CLXXI. THE DETERMINATION OF FRUCTOSE IN BLOOD.

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IN direct determinations of blood fructose by colorimetric means, use has generally been made either of the original Selivanoff reaction [Folin and Berglund, 1922; Roe, 1934], or of the van Creveld [1927] reaction [Kronenberger and Radt, 1927; Radt, 1928; Corley, 1929] which employs diphenylamine. The present communication is concerned with the latter reaction only, which was investigated with the main object of comparing the results obtained with those resulting from the application of a new principle worked out in this laboratory [Scott, 1935].

Preliminary trials of the method described by Radt [1928] as suitable for the estimation of blood fructose furnished evidence which seemed to make it desirable to modify it in certain directions. The first unsatisfactory feature is that much more amyl alcohol is required to extract the coloured product in the treated blood filtrate than is required to carry out the corresponding operation upon the standard solution, and this has the effect of upsetting the volume relationships which it is desirable to maintain between unknown and standard. It was found that the extraction was greatly facilitated by the introduction of a little solid ammonium sulphate, which process enabled the final colour in both standard and unknown to be contained in the same volume.

The second important drawback to the method lies in the choice of blood filtrate. When using whole blood the filtrate from protein, precipitated with HCl and mercuric chloride, contains substances which interfere with the colour reaction between fructose and diphenylamine and have the effect of causing the development of colour in the unknown to proceed faster than in the standard and eventually to reach an intensity beyond that which could be attributed to the fructose present. This difficulty was readily overcome by a suitable choice of filtrate, for recent work on blood sugar has provided a number of filtrates established as satisfactory for true sugar determination, and though these are much more dilute than is advisable for the estimation of the very minute amounts of fructose that appear in blood after the ingestion of such quantities of the sugar as are commonly used in tolerance tests, the subsequent concentration was an operation easily accomplished without danger of vitiating the estimation.

On general grounds a zinc filtrate appeared to be eminently suitable for the purpose, and in that the form of precipitation used by Scott gave the opportunity of making this work strictly comparable with his, and further provided a greater volume of filtrate than that available from the standard Somogyi method, exactly the same procedure was adopted as that forming the preliminary stage to the bile salt colorimetric method. The quantities used were identical with those described as being applicable to a determination upon 2 ml. whole blood.

The subsequent stages of the estimation were carried out as follows. After the acidification with acetic acid the 10 ml. filtrate (equivalent to 1 ml. blood)

were evaporated down (using a small Bunsen flame for direct heating) in a test-tube graduated at 4 ml. until the volume fell to just below the mark. It was then made up exactly to 4 ml. volume. This concentrated filtrate was then treated with 4 ml. HCl (5 parts concentrated acid + 3 parts distilled water), and 0.4 ml. 20 % alcoholic diphenylamine, the mixture shaken thoroughly, and then heated in a boiling water-bath for 15 minutes. On cooling to room temperature, 10 ml. butyl alcohol were added, followed by 2 g. solid ammonium sulphate. The tube was then stoppered and shaken up vigorously to permit extraction of the blue colour by the organic solvent. A solution of fructose (that containing 0.1 mg. fructose in the 4 ml. volume was the most suitable standard for use in tolerance tests) was similarly treated, and the butyl alcoholic layers were pipetted off for comparison in the colorimeter. Blank determinations on normal fasting bloods showed only the slightest trace of colour, which might for practical purposes be regarded as negligible. Actually it would appear to be equivalent to about 1 mg. fructose per 100 ml. blood, from the values recorded in Table I, which shows a comparison between the results ascertained by the process just outlined, and those obtained by the bile salt method.

Table I.

Sample	Estimated fructose	Estimated fructose	Type of case
	mg./100 ml. Present method	mg./100 ml. Scott's method	
1. With added fructose	11	10	Normal
2. With added fructose	16	15	Normal
3. 1 hour after fructose	13	12	Cirrhosis of liver
4. 2 hours after fructose	9	8	Cirrhosis of liver
5. 1 hour after fructose	19	18	N.A.B. Jaundice

The satisfactory agreement between the two sets of values establishes confidence in both methods as a means of determining blood fructose.

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