THE TECHNIQUE OF BLOOD GROUPING AND THE INDICATIONS FOR BLOOD TRANSFUSION.

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Blood Grouping.

It is essential that the stock agglutinating sera used are of satisfactory potency and devoid of prozone phenomenon (inhibitory action, when undiluted). Such sera should have an initial agglutinin titre of at least 1 in 100 and will then remain reliable, even at room temperature, for at least six months (sera conforming to these standards are now available commercially from Messrs. Burroughs Wellcome and Company).

An opal glass plate constitutes the best surface on which to carry out blood grouping. In cold weather, to minimise the possibility of minor degrees of "cold" agglutination, it is wise to warm the plate by holding under the warm water tap and then wiping dry before use. A drop of each of the Group II and III* sera, contained in capillary tubes of distinctive colour, is placed on the plate in areas labelled with a grease pencil, care being taken in assigning each serum to its appropriate space.

A globule of blood is obtained by needle puncture of the finger or ear lobe of the individual to be grouped and a small drop transferred to, and mixed with, each of the stock sera with a platinum loop. The amount of blood added is sufficient to colour the mixture pink, but not deep red; with the former proportions it is easier to recognize finer degrees of agglutination. The platinum loop must be heated and cooled between each addition.

The circular area covered by each red cell-serum mixture should be about half-an-inch in diameter. The plate is gently agitated so as to ensure adequate admixture of the red cells and sera, and, provided the latter are potent, agglutination, if it is going to occur, will show macroscopically within two minutes. The aid of a small hand lens of about $\times 10$ magnification is sometimes helpful, but the use of a microscope is not required. Re-examination of the plate after the sera and cells have been standing for some time—e.g. ten minutes—may suggest false agglutination owing to partial drying and rouleaux formation, in a previously negative result. No significance should be attached to such findings. Determination of group with a relatively fresh oxalated or citrated specimen of blood is quite satisfactory.

CELLS.	SERA.		SERUM.	CELLS.	
	Group II.	Group III.		Group II.	Group III.
Group I	+	+	Group I	—	—
II	—	+	II	—	+
III	+	—	III	+	—
IV	—	—	IV	+	+
Routine grouping test with red cells against stock Group II and III sera.			Confirmatory test with individual's serum against known Group II and III cells.		

* The group numbers in text and tables refer to the Moss classification.

Confirmation of a blood group may be obtained by testing the individual's serum against known Group II and III corpuscles. By this means the agglutinin content of the serum is demonstrated, in addition to the agglutino-gen factor in the red cells determined in the routine test.

Before transfusion, a direct test should always be carried out between the chosen donor's red cells and a drop of the prospective recipient's serum using the technique above described. This constitutes a check on the standard grouping and also avoids the possibility of a sub-group reaction peculiar to the two individuals concerned. When grouping sera are unavailable this direct test may alone form the basis of compatibility.

A drop of serum is easily obtained within twenty minutes by withdrawing about 2 cc. of blood from an arm vein of the patient and allowing it to clot in a tube; centrifugalization is unnecessary. A drawn out glass pipette is used to remove a bead of serum from the rim of the clot. In anæmic states the latter separates readily and the serum yield is rapid and generous. The practice of mixing donor's blood with patient's blood in a direct test is to be deprecated; clot formation is liable and the red background of the patient's blood tends to obscure minor degrees of agglutination of the donor's red cells.

An apparent incompatibility in the direct test, of a donor who should be acceptable to the patient, as judged from the standard groupings, may result from rouleaux formation or from "cold" agglutination properties in the patient's serum.

Rouleaux formation (or pseudo-agglutination) is the commonest cause of this apparent incompatibility. The serum of some patients, especially if febrile, has a marked tendency to cause rouleaux formation of admixed red cells; this tendency is associated with an increased erythrocyte sedimentation rate. The naked-eye, or lens magnification, appearance is that of a slight uniform granularity; the variation in size of the clumps of red cells seen in agglutination is absent. Even if a microscope is used it is not always possible to be certain of the exact identity of rouleaux formation. It is best distinguished in blood grouping by noting that dilution of the serum on the plate with an equal sized drop or two drops of normal saline, and re-performance of the test, results in disappearance of the phenomenon. This assured, the direct test can be passed as satisfactory and the donor bled.

"Cold" agglutination is a rarer cause of apparent unsuitability in the direct test of a compatible group donor. The patient, whose serum exhibits this property, has usually a marked grade of anæmia and the incidence of liver disease is high. At room temperature, agglutination of the donor's red cells into heterogeneous sized clumps occurs. This reaction is accentuated by carrying out the test after serum and plate have been cooled under the cold water tap. It becomes characteristically less marked and tends to disappear if both serum and plate are warmed to 37°C. immediately before. The presence of clumping in the cold and its absence or marked diminution at 37°C. identifies the phenomenon as one of "cold" agglutination. Confirmation of this is obtained by getting a similar result with the patient's own corpuscles—auto-agglutination and "cold" agglutination being always associated properties. The demonstration of "cold" agglutinins in a patient's serum need not contra-indicate transfusion from a compatible group donor; it is imperative, however, to keep the temperature of the transfused blood strictly at body heat or just above throughout the procedure.

If the problem of an unsatisfactory direct test is not solved by identifying the cause as rouleaux formation or "cold" agglutination as above detailed, a true incompatibility must be assumed and transfusion from the donor tested is contra-indicated.

This incompatibility may result from incorrect initial grouping of donor or patient, or more rarely from a true sub-group agglutinative reaction (mainly confined to Group II individuals). The routine grouping of each should be re-checked with known potent and fresh stock sera.

Indications for Blood Transfusion.

The anæmia of acute or chronic hæmorrhage comprises a large and obvious group of cases necessitating this remedy. Transfusion should be considered imperative if the hæmoglobin falls to 40 per cent. (Haldane), a level below which life is endangered. In addition to improving the patient's general condition, the blood has in some cases a definite hæmostatic effect; if the hæmorrhage has been controlled, transfusion will diminish the period of convalescence.

Before an anæmic patient is subjected to a severe surgical operation, a minimal hæmoglobin figure of 70 per cent. should, if possible, be attained. Recurrent bowel hæmorrhage from a peptic ulcer, demanding surgical intervention as a last resort, is a condition to which the above figures are particularly applicable.

In a severe infection (with or without proved septicæmia) transfusion is indicated if the hæmoglobin recedes to 65 per cent.; it aids the patient's resistance by combating the anæmia and also by supplying fresh human complement. Ulcerative colitis, a toxic state combined with blood loss, is definitely benefited by blood transfusion. The doubtful value of immuno-transfusion in septicæmia will apparently in the future be replaced by the more promising chemo-therapy.

Blood transfusion is of great value in the hæmorrhagic states and blood diseases. For hæmophilia it constitutes a specific, though temporary, remedy; in addition to being an essential pre-operative safeguard in a patient with prolonged coagulation time, the administration of normal blood (about 300 cc. to an average-sized adolescent) will have a hæmostatic effect on a bleeding focus. In hæmorrhagic disease of the new born, transfusion is curative, and in purpura hæmorrhagica, by raising the patient's platelets to above the critical level, dangerous hæmorrhage can usually be controlled. The hæmolytic anæmia of Lederer and an allied type seen in pregnancy both respond well to transfusion of blood, although in the latter condition the uterus may have to be emptied. In pernicious anæmia of severe degree, blood transfusion is sometimes indicated to tide the patient over the latent period of a few days before the beneficial action of liver therapy is apparent. It is also the main treatment for aplastic anæmia, although of transitory value only; in the chronic leukæmias, especially the myeloid variety, and in Hodgkin's disease the effect is to prolong life somewhat by combating the anæmia and by allowing of effective irradiation.

In surgical shock, blood transfusion constitutes the best method of increasing blood volume, which has become diminished owing to stagnation of the blood in the abdominal veins.

Lastly, this form of therapy has proved of value in cases of poisoning by substances having a primary effect on the hæmoglobin of the circulating erythrocytes. Coal gas, the carbon monoxide of which, by forming the relatively stable carboxyhæmoglobin, reduces markedly the amount of functioning available hæmoglobin, is the important example. A large volume transfusion of healthy blood, preceded by venesection of the patient, increases the amount of available circulating oxyhæmoglobin and may be life saving.