundantly clear that the infant affected with haemolytic disease may present widely varying signs. The pictures of hydrops foetalis, icterus gravis neonatorum and congenital anaemia of the newborn are well known, but it is not so generally recognised that the affected foetus may be stillborn, often prematurely and present no outward signs of disease. Clearly, then, only Rh negative blood of suitable ABO group should be transfused to a mother when her infant is stillborn, oedematous, jaundiced, or anaemic, unless tests have shown that she is Rh positive.

3. Compatibility tests.

It has been stated above that testing for Rh agglutinogens and anti-Rh agglutinins is at present a job for a specialist. Usually, therefore, it will be safer to obtain Rh negative blood from a blood transfusion centre or from a panel of specially tested donors. Nevertheless, wherever possible a compatibility test should be carried out with this blood using the technique described above, of which the essentials are:—

- (a) use of a weak suspension (say 1 per cent to 2 per cent) of donor's blood in iso-tonic sodium citrate or saline;
- (b) addition of one small volume (say 0.1 to 0.2 c.c.) of this suspension to an equal volume of the recipient's serum in a small test-tube (diameter 1 cm. or less);

- (c) incubation at 37°C. for not less than 2 hours;
- (d) gentle transfer of red-cell sediment to a glass slide for examination under the microscope.

4. Transfusion of infants affected with haemolytic disease.

Levine and his co-workers (1941) first reported that infants affected with haemolytic disease of the newborn showed a better response to transfusions of Rh negative blood than Rh positive blood. It has since been demonstrated (Mollison, 1943b) that Rh positive erythrocytes are usually rapidly eliminated from the circulation of an affected infant, in extreme cases within two days of transfusion, whereas Rh negative erythrocytes are not usually completely eliminated for at least 90 days after transfusion.

From the clinical point of view the results of treating affected infants with transfusions of Rh negative blood are very striking (Gimson, 1943). Personal experience of this method of treatment convinces one that early and adequate transfusion of Rh negative blood may immensely improve the prognosis in severe cases. Once the diagnosis of haemolytic disease has been made, it seems worth giving at least 100 c.c. of group O Rh negative blood, whatever the infant's haemoglobin value may be. In the face of definite anaemia, quantities up to 200 c.c. may safely be given. The drip method of transfusion, using a small cannula tied into the internal saphenous vein at the ankle, has been found most satisfactory. As a compatibility test it has been recommended that the donor's erythrocytes should be tested against the mother's serum (Mollison, 1943b). Alternatively, if the mother's erythrocytes, washed free from plasma, are used for transfusion, no compatibility test is necessary (Lloyd, 1943; Wiener and Wexler, 1943).

Application of Rh tests in the diagnosis of haemolytic disease of the foetus.

From what has already been said it is evident that in a suspected case of haemolytic disease of the foetus it will not be sufficient to show that the mother is Rh negative and the infant Rh positive, since this combination occurs in 1 in 10 of all pregnancies, and therefore may well be due to chance. The demonstration, in addition, of anti-Rh agglutinins in the mother's serum is of far greater significance, and in fact will almost always mean that the infant is affected. The most favourable time of examination of the mother's serum is seven to twenty-one days after delivery, since the immune response usually reaches its peak at this time (Boorman, Dodd and Mollison, 1942).

Although the finding that the mother is Rh positive makes the diagnosis less probable, it by no means excludes it. However, if it can be shown that the mother's serum contains no atypical agglutinins (active at 37°C.) and no exceptionally potent anti-A or anti-B agglutinins, the diagnosis then becomes exceedingly improbable.

The application of Rh tests is particularly valuable in at least two types of case, namely, unexplained stillbirths and cases of jaundice in the newborn.

Unexplained stillbirths.

When a woman has given birth to one or more stillborn foetuses, it is well worth making serological tests to discover whether she has become immunised to the Rh agglutinin. In quite an appreciable proportion of cases (it may well be 10 per cent) in which the foetus is still-born after the twenty-eighth week of pregnancy, it will be found that there is a serological cause. The importance of making this diagnosis is considerable, since the patient can then be spared the trouble and expense of useless treatment and a more accurate (if gloomy) prognosis can be given. Miscarriages (before the twentieth week of pregnancy has been reached) seem very seldom to be caused by iso-immunisation (Boorman, Dodd and Mollison, 1943).

Icterus neonatorum.

It may at times be difficult to decide from a clinical point of view during the first few days of life whether a given infant is affected with haemolytic disease of the newborn or simply with "physiological jaundice of the newborn." As is well known, in some cases of haemolytic disease of the newborn the haemoglobin value may be well maintained for the first few days of life, although it subsequently falls to a low level. During this initial period serological tests will often make it clear whether the infant is affected, and treatment may then be started without delay.

The application of Rh tests during pregnancy

In some ante-natal clinics in this country (for example, St. Helier Hospital, Surrey County Council) Rh tests are being made as a routine on all patients. This has at least two advantages from the patient's point of view (not to mention the opportunities that it provides for research). Firstly, Rh negative women can be detected and a special note made on their history sheet to the effect that they must never be transfused except with Rh negative blood of suitable ABO group. (They can, of course, receive plasma or serum transfusions without any preliminary tests.) Secondly, the sera of these Rh negative women can be tested on one or more occasions during the last few months of pregnancy so that those who have formed anti-Rh agglutinins can be detected. Arrangements can then be made to admit these women to hospital for their delivery, and preparations made to transfuse the infant soon after birth if this should prove necessary.

When anti-Rh agglutinins are found in a woman's serum during pregnancy, it will be profitable to titrate the serum at intervals to discover whether or not the titre of the agglutinins is increasing. A rise in titre will probably always mean that the infant *in utero* is Rh positive, and is therefore almost certain to be affected. A steady titre, on the other hand, does not by any means necessarily indicate that the infant *in utero* is Rh negative (i.e. that the anti-Rh agglutinins were formed in a previous pregnancy). Several cases have been seen in which the titre of anti-Rh agglutinins remained steady during the last few months of pregnancy, but nevertheless the foetus, when born, proved to be Rh positive and affected (Boorman, Dodd and Mollison, 1943).

The application of Rh tests in prognosis

In the usual case of haemolytic disease of the foetus in which the mother is Rh negative and the affected infant Rh positive, the fate of any subsequent siblings in the family depends upon whether the father is heterozygous (*Rhrh*) or homozygous (*RhRh*), for only if he is heterozygous is there any chance of the birth of an Rh negative (unaffected) sibling. In practice, families in which the birth of an affected foetus is followed by the birth of a normal infant are uncommon (Race, *et al*; Boorman, *et al*, 1943). However, some assistance in forming a prognosis may be obtained in two ways.

- (i) Any previous siblings are tested. If there is an Rh negative child amongst them, the father is clearly heterozygous.
- (ii) The father's Rh subgroup may be determined. By the use of appropriate sera it will often be possible to determine whether or not he is heterozygous (*Rhrh*) (Race and Taylor).

SUMMARY

1. Although in most cases (say, 95 per cent) of haemolytic disease of the newborn, the mother is Rh negative, in other genuine cases (say 5 per cent) the mother is Rh positive and has become immunised to some red cell antigen other than the typical Rh agglutino-gen. The finding that the mother is Rh positive, therefore, by no means excludes the diagnosis. The other antigens most commonly responsible appear to be variants of Rh and the agglutinogens A and B.

2. To support the diagnosis of haemolytic disease of the newborn it is not sufficient to show that the mother is Rh negative; it must also be demonstrated that her serum contains anti-Rh agglutinins (although failure to demonstrate such agglutinins does not exclude the diagnosis). When the mother is Rh positive, immune agglutinins other than anti-Rh must be sought in her serum.

3. Serological tests are of most value in:—

- (a) cases of jaundice of the newborn, in deciding between the diagnosis of "physiological jaundice" and "icterus gravis neonatorum."
- (b) Cases of stillbirth to decide whether the foetal death can be attributed to haemolytic disease.
- (c) Cases of haemolytic transfusion reaction in which the cause of the reaction is in doubt.

4. Most of the serious haemolytic transfusion reactions caused by the development in the recipient's serum of anti-Rh agglutinins occur in recently delivered women. Obstetricians must, therefore, always have this possibility in mind and must never use blood of unknown

Rh group when there is a possibility that the infant may be affected with some form of haemolytic disease of the foetus.

5. The early and adequate transfusion (with group O Rh negative blood) of infants affected with haemolytic disease of the newborn produces most encouraging results. It is desirable to test the donor's erythrocytes against the mother's serum before transfusion.

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THE MEDICO-LEGAL IMPORTANCE OF THE BLOOD GROUPS

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The medico-legal importance of the agglutinin factors of the red blood corpuscles designated A and B (O indicating absence of these factors), and the agglutinin factors of the serum designated *a* and *b* may be divided into two headings.

1. The determination of the presence of one or both of these factors in blood, in secretions and in excretions.
2. The utilisation of these agglutinin factors together with the agglutinin factors, M and N, in the determination of non-paternity.

Blood Stains

The essence of the examination is to establish the group of the stain upon important exhibits and to compare the result with the group of the blood of the victim, obtained either from post-mortem blood or from articles known to have been stained with the victim's blood, e.g. clothing, or, if alive, from samples of his blood. If permission is granted, and it is usually not granted, a comparison is made with the blood from the accused person. Thus, to quote an example, if the blood upon certain exhibits belongs to group A and the injured person is group A, then the blood upon the exhibits could have come from him, but of course could have come from any other person of group A. The importance of the findings depends greatly upon the evidence available regarding the significance of the exhibits.

If the accused's blood is available for test and is of group B for example, then an explanation as to how articles amongst the exhibits belonging to the accused, e.g. knife or clothing, are stained with blood of group A is a matter for the court.

The accused is in a dilemma, if asked to give his blood for test; the result may be greatly in the accused's favour if his group is the same as the injured man's, but if different the result will tell against him.

It is not possible to obtain a specimen of blood without consent, for no accused person need in law provide evidence which may be used against him, but I have known cases where articles known to have been stained with material from the prisoner have been seized by the police, examined, and the results given in evidence.