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## A RAPID AND SIMPLE METHOD OF TESTING DONORS FOR TRANSFUSION \*

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The increasing popularity of transfusion as a therapeutic measure has brought with it the need of a test whereby the suitability of donors may be rapidly determined. There are already many instances on record of serious symptoms or death following the rapid breaking down of blood after transfusion. The evidence is convincing that such instances may be avoided if a donor is selected beforehand by means of certain simple tests carried out *in vitro*. Unfortunately these, as performed at present, require time and labor. It is with the aim of providing a test rapid and simple enough to be employed even in emergencies that the following method has been devised:

### METHOD

**Collection of the Blood.**—The blood is taken from the patient and the prospective donors in a 1:10 mixing pipet, such as is used in counting leukocytes. The pipet is rinsed beforehand with 10 per cent. sodium citrate in water; the citrate solution is drawn up to the mark 1; the pipet is rapidly filled with blood from a puncture of the ear or finger; and without pause the mixture is expelled into a small, narrow test tube. There is thus obtained a citrated blood containing slightly less than 1 per cent. of citrate. The pipets which we have employed hold only 0.25 c.c. of fluid. This much blood is easily obtained from a single puncture. There is no objection to increasing the flow by pressure. Should it cease before the pipet is full, the blood must be at once expelled into a test tube, in order that it may mix with the citrate and clotting be avoided. The mixture is then taken up again, a new puncture made, and the pipet completely filled. After each blood is obtained, the pipet is rinsed with citrate, then with distilled water, then with fresh citrate, and it is ready for another blood. If several donors are to be tested, two pipetfuls of citrated blood should be obtained from the patient. It is best to take them from different puncture wounds, in order to avoid a possible clotting in the pipet.

**Mixing.**—The mixing is done in pipets with a capillary end—the so-called Wright pipets obtained by drawing out glass tubing in the flame. The citrated bloods are used as such, and two combinations are made of the patient's blood with that of each prospective donor—a mixture containing nine parts of the patient's blood to one of the donor's, and a mixture of equal parts of the two. The proportions used need be only approximate. In case of emergency the first of the

mixtures will suffice, since by its use the most dangerous possibility, namely, that the blood of the recipient may destroy that of the donor, can be ruled out. Following the technic usual with Wright pipets, the capillary tube is marked, blood is drawn to the mark, and each column of the blood is separated with an air bubble from the next that is drawn up. To insure proper mingling, each mixture should be expelled on a slide, or Widal plate, and then drawn high in the pipet, which may be sealed off in the flame in case the examination is not to be made for some time.

**Incubation.**—No incubation in the ordinary sense is necessary. The pipets are kept at room temperature, and readings are begun after two minutes, if there is need to hurry. The readings are for agglutination, and even within two minutes this is plainly evident, except when the agglutinating forces are notably weak. In the final choice of a donor it is safest to rely on results obtained after the mixtures have stood for fifteen minutes. But the ruling out of individuals with unfit blood may be begun practically at once.

**Readings.**—The capillary end of each pipet is broken, a small drop of the blood expressed on a slide, a large drop of normal salt solution superimposed without mixing, a coverslip put on, and the preparation examined for agglutination under the microscope. Fresh preparations can be made at intervals if desired. The salt solution is not absolutely necessary; but very clear pictures are obtained as the blood spreads in it. When agglutination has occurred, the red cells show a characteristic clumping, sometimes in small masses, often in large ones that are very evident macroscopically. The clumps in each preparation are fairly uniform in size. The picture is absolutely different from that in mixtures of nonagglutinating bloods under similar conditions. In these, the cells lie free or in rouleaux, just as in a single blood. But in agglutinating mixtures the cells are stuck together "every which way," and, where the film is thin, they do not separate but lie connected with one another in irregular heaps. If pressure is put on the coverslip, a very characteristic phenomenon may sometimes be seen. The agglutinated cells pull out in strands as though they consisted of some sticky substance. The most striking pictures are encountered when there are nine parts of an agglutinating blood to one that is agglutinated. Here large discrete masses lie scattered amid unclumped red cells.

If there is no clumping in the preparations made after the mixtures have stood fifteen minutes, the assumption is warranted that the bloods do not agglutinate or hemolyze each other. The experience of previous workers has taught that in such instances transfusion is safe. But if clumping is present in the 9:1 mixture and to a less degree or not at all in the 1:1 mixture, it is certain that the blood of the patient agglutinates that of the donor and may perhaps hemolyze it. Transfusion in such cases is dangerous. Clumping in the 1:1 mixture with little or none in the 9:1 indicates that the plasma of the prospective donor agglutinates the cells of the prospective recipient. The risk from transfusing is much less under such circumstances, but it may be doubted whether the blood is as useful as one which does not and is not agglutinated. A blood of the latter kind should always be chosen if possible.

For practical purposes these findings suffice. But if there is a desire to know whether *both* bloods contain agglutinins, a 1:9 mixture should be made. If this and the 9:1 mixture show large clumps, whereas the clumps are smaller when the bloods are mixed in equal parts, two agglutinins must be present.<sup>1</sup> Should there be only one agglutinin, little clumping or none will be observed when the blood containing the agglutinin is diluted with nine parts of the other blood.

**Difficulties.**—The single technical difficulty of the method is that of clotting, and to avoid it the blood should be taken as rapidly as possible. With normal blood, trouble is seldom experienced, but in pathologic instances a thin web may form in the test tube into which the blood is expelled. The clotting

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1. This phenomenon has been described by Ottenberg in an interesting study of the conditions under which human bloods show agglutination. He used mixtures of defibrinated whole blood. (Ottenberg, R. Jour. Exper. Med., 1911, xiii, 425.)

begins in the calibrated tube of the pipet which, as the blood is taken, is swept free of citrate; and the use of a strong citrate (15 per cent.) will not prevent it. But the clot is always thin and may be picked out of the blood and the latter used for the tests. In the pipet even small clots are troublesome, since they form a locus for new ones when another blood is taken. They are best dissolved out with 5 per cent. potassium hydroxid.

The likelihood that something else may be mistaken for agglutination is practically nil. The presence of fibrin strands and clumps of platelets and white cells absolutely differentiates bits of clot. Dried blood will not be found except in case of carelessness, and it is unlikely to cause confusion. Its nearly homogeneous, cheesy appearance under the microscope is sufficiently distinctive.

#### COMMENT

The test here described has some features in common with two of the methods already in use. Therefore, to point out wherein it is an advance over these will not seem amiss. Epstein and Ottenberg<sup>2</sup> use Wright's pipets, collecting blood in them for serum, and making the ultimate test mixtures in them. But the mixtures consist of serum and washed red cells; they are incubated for two hours, and the readings are macroscopic. Weil<sup>3</sup> employs citrated bloods, making, in test tubes, three mixtures of the same relative proportions as ours. Two c.c. of each blood are required, thus making necessary aspiration from a vein; the tubes are incubated for an hour, and the readings are macroscopic. By means of the test of Epstein and Ottenberg, aspiration from a vein is avoided; and by Weil's method the need to separate serum, wash the red cells and make reciprocal observations is done away with. In both instances, however, the time and the labor of testing donors is very considerable. Using our method, it is only necessary to obtain citrated bloods from a finger-prick, make two mixtures of them in capillary pipets, and, by reading with the microscope, the test is finished within a few minutes.

The method involves several important assumptions. They may be summed up in two questions: 1. Is the presence or absence of agglutination an index to the injurious qualities of a blood? 2. Are the microscopic findings with mingled, citrated bloods that have stood fifteen minutes at room temperature as certain in their indication as macroscopic findings after longer periods with mixtures of serum and 5 per cent. washed red cells—that is to say, as certain as the test most used at present?

The first question can be answered from the literature. Leaving from consideration disease in the donor, two injurious influences must be thought of when a foreign blood is introduced into the human body, namely, hemagglutination and hemolysis. The latter is far the more serious. Moss<sup>4</sup> has found in extensive observations that agglutination frequently occurs without hemolysis, but that hemolysis is always associated with or preceded by agglutination. This conclusion he has substantiated with seventy-five successful transfusions<sup>5</sup> in which the donors were selected by means of the agglutination test. It is well

known that human beings fall into four groups as regards the agglutinins in their serum and the capacity of their corpuscles to be agglutinated. Moss was careful to select for transfusion individuals of the same group. Without going deeply into the matter we may say that, when serum and washed cells are used, reciprocal observations are necessary to determine whether two individuals belong to the same group. But in mixtures of whole citrated bloods, agglutination is only absent when this is the case.

In our test, agglutination is looked for with the microscope. This does not mean that macroscopic readings are impossible, for when clumping is at all outspoken in the slide preparation, it is plainly evident to the naked eye. Observations can, in emergency, be made in this way. But with the microscope, as one would naturally suppose, the readings are more sensitive and more precise. When antirabbit goat serum is mixed with rabbit red cells, clumping is observable microscopically in dilutions of serum much higher than those in which it is evident macroscopically in the test tube. This can be noted also with dilutions of an agglutinating human serum mixed with human red cells and with undiluted human serums weak in agglutinins. An agglutinative clumping is rarely doubtful microscopically, for the cohesion of the cells, even when only two or three are concerned in each clump, is absolutely different from rouleaux formation. In the test tube an agglutination is often difficult to distinguish from sedimentation; and the shaking which throws up the clumps into the fluid that they can be viewed may break them so that they do not reform.

Our experience is that if agglutination occurs at all it will be noticeable microscopically within five minutes. But it is better to let the mixtures stand longer. We have no hesitation in saying that agglutination can be told with the microscope as certainly after fifteen minutes as in two hours with the old macroscopic method. The explanation of this is probably simple. The clumps which become visible in the test tube after an hour or more are in general not the primary clumps of an agglutination, but the result of the massing together of many such clumps, which themselves are often small. Such secondary massing requires time, whereas the primary clumps form almost immediately.

That agglutination is sometimes better at room than at body temperature is well known.<sup>6</sup> Repeated trial has shown that the incubation of our agglutinating mixtures seldom increases the rapidity of the reaction. In some instances agglutination was much more pronounced after fifteen minutes at room temperature than in two hours at body heat. An altered distribution of the agglutinin may account for this.

It has been repeatedly shown that an excess of red cells may mask the presence of an agglutinin or hemolysin, and that hemolysis is much influenced by the presence of neutral serum. Agglutination is practically independent of this latter factor.<sup>7</sup> The influence of small amounts of sodium citrate does not seem to have been determined. The following is one of several experiments dealing with these points in their relationship to our test:

#### PROTOCOL OF EXPERIMENT

By venous puncture some cubic centimeters of a human blood, A, were taken with citrate (1 part 10 per cent. citrate to 10 parts blood) and some obtained for serum. Graded

2. Epstein, A. A., and Ottenberg, R.: A Method for Hemolysis and Agglutination Tests, *Arch. Int. Med.*, May, 1909, p. 286.

3. Weil, Richard: Sodium Citrate in the Transfusion of Blood, *THE JOURNAL A. M. A.*, Jan. 30, 1915, p. 425.

4. Moss, W. L.: *Johns Hopkins Hosp. Bull.*, 1910, xxi.

5. Moss, W. L.: *Am. Jour. Med. Sc.*, 1914, cxlvii, 698. See also, for the bearing of agglutination on transfusion, Ottenberg, Reuben, and Kaliaki, D. J.: Accidents in Transfusion, *THE JOURNAL A. M. A.*, Dec. 13, 1913, p. 2138.

6. Paltauf, R., in *Kolle-Wassermann's Handbuch der pathogenen Mikroorganismen*, ii.

7. Hektoen, Moss, Ottenberg.

dilutions of the serum were made with salt solution, and mixed in small test tubes, as also in Wright's pipets, with equal parts of 5 per cent. suspensions in salt solution of certain washed red cells, *B* and *C*, which the undiluted serum agglutinated.

Dilutions of the plasma of the citrated blood *A* were made, similar to those of the serum, but the proportion of red cells was kept the same as in the original citrated blood. For this the citrated blood was distributed in a number of graduated tubes, centrifugalized rapidly, the supernatant fluid measured and diluted appropriately, and, when the red cells had been suspended and centrifugalized, all of the fluid was pipetted away except the original volume, and the cells were resuspended. The diluent used was sodium citrate in salt solution (1 part 10 per cent. sodium citrate to 9 parts 0.95 sodium chlorid). Nine parts of each of the combinations thus obtained were mixed in Wright pipets with one part of the whole citrated bloods *B* and *C*. The microscopic findings after fifteen minutes were compared with those in the serum mixtures after one and two hours. They gave identical results. Agglutination occurred when dilutions of serum or plasma up to 1 part to 7 of the diluent had been used for the mixtures, but not when 1 part to 15 had been employed. It was slight with 1:7 plasma and no better marked with 1:7 serum.

The experiment shows that a weak agglutinin may be demonstrated quite as well with mixtures of citrated whole bloods as with serum and a diluted suspension of washed red cells in salt solution. The interference of neutral red cells does not constitute a difficulty.

#### CONCLUSION

The test here described compares well in delicacy with those already in use, but we wish to insist only on its practicability. It enables one to determine within a few minutes, so far as agglutination and hemolysis are concerned, whether or not the blood of a donor is suitable for transfusion.