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A STUDY OF METHODS FOR THE DETERMINATION OF REDUCING SUGAR IN BACTERIOLOGICAL MEDIA II

E. J. MCCREARY AND H. G. SMITH

Previous work of Magee and Smith¹ has shown that the determination of reducing substances in beef-broth media by the Shaffer-Hartman micro method, following clarification with lead subacetate, gives results that are extremely variable. It was found impossible to completely recover known quantities of glucose added to the medium.

In the present series of experiments, and with a modification of the Shaffer-Hartman method, the findings of Magee and Smith have been confirmed. Attempts to determine reducing substances following the use of other protein precipitants have been partially successful. When Lloyd's reagent is used for clarification, variations are less than after the use of lead subacetate and zinc hydroxide. The latter two precipitating agents produce similar variations but the average of all values is 10 percent lower after the use of zinc. Recoveries of added glucose are as variable after clarification with Lloyd's reagent as after zinc hydroxide and the average percent recovery is the same.

Determinations on pure glucose solutions after treatment with the precipitating reagents show a loss of 5 percent after zinc, a 10 percent loss after Lloyd's reagent. An average recovery of 201 percent followed the use of lead subacetate but the variations in a series of determinations were greater than after the other precipitants.

Dilution of the medium five or ten times before treatment with the clarifying reagent materially reduced the variability. The least variations and the best recoveries of added glucose were obtained following the use of zinc hydroxide or copper sulfate.

The inability to obtain accurate determinations of reducing sugar in the medium is probably the result of variations in the amount of interfering substances removed by the clarifying agent as well as of variations in the amount of glucose adsorbed by the precipi-

¹ Magee, M. Catherine, and Smith, H. Gregg, A Study of Methods for the Estimation of Reducing Sugar in Bacteriological Media, *Jour. Bacteriol.* 19: 125-132. 1930.

Reducing Substances in Bacteriological Medium. (All Values Expressed as Mg. Percent)

TREATMENT	No. OF DETNS.	AVERAGE VALUE	PERCENTAGE VARIATION	AVERAGE RECOVERY OF ADDED GLUCOSE		VARIATIONS FROM 100 PERCENT RE- COVERY PER- CENTAGE
				No. OF DETNS.	PERCENTAGE	
Standard glucose.....	--	---	---	13	101.9	2
None.....	4	73.4	0.4	20	107.8	+ 32.3
Lead subacetate.....	21	89.5	19.0	20	94.8	- 23.2
Lloyd's reagent.....	7	73.4	7.1	7	91.5	+ 12.9
Zinc hydroxide.....	44	79.5	20.1	--	---	- 28.9
Medium diluted 1-5.....	12	72.1	4.8	12	81.5	+ 3.7
Lead subacetate (medium 1-5).....	12	75.9	6.8	12	87.3	- 19.5
Zinc hydroxide (medium diluted 1-5).....	12	56.6	3.4	12	106.8	---
Zinc hydroxide (medium diluted 1-10).....	12	81.2	4.0	11	108.0	- 29.4
Copper sulfate (medium 1-5).....	12	59.6	3.8	12	104.8	- 18.5
Fermentation with yeast followed by copper sulfate.....	12	35.2	4	--	---	+ 13.2
						+ 9.7
						+ 6.4

tate. There seems to be no explanation for the fact that values obtained after clarification are higher than those obtained without clarification. The favorable influence of dilution is probably due to lessened adsorption of reducing material on the precipitate.

Copper sulfate removes all non-sugar reducing substances from blood filtrates but does not do so from beef broth medium. Determinations after copper precipitation gave an average of 59.6 mg. per 100 cc. After fermentation with yeast an average of 35.2 mg. was obtained, a difference of 24.4 mg. percent, indicating that glucose constitutes 40 percent of the reducing substances of the medium.

Further investigations leading to an accurate method for determining reducing sugar in beef broth medium are necessary.

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