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CARBOHYDRATE METABOLISM

GLUCOSE DETERMINATION IN BIOLOGICAL FLUIDS

There are several methods performed for current glucose analysis. These are:

- Methods which use enzymes as reagents (e.g., hexokinase, glucose oxidase, glucose 6-phosphate dehydrogenase).
- Chemical methods - the most used is the orto -toluidine method.

The enzymatic methods are more specific for glucose determination and for that reason are widely used. The chemical method, using o-toluidine is performed were the enzymatic test is not available.

Each laboratory is recommended to confirm the validity of the reference intervals for the population it serves.

Blood specimens must be carefully processed and preserved because blood specimens left at room temperature lose glucose at about 10 mg/100 ml/1 hour. This loss is due to glycolysis by bacterial contaminants or by the cells in the blood. If the blood is collected and handled aseptically, and the serum separated immediately from the cells and stored at 4°C (refrigerator), the glucose concentration does not vary for at least 8 hours.

Blood is usually collected in tubes containing 1 mg NaF / ml of blood if immediate processing and refrigeration is not possible.

A. Glycemia determination using non-specific methods

Glycemia determination with o-toluidine

Principle

In acetic medium, in the presence of thiourea, at warm, glucose condensates with o-toluidine to form a green colored compound, which absorption (at 630 nm) is proportional to the glycemia level.

B. Enzymatic Methods for glycemia determination

1. Hexokinase method

This method employs the enzyme hexokinase to catalyze the formation of glucose-6-phosphate (G6P) from glucose. The reactions implied in the determination (optical test) are:

$$\text{glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{glucose-6-phosphate} + \text{ADP}$$

$$\text{glucose-6-phosphate} + \text{NADP}^+ \xrightarrow{\text{G6P dehydrogenase}} \text{6-phosphogluconate} + \text{NADPH} + \text{H}^+$$

The formation of NADPH is accompanied by an increase in absorbance at 340 nm. As one may see from the reaction above, for each mole of NADPH formed, 1 mole of glucose has been reacted upon. Thus the change in absorbance measured

spectrophotometrically at 340 nm is used to measure the concentration of glucose present.

2. Glucose - dehydrogenase method

The method is high specific, no other interference exists. Glucose is transformed in gluconolactone by action of the glucose dehydrogenase enzyme (GDH). The GDH enzyme acts only on the β form of the glucose and, consequently, other enzyme is necessary to transform α-glucose in the β form (glucomutase). The NADH and D-gluconolactone result from the transformation of the glucose under catalytic action of GDH. D-gluconolactone is continuously transformed in gluconic acid and it is eliminated. The variation of the absorbance at 340 nm is proportional with the glucose concentration.

$$\alpha\text{-D-glucose} \xrightarrow{\text{glucomutase}} \beta\text{-D-glucose}$$

$$\text{D-glucose} + NAD^+ \xrightarrow{\text{glucose dehydrogenase}} \text{D-gluconolactone} + NADH + H^+$$

$$\text{D-gluconolactone} + H_2O \longrightarrow \text{D-gluconic acid} + H^+$$

3. Glucose oxidase method. Experimental part

This method employs the enzymes glucoseoxidase and peroxidase, and an oxidative reaction:

$$\text{glucose} + H_2O \xrightarrow{\text{Glucose oxidase}} \text{gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{1,7-dihydroxynaphtylene} \xrightarrow{\text{peroxidase}} \text{a red dye}$$

The red color is spectrophotometrically read at 555 nm and the absorbance is directly proportional with the glucose concentration.

Other instruments may use the polarographic oxygen electrodes, which measure the rate of oxygen consumption after the addition of the specimen to the glucose oxidase. This method is not to be used for urine.

An electrode can be used, which has been made selective for glucose by incorporating glucose oxidase in the membrane. Glucose diffuses through this membrane and upon contact with the glucose oxidase forms hydrogen peroxide. The hydrogen peroxide then diffuses through a second membrane and reaches the electrode where oxidation occurs and a current is generated.

Reagents

1. Working reagent:
   - phosphate buffer, 0.5 mol/l, pH 7.50
   - phenol, 7.5 mmol/l
   - glucoseoxidase, 12000 U/l
   - peroxidase, 660 U/l
   - 4-aminoantipyrine, 0.4 mmol/l

The reagent is stable 3 months at 2-8^{0}C or 3 weeks at 20-25^{0}C.

2. Glucose standard, 100 mg%

Serum and standard are diluted 10 times with distilled water.

Procedure

Pipette into 3 test tubes as follows:

Reagents, μl	Sample	Standard	Blank
Working reagent	1000	1000	1000
Diluted standard	-	100	-
Diluted serum	100	-	-
Distilled water	-	-	100

Incubate the tubes at 37^0C for 15 minutes or at 25^0C for 30 minutes.

Read the absorbance of sample and standard at λ = 546 nm, zeroing the spectrophotometer with the blank. The color is stable for 60 minutes. The response is linear up to a glucose concentration of 400 mg%.

Calculation

Calculate serum glucose concentration using the formula:

$$\text{mg glucose/ 100 ml} = \frac{E_{sample}}{E_{standard}} \times C_{standard} = \frac{E_{sample}}{E_{standard}} \times 100$$

Normal values:

Fasting males: 75 - 110 mg%

Fasting females: 65 - 105 mg%

Pathological conditions

Hyperglycemia: diabetes mellitus, Cushing syndrome (glucocorticoids excess), acromegaly (growing hormone excess), feochromocytoom (catecholamine excess), sever hyperthyroidism, stress, shock states, pancreatitis, pancreatic carcinoma, pancreatectomia.

Hypoglycemia: insulin supra-dose, ingestion of high quantities of alcohol, gastric resection pancreatic tumors that secrete insulin (insulinoma).

2. Glucose tolerance test

The glucose tolerance test measures the ability of a person to appropriately respond to a heavy dose of glucose. Normally, upon receiving such a dose, insulin is secreted from pancreas in an amount necessary for the metabolism of glucose. The degree and timing of the rising and falling of the blood glucose after glucose administration gives an indication of the ability of the individual to respond appropriately.

The test is performed after it has been found that a person has a fasting glucose above that found for most non diabetic individuals (115-126 mg%). It may also be performed to determine hypoglycemia, an abnormal response to glucose load which results in a blood glucose concentration much below the normally accepted range.

Reference Interval:

Non diabetic subjects: all urine specimens are negative.

Highest blood serum glucose is normally achieved within 30 - 60 minutes following dose.

Concentration at peak < 180 mg%

At one hour, the blood glucose ≤ fasting level

Method - oral glucose tolerance

1. The test requires a specified carbohydrate diet for 3 days prior to the test. The test will take about 4 hours. Several blood specimens will be taken during this time and the patient will have to remain near the laboratory.
2. The patient is given a carbohydrate diet containing 1 grams of carbohydrate per kilogram of body weight with instruction to adhere to it for 3 days prior to the oral glucose tolerance test.
3. At 8 P.M. of the evening prior to the test, the patient is not to eat or smoke and may not drink anything but water until otherwise informed.
4. The test is started between 7 A.M. and 9 A.M. on the day of the test, by collecting a fasting venous blood specimen and a urine specimen for glucose determinations.
5. The patient is then given a solution to drink which contains100 grams of glucose, which may be flavored with lemon juice or any other non caffeine flavor. Glucose preparations for this purpose are commercially available. The patient is instructed to drink the solution within a 5 minute period.
6. The time is noted when the patient has completed drinking the glucose solution.
7. Blood and urine specimens for glucose determination are collected at intervals of 30; 60; 120 and 180 minutes following the ingestion of the glucose solution.
8. If hypoglycemia is being studied, the test may be extended to 5 hours by collecting specimens at 240 and 300 minutes.
9. Patients are to be watched carefully during this test for symptoms of hyperglycemia or hypoglycemia and a physician notified if symptoms become marked.

Glycemia depends also on age. The following table presents a normal glycemia curve during an oral glucose tolerance test:

	Fasting glucose (0 hour)	glycemia after 1 hour (max)	glycemia after 2 hours
Normal	75-115 mg%	< 140 mg%	75-115 mg%
Low glucose tolerance	115-126 mg%	140-200 mg%	126-200mg%
Diabetes type 2	>126 mg%	>200 mg%	>200 mg%

Fasting and two-hour postprandial blood glucose

Ordinarily these are the principal tests performed to monitor glucose metabolism. A fasting blood glucose and one taken 2 hours after breakfast are useful in evaluating diabetes. The fasting blood glucose should be less then 115 mg% and the blood glucose taken 2 hours after the meal should be less than 140 mg%. Blood glucose results higher than these may not be interpreted as due to diabetes until hormonal disorders, diet, and possible interfering medications have been considered.