[CONTRIBUTION FROM THE EVANS MEMORIAL HOSPITAL]

THE RELATIVE REDUCING POWERS OF SOME COMMON SUGARS

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In the study of the group of the so-called reducing sugars, it has long been recognized that the velocities of reduction, or reducing powers, of the several members exhibit somewhat wide divergences from one another. The literature contains many records of investigations of the several relationships. The majority of these are based upon some form of alkaline copper reduction, although there is a wide diversity in the composition of the reagents selected. The time element shows similar permutations, ranging from the partial reduction obtained by heating or boiling for a specified period to demonstrated complete reduction where the time is not recorded. Further, the amount of sugar determined influences the equivalence so that frequently limiting values must be given for high and low concentrations.

With the development of the methods of blood analysis, new analytical procedures were formulated to permit the accurate quantification of amounts of sugar of the order of tenths of a milligram. Both the Folin-Wu[1] and Lewis-Benedict[2] procedures and their many modifications have been extensively used. Both were designed for the estimation of glucose, the characteristic sugar of the blood. The introduction of a variety of diagnostic tests based upon the concept of a "threshold of tolerance" for carbohydrates has in more recent years led to the use of a variety of sugars. The earlier tests depended upon the appearance of the reducing body in the urine under the excitation of a dose of sugar in excess of the individual tolerance. Later, as the methods of blood chemistry were developed, the levels of blood sugar assumed dominant importance, and the magnitude of change in level under the stimulus of a carbohydrate test meal became the criterion. The normal blood sugar is, of course, glucose; but the magnitude of the test dose and the gastro-intestinal disturbances implicit thereto has led to the use of other sugars for the purpose. In a recent investigation by one of us[3] on the diagnostic significance of blood-sugar curves, so-called, galactose was the sugar selected for the study. Recognizing that the reduction capacity was not identical with that of glucose, and that a mixture of the two would be present in the blood stream after a test meal, a brief investigation already reported[3] was carried out to determine the relative capacities. So striking was the difference, and so potentially significant to the

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[1] Folin and Wu, *J. Biol. Chem.*, **38**, 81 (1919).

[2] Lewis and Benedict, *ibid.*, **20**, 61 (1915).

[3] Rowe and Chandler, *Endocrinology*, **8**, 803 (1924).

evaluation of true blood-sugar levels, that the present investigation embracing other common sugars was carried out.[4]

Method

The sugars used, with the exception of one sample of Kahlbaum glucose "Zür Analyse," were Pfanstiehl products of the highest obtainable grade. The purity was confirmed by the polariscope, by osazone formation, and by the melting points of the sugars. To be certain that the standards were anhydrous, combustion analyses were made and carbon and hydrogen determined.[5]

Solutions were made by dissolving 100 mg. of sugar in 100 cc. of pure, distilled water. Mixtures were made by diluting these stock solutions with one another. All boiling tubes were calibrated by weight, a most important precaution. The use of a stopwatch and the quenching in very cold water were found to be necessary refinements.

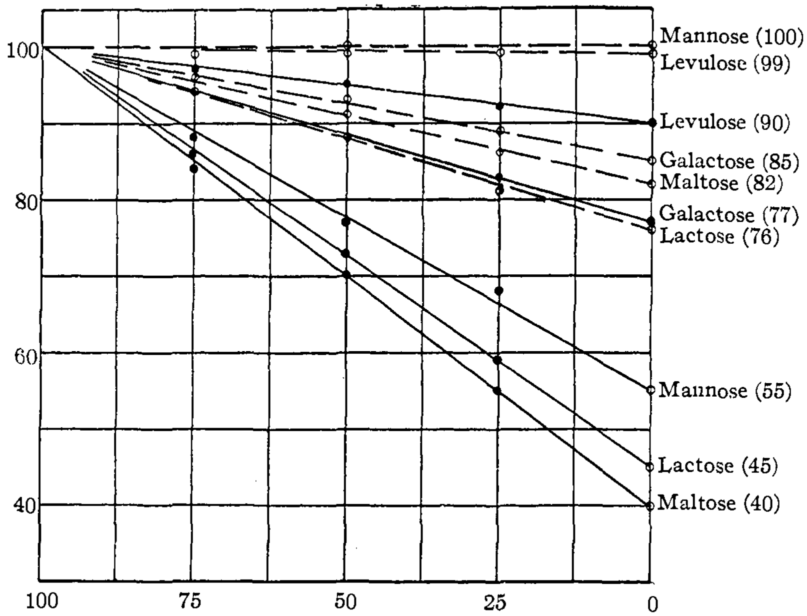

Fig. 1.—Equivalent reductions of mixtures of glucose with other sugars, in terms of glucose.

The data obtained with the several solutions using the Folin-Wu (solid line) and Lewis-Benedict[6] (broken line) reagents is best shown graphically. The functions, as might be anticipated, are rectilinear, observed and calculated values agreeing within the limit of error. By the Folin-Wu method

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[4] Several partial comparisons have appeared in the literature and most recently Greenwald, Samet and Gross [*J. Biol. Chem.*, **62**, 397 (1924)] have tabulated results with a number of sugars and several methods.

[5] The authors take much pleasure in expressing their indebtedness to Mr. E. L. Kochmann, by whom all of these analyses were performed.

[6] The modified form [Benedict, *J. Biol. Chem.*, **34**, 203 (1918)] was used.

and after six minutes' boiling, the reduction velocity of the levulose approximates that of the glucose, galactose has but three-fourths the reducing power, and mannose approximately one-half. With the disaccharides the results obtained appear to be below the truth, for the reducing powers of equal weights should be but slightly more than one-half that of glucose (52.6%). If the value for pure lactose and maltose be recalculated on this basis, assuming no hydrolysis, the powers of their reducing groups become, respectively, 86 and 76, or between the levulose and galactose values.

With the Lewis-Benedict method and after ten minutes' boiling the values are different from those obtained by the copper method. Perhaps the most striking change is found with mannose, which equates directly with glucose under the conditions of this method. The longer period of boiling is a factor, but this fails to account for the increase in either the levulose or mannose. With the disaccharides there is evidently hydrolysis. That maltose gives a higher value than lactose is strongly confirmatory of this, as the latter hydrolyzes to a less active mixture than does the former. Assuming that the disaccharides are split before absorption from the intestine, the picric acid method would give dependable values for blood-sugar levels except with test meals of lactose and galactose. This statement ignores other difficulties apparently incident to the method.

To determine the time factor, the solutions were tested by the Folin-Wu method using longer boiling times. The results are given in Table I.

TABLE I

TIME FACTOR IN REDUCTION

Glucose standard = 100

Sugar	Sugar 12 min. Glucose 6 min.		Sugar 12 min. Glucose 12 min.		
	Observed reading	% Increase	Obs.	Calcd.	Diff.
Glucose	105	+5	..	..	...
Levulose	90	+0	88	86	+2
Galactose	88	+14	85	84	+1
Mannose	92	+67	87	88	−1
Lactose	59	+31	56	56	0
Maltose	50	+25	47	48	−1

The marked increase shown by mannose with the Lewis-Benedict method is here substantiated. The disaccharides give some evidence of hydrolysis.

By means of the values here determined, it should be possible to compute approximately the true blood-sugar level where a test meal consists of sugar other than glucose. To verify this conclusion a set of measurements were made, using blood serum and adding known amounts of the foreign sugar to it. The averages of these data are collected in Table II. The individual variations were of the order of ±1 or 2%.

TABLE II

RECOVERY OF SUGARS ADDED TO SHEEP'S BLOOD

Control plus	Weight mg.	Obs.	Calcd.	Diff.
Control	..	54	...	...
Glucose	25	79	78	+1
Glucose	50	104	103	+1
Galactose	25	72	73	−1
Galactose	50	92	93	−1
Mannose	25	67	68	−1
Mannose	50	81	82	−1

From the above it is evident that the reduction coefficients of the several sugars as determined in aqueous solution are equally valid when applied to blood plasma.

By use of them, as stated above, it should be possible to secure a closer approximation to true blood-sugar levels when carbohydrate test meals have been given. Absolute values could be obtained only if it were known that the control blood-sugar level remained unchanged throughout the test. Such an assumption lacks justification, for with the ingestion of the test meal, the basal state is destroyed. Moreover, two hours, the usual period for a blood-sugar curve determination, is rather too long for the maintenance of a static condition of circulating carbohydrate. The proposed calculation would give significantly increased levels, however, particularly in those cases where doses of heroic proportions of sugars other than glucose have been administered.

Summary

1. No two of the simple reducing sugars have the same reaction velocity. With the methods of blood analysis in which time is specified and the reaction is incomplete, this may introduce an error of significant magnitude.
2. The reduction coefficients for several sugars in aqueous solution have been determined by both the Folin-Wu and modified Lewis-Benedict methods.
3. In mixtures of sugars, the reduction is proportional to the relative amounts of the several compounds and the reducing power of each.
4. The time element of reduction has been investigated and found to be highly variable.
5. Reduction values obtained in aqueous solution are shown to be the same as in plasma.

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