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Determination of Glucose in Human Vitreous Humor

Various Analytical Methods Give Different Results

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Summary. In twenty cases, human vitreous humor glucose concentrations were measured with five different methods in common use. Striking differences in glucose values were obtained by the various analytical procedures. The reasons for these discrepancies remain obscure. Before interpretation of a given vitreous value, therefore, the analytical method employed must be known.

Zusammenfassung. In Glaskörper-Flüssigkeit wurde die Glucose-Konzentration mit fünf verschiedenen Methoden, die im allgemeinen Gebrauch sind, in zwanzig Fällen bestimmt. Es wurden hierbei erhebliche Differenzen in den Glucose-Werten mit den verschiedenen Analysenmethoden erhalten, wobei die Ursachen für die Diskrepanzen unklar sind. Bei der Beurteilung von Glucose Konzentrationen im Glaskörper muß daher die angewandte Bestimmungsmethode berücksichtigt werden.

Key word: Glucose, determination in human vitreous humor.

In the living body, an intimate exchange of constituents exists between blood and vitreous humor. Vitreous humor is however anatomically isolated, and is better protected from postmortem changes than blood. Biochemical investigations on vitreous humor are used in forensic medicine for evaluation of antemortem abnormalities. Glucose concentration in vitreous humor is of particular interest when fatal hypoglycemia or diabetes are suspected.

In humans, the glucose concentration in vitreous humor is markedly lower than in blood, and there is no difference in glucose concentration between the two eyes [1]. Postmortem, a definite but variable fall in vitreous glucose occurs, and zero glucose levels may be found a few hours after death [2, 3, 4]. Therefore, a high postmortem vitreous glucose value indicates an antemortem hyperglycemia. A low postmortem value may represent an antemortem hypoglycemia or, simply, the natural postmortem glucose degradation.

The glucose concentration in vitreous humor can be measured by different methods. It is an exception, however, that the chemical principle of the analytical method is given [1]. The apparatus used for assay is also seldom mentioned.

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The aim of this paper is to compare five different analytical methods, and to show that the analytical principle of a method must be known before an analytical value can be correctly interpreted.

Material and Methods

Twenty subsequent cases of sudden death submitted to the Institute of Forensic Medicine, University of Oslo, were studied. The postmortem interval varied between ten hours and 3 days. The vitreous humors were obtained by inserting a 20 x 1½ Vacutainer ® needle into the center of the eyeballs through the outer canthus, extracting two to four millilitres of vitreous humor by means of a No. 3274 Vacutainer ® tube (Becton, Dickinson AB, Stockholm, Sweden). In five cases samples were taken separately from both eyes. The samples were directly frozen and stored at -18°C. They were immediately analysed when thawed, as preliminary tests showed that repeated thawing and freezing caused the glucose concentration to decrease. It has earlier been shown [4] that glucose concentrations do not vary significantly when vitreous humor is kept frozen up to 20 months. The following five methods were used:

1. The test strip Dextrostix ® (Ames Company, Division Miles Laboratories, Ltd., Elkhart, Indiana, USA). The reaction is based on a special modification of the glucose oxidase/peroxidase principle.

2. The glucose oxidase/peroxidase reaction after deproteinization by dialysis in a single-channeled AutoAnalyzer ® (Technicon Corporation, Tarrytown, New York, USA).

3. The glucose oxidase/peroxidase reaction after previous precipitation of proteins with perchloric acid (0.33 mol/l, pH 2.7, buffered with glycine).

4. The glucose oxidase/peroxidase reaction after previous precipitation of proteins by zink hydroxide according to Hjelm and de Verdier [5].

5. The ortho-toluidine method described by Hultman [6], and Hyvärinen and Nikkilä [7], after precipitation of proteins with trichloracetic acid (0.18 mol/l).

The glucose oxidase/peroxidase reagent used in methods 2,3, and 4 was obtained from AB Kabi, Stockholm, Sweden.

Test serum, Seronorm ® (Nyegaard & Co. A/S, Oslo, Norway. Batch no. 127), was used as control. It has a recommended glucose concentration of 6.9 mmol/l.

Analytical grade D-glucose, α-D-glucose-1-phosphate, α-D-glucose-6-phosphate, D-gluconic acid, D-glucuronic acid, D-glucosamine and hyaluronic acid (from umbilical cord) were obtained from Sigma Chemical Company, St. Louis, Missouri, USA.

Results

Glucose concentrations in the vitreous humors, determined by the five different methods, are listed in Table 1.

Methods 1,2, and 3 gave similar results, no glucose measured except in one case [no. 18].

Method 4 gave positive glucose values in sixteen of the twenty cases investigated. A relatively high concentration was found in case no. 18 where the previous methods showed small amounts. An even higher value was observed in case no. 11, where the previous methods gave zero results.

Method 5 gave generally higher glucose values than those observed by the other methods.

In five cases (no. 6–10) vitreous humor were taken from both eyes and these samples were analysed simultaneously. No significant differences were found between the two eyes. These results are not included in Table 1.

All the five methods gave glucose values of the test serum within an acceptable limit, if permitting a 5% coefficient of variation.

Table 1. Vitreous humor glucose concentrations (mmol/l) in 20 cases of sudden death and more than 10 hours post mortem time as observed with five different analytical methods

Methods	1	2	3	4	5
Case Number	Dextrostix ®	Dialysis, glucose-oxidase / peroxidase reaction in the AutoAnalyzer ®	Perchloric acid, manual glucose oxidase / peroxidase reaction	Zinc hydroxide, manual glucose oxidase / peroxidase reaction	Trichloroacetic acid, manual ortho-toluidine reaction
1	0	0	0	1.3	2.1
2	0	0	0	1.0	0.8
3	0	0	0	0.6	1.3
4	0	0	0	0.8	1.5
5	0	0	0	0	3.1
6	0	0	0	1.3	10
7	0	0	0	1.7	16
8	0	0	0	0	1.1
9	0	0	0	1.0	1.7
10	0	0	0	1.0	6.7
11	0	0	0	3.5	30
12	0	0	0	0.8	1.7
13	0	0	0	1.8	14
14	0	0	0	0.8	2.5
15	0	0	0	0	1.2
16	0	0	0	1.2	6.4
17	0	0	0	0.9	1.3
18	Traces	0.4	1.0	2.7	27
19	0	0	0	0	1.2
20	0	0	0	1.1	6.0
Seronorm ®	7.3	6.9	6.8	7.5	7.1

α-D-glucose-1-phosphate, α-D-glucose-6-phosphate, D-glucoronic acid, D-glucosamine and hyaluronic acid gave all zero results with the five methods, except D-glucosamine analyzed with method 5. A glucose concentration, approximately 20 % of the original glucosamin concentration, was measured with this method.

Discussion

Except for using Vacutainers ® instead of syringes, the sampling procedure seems well established [4].

With a post mortem time of more than 10 hours the results obtained with method 1,2, and 3 are reasonable, since low concentrations or absence of glucose would be expected (2–4).

The glucose oxidase reaction, used in methods 1–4, is specific for glucose [8]. But false low values may occur if other reducing agents are present during the final indicator reaction. Most likely any reducing substances, originally present in vitreous humor, are removed by the dialysis in the AutoAnalyzer ® (method 2), and probably also by the protein precipitants used in methods 3–5. False high values might occur if oxidizing agents were present during the indicator reaction, but the literature gives no evidence of oxidizing substances in vitreous humor. The results obtained with methods 1,2, and 3 are therfore most likely the true glucose concentration in the samples at the time of analysis.

Method 4 would also give false high or low values if oxidizing or reducing substances respectively, were present during the final indicator reaction. Taken into account the high specificity of the glucose oxidase reaction, the higher glucose values measured by this method likely represent true glucose concentrations in the test tubes. It is therefore probable than zink hydroxide treatment liberates glucose from some complex present in the vitreous humor.

In method 5, the ortho-toluidine method [6,7], the last step in the procedure is boiling the sample and the colour reagent with a mixture of acetic acid and acetic acid anhydride. This treatment may well cause hydrolysis of some aldose-containing substance in the vitreous humor. The ortho-toluidine/glucose reaction is not specific for glucose and any aldose present in the sample would give a similar colour reaction. The very high values found with the ortho-toluidine method may indicate the presence of other aldoses (although none other than glucose have been reported in vitreous humor) or that the last step of the procedure is more effective than zink hydroxide in releasing glucose from a glucose containing substance. The ortho-tuluidine method is recently, in a modified version, claimed as „the method of choice" for glucose determinations in biological fluids [8].

It should be noted that the test serum gives acceptable glucose values with all the five methods investigated.

Vitreous humor contains several substances [9–13] that might have influenzed the glucose values obtained with methods 4 and 5. Of the 6 substances studied, only glucosamine gave a positive reaction, and only with method 5. This reaction is to our knowledge not reported earlier. But glucosamine is hardly present in vitreous humor in amounts sufficient to explain the high glucose values measured with this method, and cannot be responsible for the positive reactions obtained with method 4. The substance responsible for the positive reactions obtained with two of the methods probably contains glucose, and seems to be unknown and unreported – a challenge to further investigation. It is not clear why the glucose concentration in the different vitreous humors should vary to such an extent measured with the same method. One possibility is that the concentration of an obscure source of glucose varies individually. Another explanation might be that a varying degree of degradation occurs during post mortem time, thus making the substance more or less acceptable to hydrolysis.

From a practical, forensic point of view it is noteworthy that the Dextrostix ® gave results comparable with methods 2, and 3. The one case (no. 18), that showed the vitreous humor to contain glucose with this method, was the only case that was positive with all the methods, indicating a high antemortem glucose level.

This study shows that, when measuring glucose in vitreous humor post mortem, the chemical principle of the method must be noted, because the interpretation of the results depends on the method used.

References

1. Swift, P. G. F., Worthy, E., Emery, J. L.: Biochemical state of the vitreous humor of infants at necropsy. Arch. Dis. Childh. 49, 680–685 (1974)
2. Coe, J. I.: Postmortem chemistries on human vitreous humor. Amer. J. Clin. Path. 51, 741–749 (1969)
3. Coe, J. I.: Use of chemical determinations on vitreous humor in forensic pathology. J. Forens. Sci. 17, 541–546 (1972)
4. Sturner, W. Q., Dempsey, J. L.: Sudden infant death: Chemical analysis of vitreous humor. J. Forens. Sci. 18, 12–19 (1973)
5. Hjelm, M., de Verdier, C.-H.: A methodological study of the enzymatic determination of glucose in blood. Scand. J. Clin. Lab. Invest. 15, 415–428 (1963)
6. Hultman, E.: Rapid specific method for determination of aldosaccharides in body fluids. Nature (London) 183, 108–109 (1959)
7. Hyvärinen, A., Nikkilä, E. A.: Specific determination of blood glucose with o-toluidine. Clin. Chim. Acta 7, 140–143 (1962)
8. Henry, R. J., Cannon, D. C., Winkelman, J. W.: Clinical Chemistry: Principles and Technics. Hagerstown, New York, Evanston, San Francisco, London: Harper & Row 1974
9. Meyer, K., Palmer, J. W.: The polysaccharide of the vitreous humor. Biochem. J. 107, 629–634 (1934)
10. Varma, R., Varma, R. S., Allen, W. S., Wardi, A. H.: On the carbohydrate-protein linkage group in vitreous humor hyaluronate. Biochim. Biophys. Acta 263, 584–588 (1974)
11. Wardi, A. H., Allen, W. S., Turner, D. L., Stary, Z.: Hyaluronatepeptide linkage group. Biochim. Biophys. Acta 192, 151–154 (1969)
12. Falbe-Hansen, I., Degn, J. K.: Sialic acid in the aqueous humor and the vitreous of normal human eyes and of eyes with malignant melanoma of the choroid. Acta Ophthal. 47, 972–983 (1969)
13. Day, R.: Polysaccarides in ocular tissue. Amer. J. Ophtal. 33, 224–226 (1950)
14. Adler, F. H.: Physiology of the Eye. St. Louis: V. C. Mosby Company 1959

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