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Reprinted from The Archives of Internal Medicine
May, 1909

CHICAGO
AMERICAN MEDICAL ASSOCIATION, ONE HUNDRED AND THREE DEARBORN AVENUE
1909

A METHOD FOR HEMOLYSIS AND AGGLUTINATION TESTS

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The established methods of testing for agglutination and hemolysis have been developed chiefly in animal experimentation, and their application to work with human blood is encumbered with certain hindrances. In all serum reactions there is great need for control tests. The quantity of blood which it is necessary to take from patients for these tests limits the work to a certain extent and, therefore, interferes with the drawing of definite conclusions.

To overcome these obstacles we have developed a method whereby it is possible to perform a large number of tests with a very small amount of blood. In principle the method is identical with that now used by all investigators in this field. In details of technic it is an application of the method used by Wright in his work with opsonins. It has for its main object the reduction to a minimum of the amount of serum necessary for each test.

To obtain the blood either of two methods may be used. The method in general use consists of aspiration from one of the veins at the bend of the elbow with a small syringe (10 to 15 cubic centimeters' capacity). The syringe must be washed out previously with normal salt solution and the usual aseptic precautions must be observed. One or two cubic centimeters of the blood are put in a tube containing an excess of salt-citrate solution (0.5 per cent. sodium citrate, 0.85 per cent. sodium chlorid). The red blood cells are subsequently centrifugated, washed and made up to any desired percentage suspension in normal saline solution. The remainder of the blood is placed in small slanted test-tubes and the serum allowed to separate by clotting.

The alternative method is reserved for cases in which, for any reason, the taking of blood in the above manner is not allowed or is impossible (as in infants). Blood is obtained by pricking the lobe of the ear rather deeply with a Hagedorn needle. For the red cell suspension the drops of blood are taken up with a dropper and expelled into a tube of salt-citrate solution, centrifuged, washed and made up to the desired per-

centage. For the serum, capsules, six or seven centimeters long, made of glass tubing about four millimeters in caliber, and drawn out into capillaries at both ends, are used. These capsules fill themselves with blood by capillary action (Fig. 1). When the tube is three-quarters full the unused free capillary end is sealed in a small flame. The blood is allowed to clot and the capsules are centrifuged. After each capsule is nicked with a file it is broken open and the serum can be pipetted off.

For making the mixtures small pipettes (four to five millimeters' caliber), fitted with rubber nipples, are used (Fig. 2). The tips of these pipettes, drawn to a length of two or three inches, are marked at

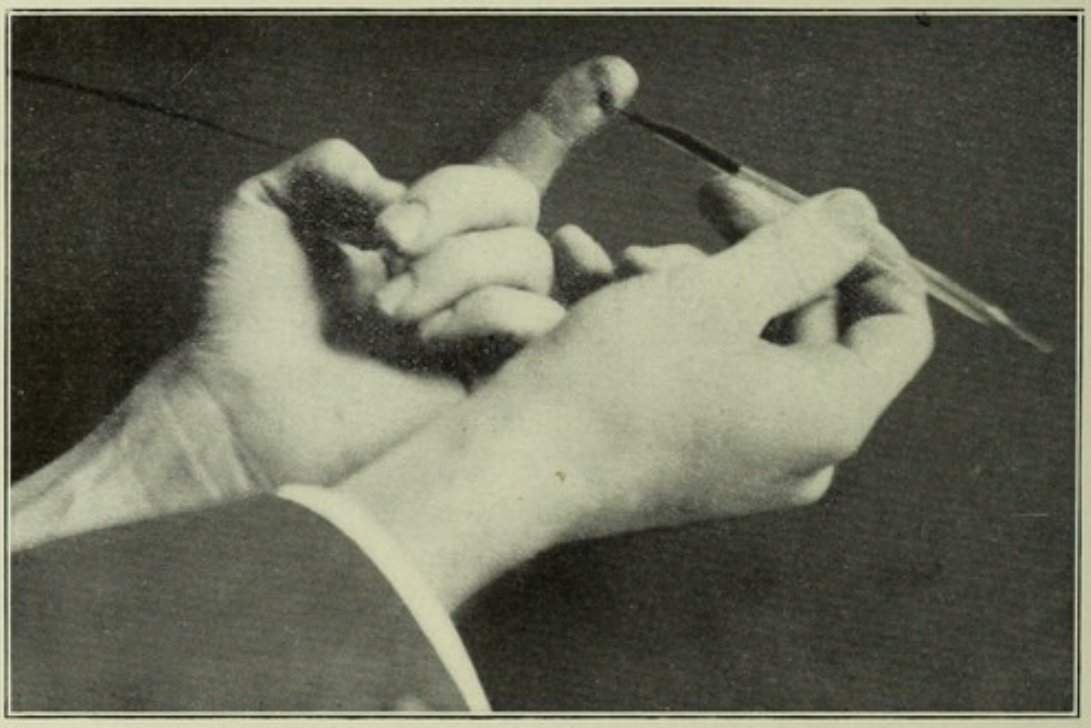

Fig. 1.—Method of drawing blood from the finger.

an arbitrary point with a blue pencil. The suspension of erythrocytes is drawn up to the mark. By drawing this a little farther into the pipette one allows a small bubble of air to enter the tip, and then, in a similar manner, one or more volumes of the serum are drawn into the same pipette. Thus definite proportions of the ingredients can be accurately measured. By running the cell suspension and serum gently up and down in the pipette they become thoroughly mixed. The entire mixture is then drawn up into the body of the pipette and the tip is sealed in a flame. The pipettes may be kept upright by sticking them

into a tumbler of sand. These narrow tubes serve in every detail the purposes of the larger test-tubes generally used.

The glassware should be absolutely clean and dry, but need not generally be sterile.

As to the time and manner of incubation and observation the well-established rules are followed. In studying hemolysis with human red

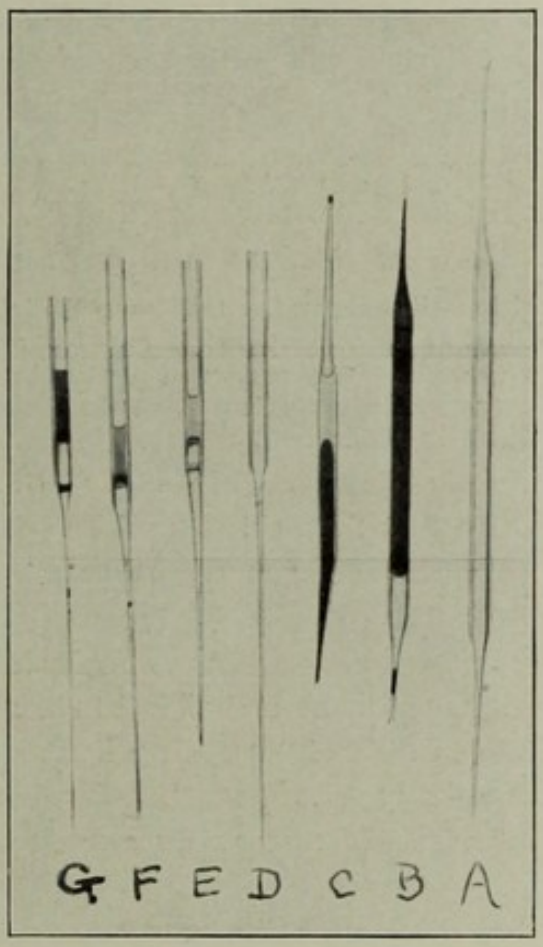

Fig. 2.—Apparatus: A, empty capsule for collecting blood; B, capsule full, lower end sealed, ready for centrifuging; C, capsule centrifuged, serum separated; D, empty pipette for making mixtures; E, pipette, with mixture of red cells and serum which has stood upright for several hours; cells have settled; F, same, showing fairly marked hemolysis; G, same showing very strong hemolysis.

blood cells, it is essential that the mixtures be made within twelve or at most twenty-four hours of the time of collecting the blood. Otherwise the cells become abnormally vulnerable to hemolytic agents. At the end of the two hours in the thermostat most of the cells have usually settled

to the bottom, and pronounced hemolysis can be seen. For finer grades of hemolysis it is usually necessary to allow the tubes to stand twelve to twenty-four hours (in the ice-box).*

Agglutination (which, when it occurs, is rather prompt) can be readily observed in the gross by the clumping and sedimentation of the erythrocytes.

The importance that direct blood transfusion has recently assumed, and the necessity of testing for hemolysis as well as agglutination before each transfusion, should make this method one of clinical value. The ease and rapidity with which experiments can be made by this method make it of value in scientific work. The results obtained have been compared in parallel tests with those of the standard method and found to be identical.

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*When it is desired to make comparative tests as to the intensity of hemolysis, the total amount (as well as the proportions) in each test ought to be the same. In this case it is easy to calibrate any number of pipettes by simply transferring a small drop of fluid (normal salt solution) from the tip of one to the tip of another.