liminary report, it was found that vaso-tone efficiency was 50 per cent. greater at 68° than at 86°. It is believed that this test will measure with reasonable accuracy the efficiency of the splanchnic vaso-tone and that this is an important indication of the efficiency of a body and related closely to vitality. It is realized that the test does not measure other important factors of physical and mental efficiency, and will not, for instance, reveal the structural condition of the heart. It does, however, open a new field for the measurement of the results of work calculated to improve physical condition.

74 (1006)

On the preservation in vitro of living erythrocytes.

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The length of life of the functioning red blood cell is not known, but there is indirect evidence that it is several weeks at least. *A priori* one might suppose that if these elements were kept in the cold outside the body their period of survival would be much longer. But as a matter of fact in citrated plasma or defibrinated blood the erythrocytes of many species begin to break down within a week or ten days; and washed erythrocytes in normal salt solution or Ringer's fluid do not last even so long.

In previous papers we have shown that the early hemolysis of washed erythrocytes is attributable in large part to injury during washing; and that this injury can be prevented by the presence of a very little gelatin in the wash fluid (1/8th of 1 per cent.). But even when protected during washing the erythrocytes do not remain intact *in vitro* nearly so long as they are supposed to in the circulation. We have addressed ourselves to the problem of their preservation.

For reasons which need not here be entered into, our first experiments were made with solutions of inorganic salts to which non-protein colloids were added. But it was found that though gelatin will protect red cells against injury during washing it has

no preservative effect; agar proved toxic, as shown by early laking; and dextrin had only slight preservative qualities, except in concentrations which caused a browning of the blood pigment, presumably to methemoglobin. Soluble starch, the watery extract of coagulated blood serum, beef albumen, an aqueous solution of the alcohol-soluble constituents of blood serum, and even serum water made up to isotonicity with sodium chloride were all non-preservative. The sugars alone have proved well suited to our purpose. In a dextrose-Ringer's mixture containing 2½ per cent. of dextrose the erythrocytes of the sheep have been kept intact for a period of two months; and those of man for a month in a somewhat similar solution. The limits of the method have not yet been reached. The sugars which have proved best are saccharose and glucose. Isotonic mixtures are better than hypertonic. For the blood of each species a different preservative solution is required. Thus, the blood of the dog keeps best in a sugar-Ringer's-dextrin mixture, whereas for other bloods dextrin is useless if not harmful. It is interesting that though sugars and dextrin are *preservative* they are not *protective;* red cells handled in solutions of them undergo as much injury as in ordinary Ringer's.

Are the cells kept intact in the preservative mixtures to be considered as surviving? They can be washed repeatedly; will take up and give off oxygen; and those of the sheep when used for the Wassermann reaction behave exactly as do the freshly drawn cells of the same animal. But this is not sufficient evidence of viability. To determine the matter bleedings followed by transfusions have been performed, the blood of a number of animals being replaced so far as possible with red cells preserved for many days *in vitro* and suspended in salt solution. The results of such experiments with rabbits show that the washed and preserved blood cells remain alive. Following a disturbance in the blood count in the first few hours, associated with the replacement of more than half the total blood and due to discrepancy between the number of red cells taken out and that put in, to readjustment of the blood volume, etc., there is practically no change in the count, or in the readings of hemoglobin. Bile has never been found in the urine after the transfusions, nor hemo-

globin with any certainty. The guaiac test is sometimes faintly positive after the first twenty-four hours but so it sometimes is in control rabbits bled and injected with Ringer's solution, and at times even in normal rabbits. There is no rise in temperature and immediately after the operation the transfused animals are lively and seem quite normal. Control rabbits in which the blood is replaced by Ringer's solution recover very slowly from the profound anaemia. Animals of which the blood is replaced by blood collected and kept in Ringer's citrate may show severe disturbance associated with hemoglobin in the urine. And an animal transfused with blood kept in Ringer's solution had intense hemoglobinuria and died in convulsions. There is no doubt that washed erythrocytes left in a sugar-Ringer's mixture are really preserved alive, and preserved better than in plain Ringer's or in plasma-Ringer's-citrate.[1]

Weil has kept guinea-pig blood and dog blood several days in plasma with a minimum of citrate and then revived exsanguinated animals with it.[2] Do red cells survive longer in the preservative solutions we have devised than in such plasma citrate? There is no doubt that human cells and sheep cells do. In plasma-citrate these rapidly disintegrate. Rabbit's cells last longer but in their case we have had better results with the preservatives than with a plasma-Ringer's-citrate. On the other hand, it is possible that an optimum plasma-citrate medium has not yet been found. Our most recent experiments demonstrate that the blood of some species when allowed to flow directly into a large excess of a preservative fluid in which citrate is present can be kept for long periods. And since the cells soon settle out, and practically all the preserving fluid can be pipetted off previous to the employment of the blood for injection this constitutes a great simplification of method.

[1] Locke used one tenth of one per cent. of dextrose in the solution which bears his name. This amount of sugar is far too little for any preservative effect on the red blood cell.

[2] R. Weil, *Jour. Am. Med. Assn.*, 1915, LXIV, 425.