Acholuric Jaundice.

Red Cell Fragility Tests.

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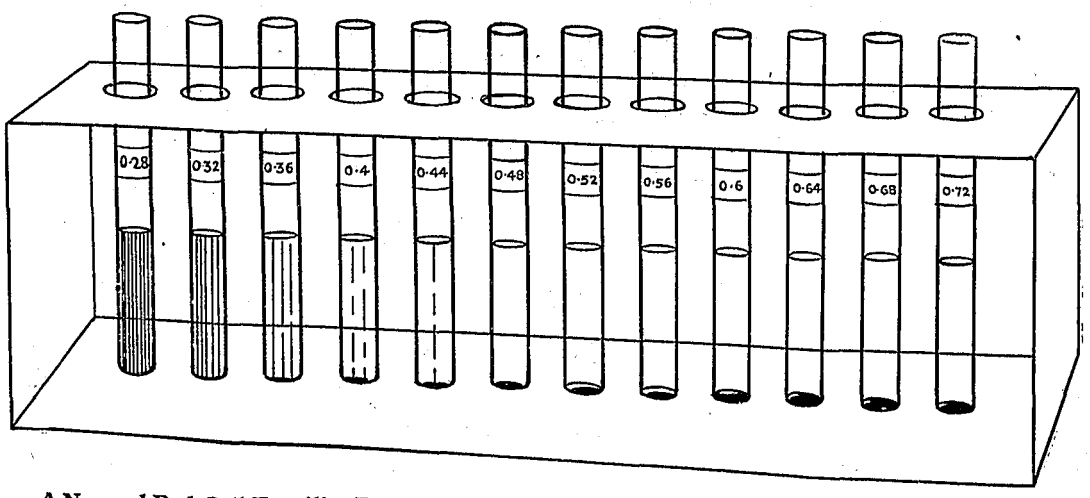
ONE of the characteristics of the blood in the disease variously described as Acholuric Jaundice, Congenital Haemolytic Anaemia and Acholuric Family Jaundice, is an increased fragility of the red cells. This increased fragility can be demonstrated in solutions of saline and briefly this is the principle of the red cell fragility test. This particular determination is one of the essential measures in the complete laboratory investigation of the disease. The test requires careful technique coupled with scrupulous cleanness of apparatus. In the healthy individual there is a continual destruction of the old and worn-out red blood cells; this is a normal process. With certain pathological conditions this destruction of the red cells may be greatly increased, the increased haemolysis may be brought about in a number of ways; in the case of acholuric jaundice it is thought to be due to an abnormal fragility of the red cells themselves. When circulating in the blood stream the red cells are suspended in a fluid of a complex nature, called plasma—in other words, the fluid part of the blood. Plasma has an osmotic pressure equal to a 0.85 per cent. solution of sodium chloride, which is usually known as physiological saline, therefore cells suspended in saline solutions of this strength are unaltered. Raising the strength of the sodium chloride has the effect of shrinking or crenating the cells. On the other hand, a reduction in the saline content much below the level of 0.85 per cent. will cause the cells to show haemolysis. Actually what happens is that the cell envelope ruptures and its contents, haemoglobin, goes into solution. This is precisely the process which occurs in a determination of the fragility rate, when the blood cells under investigation are suspended in a series of saline solutions of reducing strengths. The exact percentage of saline at which haemolysis commences and is complete being together with the degree of haemolysis in the intervening tubes the essential points. Commencement of haemolysis is shown by the escape of haemoglobin from some of the cells into the clear saline, while its completeness is shown by the disappearance of all the red cells.

Collection of Specimen.

While it is possible to carry out a rough test on capillary blood, for an accurate determination of the degree of haemolysis, often a very important point, venous blood is required. Whole blood, that is, blood which has been prevented from clotting by the addition of an anticoagulating reagent, is necessary—about 5 c.c. of blood should be obtained. The choice of an anticoagulant for the blood is another important point; many workers recommend the employment of heparin, but from a ward point of view this is a trifle impracticable as it is rarely available. In practice excellent results may be obtained by the use of an anticoagulating mixture advocated by Wintrobe; it is isotomic and is suitable for most other haematological tests as well as the determination of fragility rates. This mixture consists of 4 mgms. of potassium oxalate and 6 mgms. of ammonium oxalate for 5 c.c. of blood. Most laboratories will have small tubes already prepared for ward use. It is essential that approximately 5 c.c. of blood should be obtained, the amount of dried anticoagulant in the tubes is designed to take care of just this amount of blood. While it is desirable that the specimen should be sent to the laboratory as quickly as possible, fragility determinations can be made with confidence three hours after the collection of the specimen.

Determination of Fragility of Rate.

Unless the ward test room is unusually well equipped, the test is rather one for the laboratory than the test room, not only does it require apparatus not usually available in the ward test room, but it is easily influenced by quite a number of purely physical factors and care must be taken to avoid erroneous results due to fallacies of technique. Accurate saline solutions, distilled water of known *p*H, careful measurement of amounts, proper mixing of the solutions are all important points. The CO_2 content of the blood specimen itself must be eliminated by oxygenation before the test is set up. Of the methods available the one most frequently employed is that of Creed and it consists of a rack of twelve tubes which contain various amounts of 1 per cent. sodium chloride and distilled water, giving a series of concentrations of saline from 0.72-0.28 per cent. This dilution range is usually effected by means of carefully measured drops. To each tube is added one drop of blood, the saline solution and blood being properly mixed. The rack is allowed to stand for 20 minutes then the cells are spun to the bottom of the tubes by means of a centrifuge, leaving the supernatant fluid clear, if no haemolysis has occurred. The degree of haemolysis in the tubes in which it has occurred is assessed by means of comparison with a series of standards prepared from the same blood. Haemolysis is shown by the presence of haemoglobin in the supernatant fluid.



A Normal Red Cell Fragility Rate Determination, the shaded areas in the first tubes indicate where haemolysis has occurred.

previous page next page