LXXX. ESTIMATION OF SUGAR IN SMALL QUANTITIES OF BLOOD.

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THE method is a modification of the one described by Shaffer and Hartmann [1921]. It has been in use since 1921 and has found its way into several laboratories in Holland.

Solutions

1. Phosphotungstic acid ¹ ...	...	...	5 g.
Sodium sulphate (pro analysen)	...	...	20
Sulphuric acid (pure)	...	...	2
Water to	...	...	1000 cc.
2. Copper sulphate (crystals)	...	...	1.5 g.
Tartaric acid	...	...	3.75
Sodium carbonate (anhyd.)	...	...	40.0
Potassium iodide	...	...	2.0
Potassium iodate	...	...	0.2
Water to	...	...	1000 cc.

Procedure. The blood is mixed with 25 volumes of solution 1. For one determination and a blood-sugar content between 0.07 and 0.20 % one can take conveniently 6.8 cc. of solution 1 and add 0.2 cc. of blood. When one expects more than 0.2 % of blood-sugar one should take 50 of solution 1. In rare cases the blood is to be diluted still more. When the blood-sugar is lower than 0.07 % the blood should be diluted with only 10 volumes of solution 1 and more blood should be taken. For double determinations the amounts of blood and solution 1 should be doubled. After thorough mixing the haemoglobin begins to settle to the bottom as haematin within a few minutes. It then gives a clear and colourless filtrate when filtered through paper. The paper must be starch-free and should not give off any fibres to the filtrate.

5 cc. of the filtrate are carefully pipetted into a large test-tube of 23 mm. diameter; 5 cc. of solution 2 are added and the tube is covered with a well-fitting glass cover in the form of a small crystallising dish. The test-tube is

¹ I always used a specimen from Merck.

placed in a boiling water-bath for 15 minutes. I had special baths made for the purpose in which six tubes could be heated at the same time (care should be taken that there is room to move the burner freely to and fro underneath the bath without lifting the bath itself).

Exactly 15 minutes after the beginning of the heating the tube is cooled in water. 5 cc. of *N* sulphuric acid are now added and mixed well with the contents; the tube is again covered and shaken at intervals during 2 minutes, after which time the liberated iodine is titrated with thiosulphate (0.08 *N* is a convenient strength; it deteriorates less than the weaker solutions, but it should be kept in a microburette).

For calculation of the amount of reducing substance in the form of glucose the amount of copper reduced is calculated from the formula

$$W = D \times 63.56,$$

where *W* is the amount of copper; *D* is the difference between the determination and the value of a blank (where instead of 5 cc. of filtrate 5 cc. of solution 1 are used) expressed in cc. of *N* thiosulphate; and 63.56 is the atomic weight of copper.

The amount of reducing substance as glucose is read from Table I.

Table I. (*Reduction table.*)

Copper mg.	Glucose mg.	Interpolation table to be used between 0.05 and 0.500 mg. of copper	
0.050	0.090	$\frac{0.026}{50} \times 2 = 0.001$	
0.100	0.116		
0.150	0.142	4	02
0.200	0.168	6	03
0.250	0.195	8	04
0.300	0.221	10	05
0.350	0.247	12	06
0.400	0.273	14	07
0.450	0.300	16	08
0.500	0.327	18	09
0.550	0.358	20	10
0.600	0.395	22	11
0.650	0.447	24	13
		26	14
		28	15
		30	16
		32	17
		34	18
		36	19
		38	20
		40	21
		42	22
		44	23
		46	24
		48	25
		50	26

In Table II are given values of blood-sugar found with the use of the method described in comparison with MacLean's method [1919], which was tested in this laboratory by Bahlmann [1920].

Table II. *Comparison with MacLean's method.*

	Mg. glucose per 100 cc. of blood	
	MacLean	Author's method
1	65	73
2	71	63
3	124	116
4	158	164
5	178	159
6	253	270
7	398	408
8	488	483

Wetselaar [1924] working with the method described obtained the following results (Table III) (reducing his values of 0.005 *N* thiosulphate to mg. of glucose by Table I):

Table III. *Values derived from Wetselaar's work.*

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Glucose taken mg.	Glucose found mg.
0.090	0.088
0.112	0.113
0.135	0.138
0.168	0.168
0.179	0.183
0.224	0.222
0.258	0.264
0.280	0.280
0.314	0.313
0.336	0.339
0.370	0.371
0.392	0.391
0.426	0.433
0.448	0.455

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 MacLean (1919). *Biochem. J.* **13**, 133.
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