REVISION OF A METHOD FOR THE DETERMINATION OF HIGH BLOOD GLUCOSE VALUES, BASED ON THE HAGEDORN AND JENSEN TECHNIQUE

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(Received September 29th, 1960)

Except in the U.S.A.[1], the titrimetric procedure for the determination of blood glucose of HAGEDORN AND JENSEN[2] is well known. In The Netherlands about 100 out of approximately 180 clinical chemical laboratories, participating in national trials, are using it[3]. Although this method still retains the names of its original authors, it has been significantly altered in later years[4,5]. The principle is that, after removal of the proteins, glucose is oxidized by heating at 100° for 10 or 15 min with a known excess of potassium ferricyanide in a strongly alkaline solution (sodium carbonate[2] or a mixture of sec. and tert. potassium phosphate[4,5]), followed by iodometric back-titration of the unchanged ferricyanide with sodium thiosulphate.

Without changing the ratio of blood to reagents which is commonly used (*viz.*, 0.1 ml of blood: 2 ml of *N*/200 ferricyanide reagent), blood glucose values higher than 348 mg/100 ml (phosphate-containing reagent) or 385 mg/100 ml (carbonate-containing reagent) cannot be determined. In these cases glucose and ferricyanide are present in equivalent quantities. On several occasions, methods have been described for making estimations of blood samples with glucose levels higher than the limits mentioned above, based on, *e.g.*, dilution of the protein-free blood filtrate[5] or increasing the quantity of ferricyanide in each test. For an extensive survey see HINSBERG AND LANG[6]. All these methods only apply, however, if one knows beforehand that the glucose level is too high to be handled in the ordinary way. In practice, where this is seldom realized, it has become customary—at least in this country—to add another portion of 2 ml ferricyanide reagent and heat once more for 10 min, if the mixture of blood filtrate and ferricyanide reagent has decolorized on heating (indicating total consumption of the ferricyanide due to an unexpected high glucose content of the blood tested). It is even said to be permissible, if after the second addition the fluid becomes colourless again, to continue adding the reagent and heating. This extension of the method was not introduced by HAGEDORN AND JENSEN or any of the authors referred to above, who published modifications. However, it has been described in some recent handbooks[7-10], although, regrettably, not with as many details as appears to be necessary now, as the exact moment for the second addition of the ferricyanide reagent is not mentioned.

Recent work, carried out on behalf of the Dutch "Clinical Standardization Committee", organized by the Dutch Association for Clinical Chemistry and the

National Institute of Public Health, to draw up standardized method sheets for clinical chemical determinations[11], lead us to the supposition that in many laboratories the directions given by the handbooks referred to above could be employed incorrectly.

METHOD

The results, published here, have been obtained with the phosphate-containing ferricyanide reagent [4,5]. According to experiments, as yet unpublished, essentially the same phenomena were observed using the carbonate-containing reagent.

RESULTS

It appeared that, whichever glucose concentration one chooses, provided that it is higher than 348 mg /100 ml, the results found were far below the correct values if the second portion of 2 ml (or 4 ml when necessary) of ferricyanide reagent was added after the mixture of the blood filtrate and the first 2 ml of reagent had been heated for the full 10 or 15 min. On the other hand, the expected values were found if 4 ml (or 6 ml when necessary) of the reagent were added at once (Table I).

An explanation can be readily given. A heating time of 5 min at 100° (including a lag in the temperature rise of about 2.5 min: Fig. 1) practically suffices to complete the oxidation–reduction (Fig. 2). However, when glucose is heated in a medium containing ferricyanide and alkali, this substance is exposed to two separate reactions, *viz.*, oxidation by the ferricyanide and decomposition by the alkali. If an excess of ferricyanide is present, the former reaction prevails. If, on the other hand, the amount of glucose is larger, the excess glucose is transformed into products which are not, or nearly not, oxidized by ferricyanide. This phenomenon has also been described on another occasion[12]. In Fig. 3, results are illustrated of experiments where glucose solutions were heated with a "ferricyanide reagent" without ferricyanide (*i.e.*, containing the alkali only) after the HAGEDORN AND JENSEN technique for various intervals (including the lag time in the temperature rise), after which the required quantity of a ferricyanide solution (without alkali) of the concentration of the regular ferricyanide reagent was added; heating was continued for 10 min and the tests were completed in the ordinary way. After 2 min heating with the alkali (*cf.* the lag time in the temperature rise) a sharp drop in the recoverable quantity of glucose occurs. In the actual tests (*i.e.*, with the complete ferricyanide reagent) the loss of glucose becomes serious, for reasons not fully understood, after 3 min heating (Fig. 4). If, therefore, the second portion of ferricyanide reagent is added before this moment, nothing would be lost.

DISCUSSION

Unfortunately, one cannot always predict by the colour intensity of the fluid after 3 min heating, whether the glucose content of the test will be so high as to make a second addition of ferricyanide reagent necessary (Table II). For this reason it would be necessary to wait 4 or 5 min. There are, however, several ways of dealing with this problem.

The best solution would be to reject the regularly performed tests of blood samples,

TABLE I

RESULTS OF THE HAGEDORN–JENSEN METHOD APPLIED TO HIGH GLUCOSE CONCENTRATIONS

mg glucose/100 ml

	*Ferricyanide added**					
	"2 ml"	*"2+2 ml"*	*"4 ml"*	*"2+2+2 ml"*	*"2+4 ml"*	*"6 ml"*
Standard solution**						
380 mg glucose/100 ml		358	386			
500 mg glucose/100 ml		388	498			
670 mg glucose/100 ml		396	658			
930 mg glucose/100 ml					506	944
Blood A	114					
Blood A + standard solution 350 mg glucose/100 ml = blood 464 mg glucose/100 ml		368	467			
Blood A + standard solution 450 mg glucose/100 ml = blood 564 mg glucose/100 ml		405	571			
Blood B	57					
Blood B + standard solution 650 mg glucose/100 ml = blood 707 mg glucose/100 ml		592		***		705
Blood B + standard solution 850 mg glucose/100 ml = blood 907 mg glucose/100 ml		666		***		901

* The various ways in which the ferricyanide reagent was added, were:

"2 ml" = regular method
"2+2 ml" = regular method, after 10 min heating a second quantity of 2 ml was added and the heating continued for an additional 10 min
"4 ml" = 4 ml added at once
"2+2+2 ml" = regular method, after 10 min heating a second quantity of 2 ml added and the heating continued for an additional 10 min, then a third quantity of 2 ml added and the heating continued for a third period of 10 min
"2+4 ml" = as for "2+2 ml", but the second time 4 ml added at once
"6 ml" = 6 ml added at once

** Deproteinizing reagents (zinc sulphate and sodium hydroxide) and a preserving agent (benzoic acid) have no influence

*** As the loss of glucose, by 10 min heating for a blood sample with a concentration of even > 900 mg glucose/100 ml is so great that the remaining glucose concentration is < 700 mg/100 ml, the intended third addition of 2 ml ferricyanide reagent after the second 10 min heating appeared to be superfluous

if the glucose content appears to be higher than 348 (385) mg/100 ml, and repeat them using less blood or more ferricyanide reagent for each test.

The second best way would be the addition, after 3 min heating, of 4 ml ferricyanide reagent to all tests which are decolorized or only faintly coloured by that time. In this manner glucose concentrations up to more than 1000 mg/100 ml could be determined without appreciable losses and the tests, once set up, would be saved. This method would, however, introduce much unnecessary work.

A third possibility is the following. If it is necessary to save the tests, which often may be the case in a clinical chemical laboratory, *e.g.*, for lack of blood or time, one

TABLE II

DECOLORIZATION OF THE MIXTURE OF BLOOD FILTRATE WITH A HIGH GLUCOSE CONTENT AND 2 ml FERRICYANIDE REAGENT DURING THE HEATING

— not decolorized + decolorized

Heating time minutes	*Glucose concentration mg/100 ml*				
	400	*500*	*600*	*750*	*1000*
1	—	—	—	—	—
2	—	—	—	—	—
3	—	—	—	+	+
4	—	+	+	+	+
5	+	+	+	+	+

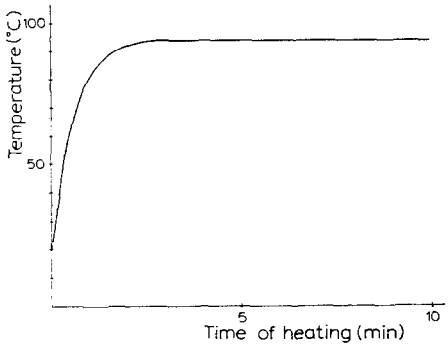

Fig. 1. Temperature during the oxidation–reduction of glucose–ferricyanide; diameter of the tube 3 cm.

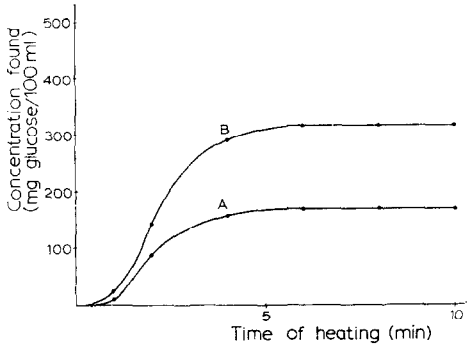

Fig. 2. Oxidation-reduction rate of glucose–ferricyanide: A, 175 mg glucose/100 ml; B, 320 mg glucose/100 ml.

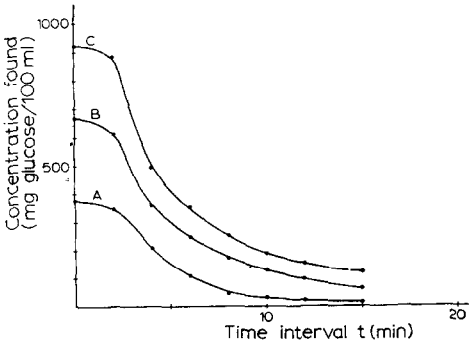

Fig. 3. Concentrations found for glucose solutions after pre-heating them for various intervals (t) in an alkaline medium identical with that used in the HAGEDORN AND JENSEN test. A, 375 mg/100 ml; B, 660 mg/100 ml; C, 920 mg/100 ml.

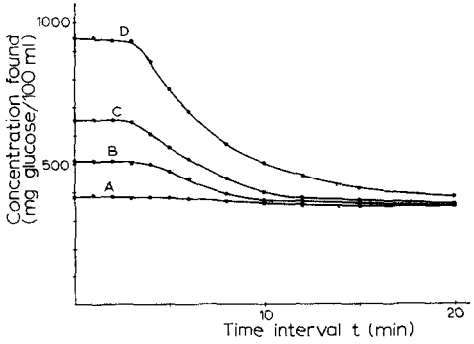

Fig. 4. Concentrations found for glucose solutions, if the second ferricyanide addition was given at various intervals (t) after the beginning of the heating. After each second addition the heating was continued for an additional 10 min. A, 380 mg/100 ml; B 515 mg/100 ml; C, 670 mg/100 ml; D, 925 mg/100 ml.

can add, after heating 5 min, a second quantity of ferricyanide reagent to those tests which are decolorized by then, provided that it is fully realized that in doing so the values found will be too low, *viz.*, from about 5% for 500 mg/100 ml to about 15% for 950 mg/100 ml (Fig. 4). If, for the second time, not more than 2 ml ferricyanide reagent is added, one admittedly lowers the maximum value to be found by the method (696 or 770 mg/100 ml, respectively), but the percentage in which this value is wrong as well. Moreover, the concentration range up to about 700 mg/100 ml covers by far the greater part of all values likely to be found and in values of 700 mg/100 ml an inaccuracy of 10% may be regarded as without clinical significance.

The choice of method must be made by the individual investigator himself, taking into account all essential points pertaining to the test concerned.

SUMMARY

An extension of the HAGEDORN–JENSEN method for handling blood samples with high glucose values is criticized. This modification, which is frequently used—at least in The Netherlands—consists of the addition of a second quantity of ferricyanide reagent to the test, after one has observed, by the decolorization of the fluid, that the first quantity was too small. It is shown that current descriptions of this procedure are insufficiently detailed, as they do not give the exact time for the second addition. If one does not give attention to this point, the results found may be grossly incorrect (up to 30% for a real value of 1000 mg/100 ml). Blood glucose values of diabetic patients in coma, formerly obtained by this method, must be regarded therefore with suspicion. While retaining the essentials of the method, several improvements are suggested.

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