Biochemical Examination of Diabetes Mellitus

General Medicine

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1 Glucose homeostasis

Blood glucose is normally maintained within narrow limits. Typical fasting values are 3.9 – 5.5 mmol/l and postprandial values are below 9.99 mmol/l in whole blood. The nervous system is totally dependent on glucose for energy, therefore it is critical to maintain a steady supply to this tissue.

Blood glucose is regulated by insulin, which is the major hypoglycemic hormone, while other hormones such as glucagon, cortisol, epinephrine and growth hormone antagonize the effect of insulin. The liver also plays an important role in blood glucose homeostasis. The two types of carbohydrates that contribute to human nutrition − starch and various disaccharides − are all absorbed as glucose, galactose or fructose after digestion. Next, they are transported by portal vein to the liver where galactose and fructose can be converted to glucose. Some of the glucose can be utilized for energy by the liver and other cells. The liver stores excess glucose as glycogen after feeding, while in the fasting state it maintains blood glucose through glycogenolysis and gluconeogenesis from amino acids, lactate and glycerol (Fig. 1).

**Delivery of glucose**

**Uptake of glucose**

Fig. 1: Glucose delivery to blood plasma and its uptake by tissues (according to Bartoš V., Pelikánová T. a kolektiv: Praktická diabetologie. Maxdorf, Jesenius, Praha, 1996).
### 1.1 Changes of blood glucose concentration

- Blood glucose value < 2.8 mmol/l defines **hypoglycemia**, which threatens delivery of glucose into CNS. It can occur e.g. due to tumors of pancreas producing insulin (insulinoma), overdoses of insulin or antidiabetic drugs, some congenital defects of metabolism, and also during starvation. Severe hypoglycemia causes seizures and coma.

- Blood glucose above the reference value constitutes **hyperglycemia**. It is a classical laboratory sign of diabetes mellitus. Transient hyperglycemia, however, can be caused by a number of physiologic and pathologic states (myocardial infarction, surgery, stroke, and trauma); usually does not last longer than one week.

### 2 Diabetes mellitus

Diabetes mellitus is a chronic condition with high morbidity and mortality, and with prevalence markedly increasing during the last decade. Currently, there are more than 800,000 diabetics in Czech Republic. Diabetes mellitus represents a heterogenic group of chronic metabolic diseases, whose basic symptom is **hyperglycemia**. It results from **lack of insulin, or insufficient effect of insulin, or combination of both**.

Due to lack of insulin the transport of glucose from blood to the cell across the cell membranes is impaired, resulting in **hyperglycemia** and lack of glucose inside the cells. Gluconeogenesis and glycogenolysis are stimulated. Next, lipolytic cleavage of triacylglycerols to fatty acids and glycerol in adipocytes increases. Breakdown of fatty acids by \(\beta\)-oxidation yields excess of acetyl-CoA, which in the liver gives rise to ketone bodies – acetoacetic acid, 3-hydroxybutyric acid, and acetone. Acetoacetic and 3-hydroxybutyric acids can serve as energy source for the muscle and brain instead of glucose. If, however, production of ketone bodies exceeds their utilization by peripheral tissues, **ketoacidosis** results. Since ketone bodies are soluble in water and can be excreted into urine, ketoacidosis is accompanied by **ketonuria**. Surplus of glucose also passes to urine, leading to **glycosuria**. Urinary excretion of glucose, due to its osmotic activity, requires also higher excretion of water, which forms basis for **polyuria** (Fig. 2).

These metabolic alterations explain the characteristic symptoms of insulin-dependent DM, such as thirst and polyuria; anorexia and weight loss is also found.

Acute and especially chronic hyperglycemia is associated with impaired function of many organs, in particular the kidneys, eyes, vascular and nervous systems.

#### 2.1 Classification of diabetes mellitus

**Diabetes mellitus:**
- Type 1 diabetes mellitus (formerly insulin-dependent diabetes mellitus – IDDM)
- Type 2 diabetes mellitus (formerly non-insulin-dependent diabetes mellitus – NIDDM)
- Other specific types of diabetes mellitus (e.g. genetic defect of \(\beta\)-cells, genetic defects of insulin action, diseases of exocrine pancreas)
- Gestational diabetes mellitus

**Borderline disorders of glycoregulation:**
- Impaired fasting glucose (IFG)
- Impaired glucose tolerance (IGT)
Type 1 diabetes mellitus

Type 1 diabetes mellitus is characterized by an absolute lack of insulin. Secretion of endogenous insulin is almost or completely absent, and the patients are vitally dependent on injections of exogenous insulin. The patients are prone to ketoacidosis.

The disease is caused by an autoimmune-induced selective destruction of insulin-producing β-cells in the pancreatic Langerhans islets in genetically predisposed individuals. Viral infections or contact with other exogenous or endogenous agents may trigger the autoimmune reaction.

Type 1 DM is a less common form of diabetes, occurring in about 7% of the patients. The classical signs of Type 1 DM are thirst, polyuria, polydipsia and weight loss.

The clinical picture of Type 1 diabetes depends on aggressiveness of the autoimmune process. In childhood or adolescence when majority of cases are diagnosed, the destruction of β-cells is usually very rapid and the classical acute signs of diabetes (including the
ketoacidosis) develop. In a more advanced age the onset of disease is often fairly slow and it takes more time until the patient reaches the complete dependence on exogenous insulin. The secretion of insulin may be diminished for years but still sufficient to prevent ketoacidosis. Therefore, the course of the disease rather resembles Type 2 diabetes; according to some recent reports about every seventh patient originally classified as Type 2 diabetic in fact suffers from slowly progressing Type 1 diabetes – latent autoimmune diabetes of adults, LADA (Table 1).

Type 2 diabetes mellitus

Type 2 is the prevailing form of diabetes mellitus. The patients are not vitally dependent on exogenous insulin because production of their own insulin usually is not decreased. The cause of this disease lies in disorder of action of insulin. Decreased cellular effects of insulin on the target organs are referred to as insulin resistance, due to a disorder at the level of insulin receptor or transduction of signal from the receptor inside the target cell. Initially, due to insulin resistance the absolute values of insulin are higher than normal. Later, however, a disorder of insulin secretion also appears as the β-cells gradually lose their ability to respond to hyperglycemia with increased insulin production. Onset of Type 2 diabetes mellitus is usually in adulthood, typically in age above 40. Unlike the Type 1 the patients are not prone to ketoacidosis. 60 to 90 % of the patients are obese (Table 1).

Tab. 1: Characteristic features of Type 1 and Type 2 DM:

<table>
<thead>
<tr>
<th></th>
<th>Type 1 DM</th>
<th>Type 2 DM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LADA</td>
<td></td>
</tr>
<tr>
<td>insulin secretion</td>
<td>absent</td>
<td>insulin resistance, disorder of insulin secretion</td>
</tr>
<tr>
<td>typical onset in</td>
<td></td>
<td></td>
</tr>
<tr>
<td>childhood and</td>
<td></td>
<td></td>
</tr>
<tr>
<td>adolescence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ketoacidosis</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>often low BMI¹</td>
<td>–</td>
<td>often high BMI¹</td>
</tr>
<tr>
<td>autoantibodies</td>
<td>positive²</td>
<td>autoantibodies positive²</td>
</tr>
<tr>
<td>C-peptide² negative</td>
<td></td>
<td>autoantibodies absent²</td>
</tr>
<tr>
<td>immunoreactive insulin² negative</td>
<td>decreased</td>
<td>immunoreactive insulin² increased</td>
</tr>
</tbody>
</table>

Gestational diabetes mellitus

It is defined as a disorder of glucose homeostasis that originates during pregnancy. In some women a true diabetes mellitus may develop later after delivery.

¹ BMI – body-mass index
² See part 3.8. Other laboratory examinations of diabetic patients.
Impaired glucose regulation

Impaired glucose regulation refers to a metabolic state intermediate between normal glucose homeostasis and diabetes. It includes impaired fasting glycemia (IFG) and impaired glucose tolerance (IGT). IFG refers to fasting glucose concentrations that are lower than those required to diagnose diabetes mellitus but higher than the non-diabetic (“normal”) reference value. IGT is an asymptomatic state diagnosed on the basis of the response of blood glucose to the glucose load (OGTT, see below).

3 Basic Biochemical Examinations in Diabetic Patients

3.1 Blood glucose

Estimation of blood glucose concentration (glycemia) is a laboratory examination providing basic information on patient’s saccharide metabolism. Either capillary or venous blood is taken, and glucose level is estimated in the whole blood, plasma or serum. Glycemia values in the whole blood are 10-15 % lower than in plasma/serum (depending on hematocrit); arterial blood gives 10 % higher glycemia values than venous blood (arterio-venous difference). In order to stop glycolysis (consumption of glucose by red blood cells) sodium fluoride, NaF, is added to the sample vessels (2.5 mg per 1 ml of the whole blood).

Examination of blood glucose concentration is fully informative only if time interval between taking the blood sample and last food intake is known.

Examination of glycemia is performed:

- **as fasting glycemia** (blood taken at least 8 hours after the last meal) – screening test for finding diabetics in population, for confirmation of diagnosis of DM, or for monitoring of therapy of DM;
- **as random glycemia** (blood taken regardless of food intake) – in cases of suspect hypoglycemia or hyperglycemia;
- **after meal – postprandial glycemia** (1 hour after meal containing saccharides) – for monitoring of therapy of DM;
- **as glycemic profile** - glycemia is estimated several times per day, typically between main meals, sometimes also after meals or during night.

3.2 Principles of glycemia estimation

3.2.1 In the laboratory

Measurement of glucose concentration can be based on different principles; but enzyme methods are the ones most widespread. In general, any enzyme that metabolizes glucose can be employed for glucose estimation. The recommended routine technique utilizes coupled enzyme reactions of glucose oxidase (GOD) and peroxidase (POD) (Fig. 3a).

In the first reaction the enzyme glucose oxidase catalyzes glucose oxidation with air oxygen producing gluconic acid in the form of its inner ester - gluconolactone. The glucose solutions normally consist of 36 % of α-anomer and 64 % of β-anomer. GOD is highly specific for β-
Diabetes mellitus

D-glucopyranose. Therefore, in order to achieve oxidation of both anomers, conversion of \( \alpha \) to \( \beta \)-anomer is necessary, which, however, occurs spontaneously during sufficiently long incubation. The other product of the glucose oxidase reaction is hydrogen peroxide in amount equivalent to that of glucose.

In the next reaction catalyzed by peroxidase the hydrogen peroxide reacts with a suitable chromogen, e.g., derivative of phenol that is oxidized to a reactive intermediate, which, in turn, reacts with another compound, such as 4-aminoantipyrine, yielding a stable soluble dye (Fig. 3a), whose absorbance is measured.

Alternatively, it is possible to measure decay of oxygen consumed in the glucose oxidase reaction, by means of electrochemical techniques (oxygen electrode or an enzyme electrode).

The hexokinase method (Fig. 3b) is also a highly specific one. Glucose is first phosphorylated with ATP to glucose 6-phosphate, which is then oxidized with NADP\(^+\) to 6-phosphogluconolactone in the reaction catalyzed by \textit{glucose-6-phosphate dehydrogenase}. Reduction of NADP\(^+\) to NADPH can be followed directly as an increase of absorbance in the UV region (the Warburg optical test).

**Fig. 3: Principles of methods for estimation of glucose**

**a) Glucose oxidase method:**

\[
\text{\( \beta \)-D-glucopyranose} + \text{O}_2 \xrightarrow{\text{glucose oxidase}} \text{D-glucose-1,5-lactone} + \text{H}_2\text{O}_2
\]

\[
\text{4-aminoantipyrine} + \text{phenol} \xrightarrow{2\text{H}_2\text{O}_2, \text{peroxidase}} \text{quinonimine (coloured product)}
\]

**b) Hexokinase method:**

1. \[
\text{glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{glucose 6-phosphate} + \text{ATP}
\]

2. \[
\text{glucose 6-phosphate} + \text{NADP}^+ \xrightarrow{\text{glucose-6-phosphate dehydrogenase}} \text{6-phosphogluconate} + \text{NADPH} + \text{H}^+
\]
3.2.2 Outside laboratory

Glycemia is one of the parameters often examined even without laboratory. A rapid approximate estimation of glycemia is common in the emergency care. Also, in insulin-treated patients it is advantageous to monitor glycemia regularly by means of a personal glucometer and adjust the treatment accordingly. The concentration of glucose in blood is one of the most common parameters estimated in place where the care is provided to the patient (point of care testing, POCT). However, it should be stressed that although the POCT methods improve the patient’s quality of life and comfort, they cannot replace the regular medical and laboratory examinations.

The techniques of rapid glycemia estimation utilize several principles. The starting material is usually a droplet of full capillary blood that is applied on a test strip. The oldest, although still used test strips are based on the same principles as the photometric glucose measurement. The capillary blood passes through several layers of various materials, resulting in capture of the blood cells while only the plasma penetrates to the reaction zone, which contains glucose oxidase, peroxidase and suitable chromogen. The color intensity is proportional to the glucose concentration. Evaluation can be done either by simple comparison with a color scale, or by means of a glucometer, single-purpose reflectance photometer. The visual evaluation has the advantage of being independent on any instrumental accessories. Estimation of glycemia in this way is truly only approximate, but still fully sufficient especially in the field emergency medical care.

The glucometers based on the reflectance photometry are currently largely replaced with more reliable electrochemical analyzers. Majority of present glucometers detect the product of an enzyme-catalyzed oxidation of glucose by means of an enzyme electrode (detailed principle of its functioning is beyond the scope of this text).

3.3 Oral glucose tolerance test (OGTT)

OGTT evaluates glucose clearance from the circulation after glucose loading under defined and controlled condition. The OGTT simulates oral food intake. Enteric factors such as gastric emptying, intestinal absorption and liver function may influence the results of the test; therefore OGTT shows a significant range of variation.

The OGTT is useful especially for an early diagnosis of gestational diabetes. In this case it is employed as a routine screening examination, performed in all pregnant women at the turn of first/second trimester (if there is a high risk it is also done as soon as possible following the detection of pregnancy). In other persons the OGTT is recommended as an auxiliary diagnostic test for diagnosis of DM in cases the fasting and random glycemia are inconclusive (especially if the fasting glycemia is between 5.6 – 6.9 mmol/l). If the values of fasting or random glycemia conclusively prove the diagnosis of DM, the OGTT would be a redundant burden for the patient and is therefore contraindicated. Likewise, the test is avoided in acutely ill and immobilized patients as well as in patients on a weight reduction diet.

Requirements that patients should meet:
• The patient should be on an unrestricted diet containing at least 150 g of carbohydrate/day for at least three days prior to the test.
• The patient should continue his/her usual physical activities prior to the test.
• The patient must stays sitting and refrain from smoking during the test.
Test procedure:
- The first blood specimen is collected after 8 – 14 hours of fasting.
- A standard dose of glucose (75 g dissolved in 250 – 300 ml of tea or water) is drunk within 5 – 10 minutes.
- The blood specimen are collected one (second specimen) and two hours (third specimen) after glucose loading.

This examination in healthy subjects (Fig. 4) shows a peak at 30 minutes and return to fasting levels at 120 minutes. Diabetic patients peak late (approximately 60 minutes) and/or show a plateau at 120 minutes to 180 minutes and return to baseline value after 180 minutes.

Fig. 4: Results of OGTT in healthy and diabetic subject:

If the glycemic curve is to be examined in detail, three phases can be distinguished. First, the glucose administered orally is absorbed from the intestine and glycemia rises – the ascending part. It is remarkably steep in patients after gastrectomy; in contrast, the curve is flat in cases of malabsorption.

The next, top part of the curve reflects function of the liver and the effects of insulin in the liver. In incipient DM the transformation of glucose to glycogen in the liver is impaired, that is why the glycemic curve peaks later (at 60 minutes or even later) and exceeds the value 11.1 mmol/l. In liver disease the hepatocytes fail to metabolize incoming glucose and more glucose enters the periphery. The peak again exceeds 11.1 mmol/l and the elevation lasts over 60 minutes, but unlike DM the curve returns to basal levels at 120 minutes (the bell-shaped type of curve). In hyperthyreosis the peak value of 11.1 mmol/l is also exceeded due to fast absorption of glucose from the intestine, but the subsequent return to basal levels is equally fast (the gothic type of glycemic curve).

The descending part of the curve depends on action of insulin, and a retarded and insufficient return to basal levels is the classical feature of diabetes mellitus.

In general, however, the OGTT suffers from random variations and rather poor reproducibility.
3.4 Evaluation of glycemia and OGTT

Fasting glycemia $< 5.6 \text{ mmol/l}$, and in OGTT glycemia in 120 minutes $< 7.8 \text{ mmol/l}$ are considered normal.

In contrast, fasting glycemia $\geq 7.0 \text{ mmol/l}$, or glycemia $\geq 11.1 \text{ mmol/l}$ in random sample or in 120 minutes of OGTT together with classical clinical signs (thirst, polyuria, unexplained weight loss) point to diagnosis of diabetes mellitus. For confirmation of diagnosis of DM the examination must be repeated (Fig. 5, Table 2)

Fig. 5 Evaluation of glycemia (venous plasma) in mmol/l in laboratory screening of DM

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3 According to “Laboratorní diagnostika a sledování stavu diabetu mellitu” on website of Czech Society for Clinical Biochemistry (http://www.cska.cz/Doporuceni/DM.htm)
**Tab. 2**  
*a) Evaluation of glycemia measured in venous plasma:*

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>Fasting glucose concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt; 5.6 mmol/l</td>
</tr>
<tr>
<td>Impaired fasting glycemia</td>
<td>≥ 5.6 and &lt; 7 mmol/l</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>≥ 7.0 mmol/l</td>
</tr>
</tbody>
</table>

**b) Threshold values for OGTT in venous plasma:**

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>Glycemia in 120 minutes of OGTT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt; 7.8 mmol/l</td>
</tr>
<tr>
<td>Impaired glucose tolerance</td>
<td>≥ 7.8 and &lt; 11.1 mmol/l</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>≥ 11.1 mmol/l</td>
</tr>
</tbody>
</table>

### 3.5 Glycated proteins

Glucose and other monosaccharides react spontaneously with protein to form glycated proteins. Such reactions take place in healthy individuals as well, and all soluble serum proteins as well as structural proteins can be involved. The extent of glycation depends on the average glucose level to which the protein is exposed and on the half-life of the protein.

This process of **non-enzymatic glycation** of protein molecules is referred to as the **Maillard reaction** (see Fig. 6):

- It is initiated when a free sugar aldehyde or ketone group condenses with a free amino group of proteins to form a **Schiff base (aldimine)**. This reaction is reversible and takes place over the course of hours. The transient hyperglycemias result in only minimal glycation.

- The Schiff base then slowly (within days) rearranges to form a fairly stable **ketoamine** called the **Amadori product (fructosamine)**. Concentration of fructosamine can be measured by reaction with nitroblue tetrazolium. Formation of the Amadori products is still to some extent reversible, although the equilibrium is strongly shifted to their production. The equilibrium is reached during several weeks. Concentration of ketoamines can decrease if glycemia is normalized.

- Finally, during weeks and months and independently on the glucose presence the Amadori products react with long-lived structural proteins (collagen, elastin, myelin of nerves etc.) and produce complex series of cross-linkages and adducts called the Maillard products or **advanced glycation end products (AGEs)**. These processes are **irreversible**.
Non-enzymatic glycation of proteins is considered as one of the causes of the late complications and organ damage in patients with diabetes mellitus.

Measurement of the Amadori products of protein glycation is useful in monitoring long-term glucose concentration and provides indirect information on the course of glycemia for the life time of the protein whose glycation is assessed. Routinely glycated hemoglobin and glycated serum proteins (fructosamine) are measured.

Estimation of glycated hemoglobin and glycated proteins is used in controls of diabetic patients and for detection of permanent hyperglycemia. Increased values of glycated derivatives indicate that elevated blood glucose levels prevailed during the past few weeks and that diabetes has not been well compensated.

Glycated hemoglobin (HbA\textsubscript{1c}) is considered as the best marker for monitoring of blood glucose in diabetic patients. Concentration of glycated hemoglobin informs about glycemia during the last 6-8 weeks. It is measured by chromatographic or immunochemical methods. It is assumed that in the near future the concentration of glycated hemoglobin will also become one of the main examinations for the screening and diagnostics of diabetes mellitus, especially Type 2.

**Evaluation of concentration of glycated hemoglobin (HbA\textsubscript{1c}):**

- Normal values: 20 – 42 mmol/mol
- Compensated DM: 43 – 53 mmol/mol
- Poor compensation of DM: > 53 mmol/mol

Glycated plasma proteins (fructosamines) have shorter half-life and their levels reflect average glycemia during the last 2-3 weeks. The major component is glycated albumin. Results of this estimation can be affected by hypoproteinemia. Nowadays, measurement of fructosamines is not routinely performed in diabetic patients.

**Evaluation of glycated proteins (S- glycated proteins ):**

- Normal values: 205 – 285 µmol/l
- Good compensation of DM: 285 – 320 µmol/l
- Satisfactory compensation of DM: 321 – 370 µmol/l
- Poor compensation of DM: > 370 µmol/l

Fig. 6: The non-enzymatic protein glycation (the Maillard reaction). According to Janebová a kol., Remedia 1999.
3.6 Glucose in urine

Glucose in the kidney is first filtered through glomerular membrane to primary urine, and then is reabsorbed in proximal tubules. The body aims at preserving glucose and protecting it from losses – normally the tubular reabsorption system is occupied from 1/3 of its capacity. However, if the blood glucose concentration exceeds 9 – 10 mmol/l, also referred to as the renal threshold, the capacity of tubular reabsorption is saturated, and glucose appears in the urine – glycosuria (glucosuria). Normally only very small amount of glucose – up to 0.72 mmol/ 24 hours – is excreted into urine.

Detection of glucose in urine suggests presence of diabetes mellitus; therefore, every case of glucosuria needs further examination. On the other hand, negative finding of glucose in urine does not rule out diabetes mellitus, because renal threshold varies somewhat from person to person. False positive results may be obtained in a subject with low threshold for glucose.

The finding of glycosuria is necessary to evaluate together with examination of fasting glycemia. Following cases can be distinguished:

- **Glycosuria in the presence of hyperglycemia**
  It is the typical finding in diabetes mellitus. During the course of the disease, however, the renal threshold for glucose can rise and glycosuria can even disappear. Therefore, glycosuria is unreliable as a marker upon which decisions on therapy of diabetic patients should be made. Transient ‘alimentary glycosuria’ can occur even in non-diabetic patients as a result of meal rich in saccharides or during OGTT.

- **Glycosuria in the presence of normoglycemia – renal glycosuria**
  It is a disorder of glucose reabsorption in the renal tubular cells. It may occur as an inherited disease or as an acquired disorder due to a toxic or inflammatory damage to the kidney, affecting the function of proximal tubuli.

Methods of examination of urinary glucose

The detection of urinary glucose is possible either by **non-specific chemical reactions or by test strip tests**.

The **non-specific reactions** are based on reducing properties of monosaccharides. The **Fehling’s** and the **Benedict’s** tests use cupric ions complexed to tartrate or citrate, respectively, in alkaline solution. In the presence of reducing substances the cupric ions are reduced to cuprous ions, forming green-yellow, yellow, or even brick-red cuprous precipitate. The **Nylander’s** test employs alkaline bismuth(III) nitrate (bismuth(III) nitrate-oxide, BiONO$_3$) instead of copper; the action of reducing agents produces black metallic bismuth. All these reactions demonstrate in urine not only presence of glucose, but also other reducing sugars and reducing agents in general (e.g. ascorbic acid). The examined urine must not contain proteins.

The **diagnostic strips glukoPHAN** are based on the specific enzymatic reaction with **glucose oxidase and peroxidase** (i.e., the same principle as the blood glucose determination). The test is specific for D-glucose, as the reagent pad does not react with other sugars such as fructose, galactose and lactose. Large doses of reducing substances, e.g. ascorbic acid exert an inhibitory effect leading to false low or even negative results. In these cases repeating the test at least 10 hours after the last ingestion of vitamin C is recommended. False positive results, in contrast, can be produced by contamination of the urine container.
by oxidizing disinfectants (H$_2$O$_2$, chloramine). The test for glucose must be performed with fresh urine, in order to prevent bacterial contamination; or the urine must be stored at 4 °C. Finding of glycosuria should always be followed by estimation of fasting glycemia.

### 3.7 Ketone bodies in urine

The term “ketone bodies” includes three chemicals: acetoacetic acid, β-hydroxybutyric acid, and acetone. When biochemical energy is not produced from the conversions of glucose, metabolism shifts to the oxidation of lipids. Ketone bodies are synthesized in the liver from acetyl CoA, derived from the oxidation of fatty acids. Some of the acetoacetate may be then reduced to β-hydroxybutyrate, while minor part of it undergoes a spontaneous decarboxylation to acetone and CO$_2$. All three products of excessive conversion of fatty acids are excreted in the urine. The relative proportion in which the three ketone bodies are present in urine is following: 60 – 70 % of β-hydroxybutyric acid, 30 – 35 % of acetoacetate, and about 2 % of acetone.

Estimation of urinary ketones is useful as a control of patients with Type 1 diabetes mellitus. Ketone bodies are absent from urine of appropriately treated diabetic patients. Their presence suggests metabolic ketoacidosis and is always accompanied by hyperglycemia.

A small amount of ketone bodies in urine can appear in the period of weight reduction, due to vomiting, excessive physical exercise or during starvation. These states, however, are never associated with hyperglycemia.

The test for ketone bodies in urine uses a reaction of acetoacetate and acetone with sodium nitroprusside in alkaline medium, producing a purple-colored complex (Fig. 7). This reaction is used by Legal’s and Lestrade’s tests as well as by the diagnostic strip test. Nitroprusside does not react with β-hydroxybutyric acid; therefore, a negative nitro-prusside test cannot rule out ketoacidosis completely.

**Fig. 7: Principle of the nitroprusside test for ketone bodies in urine**

\[
\text{Na}_2[\text{Fe(CN)}_5\text{NO}] + \text{CH}_3\text{CO-R} + \text{NaOH} \quad \downarrow \\
\text{Na}_2[\text{Fe(CN)}_5\text{N}=\text{CHCO-R}] + \text{H}_2\text{O} \\
\quad \text{OH} \\
\text{Colored complex} \\
R = -\text{CH}_2\text{COOH} \quad \text{acetoacetic acid} \\
-\text{CH}_3 \quad \text{acetone}
\]

### 3.8 Other laboratory examinations of diabetic patients

- **Insulin** (according to the employed techniques also called immunoreactive insulin, IRI). Because of high circadian fluctuations, interference of autoantibodies and inability to distinguish endogenous insulin from the one given therapeutically, the immunoreactive insulin is usually measured during the glucose tolerance tests.
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(OGTT). Its values are low in Type 1 diabetics, but often increased in the syndrome of insulin resistance.

- **C-peptide** is a part of proinsulin molecule that is cleaved out prior to secretion of insulin. Concentration of C-peptide in serum corresponds to amount of secreted insulin. It is not degraded in the liver and because the exogenous insulin given in injections does not contain any C-peptide, its estimation is not affected by the therapy. The C-peptide passes the glomerular membrane, therefore its concentration rises in renal insufficiency.

- **Autoantibodies.** The Type 1 diabetes is often associated with positivity for antibodies against Langerhans islets (ICA), against insulin (IAA), and especially against one isoform of glutamate decarboxylase (GAD 65).