Glycaemia/determination

Determination of blood glucose concentration is an examination that will provide basic information about carbohydrate metabolism. Capillary or venous blood is collected and glucose is determined in whole blood, plasma or serum. When determining glucose in whole blood, the values are 10–15% lower (depending on hematocrit), in arterial blood they are 10% higher than in venous (arteriovenous difference). To prevent glycolysis, NaF (2.5 mg per ml of whole blood) is added to the collection vials. ***The glucose test in blood has the necessary information value only if the time interval between blood collection and food intake is known.***

Blood glucose testing is performed:

- **fasting** (blood is taken at least 8 hours after a meal) - indicated when searching for diabetics and diagnosing DM;
- **randomly** measured blood glucose (blood is taken without a time relation to food intake) - performed when hypoglycemia or hyperglycemia is suspected;
- postprandial - **postprandial** glycaemia (1 hour after a meal containing carbohydrates) - indicated to monitor the effectiveness of DM treatment;
- as **glycemic profile** - glycemia is determined several times a day, usually before main meals, sometimes after meals and at night.

Methods for determining blood glucose

Determination of glycemia in laboratory conditions

Various methods are used to determine glucose concentration. Enzyme methods are widely used. Glucose can be determined by any enzyme that metabolizes it. Another article discusses the possibilities of Non-invasive blood glucose measurement

Glucose oxidase reaction

File:Glucose oxidase reaction.png

Glucose oxidase and peroxidase reactions

The recommended routine method uses *glucose oxidase-coupled enzyme reactions* (**GOD** , 4.html EC 1.1.3.4 (http://www.sbcs.qmul.ac.uk/iubmb/enzyme/EC1/1/3/)) and **peroxidase** (**POD**, /7.html EC 1.11.1.7 (http://www.sbcs.qmul.ac.uk/iubmb/enzyme/EC1/11/1)) *. In the first reaction, the enzyme "glucose oxidase" catalyzes the oxidation of glucose by atmospheric oxygen to form gluconic acid, which is converted to the inner ester gluconolactone. It is known that 36% of glucose is known to be in the form of the α-anomer and 64% in the form of the β-anomer. GOD is highly specific for β-D-glucopyranose. In order for both anomers to be oxidized, a mutation of the α- to β-anomer is required, which occurs spontaneously during a sufficiently long incubation. An equimolar amount of "hydrogen peroxide" is formed as a by-product of the glucose oxidase reaction.*

In **another peroxidase-catalyzed reaction**, the resulting hydrogen peroxide is reacted with a suitable chromogen, which is oxidized to a reactive intermediate, which is coupled with another substance to a stable soluble dye. An example is the oxidative coupling of a phenol derivative with 4-aminoantipyrine to a red dye, the absorbance of which is measured after the reaction equilibrium has stabilized.

Other methods utilize the measurement of oxygen depletion that occurs during a glucose oxidase-catalyzed reaction and can be monitored electrochemically with an oxygen electrode or an enzyme electrode.

Hexokinase reaction

The hexokinase method is characterized by high specificity. **Hexokinase** (EC 2.7.1.1 (http://www.sbcs.qmul.ac.uk/iubmb/enzyme/EC2/7/1/1.html)) phosphorylates glucose in the presence of ATP to **glucose-6-phosphate**. In the next step, glucose-6-phosphate is oxidized by "glucose-6-phosphate dehydrogenase" against $NADP^+$ to 6-phosphogluconolactone. The reduction of $NADP^+$ to **NADPH** can be evaluated by direct UV photometry on the principle Warburg optical test.

Determination of glycemia in non-laboratory conditions

Glycemia is one of the parameters that is often examined even without a laboratory background. Rapid guideline blood glucose determination is common in emergency care. Patients treated with insulin also preferably have their blood glucose monitored regularly with a personal glucometer, and treatment is adjusted based on the measured values. Blood glucose levels are among the parameters most commonly determined by "point of care testing" ("POCT") techniques. However, it must be borne in mind that POCT methods, although improving the quality of care and patient comfort, do not replace regular medical examinations or laboratory tests.

Rapid blood glucose methods use several principles. The starting material is usually a drop of whole capillary blood that is applied to a **test strip**.

The oldest bands were based on the same reactions as the photometric measurement of glucose concentration. The reaction zone contained "glucose oxidase", "peroxidase" and the appropriate chromogen. The evaluation was performed either visually by comparison with a color scale or using a glucometer - single-purpose reflection photometer. Most meters today use "enzyme electrodes"[1] [2].

The **first generation** sensors appeared in the 1960s. The oldest system was based on the glucose oxidase reaction. He used two electrodes, one covered with an enzyme. The oxygen concentration in the sample and the rate of its decrease during the reaction was measured by the so-called Clark method: oxygen is reduced at the platinum cathode, the current intensity between the cathode and the anode corresponds to its concentration:

$$O_2 + 4\ H^+ + 4\ e^- \rightarrow 2\ H_2O$$

Later, hydrogen peroxide production was determined electrochemically instead of oxygen consumption. Even in this case, it is a simple electrochemical reaction, this time taking place at the anode:

$$H_2O_2 \rightarrow O_2 + 2\ H^+ + 2\ e^-$$

The analyzers constructed in this way were simpler and could be miniaturized more. However, amperometric measurement of hydrogen peroxide production is influenced by a number of substances: ascorbate, uric acid, many drugs, etc. Another problem of many first-generation sensors was the dependence of the measurement results on the oxygen saturation of the sample.

The **second generation** sensors are also based on the **glucose oxidase** reaction, but instead of molecular oxygen, another substance is the electron acceptor - the so-called *mediator*. Another possibility is the oxidation of glucose to gluconolactone by another bacterial enzyme, **glucose dehydrogenase**, whereby electrons are again transferred to a suitable mediator. In both cases, the reduced mediator is reoxidized at the anode and either the current flowing between the cathode and the anode is measured (amperometric determination) or the resulting anode charge (coulumbometric determination). A number of specific test strip configurations are used, and various substances are used as mediators (eg, ferric cyano, ruthenium hexamine, osmium complexes, phenanthrolinequinone).

Links

Reference

- JOSEPH, Wang. Electrochemical glucose biosensors. Chemical reviews [online] . 2008, vol 108, no. 2, pp. 814-825, also available from < https://pubs.acs.org/action/cookieAbsent >. ISSN 0009-2665.
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