XXI. NOTES ON THE HAGEDORN-JENSEN METHOD FOR THE DETERMINATION OF BLOOD-SUGAR.

By HILDEGARDE KRAMER AND ALEXANDER STEINER¹.

From the Laboratory of the Jewish Hospital of St Louis, St Louis.

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THE Hagedorn-Jensen method for the determination of blood-sugar is frequently used not only in clinical blood analysis but also in research work. Most authors employing the method consider it quite satisfactory and accurate. Their criterion is the fact that the method yields very good duplicates. A few critical studies, however, cast some doubt upon its reliability. Thus, Duggan and Scott [1926] point out that since 0.01 cc. of 0.005 *N* thiosulphate is equivalent to 1.8 mg. of glucose per 100 cc. of blood, very slight differences in titration may cause considerable errors. This and the fact that the method employs 0.1 cc. of blood represent *a priori* limitations of accuracy. Folin and Malmros [1929] call attention to a more serious inherent source of error. They state that the cotton plug used for filtration, if too small and loose, allows some precipitate to pass through, rendering the results too high. If, on the other hand, the plug is larger and compact, it retards filtration and retains some filtrate, causing loss of sugar.

As a first step in our examination of the Hagedorn-Jensen method we performed numerous determinations, strictly following the original directions, on blood samples containing known amounts of glucose. These were prepared according to Somogyi [1930] by the addition of glucose to blood samples which were rendered sugar-free either by treatment with washed yeast or by glycolysis. Although duplicate determinations as a rule showed very good agreement, the quantities of the glucose recovered differed greatly from the amounts actually added. This points emphatically to the fallacy of the assumption that good checks between duplicate determinations may be considered as a criterion for the adequacy of a method. The criterion we have adopted in this work is the accuracy with which known amounts of glucose, added to sugar-free blood, can be recovered. The Hagedorn-Jensen method, as we shall presently show, proved to be entirely unreliable in such a test.

Next, we examined to what extent the source of error, pointed out by Folin and Malmros, may account for the faulty results. To determine the amount of sugar retained by the cotton plugs, after filtration and two washings,

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two or three plugs from parallel determinations were united and extracted with 12 cc. of water; the extract was filtered through a washed filter-paper, and the reduction of the filtrate was determined. Having found that cotton gives up reducing substances to water in measurable amounts, we employed in these experiments cotton which had been carefully washed and dried at room temperature. The results, given in Table I, show that 4 to 22 mg. per 100 cc. of the sugar is lost in the cotton filters, amounts of the same order of magnitude as Folin and Malmros found by a different technique.

Table I. *Showing that the cotton filters in the Hagedorn-Jensen method retain appreciable portions of the blood-sugar.*

No.	Blood-sugar in Hagedorn-Jensen filtrates mg. per 100 cc.	Blood-sugar retained in cotton filter mg. per 100 cc.
1	24	4
2	54	5
3	123	7
4	137	7
5	151	22
6	182	11
7	213	14
8	217	8
9	224	22
10	318	10

The magnitude of the loss depends largely upon the concentration of the blood sugar, assuming uniform bulk and density of the filter plugs. In the hands of one and the same worker the method may thus yield good duplicate determinations, while the results do not represent the actual total of the reducing substances.

Before carrying our investigations further we had to eliminate this source of error. To this end we used the Hagedorn-Jensen method in the following form. In a test-tube marked at 12 cc., 0.12 cc. of blood was precipitated according to the directions of Hagedorn and Jensen. After heat-coagulation the mixture was cooled and diluted to 12 cc. The test-tube was stoppered and well shaken, and the contents were filtered through dry filter-paper. Filter-paper thoroughly washed with slightly acidified water was used in this work. For the rest, the original procedure was followed using 2 cc. of the Hagedorn-Jensen ferricyanide reagent and 10 cc. of filtrate which corresponds to 0.1 cc. of blood. In order to prevent changes in the alkalinity of the reagent, the filtrates were always neutralised with 0.30–0.35 cc. of 0.1 *N* sodium hydroxide before the addition of the ferricyanide.

For the calculation of results we have prepared our own table with standard solutions of glucose. The original table cannot be used in accurate work, since Hagedorn and Jensen erroneously postulated that the self-reduction of ferricyanide reagents is always the same, and used a constant value for this as a correction. The fact is however that it is almost impossible to prepare two batches of Hagedorn-Jensen reagent that show the same self-reduction, and

the latter will change even in the same batch upon standing for some time. Instead we follow the procedure employed in other iodimetric methods and subtract the titration figures from the titration value of a "blank" sample of the reagent. The blank, in which water takes the place of the sugar solution, is treated exactly as the unknown. In this way self-reduction is ruled out and the difference value is equivalent to the reduction of the sugar solution. It is probably due to the arbitrary method of correction that Höst and Hatlehol [1920] also found the sugar table of Hagedorn and Jensen unsatisfactory and felt the necessity of preparing one of their own. In Table II are presented the basic figures of our table.

Table II. *Glucose equivalents of titration values in the Hagedorn-Jensen method.*

Actual amount of glucose mg.	0.005 N thiosulphate (blank titration) cc.	Blood-sugar if filtrate from 0.1 cc. of blood is used mg. per 100 cc.
0.01	0.07	10
0.02	0.12	20
0.05	0.26	50
0.10	0.53	100
0.15	0.80	150
0.20	1.07	200
0.25	1.33	250
0.30	1.60	300
0.35	1.87	350

Owing to the excellent proportionality between sugar concentration and reduction, a detailed table can easily be prepared by interpolation. Well reproducible results are obtained only up to a concentration of 350 mg. per 100 cc. blood-sugar; above this limit the values become unreliable. This difficulty we have obviated by increasing the concentration of the ferricyanide for the determination of higher concentrations of sugar. As to the lower limit, our experience agrees with that of Duggan and Scott [1926] who were unable to obtain good results below the 25 mg. per 100 cc. concentration of blood sugar (0.025 mg. of glucose).

In Table III are given examples of comparative sugar determinations by

Table III. *Showing that the original Hagedorn-Jensen method yields too low results for true blood-sugar.*

No.	True sugar* found Hagedorn-Jensen method		Amount of added sugar mg. per 100 cc.
	Original mg. per 100 cc.	Modified mg. per 100 cc.	
1	89	104	100
2	126	155	150
3	154	160	160
4	172	189	190
5	173	201	200
6	178	199	200
7	298	301	300
8	487	498	500

* True sugar = total reduction (apparent sugar)—reduction of sugar-free blood.

the original Hagedorn-Jensen method and by the technique eliminating the cotton filter. The figures represent true sugar values, obtained as the difference between total reduction and non-fermentable reducing substances. The latter were determined in the blood, after it had been rendered sugar-free by means of glycolysis or by treatment with washed yeast. To another portion of this sugar-free blood a known amount of glucose was added, and the total reduction was determined. The results obtained by the original Hagedorn-Jensen methods were computed from the original table of these authors, while those determined with our modification were read from our own table (Table II). As can be seen, the original Hagedorn-Jensen technique yields results that are too low, the discrepancies being rather irregular. From the figures of Tables I and III, it is apparent that the discrepancies are of the same order of magnitude as the losses of sugar in the cotton plugs, which shows that the technique of filtration is the essential source of inaccuracy, which renders the Hagedorn-Jensen method altogether inadequate for accurate analytical work. With the elimination of the cotton filter, and with a logical method of correcting for the self-reduction of the ferricyanide reagent, it is possible to obtain very satisfactory results.

This observation is entirely contradictory to all reports from other workers. Comparative blood-sugar determinations on record, carried out by the Hagedorn-Jensen method and several copper-reduction methods (Folin, Shaffer-Hartman, *et al.*) do not show appreciable or consistent differences in one direction or the other [Duggan and Scott, 1926; Van Slyke, Hiller and Linder, 1925; Höst and Hatlehol, 1920]. The apparent conflict however is readily explained by the fact that these authors determined apparent sugar values (total reduction), while we are dealing with true (fermentable) sugar. Somogyi has repeatedly pointed out [1930] that different reagents yield different values for the non-sugar reducing substances, and furthermore that different protein precipitants leave different quantities of these substances in the blood filtrates [Somogyi and Kramer, 1928]. In particular, ferricyanide gives much higher values for the non-fermentable reducing substances than do the copper reagents. We can corroborate the findings of Somogyi that the amount of the non-sugar reducing substances in the Hagedorn-Jensen method is 20–30 mg. per 100 cc., in the Folin-Wu method 12–15 mg. per 100 cc., in terms of glucose. The higher value in the Hagedorn-Jensen method obviously balances the loss of sugar in the cotton filter, thus concealing this serious source of error, and as a consequence the two methods frequently yield rather similar results. Comparison of total reduction instead of true sugar values, of course, could not disclose that fermentable sugar is actually lost in the Hagedorn-Jensen method.

In recent very careful experiments Herbert and Bourne [1930] found that the Hagedorn-Jensen method gives sugar values "slightly higher" than the true sugar obtained by Somogyi's method. The discrepancies vary between 1 and 10 mg. per 100 cc. According to our findings, such agreements are

possible only through coincidences in some cases in which the amounts of the reducing non-sugars, included in the Hagedorn-Jensen reduction values, happen to be the approximate equivalents of the amounts of sugar lost in the cotton filters. Since both the positive and the negative error are variable, the approach to true sugar values is bound to be quite inconsistent, and the discrepancies are frequently too great to be ascribed simply to "experimental errors."

SUMMARY.

An examination of the Hagedorn-Jensen method for the determination of blood-sugar reveals sources of error which render it inadequate for accurate work.

The method does not yield true sugar values, the equivalent of 20 to 30 mg. per 100 cc. of sugar being derived from reducing substances other than sugar. At the same time the results are considerably lower than would be expected, owing—as Folin and Malmros have shown before—to a retention of 4–22 mg. per 100 cc. of fermentable sugar in the cotton plugs employed for filtration.

The table of Hagedorn and Jensen, given for the computation of sugar, leads to an additional error. In this table a definite correction for the self-reduction of the alkaline ferricyanide reagent is applied, implying the assumption that self-reduction is a constant value for every reagent. A correction value of general validity however is impossible, as the self-reduction changes with time even in the same reagent.

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