CLINICAL PATHOLOGY IN GENERAL PRACTICE

BLOOD GROUPING

BY

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Persons whose blood group needs to be known for medical reasons fall into three classes: blood donors, blood transfusion recipients, and pregnant women.

Blood donors are almost exclusively grouped by the National Blood Transfusion Service. Relatively small numbers are grouped by the Greater London Red Cross Blood Transfusion Service and by individual hospitals. It practically never happens that a donor has to be grouped or a sample taken for grouping other than at a large transfusion centre of some sort.

Apart from the most severe obstetrical haemorrhages, and uncontrollable haemorrhages due to accidental trauma, transfusions are never carried out away from hospitals. Such obstetrical and traumatic cases will, in general, be transfused with group O, Rh-negative blood by "flying squads" without preliminary grouping. Hence blood grouping of recipients is always carried out in hospitals or is done for them by the laboratories of the National Blood Transfusion Service. The same applies to the direct matching between the blood of donors and recipients.

The blood grouping of pregnant women is carried out for antenatal clinics by hospital laboratories or by the National Blood Transfusion Service, and the necessary samples are taken at the clinics. When a pregnant woman is not attending a clinic, her own doctor should arrange for the performance of the necessary tests either at the local hospital laboratory (as for other pathological tests) or at the Regional Blood Transfusion Centre. Local arrangements vary, but information about them can be obtained from the regional transfusion director or from the medical officer of health.

Collection of Specimens

For the tests a sample of blood of at least 5 ml., and preferably 10 ml., should be taken with a dry sterile syringe into a dry sterile tube. This relatively large volume is needed in order to supply enough serum for antibody tests. If the necessary syringe and tube are not available it is best to use a Behring venule* prepared without anticoagulant or preservative. It is perhaps worth repeating that to release the vacuum in a venule the needle should be bent further away from the axis of the tube, not brought into line with it. The alternative of a syringe kept in alcohol carries the well-known risk of transmitting homologous serum jaundice, but if it is used it should be rinsed several times in sterile physiological saline.

If a woman is found to have an abnormal antibody in her serum it may be necessary to test specimens from her husband and in some cases from their children. The specimen from the husband should consist of 2 to 5 ml. of whole clotted blood. Specimens from children may have to be obtained by pricking the heel, ear-lobe, or finger with a triangular needle. At least 10 drops of blood should be taken into a small dry sterile tube; some laboratories may, however, ask for such specimens to be taken into 3.2% sodium citrate or other anticoagulant.

Where there is a possibility of haemolytic disease of the newborn, it is necessary to carry out blood grouping and

other tests on the blood of newborn infants. For certain of the tests (especially the haemoglobin estimation) a cord-blood sample is essential. As soon as the cord is cut and tied, 5 ml. of blood is taken with a dry syringe (or venule) from the maternal end of the cord and put into a tube containing dry oxalate or heparin. It is important to use a dry tube so as to obtain an accurate result in the important haemoglobin determination. A separate specimen without anticoagulant is needed for serological tests. Capillary blood from the infant is useless for haemoglobin tests if they are to be of prognostic value in haemolytic disease of the newborn. The blood should be taken by piercing the umbilical vein in the tied cord as though it was a subcutaneous vein. Any attempt to obtain blood from the open cut end of the cord is apt to lead to contamination with Wharton's jelly or even with maternal blood.

The ABO Blood Groups

The only tests which are performed on all (or nearly all) blood samples are those for the ABO groups and for the Rh factor or factors.

The ABO groups depend upon the existence on the red-cell surface of two substances or antigens known as A and B. Chemically they are very closely similar polysaccharides of high molecular weight, and the only clearly defined difference between them is that shown in agglutination reactions of the kind described below. All human red cells are classified into four groups—A, B, O, and AB—according to the presence on them of either, neither, or both of these antigens. These are detected by tests carried out with certain human sera containing antibodies in solution. When red cells carrying the antigen A are mixed with a serum containing anti-A, the surface properties of the red cell are altered in such a way that the cells stick to one another or agglutinate into large clumps. Cells carrying the antigen B react similarly with a serum containing anti-B. The antibodies in question are present in normal human sera: each serum contains all those antibodies which will not agglutinate the red cells present in the same blood. Thus anti-A testing serum is derived from group B blood, and anti-B serum from group A blood. The above facts are summarized in the Table.

Name of Blood Group	Antigens on Red Cell	Red Cell Reaction with Anti-A	Red Cell Reaction with Anti-B	Antibodies in Serum
O	—	—	—	Anti-A and Anti-B
A	A	+	—	Anti-B
B	B	—	+	Anti-A
AB	A and B	+	+	—

When any blood is tested it is necessary not only to examine the red cells by means of standardized anti-A and anti-B sera but also to test for the presence of anti-A or anti-B antibodies in the serum by the use of red cells known to be of groups A and B. Tests are in general carried out by mixing small quantities of the appropriate sera and red-cell suspensions in glass tubes. The mixtures are kept at room temperature for two hours before being examined microscopically. A working description of the technique of blood grouping is beyond the scope of this article and is given in the *Medical Research Council War Memorandum No. 9*.

In deciding what blood is suitable for a given recipient, the principal consideration is the absence of reaction between

*See the article by Dr. Martin Hynes on "Dispatch of Specimens," published on October 17, p. 876.

the red cells of the donor and the serum of the recipient. Since high titres of antibody in the serum of the donor may also on rare occasions cause trouble, it is desirable always to give a recipient blood of his own group. In an emergency, however, group O blood can without great danger be given to anyone.

The Rh Factor

Every person, whatever his or her ABO blood group, is either Rh-positive or Rh-negative—that is, the red cells either contain or do not contain an antigen known as the Rh factor, or, more precisely, as the D antigen. The corresponding antibody, anti-Rh or anti-D, is not normally present in the serum of D-negative persons, but such a person, if transfused with D-positive blood or, in the case of a woman, if she bears a D-positive child, may become immunized by the D factor, so that an anti-D antibody develops in the serum. A subsequent transfusion of D-positive blood will then give rise to haemolysis and to a severe reaction. About 50% of D-negative persons receiving a single transfusion or course of transfusions of D-positive blood will become immunized. Of D-negative women who bear D-positive children, about 1 in 20 becomes immunized. It is thus important that D-negative persons, especially women, should, if transfused, receive only D-negative blood. The serum of D-negative persons who have been immunized to the D antigen will, under suitable conditions, cause *in vitro* agglutination of D-positive red cells. Such pathological sera are used in Rh blood-grouping of transfusion donors and recipients.

It is desirable also that the blood of all pregnant women be tested for Rh as well as for ABO. Two objects are in view: one is to be ready to transfuse should there be severe haemorrhage at the time of parturition; the other is so as to recognize the presence of immunization and so to be prepared for the birth of an infant with haemolytic disease. Every woman who is D-negative should therefore have her serum tested for the presence of anti-D.

Rh-testing procedures are complicated by the fact that the Rh factor is a complex entity, though most of its details have very little direct bearing on clinical medicine. Whatever other antigens may be or may become involved in the situation, however, all transfusion recipients who are negative for the D antigen, and likewise all pregnant women who are D-negative, are liable to develop anti-D. Thus a test of the red cells of all such persons should be made with anti-D serum: for nearly all purposes this single test is the only one that need be performed on the red cells.

The tests on donors are less simple and the extra tests mainly concern two other antigens of the Rh complex, known as C and E. Most D-positive bloods contain either or both of these antigens as well as D, and D-negative persons who develop anti-D are liable to make anti-C or anti-E as well. The transfusion of blood from a donor who is D-negative but C-positive or E-positive will of course be dangerous to such a recipient. Thus it is necessary after selecting D-negative donors with an anti-D serum to eliminate the relatively rare ones who have the C or the E antigen, by further testing with anti-C and anti-E sera. There is another antigen which may give rise to difficulties in the testing of donors, recipients, and mothers. It is known as D^u , and persons who have it are liable to appear D-positive when tested with some sera and D-negative with others. When the possibility of its presence is realized this is easily confirmed or disproved. Persons who are D^u -positive may be treated for clinical purposes as D-positive.

Tests for Rh antibodies in sera are carried out by means of red cells of known Rh groups. The sera of persons who are to be transfused may contain any of the antibodies already mentioned (anti-A, -B, -C, -D, -E), as well as other rarer ones which need not be described here. Even Rh-negative blood may react with some of these. It is therefore necessary before any transfusion to test the compatibility of the red cells of each donor with the serum of the recipient.

The methods of Rh testing and of direct matching are described in "The Rh Blood Groups and their Clinical Effects," by P. L. Mollison, A. E. Mourant, and R. R. Race (*Medical Research Council Memorandum No. 27*).

Planning of Blood Grouping

Only the principles and not the practice of blood grouping (including direct matching) are described in this article. It is of the utmost importance that it should be realized both by those who do blood grouping tests and by those who without adequate training may wish to do them, that, of all pathological tests, blood grouping carries the greatest responsibility: it is the one kind of test in which a single error may lead directly to the death of a patient. Thus no one who has not received a course of training in blood grouping should ever attempt to carry out such a test. Under emergency conditions, when the temptation to do the test will be the greatest, the danger of error will also be greatest. Rather should an irate pathologist be awakened from his bed and compelled to travel many miles to the hospital. Any doctor who is going to be completely isolated from laboratory facilities should obtain training in blood grouping just as he would in emergency surgery.

Instruction of house-officers and others in routine blood grouping and direct-matching techniques can be arranged through any regional transfusion director. While the house-officer untrained in blood grouping must never attempt to carry out the test, it is important that both he and his seniors should have an idea of what is involved in blood grouping and direct matching so that they can collaborate effectively with those who are doing the tests. It is agreed by most pathologists that adequate blood grouping and cross-matching tests require incubation for two hours. Hence, in the case of planned operations which may involve transfusion, a sample of the patient's blood should be sent to the laboratory well in advance of the operation—preferably some days before. Many laboratories have special arrangements for emergency grouping and direct matching by special methods which take about half an hour. Where such provision has been made emergencies are only very rarely so acute as to demand the transfusion of group O, Rh-negative blood into an ungrouped patient. Many so-called emergencies can be foreseen, and it must be realized that with emergency direct matching the possibilities of error, though slight, are appreciably greater than in normal tests.

In certain classes of surgery it is necessary that there should be available for each patient large quantities of blood which may or may not prove to be needed. In some cases the same bottles can be matched direct against the blood of several patients, but to save opening of bottles several times, and also possible confusion between the bottles intended for different patients, it is important that the routine of notification, sampling of patients' bloods, testing, and labelling of bottles should be carefully planned by consultation between the surgeon and the pathologist.

Interpretation of Rh Blood-group Tests

Cards bearing a statement of the ABO and Rh group are given to blood donors, to most pregnant women, and to a few transfused persons. On cards given to Rh-negative (D-negative) persons in the latter two classes there may also be a statement that the serum contains anti-Rh antibodies. A woman who knows that she is Rh-negative is likely to approach her doctor for information and advice about the possible effects on her children. If the husband or prospective husband is known to be Rh-negative as well, complete reassurance can be given. The shortage of testing sera is such that it is only in exceptional cases that a special test of the husband's blood can be justified for this purpose. In an Rh-negative woman who has never had a blood transfusion, the occurrence of clinical haemolytic disease of the newborn affecting a first pregnancy is practically non-existent, though slight immunization of the mother may occur. In the second pregnancies of Rh-negative women married to Rh-positive

husbands about 1 in 20 fetuses will suffer from haemolytic disease. The proportion of affected infants will increase somewhat with successive pregnancies. Once a woman has become immunized to the Rh factor all her subsequent Rh-positive children will suffer from haemolytic disease. If her husband is homozygous Rh-positive—that is, if he has received the Rh or D factor from both of his parents—then all the children will be Rh-positive (and therefore affected by the disease), whereas if the husband is heterozygous Rh-positive (having received the Rh or D factor from one parent only and the Rh-negative or d factor from the other parent) then any subsequent child has equal chances of being Rh-positive and affected or Rh-negative and healthy.

If any child of the couple is known to be Rh-negative this shows the husband to be heterozygous. Tests carried out on the husband's blood alone can never show with complete certainty whether he is homozygous or heterozygous, but they can do so with a high degree of probability. Where, therefore, an Rh-negative woman has become immunized to the Rh factor and there is a desire to increase the family, the Rh genotype of the husband should be determined. The necessary test requires the use of scarce and valuable kinds of antisera and can be carried out only at regional transfusion centres and a few other laboratories.

The above paragraphs have been written as though the Rh or D factor were the only one responsible for haemolytic disease of the newborn. In rare cases other Rh antigens, the A and B antigens, and antigens of other blood-group systems may be responsible. Where such cases are detected the medical officer in charge of the testing laboratory will readily give the necessary advice on such matters as prognosis for future pregnancies.

It is well known that Rh-negative persons should, in general, be transfused only with Rh-negative blood. If they have become immunized to the Rh factor by pregnancy or transfusion, any further transfusion of Rh-positive blood may be fatal. It is unnecessary to alarm such persons regarding their "condition," but they must be warned always to carry their blood-group cards and to proffer these when entering hospital for any reason.

Next article on Clinical Pathology.—"Investigation of Outbreaks of Infection," by Dr. R. E. O. Williams.

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OPHTHALMIC SURGERY IN INDIA SOME ENCOURAGING FEATURES*

BY

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A visit to India was organized in 1951 by Dr. R. U. Hingorani, an Indian ophthalmic surgeon practising in London. It was primarily a pleasure trip, a sight-seeing tour, but as the party was composed mainly of medical consultants and their wives, with a core of European ophthalmic surgeons, medical conditions aroused most interest, especially the efforts made to remove that great affliction of India, disease of the eye.

The amount of suffering in India is appalling, most of it preventable. This is not surprising, for there is a great shortage of trained medical personnel. A recent estimate states that there is only one trained doctor for

every 10,000 of the population, as against one for 900 in Great Britain. In addition, the doctors are poorly distributed, being congregated mostly in the towns. Facilities for the application of modern scientific medicine are lacking, and there still exists a good deal of native prejudice. Quacks abound. Some have a useful knowledge of Indian herbs and a skill in their application, passed on from father to son, but many indulge in superstitious practices concerned with the exorcizing of demons or the placating of evil spirits, practices which are often both crude and cruel. A man in any walk of life may pick up something about cataract extraction, set up in practice, and soon acquire a reputation as an eye specialist. Government intervention is not yet considered necessary, but it must come sooner or later.

Living Conditions

Poverty abounds. Most of our party had to revise their conception of the meaning of the word "poverty." In some of the refugee camps human beings were seen with scarcely a roof to cover their head. In the village huts we visited there was sometimes little else than some cooking utensils, a few fowls, and a receptacle to hold the family's supply of rice for the year.

With poverty go overcrowding and malnutrition. In the city of Bombay some 200,000 people sleep on the streets. This is partly a matter of choice owing to the heat, but is also partly due to the fact that many have little more than an accommodation address, a room which 20 or more people use to hold their worldly possessions while they eat and sleep on the streets.

Rice is the staple food; indeed, it is almost the only one, with resulting lack of balance in diet and deficiency disease. The fields given over to the cultivation of rice may provide excellent breeding grounds for mosquitoes and the spread of malaria.

A feature of Indian life that strikes the stranger forcibly is the lack of adequate attention to sanitation. Large cities like Calcutta still have open sewers in the streets, and cows are allowed to wander about unmolested even in the main thoroughfares. Some of the villages we visited were quite unprovided with streets, lighting, and sanitation, so that the spread of epidemics in the time of the monsoons can scarcely be avoided.

Finally, there is still to be found the secluded life of many women living in purdah. This system intensifies the lack of medical attention for women and children, and thus leads to needless suffering. A recent estimate gives the expectation of life for an Indian woman as 27 years as against 67 in Great Britain.

It is not my purpose to dwell on the lethargy, poverty, ignorance, and superstition of India. Rather do I wish to draw attention to some signs of an awakening to the need for action which is going on, particularly among Indian doctors. The Government also is becoming more alive to the need to do something to remove the great scourge of disease from India.

The most serious medical affliction is tuberculosis. Next comes eye disease; and here most authorities agree that at least 90% of it is preventable. I shall refer to one or two encouraging features in the ophthalmological field.

The Gandhi Eye Hospital

The Gandhi Eye Hospital is in Aligarh, a Moslem university town of 140,000 inhabitants, in the State of Uttar Pradesh (formerly United Provinces), 84 miles south-east of Delhi. The hospital has 100 beds, and plans to increase the number to 250. It began in 1928 as the private clinic

*A lecture delivered in the Department of Ophthalmology, University of Glasgow, on May 14, 1952.