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L5. Glycemia regulation. Determination of glycated hemoglobin

Carbohydrates represent the main source of energy for cells. Glucose is the unique source of energy for the central nervous system and erythrocytes.

The organism covers the approx. 250 grams of glucose needed per day by:

- taking it from food;
- lysis of the hepatic and muscular glycogen;
- gluconeogenesis.

Penetration of glucose in the muscular, adipose tissues and myocardium is realized only in the presence of insulin (insulin dependent tissues). Liver, kidney, brain, erythrocytes, Langerhans islets and intestinal mucosa do not need the presence of insulin (insulin independent tissues).

The maintaining of glycemia (plasma level of glucose) in the normal limits is realized by complex physiological and biochemical mechanisms.

Hormones role in glycemia regulation

Different hormones participate to the maintaining of a normal level of glycemia:

- insulin, having an hypoglycemiant effect;
- glucagon, adrenalin, glucocorticoids, thyroxin, growing hormone, having an hyperglycemiant effect.

Function of the food intake, glycemia regulation implies two steps:

- Immediately after feeding: glycemia is increased and its decreasing to the normal values is realized by:
    - utilization of glucose as an energetic substrate;
    - glycogenogenesis;
    - lipidogenesis (synthesis of triacylglycerols).
- Late after the feeding (hungry state): there is a tendency of glycemia decrease, with the activation of mechanisms for maintaining glycemia to normal values. This is realized by:
    - activation of hepatic and muscular glycogenolysis and gluconeogenesis; as a difference of muscle from liver, muscle has no glucose-6-phosphate phosphatase, so it cannot liberate glucose in the blood, using it for its own needs;
    - producing of alternative energy by lipolysis with ketogenesis as a consequence.

The most frequent disequilibrium which appears in the carbohydrates metabolism is **diabetes mellitus** (relative or absolute deficit of insulin). This is characterized by:

- hyperglycemia (insufficient metabolism of glucose) and glucosuria (when glycemia passes the renal threshold of 180 mg%)
- intensification of gluconeogenesis, lipolysis and proteolysis in the insulin-dependent tissues;
- ketoacidosis (lipolysis generates excess of acetyl-CoA which is oriented to the synthesis of ketone bodies.

Methods in the clinical chemistry laboratory for investigating glucose metabolism are:

- *the fasting and two-hour post prandial blood glucose,*
- *the glucose tolerance* and, most recently,
- *the glycated serum proteins and hemoglobin.*

Urine glucose is a method used by some diabetics although home testing is increasingly being done on blood obtained from finger sticks.

Glycated hemoglobin determination (HbA_1c)

HbA_1c is a product of a slow glycosylation process between glucose (which penetrates in erythrocytes easily) and valine from N-terminal position of hemoglobin β chain. The intensity of glycosylation is direct proportional with glucose concentration in blood. The erythrocytes are exposed to plasma glucose all their lifetime (approximately 120 days), so HbA_{1c} reflects glucose medium levels in the last 6-8 weeks and is important in long monitoring of diabetic patients.

HbA_{1c} represents normally 4-6% from the total amount of Hb (values are different varying with the used method) but it increases to 15-20% in diabetes mellitus cases. HbA_{1c} determinations use different methods: ion exchange chromatography, HPLC, affinity chromatography, electrophoresis, photometric method, immunological methods.

Principle

Whole blood is mixed with a lysing reagent containing a detergent and a high concentration of borate anions. Thus an elimination of the labile Schiff's base is being achieved. The following mixture of the hemolysed preparation of whole blood with a weakly cation –exchange resin results in the binding of unglycosylated hemoglobin (HbA_0) to the resin. By means of a separator the resin is being separated from the buffer solution containing unbounded HbA_{1c}. The percentage of HbA_{1c} glycohemoglobin is determined by measuring the absorbance of the HbA_{1c} fraction and the total hemoglobin at 415 nm. The ratio of the absorbances is being compared to that of a glycohemoglobin – standard, prepared as the blood specimen.

Procedure

A. Glycohemoglobin separation
   - Leave the column with the white cap down on the table for 2 minutes (to homogenize the resin)
   - Take off the white cap
   - Gently push the filter until it touches the resin
   - Tear the tip of the column
   - Into a big test-tube collect the liquid from the column
   - Add 50 µl hemolysate
   - Let it sink into the resin
   - Add 200 µl R2
   - After the liquid enters completely into the column, add 2000 µl of R2 and collect the in the same test-tube (20 min) → THROW AWAY THE LIQUID
   - Use a new test tube!!!
   - Add 2000 µl R3 and collect the liquid in a clean test tube (it contains HbA1c)
   - The supernatant has to be poured into a cuvette. Read the absorbance at 415 nm against water (A1)

B. Total hemoglobin
   - Pipette 50 µl of the hemolysate
   - Dispense 3 ml distilled water

   Read the absorbance at 415 nm against water (At)

Calculation

$$\%HbA_{1c} = (A_1/A_t) \times \tfrac{2}{3} \times 100$$

Normal values

Non diabetics: 4-6 % HbA_{1c}
Diabetic level: 6-8 % HbA_{1c}