UNIT: Carbohydrates (Glucose)

Task

Determination of glucose

Objectives

After completion of this unit, the student will be able to:

1. Explain the various methods for determining glucose concentration.
2. Discuss the significance of abnormal glucose concentration.
3. Determine glucose concentration.

Introduction

All body tissues can utilize glucose, the principle and almost exclusive carbohydrate circulating in blood. Under fasting conditions only a few tissues depend entirely upon glucose as a source of energy. These are the brain (by far the most important glucose consumer) followed to a much lesser extent by red blood cells, platelets, leukocytes and the kidney medulla. Other tissues readily oxidize fatty acids and ketone for energy purposes. Plasma glucose levels are maintained during fasting by mobilization of liver and muscle glycogen. The liver is the main organ for the storage of excess carbohydrate as glycogen. Skeletal and heart muscles can also store minor amounts of muscle glycogen. The liver is also able to synthesize glucose (gluconeogenesis) from certain amino acids, therefore providing glucose to the tissues that are completely dependent upon glucose for viability.

In times of glucose excess (elevated blood glucose, as after a meal), glucose is enzymatically polymerized in the liver to form glycogen (glycogenesis) for storage. When the blood glucose begins to drop, the glycogen is converted to glucose (glycogenolysis) by a different set of enzymes. Thus, independent mechanisms exist for regulating the blood glucose level by means of glycogenesis-glycogenolysis reactions. When the glycogen storage capacity is saturated, glucose is converted to fat by adipose cells where it is stored.

Hormones play an important role in the regulation of the plasma glucose concentration. Insulin, the hormone produced by pancreatic Beta cells, is the only one that lowers the concentration of plasma glucose. The rate of insulin secretion into the bloodstream is governed by the blood glucose concentration.

Hormones secreted by the anterior pituitary (growth hormone), adrenal medulla (epinephrine), adrenal cortex (cortisol), Alpha cells of the pancreas (glucagon), and thyroid (thyroxine) increase the plasma glucose concentration. The net result of the multiplicity of hormonal effects, nervous system control, and feedback mechanisms is a very finely regulated system that keeps the plasma glucose concentration quite constant.

Although normal values vary with specific procedure, fasting serum/plasma glucose is usually in the range of 80-120 mg/dL. CSF values are approximately 2/3 of the current serum/plasma level.

The various disorders in carbohydrate metabolism may be grouped into several categories dependent primarily upon laboratory findings: those associated with a raised plasma glucose
concentration (hyperglycemia); a decreased plasma glucose concentration (hypoglycemia); and reducing sugars in the urine.

Hyperglycemia is demonstratively harmful to the body when it is so high that the increased extracellular osmotic pressure causes cellular dehydration. Coma can be produced by severe dehydration of brain cells. Other associated factors, such as acidosis and dehydration, may cause cellular damage and produce illness. A good example is uncontrolled diabetes mellitus. The patient may be found in a coma, with a blood glucose concentration exceeding 600 mg/dL. The accompanying acidosis, ketosis, dehydration, and electrolyte imbalance must be corrected as a lifesaving measure.

Any condition in which the plasma glucose concentration falls below the lower limits of normal by greater than 2 standard deviations (below 60 mg/dL) is called hypoglycemia. Since the brain is dependent upon an adequate supply of glucose for its energy, the clinical symptoms of hypoglycemia resemble those of cerebral anoxia, which may include one or more of the following: faintness, weakness, dizziness, tremors, anxiety, hunger, palpitation of the heart, or "cold sweat"; there may even be mental confusion and motor in-coordination. Consciousness is usually lost in an adult when the plasma glucose concentration falls below 40 mg/dL, but the rapidity of fall is also a factor. Widespread convulsions may accompany the coma or even precede it. Newborn infants are less sensitive to a decreased concentration of plasma glucose and may not go into convulsions until the plasma glucose concentration falls below 25 or 30 mg/dL.

The two-hour postprandial and glucose tolerance test (GTT) are two methods of evaluating a suspected diabetic's utilization of glucose. Both tests require a fasting blood glucose. The two-hour postprandial test is generally considered a screening procedure. The patient is required to consume a large meal (usually breakfast) with a large carbohydrate content. After two hours the patient's glucose level is again evaluated. Normally, the glucose level has returned to the normal fasting range.

The glucose tolerance test is a more involved procedure. After the fasting blood glucose is obtained, the patient consumes 100 grams of liquid glucose. Blood is drawn on the patient at regular prescribed intervals over the next 3 to 5 hours. The pattern of glucose test results are compared to patterns of known carbohydrate disorders.

Methods of Determination

Methods for the quantitative measurement of glucose in blood were introduced at the beginning of the century. The principle use of the test is for the diagnosis and management of diabetes, but it is absolutely essential for the detection and proper management of hypoglycemia, a condition encountered much less frequently.

Until 15 or 20 years ago, the majority of the quantitative tests for glucose determination depended upon the oxidation of glucose by hot, alkaline copper solutions or solutions of potassium ferricyanide. These were supplanted by the ortho-toluidine test and later by enzyme methods employing either glucose oxidase or hexokinase.

Clinical chemists turned to enzymatic methods in an attempt to obtain a “true” glucose determination because of the high specificity of an enzyme for a particular substrate. It was hoped by this approach that there would be fewer interfering substances when measuring the glucose concentration in serum.
Regardless which method of glucose determination is employed, precautions in the sample collection are required to prevent the utilization of glucose by leukocytes. The glucose loss, upon standing in a warm room, may be as high as 10 mg/dL per hour. The decrease in serum glucose concentration is negligible if the blood sample is kept cool and the serum separated from the clot within 0.5 hours of drawing. The addition of 2 mg sodium fluoride per mL blood to be collected will prevent glycolysis for 24 hours.

1. **SOMOGYI-NELSON METHOD** – A protein free filtrate is prepared using barium hydroxide and zinc sulfate. The filtrate will contain no reducing substances other than glucose (“true” glucose). The glucose reduces copper tartrate reagent, and the cuprous oxide obtained is treated with arsenomolybdate to produce a blue compound.

   This method has been abandoned because it requires too much handling and cannot be automated easily.

2. **FOLIN-WU METHOD** – Protein is removed from the sample by precipitation with tungstic acid and centrifugation or filtration. Sugar present in the filtrate reduces alkaline copper tartrate. The cuprous oxide produced in this reaction is then treated with phosphomolybdic acid to produce a blue compound. The color intensity is directly proportional to the glucose concentration.

3. **ORTHO-TOLUIDINE METHOD** – In this method, glucose reacts with o-toluidine in glacial acetic acid and heat to produce a blue-green N-glucosylamine. The absorbance of this product is measured photometrically, and glucose can be calculated.

   The method is simple and rapid. There are few interfering substances, although the reagent reacts with other aldoses (sugars with an aldehyde end group). Galactose and mannose (aldohexoses) and the aldopentoses are usually in such low concentration in plasma that they cause no problems.

4. **GLUCOSE-OXIDASE METHOD** – In this reaction, glucose oxidase reacts with glucose to form gluconic acid and hydrogen peroxide. The hydrogen peroxide is split by peroxidase and reacts with o-dianisidine. This oxidized o-dianisidine combine with sulfuric acid to form a red-orange chromogen. Results are read on the spectrophotometer.

5. **ASTRA** – The Glucose Chemistry Module determines glucose by means of the oxygen rate method employing Beckman Oxygen Electrode. Solid-state circuits in the ASTRA System determine the rate of oxygen consumption, which is directly proportional to the concentration of glucose in the sample.

6. **ACA** – Hexokinase (HK) catalyzes the phosphorylation of glucose by adenosine-5-triphosphate (ATP) to glucose-6-phosphate, which is oxidized to 6-phosphogluconalactone by glucose-6-phosphate dehydrogenase (G-6-PDH) with simultaneous reduction of nicotinamide-adenine dinucleotide phosphate (NADP). One mole of NADP is reduced to one mole of NADPH for each mole of glucose present. The absorbance due to NADPH (and thus the glucose concentration) is determined using a two-filter (340-383 nm) end point technique.

   \[
   \text{Glucose + ATP} \xrightarrow{\text{HK, Mg}^{2+}} \text{Glucose-6-phosphate + ADP}
   \]
Glucose-6-phosphate + NADP$^+$ $\xrightarrow{G-6-PDH}$ 6-phosphogluconolactone + NADPH(+H$^+$)

**Glycated Hemoglobin (HgbA$\text{1c}$)**

Diabetes mellitus, as previously stated is a condition of hyperglycemia. It is estimated this condition affects 2.5-5% of the population and is considered to be the fifth leading cause of death in the U.S. (May 1982; Report of the National Diabetes Advisory Board). The disease is associated with a number of serious micro and macro-vascular complications involving the eyes, kidneys, heart and blood vessels, and may greatly impair the quality of life or shorten the life-span of the person afflicted.

The cause and prevention of these complications continue to be discussed with particular controversy concerning the relationship between control of the glucose concentration fluctuation and the progression of the disease complications. A major reason for the continuing debate has been a lack of methods to quantify accurately and objectively the degree of altered blood glucose control over a long period of time. Traditional methods of patient history, physical exam, urine and blood glucose have limited value for monitoring individual average blood glucose status over time. In addition, the aim of therapy in diabetes is to maintain a constant normal (or near normal) level of glucose in the blood. As blood glucose levels rise, the increase in non-enzymatic glycosylation of proteins is proportional to both the level of glucose and the lifespan of the protein being affected. With consideration of RBC's lifespan, glycosylated hemoglobin has been accepted as a measurement which reflects, better than FBS determinations, the mean daily blood glucose concentration and the degree of carbohydrate imbalance over the preceding two months. (Bio-Rad Hemoglobin A$\text{1c}$ Test Instruction Manual).

Today, hemoglobin A$\text{1c}$ testing is performed to monitor diabetics suspected of having erratic control over their glucose level.

**Sigma Diagnostics Glucose (trinder)**

**Overview**

In 1956, Keston proposed the use of glucose oxidase and peroxidase coupled with a chromogenic oxygen acceptor such as o-dianisidine or o-toluidine, for the colorimetric determination of glucose in biological fluids. Keston's original procedure has undergone several modifications including one by Trinder. The Sigma method is based upon the Trinder reaction and is a modification of his original procedure.

Principle, reagent analysis, discrete analyzer applications, etc. information is available in the product insert.

*Required materials not provided.*

1. Spectrophotometer & cuvets with properties suitable for use at 505 nm.
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2. Timer
3. Glucose standard(s) and control(s)

Procedure (for room temperature assay)

1. Prepare glucose reagent (Trinder) according to instructions. Reconstituted reagent should be stored in an amber bottle to protect from strong light and is stable for 3 months when refrigerated. Bring reagent to room temperature.

2. Set the spectrophotometer wavelength at 450 nm, and absorbance reading to zero with water as reference.

3. Set up a series of labeled test tubes for reagent blank, standard(s), control(s), and patient(s).

4. Pipet 0.5 mLs of deionized water (DI) into each tube.

5. Pipet 25 microliters of sample into appropriately labeled tubes. (25 microliters of DI water in Blank’s tube.) Mix gently.

6. Add 5.0 mL of Trinder glucose reagent to each tube. Incubate for exactly 45 minutes at ambient / RT temperature (or 30 minutes at 37°C).

7. Read and record absorbance of all tubes at the 450 nm wavelength. Read tubes within 30 minutes.

8. Subtract absorbance of reagent blank from absorbances of standard, control, and patient samples to obtain change in absorbance due to glucose.

9. Determine concentration of unknown’s by calculation or by interpolation using a standard curve.
**Glucose Worksheet**

Spectrophotometer Used: __________

Wavelength: __________

<table>
<thead>
<tr>
<th>Identification</th>
<th>Absorbance</th>
<th>$\text{Abs}<em>{\text{test}} - \text{Abs}</em>{\text{blank}}$</th>
<th>Concentration (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Calculation formula(s) and examples**

<table>
<thead>
<tr>
<th>Quality Control</th>
<th>Your Results</th>
<th>Controls’ range of expected results.</th>
<th>In control? Yes / No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1 ID</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level 2 ID</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Accepting Patient Results? Reason: __________________________
Study Questions

Instructions: Legibly write your answers in the space provided. Unless otherwise indicated, each question is worth one point. Using lecture notes, reading assignments and information presented in this lab, answer the following questions.

1. What disease condition is commonly associated with persistent hyperglycemia?

2. What hormone is decreased in patients with persistent hyperglycemia?

3. List four (4) hormones that can cause an increase in blood glucose. (2 points total)

4. The glucose value of a normal two-hour postprandial glucose is
   A. grossly elevated
   B. of little value
   C. affected by lipemia
   D. much below normal
   E. within normal limits

5. What part of the body will suffer first due to low glucose concentration? Why?

6. List two (2) ways plasma glucose levels are maintained during fasting. (2 points)

7. What is the effect of insulin on blood glucose levels?

8. How is insulin secretion into the bloodstream normally regulated?

9. What special treatment should be given to a tube of blood for glucose determination? Why? (2 points)

10. Why are enzymatic methods for glucose determination generally regarded as the best for obtaining a "true" result?
11. The physiological function of glucagon is
   A. inhibit glycogenesis
   B. decrease blood glucose
   C. increase glycogen
   D. decrease glycogen
   E. increase blood glucose

12. What test procedure/methodology provides the physician the best indication that his/her diabetic patient has remained under control?

13. In your own words, briefly explain how a hemoglobin measurement evaluates glucose levels.

**Case Study: Diabetes mellitus**

A 14 year old girl who was a known diabetic developed food poisoning and neglected to take her insulin. She suffered from vomiting and diarrhea for several hours before notifying her family. Her parents rushed her to the hospital where after laboratory tests were performed, a diagnosis of diabetic ketoacidosis was made.

<table>
<thead>
<tr>
<th>substance</th>
<th>result</th>
<th>Normal? - yes / no</th>
<th>If no, reason for abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>glucose</td>
<td>514 mg/dL</td>
<td>(5140 mg/L)</td>
<td></td>
</tr>
<tr>
<td>sodium</td>
<td>123 mEq/L</td>
<td>(123 mmol/L)</td>
<td></td>
</tr>
<tr>
<td>potassium</td>
<td>5.7 mEq/L</td>
<td>(5.7 mmol/L)</td>
<td></td>
</tr>
<tr>
<td>chloride</td>
<td>92 mEq/L</td>
<td>(92 mmol/L)</td>
<td></td>
</tr>
<tr>
<td>total CO₂</td>
<td>9 mmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>urine glucose</td>
<td>positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>urine ketones.</td>
<td>positive</td>
<td></td>
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</tr>
</tbody>
</table>

1. Explain what has caused this condition.

2. Complete the above table.

3. If the following tests were performed, what general results would you expect?
   
   A. serum osmolality

   B. arterial blood gases