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A criticism and modification of the MacLean blood sugar method.

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The titrimetric method for the determination of blood sugar proposed by Maclean[1] in 1919 has been used with success in a number of researches both abroad and in this country. Its simplicity, accuracy and the small amount of blood required recommend it to those who prefer titrimetric to colorimetric quantitative determinations. Briefly, the principle of the MacLean method is:

1. Removal of the proteins by heating oxalated blood with acidified sodium sulphate, adding colloidal iron, and filtering.
2. Boiling, under standardized conditions, an aliquot part of the filtrate with a known amount of an alkaline copper solution containing potassium iodide and potassium iodate.
3. Titration of the iodine liberated upon acidification with standard thiosulphate.

Since, under the conditions defined by MacLean, the cuprous salt reduced by the sugar is oxidized by the iodine liberated, the excess iodine titrated with thiosulphate is inversely proportional to the amount of reduction. Empirical factors can, therefore, be determined converting the volume of thiosulphate used into concentration of glucose.

It was found, however, that by MacLean's method the determinations of sugar in freshly drawn blood to which no anticoagulant had been added gave values distinctly higher than those determined on the same blood which had been oxalated, (Table I).

TABLE I.

Sugar determinations of blood to which $K_2C_2O_4$ had been added and had not been added.

Method.	Dog.	Concentration of sugar in mgs. per 100 c.c. of blood.					
		$K_2C_2O_4$ present.			$K_2C_2O_4$ not present.		
		a	b	Average	a	b	Average
Original.....	A	105	100	103	89	88	89
MacLean.....	B	91	92	92	55	55	55
Modified.....	C	90	85	88	90	85	88
MacLean.....	D	93	96	95	93	93	93

[1] MacLean, H., *Biochem. J.*, 1919, xiii, 135.

This fact led to a comparison of sugar determinations made on pure glucose solutions with and without the addition of oxalate, with and without the use of colloidal iron. The amount of oxalate required to prevent coagulation was about 2 mg. per c.c. It was found that the presence of the oxalate resulted in too low sugar values, *i.e.,* too much thiosulphate was used in the titration, only in case colloidal iron was used. Experiments in which the amount of colloidal iron was progressively increased from 0 to 3 c.c. showed that increasing amounts of thiosulphate were required for titration. Direct determinations of the iron in the filtrate indicated that the reaction involved was between the thiosulphate and the ferric salts. It seemed, therefore, that the choice of colloidal iron as a protein precipitant was an unfortunate one; although, under the carefully standardized conditions described by MacLean, it led to no inaccuracy of results, providing oxalate was not used as an anticoagulant.

By using phosphotungstic acid as our protein precipitant we were able to obtain results which agreed whether the blood was oxalated or not, and which were comparable with those obtained by the Folin method.

The procedure now adopted for the precipitation of the blood proteins is as follows:

1 c.c. of oxalated blood is added to 26 c.c. of distilled water in an Erlenmeyer flask or 50 c.c. centrifuge tube. Five minutes are allowed for laking the blood. 2 c.c. of a 10 per cent. solution of phosphotungstic acid and 1 c.c. of 1 per cent. acetic acid are added. The flask or tube is then vigorously shaken. In the case of pigeon's blood, where precipitation is difficult, it is necessary to gently heat the mixture after the addition of phosphotungstic acid to complete the action. The protein precipitate may then be separated by filtration or preferably centrifugation. 20 c.c. of the water-clear filtrate are transferred to an Erlenmeyer flask, and the determination continued as recommended by MacLean.

The elimination of sodium sulphate from the solution results in the reduction taking place at a lower temperature. This necessitates the construction of a new table to take the place of the one published by MacLean for the conversion of c.c. of thiosulphate into mgs. of glucose per 100 c.c. of blood. These equivalents are given in Table II.

The method as now modified has been successfully applied to human, dog, ox, rabbit, guinea pig and pigeon blood.

TABLE II.

Table for the conversion of c.c. of N/100 $Na_2S_2O_3$ into mg. of glucose per c.c.

0.01N $Na_2S_2O_3$ c.c.	Glucose mg. per c.c.	0.01N $Na_2S_2O_3$ c.c	Glucose mg. per c.c.
0.50	0.25	4.0	1.19
1.0	0.39	4.5	1.33
1.5	0.52	5.0	1.46
2.0	0.66	5.5	1.59
2.5	0.79	6.0	1.73
3.0	0.92	6.5	1.76
3.5	1.06	7.0	2.00

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On the nature of the rhythmic contractions in the stomach and intestine.

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Rhythmic contractions in the stomach and intestine persist following the administration of nicotin in doses sufficient to prevent conduction through synapses. Attempts to account for these contractions solely as responses to nervous impulses have resulted in confusion. Certain experimental data recorded by Magnus ('05),[1] Gunn and Underhill ('14),[2] and Alvarez and Mahoney ('22)[3] indicate clearly that excised pieces of the intestinal musculature may execute rhythmic contractions in the absence of nervous influences. The present paper embodies a preliminary statement of the results of a further investigation, through the use of nicotin in massive doses, of the rhythmic

1 Mangus, R., *Arch. f. d. gesammt. Physiol.*, 1905, cviii, 1.

2 Gunn, J. A. and Underhill, S. W. F., *Quart. Jour. Exp. Physiol.*, 1914, viii, 275.

3 Alvarez, W. C. and Mahoney, L. J., *Amer. Jour. Physiol.*, 1922, lix, 421.