ACCURATE DETERMINATION OF GLUCOSE IN HUMAN SEMEN

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Summary. Human semen contains glucose which can be determined by means of glucose oxidase, provided that certain strict analytical precautions are observed. Glucose occurred in all but two of 185 samples. The mean concentration was 7.24 mg/100 ml (s.e.m. ±0.69; range 0 to 99).

INTRODUCTION

Human seminal plasma contains large amounts of fructose (Mann, 1946). Recently the simultaneous occurrence of much smaller amounts of glucose has been demonstrated by using the glucose-oxidase method (Hornstein, 1961a, b). This enzymatic method was originally regarded as specific for glucose (Keston, 1956; Teller, 1956; Hugget & Nixon, 1957), but later different substances have been shown to interfere with the determination of glucose in blood (Fales, Russel & Fain, 1961; Hjelm & de Verdier, 1963). A preliminary study confirmed the occurrence of glucose in human seminal plasma but also revealed that semen contains compounds that seriously interfere with the determination. Further studies were therefore carried out, and the results are presented in this paper.

METHODS AND RESULTS

Samples of semen were obtained from patients attending the fertility laboratories at the Karolinska Hospital and the Sabbatsberg Hospital and also from volunteers. Part of each sample was centrifuged and the plasma transferred to glass tubes and kept at −20°C until analysed, usually within 2 weeks. Control experiments revealed no change in the samples during storage at this temperature. Before analysis each sample was thawed to room temperature.

Deproteinization was performed either by mixing 0.1 ml seminal plasma with 1.4 ml 0.33 m-perchloric acid (PCA), or by using, in a modified form, the cadmium sulphate reagent according to Fujita & Iwatake (1931) and Lundquist (1949). To 0.1 ml plasma in a centrifuge tube was added 1.0 ml distilled water,
0·3 ml CdSO₄-reagent (3·4667 g CdSO₄ + 16·93 ml 1·0 N-H₂SO₄ + distilled water to 100 ml) and 0·1 ml 1·1 N-NaOH. After thorough mixing the tubes were left for 15 min. Irrespective of the method of deproteinization the precipitate was removed by centrifugation. After deproteinization and centrifugation, 0·5 ml of the clear supernatant was mixed with 5·0 ml glucose-oxidase reagent and incubated at 37° C for 60 min. After centrifugation the colour was read in a Beckman DB at 450 μ against a blank. In the blank the protein-free extract of seminal plasma was exchanged by PCA, or when CdSO₄-reagent was used, by water. When heat-inactivated enzyme preparation was used, this was also added to the blank.

The glucose-oxidase reagent was commercially available under the name of Glykosoxidasreagens Kabi (Sweden) and corresponded, according to the manufacturer, to that described by Raabo & Terkildsen (1960), that is 3 mg crude powder glucose-oxidase Sigma, 15 μg peroxidase and 33 μg o-dianisidine per ml. This reagent will be called Reagent A. For experiments concerning the interfering compounds in semen, special reagents were put at our disposal by the manufacturer. Reagent B was equal to Reagent A but had no glucose oxidase. Reagent C was equal to Reagent A but contained neither glucose oxidase nor peroxidase.

It is obvious that the occurrence of factors in semen that could compete with the chromogen for H₂O₂ would give false low values. Also the occurrence of catalase or other enzymes converting H₂O₂ to O₂ and H₂O would interfere with the estimations. In order to test this 0·05, 0·10, 0·15 and 0·20 μmoles hydrogen peroxide/ml, respectively, was added to samples of Cd⁺⁺-extract from seminal plasma. These samples were then incubated with Reagent B (without glucose oxidase) at 37° C, for 60 min and the optical density was compared with that of samples where water had been used instead of semen.

Standard solutions of glucose were always included and treated in the same way as the test samples. The glucose was dissolved in 0·33 M-PCA or, when CdSO₄-reagent was used, in water. Usually the standard sample contained 5 and 10 mg glucose/100 ml.

In order to study the possibility that glucose could be released from other carbohydrates due to enzymatic reactions, e.g. by maltase (Seth & Rao, 1962), 1 ml seminal plasma was transferred to a test tube and kept in a water bath at 37° C. The glucose content was determined at 0, 3 and 6 hr. In order to prevent bacterial growth 100 i.u. benzylpenicillin/ml were added to each test tube.

**Determination of fructose**

In some of the samples fructose was also estimated. The protein was precipitated with the CdSO₄-reagent as described above. After centrifugation 0·5 ml of the clear supernatant was transferred to a test tube and 0·5 ml indol reagent (25 mg indol in 100 ml 0·2% benzoic acid) and 5·0 ml conc. HCl added. The test tubes were left in a water bath at 50° C, for 20 min, and then cooled in running water. The yellow colour was read in Beckman DB at 470 μ (Karvonen & Malm, 1955).

All chemicals used were of reagent grade. All analyses were made in duplicate.
CRITICAL EXAMINATION OF THE GLUCOSE-OXIDASE METHOD FOR DETERMINATION OF GLUCOSE IN SEMEN

The standard curve for concentrations of 2, 5 and 10 mg glucose/100 ml water was not an absolutely straight line in the range 5 to 10 mg/100 ml. The deviation was, however, so slight that the error introduced could be neglected in semen samples containing less than 150 mg/100 ml. The accuracy of the method was ±0.5%.

Interference with the chromogen

In the preliminary experiments the seminal plasma was deproteinized with PCA. In the blank PCA replaced the semen. With regard to the possibility that the seminal plasma might contain compounds that could oxidize the chromogen and thus give false values, a blank with semen and heat-inactivated Reagent A was also used. In this series eighty-two samples were analysed. The boiled Reagent A gave 'positive' reaction in seventy-three of these. The 'mean glucose value' in these samples was 5.7 mg/100 ml, the highest value corresponding to 16.8 mg/100 ml. With the active Reagent A the mean value for the eighty-two samples corresponded to 7.6 mg/100 ml when read against the PCA-blank. There was no correlation between the 'glucose-values' obtained with the active and heat-inactivated enzyme preparations. The PCA-method for deproteinization could thus not be regarded as suitable for determination of glucose in seminal fluid.

Thirty samples were precipitated with CdSO$_4$-reagent and tested with the heat-inactivated Reagent A. None gave a positive reaction. In some of the samples a white precipitate appeared which, however, was easily removed by centrifugation.

Samples of seminal plasma treated with PCA obviously contained factors that oxidized the chromogen while Cd$^{++}$-precipitation removed these factors. In order to study the possibility of an interference with the peroxidase step in the reagent mixture, the colour developed after incubation of deproteinized semen with Reagent B (peroxidase + o-dianisidine), Reagent C (o-dianisidine) or heat-inactivated Reagent A, was compared. Semen treated with PCA gave the same extinction in all series, i.e. there was only interference with the chromogen. The samples from semen precipitated with the CdSO$_4$-reagent, on the other hand, gave no 'positive' reaction in any of the tests.

Recovery of H$_2$O$_2$. The optical density following the incubation of Cd$^{++}$-deproteinized semen samples containing 0.05 to 0.20 μmoles peroxide/ml was compared to that of the incubated controls (semen replaced by water). The mean recovery (n = 4) of H$_2$O$_2$ was 100%, indicating that no interaction had occurred.

APPLICATION OF THE GLUCOSE-OXIDASE METHOD TO SEMEN

From the results described above it is obvious that in order to get correct results with the glucose-oxidase method one should deproteinize the semen with Cd$^{++}$ in alkaline solution. This method was therefore used in the studies described below.
Recovery of added amount of glucose. This was studied by adding known amounts of glucose (1 to 10 mg/100 ml) to untreated samples of semen. The recovery was between 98 and 104%.

Amount of glucose in randomly selected samples. One hundred and eighty-five samples were randomly collected from one of the fertility laboratories and analysed for glucose. The mean concentration was 7.24 mg/100 ml with s.e.m. ±0.69 (range 0 to 99 mg/100 ml). The distribution is presented in Text-Fig. 1.

Effect of incubation on the glucose content. Twenty different samples were incubated for 0, 3 and 6 hr. The mean glucose content was 9.3, 9.0 and 10.7 mg/100 ml, respectively. This indicates that no glucose was formed during this period of time.

![Text-Fig. 1. Distribution of glucose in 185 samples of human seminal plasma.](image)

Relationship between glucose and fructose concentration. Fifty samples were randomly selected from those obtained from the fertility laboratory and both glucose and fructose were determined. Glucose was found in all but two samples. The mean value for glucose was 7.83 mg/100 ml (s.e.m. ±1.14) and that for fructose was 320 mg/100 ml (s.e.m. ±14). There was no correlation between the concentration of fructose and that of glucose.

DISCUSSION

The occurrence of small amounts of glucose in human seminal plasma has been confirmed by using the glucose-oxidase method. The specificity of this method in determining glucose is, however, limited to the first step in the reaction system. Compounds in blood and other fluids may seriously interfere with the second step (Fales et al., 1961; Hjelm & de Verdier, 1963), but this has usually been overlooked.
Preliminary investigations on the occurrence of glucose in the semen, in which a heat-activated enzyme preparation was used as blank, revealed that the seminal plasma deproteinized by perchloric acid (PCA), still contained compounds that oxidized the chromogens. These interfering substances gave rise to optical densities corresponding to a mean ‘glucose-value’ of 5.7 mg/100 ml (highest value found equal to 16.8 mg ‘glucose’/100 ml). All the interfering substances could, however, be removed by deproteinizing the seminal plasma with Cd++ in alkaline solution according to Fujita & Iwatake (1931) and Lundquist (1949). The usefulness of deproteinizing blood plasma with Zn++ or Cd++ in determination of glucose was demonstrated by Hjelm & de Verdier (1963).

Hornstein (1961a, b) examined 205 samples of human semen from men attending infertility clinics. He found about 45% of the samples contained glucose, usually between 5 and 30 mg/100 ml. The highest value found was 53 mg/100 ml. In the present study on semen samples from 185 infertility patients, glucose was found in all but two. The reason for this discrepancy is most likely the difference in the methods used. Moreover, it indicates that the PCA-treated semen may contain certain factors, e.g. glutathione, that also interfere with the second step in the reaction.

What is the significance of the glucose found in the human semen? It is known that the spermatozoa can utilize glucose as well as fructose. With regard to the small amounts of glucose usually present it does not, however, appear likely that glucose can play any significant role in the metabolism of spermatozoa. On the other hand, since human semen also contains minor quantities of certain other monosaccharides (galactose, ribose, ribulose) as well as oligosaccharides, a metabolic function of glucose and of these other sugars cannot be excluded at the present time (Mann & White, 1957; Kubicek & Santavy, 1958).

The origin of glucose in semen is not clear. Seth & Rao (1962) postulated that glucose could be formed from glycogen and/or oligosaccharides by enzymatic breakdown of these products. However we observed no increase in the glucose content of the seminal plasma after 6 hr of incubation. It therefore appears less likely that this pathway is of any significance for the occurrence of glucose in human semen.

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REFERENCES


