XVII. THE PICRIC ACID METHOD FOR THE ESTIMATION OF SUGAR IN BLOOD AND A COMPARISON OF THIS METHOD WITH THAT OF MACLEAN.

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The colorimetric method for the estimation of sugar in the blood as originally introduced by Lewis and Benedict [1915] has recently been modified by Benedict [1918], and considerably simplified. Addis and Shevky [1918], writing previous to the publication of Benedict's paper, have pointed out that the colour produced by the picric-sugar reaction is not strictly proportional in intensity to the amount of sugar present. Lewis and Benedict in their original paper state that in estimating pure sugar solutions absolutely exact results were obtained, using different amounts of sugar against the same standard, but the work of Addis and Shevky would appear to negative this conclusion. That the latter observers are correct in their statement admits of no doubt and this difference was observed by me before I knew of their paper. Addis and Shevky ascertained that the nearest approach to an exact proportion between intensity of colour produced and amount of sugar present was obtained when an amount of sodium carbonate sufficient to give a concentration of 10 % was present, and the heating was continued for 45 minutes at 100°. Such conditions are not realised in Benedict's modification and in estimations of pure sugar solutions by this method a considerable error is in fact found. In the estimation of sugar in normal bloods, however, the direct error would be of no great magnitude since the sugar content of such bloods approaches closely to that of the standard.

The second possible source of error, the presence of interfering substances in the blood, was also dealt with by Addis and Shevky. They prepared large amounts of filtrates from hyperglycaemic bloods, and, comparing the curves obtained from these under different conditions of alkali concentration and temperature with those obtained from dextrose solutions, came to the conclusion that no interfering substances were present. In these experiments, however, the heating appears to have been prolonged for 45 minutes, no readings having been carried out during the very early stages of the heating.

Benedict replying to the verbal criticism that the picric acid method yields too high results for blood owing to colour production by non-carbohydrate substances gives figures for five samples of dog's blood, tested by the picric acid method before and after treatment of the blood with mercuric nitrate. In two of these samples the apparent sugar content fell after treatment with mercuric nitrate by 19 % and 21 % respectively.

In view of the above possibilities of error in the Benedict method a direct comparison with another method of sugar estimation—that of MacLean [1916, 1919]—appeared advisable. The directions of Benedict were exactly followed, a sugar standard freshly prepared from Kahlbaum's pure glucose being used. On comparing the results obtained in human blood it was found that the Benedict method invariably gave a considerably higher percentage of sugar than that of MacLean. Since Lewis and Benedict [1915] in their original paper noted that haemolysis previous to the addition of the picric solution raised considerably the estimated percentage of sugar, the sugar of the whole blood and of the plasma was estimated in a series of human bloods by both methods with the following results.

	Whole blood. Sugar %		Plasma. Sugar %	
No.	Benedict	MacLean	Benedict	MacLean
(1)	0·114	0·089	0·103	0·099
(2)	0·137	0·104	0·118	0·113
(3)	0·130	0·081	0·112	0·090
(4)	0·176	0·139	0·167	0·147
(5)	0·155	0·092	0·113	0·102
(6)	0·132	0·082	0·114	0·090
(7)	0·157	0·099	0·135	0·110
(8)	0·100	0·067	0·069	0·070

It appears that the sugar content of the whole blood as estimated by the Benedict method is always considerably higher than the result given by the method of MacLean—the excess averaging about 45 % of the sugar present. On the other hand the Benedict results for plasma approximate closely to those obtained by the MacLean method, the excess amounting on an average to about 12 %. While by the MacLean method, the whole blood shows a slightly lower sugar content than the plasma, the Benedict estimation shows a very considerable excess of sugar in the whole blood as compared with the plasma. The difference between the results of the two methods is brought out more strongly if the blood is centrifuged or allowed to sediment in the ice chest and the plasma and corpuscular layers investigated separately.

	Plasma. Sugar %		Corpuscles. Sugar %	
No.	Benedict	MacLean	Benedict	MacLean
(1)	0·108	0·082	0·143	0·064
(2)	0·092	0·079	0·129	0·057
(3)	0·080	0·082	0·113	0·062

Under these conditions while the plasma gives an excess of sugar of 12 % by the Benedict method, the sediment richly laden with corpuscles shows an

average excess of no less than 66 % over the MacLean method. Again, the portion of the blood containing the corpuscles gives a considerably higher figure for sugar than the plasma, while with the method of MacLean, as might be expected from the greater solid content of the corpuscles, less sugar is found in the corpuscular sediment than in the plasma. It should be noted that the figures obtained above do not necessarily represent the original con tent of sugar in the blood, for several of the specimens had stood in the labora tory for some time before the estimation was carried out.

These results appeared to suggest the presence of an interfering substance in the corpuscles giving a colour reaction with picric acid and raising the supposed sugar content of the whole blood. To some extent they paralle the results obtained by Hunter and Campbell [1917] in creatinine estimations by the Folin method.

A consideration of the very different results obtained by the two methods in whole blood or in blood corpuscles indicates that one of the methods must give entirely fallacious results. If, for purposes of argument, we assume tha the error lies in MacLean's method we are forced to accept the extraordinary view that blood corpuscles contain some substance which prevents the sugar present from reducing its equivalent amount of copper when heated in alkaline copper solution. That such a view is untenable is proved by the fact that in the case of blood which has been incubated for a period sufficiently long to allow glycolysis to destroy all the sugar present, any sugar which is added can be recovered quantitatively. If the blood corpuscles contained a substance which interfered with this method, it is difficult to understand why they should not prevent the added sugar from reacting in the normal way. On the other hand a blood so treated gives by Benedict's method a greater percentage of sugar than has been added. Instead of giving values of too low an order, it is highly probable that MacLean's method tends if anything to yield results slightly on the high side, and the different values obtained in the two methods cannot be explained on the supposition that MacLean's method really gives low results.

To prove however that Benedict's results are too high, experiments were carried out to determine whether any direct evidence of the presence of inter fering substances could be found in the blood.

If the whole colour reaction was due to sugar, identical readings against a standard sugar solution should be obtained at any stage of the reaction. To test this point samples of the same blood filtrate were heated with a sugar standard for short periods of time, cooled, and rapidly read on the colorimeter. The standards contained as nearly as possible the same amount of blood sugar as the filtrates. Samples of plasma and sedimentated corpuscles were also examined. The concentrations of picric acid and alkali were the same in the sugar standard as in the filtrates to be tested. The results obtained were plotted as curves (Fig. 1). It is obvious from these curves that, while the plasma gives only a slight though distinct variation, the whole blood gives *high initial*

readings falling sharply with prolongation of the heating, and the sedimented corpuscles give even higher readings at the outset with a similar rapid fall. The only possible explanation of this result is the presence of a substance differing from glucose and mainly concentrated in the corpuscles which reacts with picric acid solution more rapidly than sugar, and by its additive effect leads to the ultimate high readings obtained by the Benedict method. This substance is present to a small extent only in the plasma, and consequently estimations carried out on plasma by the Benedict method in normal bloods show only slight excess over those obtained by the method of MacLean. In hyperglycaemic bloods even the results obtained from plasma are likely to be unsatisfactory, owing to the lack of direct proportion between the intensity of colour produced and sugar present under the conditions of the test.

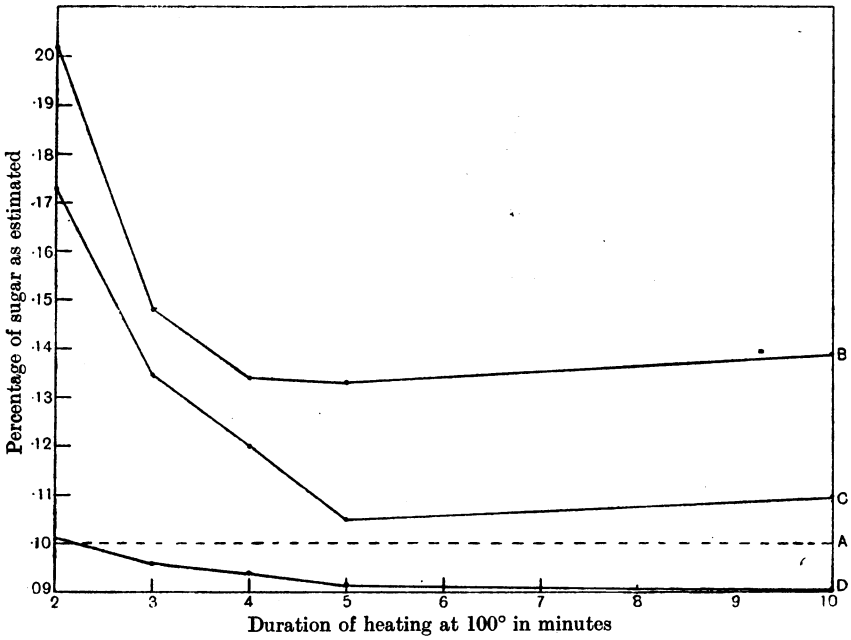

Fig. 1

A glucose standard. B corpuscles C whole blood. D plasma.

Conclusions.

1. The Benedict method for the estimation of sugar in blood gives results which are too high and shows an average figure about 30 to 50 % in excess of that found by MacLean's method.

2. The high results appear to be chiefly due to the presence of an interfering substance or substances mainly concentrated in the corpuscles but present to some extent in the plasma also; this substance reacts with the picric solution at an early stage of the heating. Creatinine probably plays a large part in this reaction.

3. On account of the influence of this interfering factor the accurate estimation of sugar in whole blood by the picric acid method as described by Benedict is impossible.

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