Introduction

Diabetes mellitus (DM) is a chronic disease that presents a serious socioeconomic problem in modern society due to its widespread prevalence and the risks of developing a number of complications. Therefore, it is necessary to improve existing methods and search for new ones for diagnosing and treating DM, which requires a detailed study of the mechanisms of the development of the disease, as well as the development of reliable and simple methods and criteria for detecting precursors of complications. The solution to these problems is made possible by combining numerous results and knowledge.

DM is a chronic endocrine disease caused by an increased content of free glucose in the body, resulting in a metabolic disorder in the organism. According to the World Health Organization, the move of DM from eighth to seventh place in the list of the most common causes of death worldwide is predicted by 2030 due to an increase in the death rate of the world's population from this disease by approximately 54% compared to 2010 [1]. At the same time, the number of patients with DM has increased since 1980 (108 million people) by 2014 by about 4 times (422 million) [2, 3].

Glucose is a monosaccharide that plays an important role in the functioning of the living body. Glucose is a source of energy for metabolic processes in the organism [4, 5]. The normal level of free glucose in blood (glycaemia) is 3.3–5.5 mmol/l (60–100 mg/dl) [4]. Beta cells in the pancreas produce the peptide hormone insulin to regulate glucose metabolism. The insufficient production of insulin by the pancreas or an abnormal reaction of the organism cells to the produced insulin leads to an excess content of glucose in the blood. Long-term elevated glucose content in blood (hyperglycemia) (above 11 mmol/l (200 mg/dl)) leads to excess glucose content in the interstitial fluid and then violates the metabolism in the organism, causing DM development [2, 6–13].

It was supposed that hyperglycemia could cause the increased oxidation of glucose and formation of superoxide radicals in mitochondria [14–16]. Anions of superoxide radicals are benign and nonreactive; however they turn into highly reactive free radicals under some conditions, mainly in the presence of reductive-oxidative transition metals, such as copper or iron [14, 17]. Such transformation occurs via metal-mediated Fenton or Haber-Weiss reactions (Equations (28.1) and (28.2) respectively) [14]:

\[
\text{Fe}^{2+} + \text{Cu}^+ + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{Cu}^{2+} + \text{HO}^+ + \text{HO}^- \tag{28.1}
\]

\[
\text{Fe/Cu}^+ + \text{H}_2\text{O}_2 \rightarrow \text{O}_2^- + \text{HO}^+ + \text{HO}^- \tag{28.2}
\]

Hyperglycemia-induced overproduction of superoxide activates hyperglycemic damage in endothelium cells of aorta. The damaging action of superoxide results in the redirection of glucose metabolism from the glycolytic path to alternative metabolic paths including the formation of the advanced glycation end products (AGEs).

Since the glucose level in blood is related to its level in the interstitial fluid, hyperglycemia leads to the metabolic disbalance and disorder of the organs functioning [13, 18–21]. Thus many papers are aimed at determining the concentration of...
Classification of DM

DM has two main types: type I is insulin-dependent diabetes (“immune-mediated diabetes,” “diabetes of the young”), while type II is insulin-independent diabetes (“diabetes of the aged”) [9, 10, 20, 71]. Gestational DM is separately considered, being diagnosed during pregnancy and not observed before it. There are also specific types of DM induced by other causes, for example, monogenic diabetes syndrome, when the mutant genes are inherited (diabetes of newborns); diabetes induced by the diseases of exocrine pancreas (such as cystic fibrosis); and diabetes caused by chemical agents (for example, when medicines are used in HIV/AIDS treatment or after transplantation of organs) [9, 21].

DM of types I and II is the most widespread. Insulin-dependent DM of type I develops due to destruction of the pancreas beta cells, which disturbs insulin production. A reduced amount of produced insulin means that it is insufficient to process the amount of glucose received by the organism. The glucose content increases in the organism, chronic hyperglycemia arises, and the metabolism is disturbed in many organs [2, 6–12, 20, 71, 78, 81–83]. Type I DM is more widespread among the young people, which is why it is also called the “diabetes of the young.” Type I of DM accounts for about 5%–10% of the total number of diabetes cases [2, 9]. Type I DM is characterized by such symptoms as polydipsia (thirst), polyuria (excess excretion of urine), loss of weight, permanent hunger, tiredness, and visual impairment [2, 9, 19, 20, 83].

Type II insulin-independent DM develops due to cell resistance to the produced insulin, and insulin deficiency also takes place in the organism. Type II is more widespread and accounts for about 90%–95% of all diabetes. In this case, the disease develops in middle and old age, and that is why this type of disease is called “the diabetes of aged”; however, this type of diabetes has been appearing more often in children in recent times. DM is largely caused by physical inactivity and overweight [2, 9, 10, 20, 78]. The symptoms of types I and II of DM are similar, but they are often less expressed in the second case, which is why the disease can be diagnosed a few years after it has started, when complications have already arisen [2]. An additional symptom of DM of type II is obesity [10]. It is important to determine the diabetes type in time for prescription of the most efficient therapy [9].

A diabetes-related hypoglycaemic coma (crisis) can happen under the excess injection of insulin or its excess production caused by medical preparations, when the glucose content in blood falls below 3.9 mmol/l (70 mg/dl). In this case, the body has not enough energy to support normal vital activity, leading to trembling, weakness, tachycardia, irritability, hunger, and loss of consciousness, and a fatal outcome is possible [9]. Genetic predisposition is the main cause of DM development [9, 20]; in other cases, diabetes is mainly caused by different diseases disturbing the metabolism in the body, as well as low physical activity [9].

The development of DM accelerates the processes occurring with organism aging [21, 77]. Water content in skin decreases with age, and, correspondingly, the ratio of fibrous structures and the base substance changes. The ratio decreases due to an increase in collagen content and decrease of glycosaminoglycans concentration. The content of hyaluronic acid greatly reduces. The chemical and physical properties of collagen change: the number and strength of intra and inter-molecular crosslinks increases, the swelling ability and elasticity reduces, the resistance to collagenase develops, and the structural stability of collagen fibers rises, i.e., the maturation of fibrillar structures of the connective tissue progresses. Collagen aging is caused by metabolic processes that occur in the organism and affect the molecular structure of collagen. When connective tissue lesions take place, all structural parts of the tissue are damaged: fibrils, cells, and intercellular substance [84].

The structure of collagen fibers in studies of the human sclera aging was investigated using wide-angle X-ray scattering. The increase of rigidity of the scleral matrix, the decrease in mechanical anisotropy and the degree of fiber straightening...
in the peripapillary sclera with age were observed [85]. The organism aging is accompanied by functional and structural modifications of macromolecules [86]. The changes occurring in organism tissues under the development of DM are investigated in laboratory animals using experimental models of diabetes [10–12, 19, 47, 48, 50, 53–55, 87–93] and directly in patients in the course of clinical examination [13, 43, 94–103].

### Experimental models of DM

The chemically induced models of diabetes are widely used due to their relatively cheapness and simple induction of diabetes development in rodents, and similar models can also be implemented in highly developed animals [10–12, 19, 47, 48, 50, 53, 87–93, 104]. The most widely used and well-known methods of chemical diabetes induction are implemented by two compounds: alloxan [19, 47, 48, 50, 53, 54, 87, 105] and streptozotocin [55, 89–93, 104, 106–108]. They accumulate mainly in the pancreas and provoke the production of radicals that lead to the destruction of the pancreas beta cells and, therefore, disorder of the insulin production [10, 11, 12, 91, 106]. Since streptozotocin and alloxan have a similar structure to glucose, their accumulation occurs by means of glucose transporters in the pancreas, which is why animals are more susceptible to them after fasting [10, 12, 109]. Thus, streptozotocin or alloxan injection in animals induces insulin-dependent (type I) diabetes [12, 106]. Since alloxan and streptozotocin solutions are relatively unstable, they should be prepared immediately before the injection. It should be noted that these substances can be toxic for other organs [10, 109, 110]. Besides destruction of the beta cells, alloxan and streptozotocin are able to modify biological macromolecules and to fragment DNA [10, 11, 12, 106].

Dunn et al. were first to describe the alloxan model of diabetes in 1943 [12, 106, 111]. A single dose of alloxan varies from 40 to 200 mg/kg of body mass for rats and from 50 to 200 mg/kg for mice, depending on the breed [10, 11, 50]. Alloxan is injected subcutaneously, intraperitoneally, or intravenously [11, 50, 92].

Rakieten published a paper in 1963 reporting that streptozotocin can induce diabetes [12, 91, 112]. The maximal single dose of streptozotocin varies from 35 to 65 mg/kg of body mass for rats [10, 113] and from 100 to 200 mg/kg for mice [10, 113, 114] depending on the breed [10, 115]. There is also a method for injecting streptozotocin in animals in small doses (from 20 to 40 mg/kg) over 5 days [10].

In contrast to animals, human beta cells of the pancreas are resistant to the toxicity of alloxan and streptozotocin [116]. It is interesting that the variation of glucose content in blood during the first week after alloxan or streptozotocin injection is nonlinear (Figure 28.1); the glucose content in blood increases during about 3–4 hours after the injection. It is accompanied by morphological changes in beta cells: dilatation of rough endoplasmic reticulum, intercellular vacuolization, diminution of insulin content and secretor granules, reduction of Golgi apparatus area, and mitochondria swelling. Then the hypoglycemic stage starts, when the glucose content in blood falls in a few hours, and hypoglycemia can lead to a fatal outcome.

![FIGURE 28.1 Phases of free glucose variation in blood after the injection of alloxan (I-IV) and streptozotocin (II-IV)](image)

Such serious transient hypoglycemia appears during insulin accumulation as a result of the cell membrane rupture and the secretory granule intoxication. In addition to the morphological changes, the nuclei of beta cells become irreversibly pyknotic [12]. The permanent diabetic hyperglycemic phase comes about 6 days after the injection, characterized by loss of beta cells integrity during 12–48 hours and morphologically full degranulation [10, 12]. Thus, it is important to choose the time for measuring the glucose content in blood in diabetes modeling.

Animals with the model diabetes have such symptoms as polydipsia, polyuria [83], and loss of body weight [10, 83], which is typical for type I DM.

Besides the listed models of type I diabetes, there are other ones which are used less widely, for example, the autoimmune modes. The lines of biobreeding (BB) rats and mice with “spontaneous” DM (“nonobese diabetes”), in which the prediabetic state of the organism develops from birth or in some time after birth, are created [10, 82, 83, 117–119]. In Akita mouse lines, in which diabetes genetically develops a few weeks after birth, diabetes is also induced using viruses [10].

Mono- and polygenic models of obesity, the rats of the Zucker line, etc. are used to model type II diabetes [10, 120]. The most widely used monogenic models of obesity are characterized by defects in the transmission of signals of the hormone leptin. Since leptin is responsible for the feeling of satiety, the absence of functional leptin in animals leads to hyperphagia (overeating) and subsequent obesity. The Lepob/ob and Lepr<sup>∆</sup>DM line of mice, the Zucker line rats have a lack of leptin and have no leptin receptor. These models are often used for testing new methods for treatment of type II diabetes [10, 120–123].

Leptin-deficient (Lep<sup>ob/ob</sup>) mice begin increasing in mass from 2 weeks of age; hyperinsulinemia develops, hyperglycemia appears within 4 weeks, and the concentration of free glucose in the blood continues to grow, achieving its maximum in 3–5 months [10, 124]. The volume of the pancreas significantly
Glycation of proteins

The main complications of DM are connected with glycation of proteins. Glycation is a result of interaction between proteins and glucose molecules, which leads to a change in protein structure and the restriction of tissue functioning [20, 41, 44, 46, 131]. Protein glycation is initiated by nonenzymatic reaction between the carbonyl group of sugars and the amino group of proteins, which correspondingly, leads to the formation of crosslinks between protein molecules [18, 42, 45, 132]. The result of glycation is the Mallard reaction [133, 134], which includes two stages. The interaction of protein with glucose via the adducts of the Schiff’s base leads to the formation of a stable Amadori product. Further incubation with glucose leads to transformation of the Amadori product into the advanced glycation end product (AGE) [18, 134–136]. The glycation can be accompanied by both attachment of the AGE to the protein receptor and protein modification [86]. The mechanisms of collagen glycation involve complex processes accompanying the occurring reactions [42, 45, 135, 137, 138]. Such AGEs as Nε-carboxymethyllysine (CML) and pentosidine accumulate in skin collagen with age and DM development. Pentosidine is a fluorescent molecule, which can be detected by the methods of fluorescence analysis [45, 86, 135, 137, 139–142] or confocal Raman spectroscopy [143]. Glucosepane is related to retinopathy, neuropathic, and nephropathic complications [98, 141, 142]. Pyrroline [86], glyoxal [139], and glucosepane [143] are also defined as AGEs; other AGEs and the substances facilitating their accumulation are also known [86, 142].

Since proteins constitute a considerable part of many tissues, glycation of proteins leads to changes in tissue structure, restriction of tissue functioning [7, 42–46, 86, 131, 139], metabolic disbalance and, as a result, malfunction of organs [18]. Since the structure of tissues determines their optical properties, it is possible to monitor their changes by optical methods, such as fluorescence spectroscopy [42, 44, 50, 68, 70, 95, 100–103, 137, 139, 140, 142, 144–150], spectrophotometry in a wide spectral range [47–49, 55, 70, 100, 102, 151, 152], refractometry [43, 154], electron microscopy [19, 89, 94], confocal microscopy [96], laser speckle contrast imaging (LSCI) [53, 54, 70, 106, 155], multiphoton microscopy [131], Raman spectroscopy [51, 52, 68, 143, 150, 156], laser Doppler flowmetry [46, 68, 100–102, 157], optoacoustic spectroscopy [65, 158], OCT [79], and THz spectroscopy [70].

To study protein glycation, tissue and cell samples taken from objects with natural or model DM (in vivo glycation), as well as samples glycated in in vitro conditions, are used. For example, studies of in vitro glycation are conducted for the human placental IV type collagen, which is performed using fluorescence analysis, densitometry, and electrophoresis [159]; for the collagen of bovine skin, using multiphoton microscopy [42]; for hemoglobin, using OCT [161–163], IR spectroscopy, refractometry [154], and biochemical analysis [160]; for albumin, using fluorescence spectroscopy [86], THz spectroscopy [164] and refractometry [154]; for collagen of tendon, using biomechanical and biochemical analyzes [165]; and for collagen hydrogels, using multispectral fluorescence life time imaging (FLIM) [166]. Glycation was provided by incubation tissue and liquid samples in solutions of ribose [45, 159, 165, 166], glucose [86, 154, 159, 160, 161, 164], fructose [86, 164], glycolaldehyde [132], or glyoxal [139]. All solutions show a sufficiently effective protein glycation during 10–11 days of incubation and change in tissues’ mechanical properties due to the formation of collagen cross-links. Increase of glucosapane concentration in the skin with age and with the development of hyperglycemia in patients with type I DM was described [98].

Studies of the fluorescent properties of the glycated hemoglobin and tissues have been performed to determine the degree of protein glycation [42, 44, 50, 137, 139, 140, 144–148]. A refractometry-based biosensor was offered for studying the glycated hemoglobin in human blood [167]. The modification of vascular walls at protein glycation [168], the refractive properties of erythrocytes of patients with DM and healthy volunteers [43], and the optical properties of skin in diabetes [46, 147, 148] were also investigated. Many papers are focused on the development of methods of monitoring of DM and its different complications, which are based on the detection of changes of the tissue optical properties with diabetes development [7, 18, 42–46, 49, 56, 57, 68, 93].

The increased deformation, maximum load, tension, Young modulus of elasticity, and viscosity indicating that glycation increases the stiffness of the tendon matrix were observed after 8 months of glycation of the rabbit Achilles tendon in ribose solution [165]. The tendon glycation led to significant reduction in the soluble collagen content and a significant increase in insoluble collagen and pentosidine content. Thus, the obtained results showed that the collagen crosslinks formed by glycation directly increase the stiffness of the matrix and change other mechanical properties of the tendon.

The literature analysis shows that the study of glycated tissues is demanded and is a promising field of research in terms of developing of new technologies for noninvasive or
least-invasive monitoring of severe complications, their pre-
vention, and treatment maintenance.

**Optical and structural properties of tissues at glycation and DM development**

**Blood and cardiovascular system**

The DM development is related to increased risk of macro-
and microvascular complications (angiopathy) [9, 10, 14, 20,
21, 76–78, 98], such as nephropathy and neuropathy [9, 10,
14, 21, 74] and retinopathy that leads to blindness [9–11, 14,
21, 38, 74, 79]. DM significantly enhances the risk of vascular
diseases that finally lead to brain stroke [5, 14, 20, 21, 74,
77]. Hyperglycemia accelerates the atherosclerotic processes
[14, 75, 77]; atherosclerosis and the corresponding loss of
elasticity in coronary artery walls induce stenosis and, there-
fore, the reduction of blood supply to the cardiac muscle,
which finally leads to cardiomyopathy, angina pectoris, and
increased risk of myocardial infarction [13, 14, 77, 78].
The cause of serious complications is often the glycation of pro-
teins in blood and in vascular walls permanently washed by
blood in the presence of high level of free glucose in it [49,
156, 159, 161].

For determination of glycated albumin or hemoglobin,
different methods are used such as ion exchange, liquid or
affine chromatography, electrophoresis, immunochemical,
and colorimetric methods [86, 169]. Firstly, these methods are
invasive, because blood collection from the patient’s vein is
needed. Since the mean lifetime of erythrocytes is 3 months,
only information about glycation accumulated during those 3
months can be collected. Moreover, the measurement accu-
ricacy is affected by the increased content of glucose, lipids,
bilirubin, and other substances [169]. The level of free glucose
in the blood is also influenced by external factors, for example,
physical activity and food intake, which limits the applicabil-
ity of many diagnostic methods where blood measurements
are required [8, 9]; therefore, it is necessary to develop new
algorithms for processing the obtained data to reduce the influ-
ence of external factors.

Optical methods for diagnosing the state of tissues and
organs are widely used in biology and medicine, for example,
to determine the degree of their oxygenation and blood perfu-
sion [170, 171]. The safety of these methods and the ability to
obtain information in real time explain their widespread use
and the constant expansion of the field of application in medi-
cine [22, 46, 56, 172–175]. It is possible to get information
about the composition, structure, and properties of tissues to
investigate metabolic processes, avoiding the negative effect
on them using optical radiation [170]. An essential advantage
of visible and NIR optical radiation is its capability to pen-
etrate sufficiently deep into tissues. The wavelength range
from 600 to 2500 nm includes four “transparency windows,”
within which the light attenuation by tissues is minimal.
Since many tissues contain a lot of water, the spectral “trans-
parency windows” correspond to the wavelengths at which
the light absorption by water is minimal. The first “transpar-
ency window” includes the wavelengths from 650 to 950 nm;
the absorption of light by water is relatively weak in it, but
the light absorption by hemoglobin and myoglobin is high.
The second “transparency window” is placed between two
bands water absorption and corresponds to the wavelengths
from 1100 to 1350 nm. The third “window of transparency” is
in the wavelength range from 1600 to 1870 nm and the fourth
“window” is from 2100 to 2300 nm, which is conve-
nient for the investigation of collagen-containing tissues [171,
176–178].

The possibility of Raman spectroscopy to determine the
degree of hemoglobin glycation was shown [156]. The sig-
nificant difference in the refractive properties of erythrocytes
in diabetic and healthy patients was observed in microscopic
studies using the Nomarsky interference microscope combing
a two-beam interferometer and a polarization microscope
for enhancing the contrast of phase images [43]. The quan-
titative analysis of the glycated hemoglobin concentration
in human blood using the spectral analysis in the infrared
wavelength range from 780 to 2498 nm was proposed in the
Reference [49]. The authors of Reference [50] measured the
fluorescence of blood plasma 12 days after the alloxan injec-
tion in rats. It was shown that the shape of the fluorescence
band at an excitation wavelength of 320 nm is most indicative
of hyperglycemia in blood plasma samples due to the forma-
tion of a protein fluorescent bond due to nonenzymatic glyca-
tion. In Reference [154], the optical properties of albumin and
hemoglobin in aequous solutions of glucose were investigated.
The increase in the refractive index and the reduction of the
absorption of the solutions with the increase in glucose con-
centration in the solution were obtained. The probable cause
is protein–glucose binding. A decrease in the albumin absorb-
ance in the THz range of wavelengths with increase in the
incubation time of albumin in glucose and fructose solutions
(glycation in vitro) was observed [164]. Also the slow glycation
in the glucose solution in comparison with the fructose one
and the dependence of albumin glycation rate on the pH of the
sugar solution were obtained in this work.

The applicability of Raman spectroscopy for the assessment
of glycated hemoglobin in vivo, carried out for ear, hand, and
forehead skin sites, was shown by the authors of Reference
[52]. The porphyrin conformations of erythrocyte hemoglobin
were observed in diabetes using Raman spectroscopy [51]. A
reduction in erythrocyte membrane viscosity was obtained
in diabetes by spectroscopy of electronic paramagnetic reso-
nance. A change in permeability of the erythrocyte plasmatic
membrane was found, namely, a higher rate of Na-H exchange,
the activity of Ca$^{2+}$-dependent K$^+$-channels, and a decrease
of the Ca-ATPase activity in patients with II type of DM [51].
It was supposed that the change of permeability and viscosity
of the erythrocyte plasmatic membrane can cause the confor-
mation change of the hemoglobin porphyrin, the reduction of
oxygen transport by hemoglobin, and binding activity under
DM [51].

A noninvasive method and a device for assessing the gly-
cated hemoglobin concentration based on light reflection spec-
tra measured from blood samples were proposed in the patent
[151]. The device consists of two measuring units, one of
which contains a Raman spectrometer, and the other a spectro-
photometer recording the absorption spectra. The possibility
of using absorption spectroscopy in the spectral range 200–850 nm for the assessment of glycated hemoglobin amount was demonstrated in Reference [152]. A correlation between results obtained by the spectroscopic method and the standard method of high-efficiency liquid chromatography was found.

The method of quantitative analysis of the glycated hemoglobin concentration in hemolysate samples of human blood using NIR spectroscopy and subsequent processing the spectra by the method of moving window partial least squares (MWPLS) was proposed in Reference [49]. This method allows one to find the optimal spectral ranges for determining the content of nonglycated and glycated hemoglobin. The optimal wavelengths for analysis were in the NIR from 958 to 1036 nm for native hemoglobin and from 1492 to 1858 nm for glycated hemoglobin.

The optical characteristics of erythrocytes from diabetic patients were noninvasively studied by three-dimensional quantitative phase imaging based on common-path diffraction optical tomography (cDOT) [179]. The morphological (surface area, volume, and sphericity), mechanical (membrane fluctuations), and biochemical (concentration of hemoglobin) parameters of individual cells were quantitatively determined from the measured three-dimensional tomograms of the refractive index and two-dimensional time-dependent phase images. Statistically significant changes in biochemical and morphological parameters of erythrocytes of diabetic and nondiabetic patients were not obtained; however, the lower deformability of the membrane of diabetic erythrocytes was demonstrated.

Since the AGEs are dissolved in the blood plasma, they interact with the endothelium, and, hence, affect the endothelial functioning with tissue hypoxia and hyperperfusion [21, 77, 132, 135]. Thus, the AGEs accumulate in vascular walls with formation of the crosslinks [135]. The corresponding damage to the endothelium leads to the development of atherosclerosis [14, 21, 135, 168]. The risk of ischemia increases in the presence of DM [21, 78, 97].

In comparison to hemoglobin, which is subject to glycation over the course of 3 months, the glycation of other proteins lasts longer; therefore, the diagnostics of such proteins should provide more information about organism condition when DM develops.

The exhaustion of lectin content in the mice endothelium with 4-week streptozotocin-induced diabetes was found using the histological sections stained by the lectin antibody (Figure 28.2) [89]. Poorer perfusion recovery after ischemia was obtained in diabetic outbred mice compared to linear mice. Thus, the result can depend on the animals that are used in the study. The study [97] of human arium samples in vitro, carried out after reoxygenation and perfusion modelled ischemia, showed stronger cell necrosis and apoptosis expressed in patients with type I and II DM. The apoptosis of myocar
dial cells was observed in rats with streptozotocin-induced diabetes [90].

DM disturbs reperfusion mechanisms, i.e., the activation of earlier existing arterial collaterals and the generation of new vessels (angiogenesis, arteriogenesis), which hampers recovery after an ischemic stroke [77, 78]. A smaller number of vessels and the accumulation of the AGE CML in muscles and blood of all outbred mice with diabetes was found [89]. A topical application of an iohexol solution (X-ray contrast solution “Omnipaque®”) on blood flow in the pancreas of rats with alloxan-induced diabetes studied using laser speckle contrast imaging (LSCI) showed an increase in blood flow rate compared to the control group of rats, indicating the increase of endothelial permeability at the development of disease [53]. The endothelium-dependent skin microvascular vasodilator response was found to be significantly impaired in patients with type I diabetes compared to healthy patients using LSCI coupled with physiological postocclusive reactive hyperemia and pharmacological iontophoresis of acetylcholine as a local vasodilator stimulus [155]. An increase in cutaneous microvascular permeability was obtained in mice with alloxan diabetes by spectral imaging and the optical clearing skin window [131].

A decrease in the elasticity of arterial walls under the development of type II DM was demonstrated by the rate of ultrasound propagation and elastic modulus of arteries [77, 180]. A decreased blood flow rate during diabetes development was
Diabetes mellitus-induced alterations of tissue optical properties

observed by laser Doppler flowmetry [46, 157]. An anomalous thickening of vascular walls in skin was also found [46].

Studies of assessment of noradrenalin impact on the blood flow of mice with alloxan diabetes were carried out [106]. The decrease of arterial and venous blood flow without subsequent recovery caused by noradrenalin injection was observed using the LSCI in mice with 2- and 4-week alloxan diabetes (see Chapter 29, Figure 29.9).

Since the heart is the main organ of the cardiovascular system, through which the process of blood circulation is implemented in the organism, it is directly affected by the negative effect of the disease with numerous complications [13, 14, 77, 78]. Changes in adipocyte size and an excess of lipid accumulation on the heart epicardium in patients with DM were found [13]. Such changes lead to the development of heart disease. The optical measurements demonstrate the reduction of the glycerol diffusion rate in ex vivo myocardium of rats with 2 weeks [48] and 1 month [105] of alloxan DM, which indicates the change of cardiac muscle tissue structure in the first weeks of the development of alloxan diabetes in rats.

The possibility of assessment of the functional condition of the microcirculatory system in patients with DM using such noninvasive optical methods as laser Doppler flowmetry, diffuse reflection spectroscopy, and fluorescence spectroscopy was shown [100, 102]. The obtained data have demonstrated that the combined application of these three diagnostic technologies allows one to reveal and predict the development of trophic disorders and the syndrome of diabetic foot at early stages. The use of wavelet analysis for evaluating the regulatory mechanisms of peripheral blood flow during the heat tests makes it possible to study the change of vascular tonus autoregulation and the regulation of bypass blood flow by sympathetic fibers. This allows for indirect consideration of blood flow innervation and can indicate the presence of neuropathies [100]. The variation of hemoglobin concentration under the impairment in the microcirculatory layer of the foot skin due to diabetic microangiopathy can be detected in the skin optical reflection spectra due to changes in the scattering and absorption properties of skin [102].

Diabetic abnormalities in the structure of various tissues

Type I DM develops due to a disorder of the pancreas functioning that leads to development of chronic hyperglycemia and to glucose excess in the interstitial fluid, and then to disturbance of the metabolism and functioning of many organs [2, 6–12, 18, 21, 69, 81–83, 106, 111]. Since all vital organs, such as cerebral tissues, myocardium, and eye retina, are strongly supplied with blood and therefore with glucose, so they are glycated first in patients with DM. Multiple studies of the impact of DM on different properties and functioning of tissues and organs have been conducted for finding new effective methods for DM diagnostics and treatment and preventing its serious complications.

Since the pancreas is the primary organ to suffer from diabetes, many pancreatic diseases develop in diabetes [21]. For example, the swelling of the pancreas and liver mitochondria was fixed 12 days after intra-abdominal injection of streptozotocin to rats, leading to damage of mitochondria and ATP deficiency [92]. The influence of DM on rat internal organs was studied [19]. Hyperglycemia, polyuria, and polydipsia were fixed in the rats after the intra-abdominal injection of alloxan. The histological sections of the spleen, liver, pancreas, and kidney tissues were taken on the 15th day after alloxan injection. Morphological changes of different degrees of severity in comparison with the control group of rats were observed in tissues after the histological analysis of tissues. The authors obtained a significant decrease of glycogen accumulation in the liver, the presence of perivascular fibrous inductions, and a decrease in the size and the number of pancreatic islands in the pancreas [19]. The necrosis of individual cells in kidneys was revealed with the development of diabetes. These results confirm that alloxan diabetes results in the structural changes of tissues and organs caused by an accumulation of glycogen 2 weeks after the intra-abdominal injection of alloxan in rats.

Since the high concentration of free blood glucose leads to the growth of its level in interstitial fluid, glycation affects not only the blood proteins, such as hemoglobin and albumin [86, 156, 160, 161, 164, 181], but also other proteins of the tissues. For example, collagen glycation leads to fibrosis development [72, 182]. In its turn, the structural changes of tissue proteins lead to changes in their optical properties.

Studies of the fluorescence properties of the AGEs of skin, hemoglobin, cornea, articular tendon, and aorta [42, 44, 137, 139, 140, 145, 183] and corresponding nonlinear susceptibilities of tissue compounds [42, 144, 184] showed that glycation facilitates an increase in tissue fluorescence intensity [42, 137, 139, 140, 145, 183] and a decrease in second harmonic generation (SHG) intensity [42, 144, 184] (see Figure 28.3).

The authors of Reference [95] have studied skin in vivo autofluorescence and brain magnetic resonance (MRI) images in patients with type II DM. An increase in autofluorescence was found both with age and with DM development. The corresponding reduction of the gray-matter volume in the brain was observed in DM patients, which is the cause of cognitive defects of various degrees.

It was found that the length and density of nerve fibers are significantly smaller in patients with recently diagnosed type DM than in the control group [96]. Such studies have been performed by skin biopsy and confocal microscopy of the eye cornea, for which the loss of nerve fibers and the accompanying injury of nerve conductivity were found, indicating early pathological changes in both large and small nerve fibers.

In turn, the damage of nerve fibers (neuropathy) can lead to a neuropathic foot ulcer, the most severe skin injuries arising when the reduced recovery capability of diabetic skin gives rise to infection, gangrene formation, and, finally, leads to the amputation of lower extremities [72, 73]. The authors of Reference [95] suggested that skin autofluorescence can indicate the formation of AGEs of other cell proteins, e.g., in neurons.

Since proteins are the main components of many tissues, protein glycation leads to significant changes in tissue structure [42, 45]. Since tissue permeability for chemicals is largely determined by tissue structure and its changes caused...
by pathological processes, such as glycation, the change of the molecule diffusion rate in tissue during a certain time interval can therefore indicate the change of the tissue structure and, thus, can be used as a biomarker of the degree of tissue glycation [48, 114, 185]. The study of the permeability of tissues for different molecules is aimed at getting information about the mechanisms of tissue interaction with different chemicals, drug transport in tissues, and agent impact on the morphological, optical, functional, and diffusion properties of tissues [56, 57, 61, 105, 172, 173, 186–188]. These data are necessary for effective application of different pharmacological preparations to treat DM and for developing noninvasive optical methods of disease diagnostics and monitoring [56, 189], since efficiency of treatment and diagnostics is determined by drug (agent) diffusion rate, i.e., the time needed for drug (agent) molecules to reach the target part of the organism.

The slower glucose diffusion in a kidney sample of a diabetic mouse as compared to a nondiabetic one was obtained [55]. It was supposed that the diabetic kidney has a denser structure due to tissue glycation. The optical clearing efficiency of kidney, cornea, and skin samples in THz wavelength range was higher for nondiabetic samples than for diabetic ones at application of glycerol solutions of different concentration [55], which can be associated with lesser water flux in glycated tissues, as tissue dehydration is the major mechanism of optical clearing for THz waves.

About 80% of glucose in the organism is transported to muscles [20]. The reduction of insulin production under DM leads to the malfunction of glucose absorption by muscle cells, which leads to muscle dysfunction and reduction in muscle mass [190]. A 35% reduction of mitochondria size in skeletal muscles of patients with obesity and type II DM was observed using electron microscopy [94]. Vacuoles growth in muscle fibers and lowering of NADH:O₂-oxidoreductase was also found in patients with type II DM [94]. The authors concluded that the bioenergetic capability of the mitochondria of skeletal muscles is disturbed in type II DM.

Larger fat cells were found in patients with DM as compared to healthy people [99]. It was demonstrated that the main phenotype of white fat tissue in humans with type II DM without obesity is adipocytes hypertrophy, which can lead to the inflammation of fat tissue, the release of fatty acids, the deposition of ectopic fat, and stronger sensitivity to insulin.

Using a fluorescence microscope, it was found that the mean cross-section of adipocytes of the fat tissue extracted from patients during surgical operation is higher in diabetes than in the control group (see Figure 28.4) [103]. A reduction of fibrous tissue formation and an increase of adipocyte hypertrophy was also obtained in patients with DM and obesity. It is noted that hypertrophy is a cause of fat tissue dysfunction [103].

Observed changes in ocular tissue autofluorescence in patients with type II DM having no evident signs of diabetic retinopathy indicates the accumulation of AGEs in the eye tissues [191].

The studies of air expired by patients with type II DM, prediabetic patients, and a control group of patients observed a correlation between the index of insulin sensitivity (1/ISI₀.₁₂₀) and the concentration of the isotope ¹³C (δ₁₃C(‰)) in the expired air (Figure 28.5), which could serve as a marker for noninvasive assessment of both the development of type II DM and prediabetic condition in humans [192]. To obtain such a marker, the patient was orally administered ¹³C-labelled D-glucose, which is metabolized and produces ¹³C-labelled carbon dioxide (¹³CO₂), which gets to the lungs via the blood flow and then is expired [192].

**Skin pathologies induced by DM**

Skin is subjected to various infections during DM development; skin fibrosis is observed, the skin becomes more dry and vulnerable due to functioning disorder of leucocytes,
Diabetes mellitus-induced alterations of tissue optical properties

and patients develop skin itching. These symptoms mean the presence of DM complications caused by hyperglycemia and protein glycation. There are many skin diseases indicated by DM [20, 72, 73]. Such structural changes as pore enlargement, dermis thinning, volume loss of collagen, flattening of the dermis–epidermis junction, and atrophy of blood vessels occur in the skin with age [193]. These age-related changes are enhanced by the development of DM [77]. Increased skin autofluorescence can indicate age-related changes [147], which are enhanced at DM with nephropathy and retinopathy complications [148]. The excitation light wavelength varied from 300 to 420 nm with the excitation peak at 370 nm with autofluorescence in the range of 420–600 nm [148].

As seen from the Refs. [95, 147], the increase of fluorescence intensity is associated with DM development and aging, and thus it is important to distinguish the contribution of DM and age to the signal. An increase in the glycated collagen amount of only 33% was obtained in patients not suffering from DM at the age of 20–85 years [194], while the fluorescence of the AGEs in DM increased five-fold, strongly correlating with age. Collagen glycation increased by three-fold in patients with DM compared to nondiabetic subjects, strongly correlating with the concentration of glycated hemoglobin in blood, but not with age. Thus, appropriate glycation levels in tissues can be identified for a certain age, but these indicators are significantly increased with DM development. Therefore, it is possible to have criteria differentiating changes associated with DM or age.

Elevated skin autofluorescence in patients with insulin resistance and/or DM, as well as in healthy elderly and middle-aged subjects, was observed [150]. Changes in the skin hydration state, degradation of type I collagen, and greater glycation related to DM and aging were evaluated by Raman spectroscopy. A weak positive correlation between the Raman peaks ratio (855/876) related to glycated proteins and the skin autofluorescence was found [150]. Using confocal Raman spectroscopy, the increase in the amount of such AGEs as pentosidine and glucosepane in the skin dermis of elderly women with type II DM and healthy elderly women in comparison with healthy young women was obtained [143].

Many papers are devoted to the study of structural and optical parameters of skin in DM development and its modeling. Unfortunately, the conclusions based on different studies are often contradictory [69]. The reduction of stratum corneum hydration was shown in vivo in humans and mice during DM.

FIGURE 28.4 Histogram, microscopic images, and plots describing the increased size of adipocytes of the visceral (VAT) and subcutaneous (SAT) adipose tissue of the patients suffering from DM and control group (NDM) [103].

FIGURE 28.5 Distribution of $^{13}$C isotope concentration in the expired air ($\delta_{DOB}^{13}$C(‰)) and the insulin sensitivity index (1/ISI$_{10,120}$) depending on the level of glycated hemoglobin HbA1c (%) in the blood of the control group (Normal), prediabetic patients (Pre-diabetes), and of the patients with type II DM (Type 2 diabetes) [192].
development [195, 196], which was not related to disorder of the barrier function of the epidermis. THz imaging applied for early screening of diabetic foot syndrome showed less water concentration in diabetic subjects [80]. However, it was noted that the transepidermal water loss (TEWL) does not increase in the case of DM [195, 197]. However, long-term hyperglycemia disturbs the barrier function of the skin and its permeability [196].

A reduction in keratinocyte proliferation in the epidermis of mice with modeled DM was observed [69, 197, 198], while no such changes were obtained in the experiments in rats and mice of other ages [196, 198]. Contradictory results were obtained in the studies of epidermal thickness at DM development [69]. The authors of [196, 198, 199] conclude that epidermis thickness does not change under the conditions of long-term hyperglycemia in rats and DM in humans and mice; however, for another DM model and ages, the thickness decreases [197, 198, 200] or increases [201]. The increase of skin thickness in patients with DM was measured ultrasonically [202]. The different conclusions could be caused by different ages of mice under study and DM animal model. Using microscopy and histology, changes in collagen distribution in the dermis of DM patients with complications were observed in contrast to those having no complications [203]. The inconsistent results require one to specify the experimental conditions.

AGEs formation reduces the solubility and elasticity of collagen, thereby enhancing its stiffness. [72, 204]. The results presented in Reference [205] confirm that the accumulation of fragmented skin collagen and the presence of molecular cross-links during DM development impairs the structural integrity and mechanical properties of skin collagen. The pronounced crosslinks of collagen fibers were demonstrated in the studies of diabetic skin aging. Atomic force microscopy (AFM) has shown that the collagen fibrils of skin are disordered and fragmented (see Figure 28.6(a), b), and their key mechanical properties are essentially changed in the case of diabetes [205]. The quantitative analysis of AFM data has demonstrated that the mean roughness of collagen fibrils as a measure of their arrangement increases in diabetic dermis by 176% (Figure 28.6(c)) from 16 nm to 29 nm; this can be related to the increased concentration of matrix metalloproteinases, which provoke fragmentation of collagen fibrils. Fibril fragmentation impairs the structural integrity of collagen and, thus, changes the mechanical properties of skin dermis. The basic mechanical properties of the dermis, such as tensile strength (Figure 28.6(d)) and traction force (Figure 28.6(e)), were increased by 197% and 182% respectively, while the deformation of collagen fibrils was reduced by 58% (Figure 28.6(f)) in the diabetic dermis in comparison with the nondiabetic one.

A significant reduction in the number of collagen fibers and disorder of skin collagen fibers caused by alloxan diabetes was observed using two-photon imaging [131]. Classical histological analysis also showed that DM led to a change in skin filamentous structure.

The mechanical properties of collagen hydrogel were studied using FLIM after its incubation in ribose and glutaraldehyde solutions, which contribute to the formation of collagen cross-links [166]. Correlations between the mechanical properties of collagen and the fluorescence lifetime were observed in the case of collagen incubation in glutaraldehyde in contrast to ribose. It has been found that the degree and nature of collagen cross-linking significantly affects tissue elasticity also under the action of ribose.

In vitro glycation of murine skin in the glyoxal solution showed an increase of TEWL, and significant increase of saturated fatty acids concentration in the epidermis; moreover, the barrier function of the epidermis was impaired [139]. The skin immersed in the glyoxal solution became a yellow color. The presence of AGEs in the skin was detected by increased autofluorescence [139]. The yellow color could be caused by nonenzymatic glycation of dermal collagen. Yellowish nails and skin are observed in diabetic patients [21].

The results of a study of glucose diffusion in ex vivo skin of mice with alloxan diabetes by measuring the collimated transmittance of visible light through the skin samples are presented in Reference [47]. The glucose diffusion rate in the skin of mice with alloxan diabetes was up to 2.5 times slower as compared to the control group of mice (Figure 28.7). Similar results were received for the aqueous glucose solutions of three different concentrations: 30%, 43%, and 56%.

Reduction of glycerol molecule diffusion rate in ex vivo skin samples taken from rats with 2 [48] and 4 [105] weeks of alloxan diabetes was observed. Two weeks of the streptozocin diabetes caused the reduction of glycerol diffusion rate in skin ex vivo [107]. In vivo OCT studies also showed slower OC of skin under action of glycerol solution in DM animals [105]. These results are illustrated by data presented in Figure 28.8 and summarized in Table 28.1. The typical kinetic curves of collimated transmittance spectra of rat skin and myocardium ex vivo samples of nondiabetic and diabetic animals measured during tissue sections immersion in a 70%-glycerol solution are shown in Figure 28.8. The collimated transmittance of both tissues increases with time and reaches saturation.

Transmittance is significantly increased for all wavelengths of both tissues and much faster for myocardium. It is seen that collimated transmittance of diabetic tissues grows much slowly than for nondiabetic tissues. In Table 28.1, mean values of thickness and weight of ex vivo rat tissue samples before and after immersion in a 70%-glycerol solution and tissue permeability (P) and diffusion (D) coefficients for glycerol in the skin and myocardium of nondiabetic and diabetic groups, evaluated from collimated transmittance kinetic curves as shown in Figure 28.8, are presented. Both skin and myocardium permeability for glycerol is decreased in diabetic groups. Transverse shrinkage (less sample thickness) and dehydration (less sample weight) in all tissue samples caused by osmotic pressure induced by glycerol are seen.

The characteristic time τ and OC efficiency of in vivo rat skin under the action of a 70%-glycerol solution was estimated [105]. The degree of the OC was defined as the ratio of tissue optical attenuation coefficient μ before and after glycerol action, for nondiabetic animals μ was reduced up to (46 ± 19%) and for diabetic (alloxan type), was up (37 ± 16%). The OC characteristic time was found as τ = (2.7 ± 0.4) min for nondiabetic and (8.9 ± 7.7) min for diabetic animals.

The increase of light penetration into tissue under the action of glycerol solution is observed for both ex vivo and in vivo studies. The slowdown of skin OC in the diabetic group was
established in two independent sets of measurements ex vivo (spectral collimated transmittance) and in vivo (OCT).

The reduction of tissue permeability to glycerol molecules during development of diabetes must be related to alterations in tissue morphology [19] and modification of structure, including the degree of fibril packing, loss of axial packing of the collagen fibrils due to the twisting and distortion of the matrix by the glycation adducts [144, 170, 171], the cross-linking of proteins, changes in free and bound water content in tissues, and increase in sarcoplasm viscosity [19, 41, 170].
These structural modifications also lead to changes of tissue optical properties, specifically to the increase of scattered light intensity [144]. A decrease in tissue permeability for glycerol molecules during the development of diabetes should be associated with changes in tissue morphology [19], including the degree of fibril packing, loss of axial packing of collagen fibrils due to twisting, and distortion of the matrix due to the appearance of glycation adducts [144, 170, 171], protein crosslinking, changes in the content of free and bound water in tissues, and an increase in the viscosity of sarcoplasma [19, 41, 170]. These structural modifications also lead to changes in the optical properties of tissues, and in particular to an increase in the intensity of scattered light [144].

Optoacoustic spectroscopy was used to study AGEs in skin at in vitro glycation [183]. The principle of optoacoustic spectroscopy is based on the transformation of energy of
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Time-modulated or pulsed optical radiation interacting with object into thermal and further acoustic waves [206]. A spectrally nonselective detector of absorbed energy at different wavelengths is a microphone or ultrasonic transducer [206]. The conditions of physiological hyperglycemia were provided by immersion of porcine skin samples in ribose solution over the course of 17 days [183]. An increase in optoacoustic signal of glycated skin at light absorption in the range 540–620 nm with time of incubation was observed [183]. The increase in the optoacoustic signal can be related not only to high light absorption by glycated skin, but also to the increased efficiency of light-to-sound conversion due to greater tissue elasticity caused

![FIGURE 28.8 Typical time dependences of collimated transmittance spectra of ex vivo Wistar rat tissue samples for nondiabetic (a, c) and diabetic (alloxan model) (b, d) animal groups during OC using a 70%-glycerol solution; sections with the initial thicknesses (0.79 ± 0.04) mm of nondiabetic (a) and (0.81 ± 0.01) mm of diabetic (b) skin; and sections with the initial thicknesses (0.58 ± 0.02) mm of nondiabetic (c) and (0.76 ± 0.03) mm of diabetic (d) myocardium [105].](image)

### TABLE 28.1

Mean values of the thickness and weight of ex vivo Wistar rat tissue sections before \((l_0 \text{ and } W_0)\) and after \((l \text{ and } W)\) immersion in 70%-glycerol solution; tissue permeability \((P)\) and diffusion \((D)\) coefficients of glycerol measured in skin and myocardium samples of nondiabetic and alloxan/streptozotocin diabetic animal groups

<table>
<thead>
<tr>
<th>Group</th>
<th>(l_0/l), mm</th>
<th>(W_0/W), mg</th>
<th>(P), cm/sec</th>
<th>(D), cm²/sec</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Skin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nondiabetic [48]</td>
<td>((0.53±0.11)/(0.55±0.13))</td>
<td>((271±68)/(183±21))</td>
<td>((1.68±0.88) \times 10^{-5})</td>
<td>((8.33±2.60) \times 10^{-5})</td>
</tr>
<tr>
<td>Diabetic/alloxan [107]</td>
<td>((0.56±0.04)/(0.57±0.07))</td>
<td>((270±32)/(203±73))</td>
<td>((1.20±0.33) \times 10^{-5})</td>
<td>((6.77±2.11) \times 10^{-5})</td>
</tr>
<tr>
<td>Diabetic/streptozotocin [107]</td>
<td>((0.47±0.05)/(0.57±0.06))</td>
<td>((198±17)/(161±1))</td>
<td>((1.36±0.82) \times 10^{-5})</td>
<td>((6.97±4.37) \times 10^{-5})</td>
</tr>
<tr>
<td><strong>Myocardium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-diabetic [48]</td>
<td>((0.68±0.11)/(0.51±0.09))</td>
<td>((210±37)/(146±35))</td>
<td>((1.18±0.61) \times 10^{-5})</td>
<td>((7.90±3.61) \times 10^{-5})</td>
</tr>
<tr>
<td>Diabetic/alloxan [48]</td>
<td>((0.58±0.06)/(0.47±0.07))</td>
<td>((214±41)/(153±36))</td>
<td>((0.86±0.32) \times 10^{-5})</td>
<td>((5.14±2.10) \times 10^{-5})</td>
</tr>
<tr>
<td>Diabetic/alloxan [105]</td>
<td>((0.96±0.30)/(0.80±0.26))</td>
<td>((377±139)/(315±138))</td>
<td>((0.88±0.50) \times 10^{-5})</td>
<td>((7.74±4.40) \times 10^{-5})</td>
</tr>
</tbody>
</table>
by the crosslinks that appear between the collagen molecules. It was shown [158] that photoacoustic tomography can be used to detect vascular dysfunction in diabetes due to its unique peripheral hemodynamic response to occlusion and a lower level of SO\textsubscript{2}, compared to that for healthy patients. In Reference [183], optoacoustic measurements were compared with nonlinear microscopic imaging of glycated skin (see Figure 28.9). An increase of intensity of autofluorescence and decrease of SHG-signal were demonstrated for the glycated skin and other collagenous tissues (see Figure 28.3 and Figure 28.9) [42, 183], which demonstrate modification of tissue structural properties and corresponding increase of optoacoustic signal.

A patent [149] proposes a device with an operation principle based on autofluorescence detection, by which it is possible to determine the collagen AGEs and to measure their concentration in sclera, oral mucosa, and skin for diagnosing DM or prediabetes. The degree of tissue glycation is quantified from the autofluorescence spectra.

The possibility of combined measurements of blood perfusion and skin fluorescence was evaluated using laser Doppler flowmetry for diagnostics of complications of type II DM in the lower extremities of patients [101]. The blood microcirculation was topically stimulated by heating. The studies showed that the patients with DM had an enhanced fluorescence and lower perfusion response to local heating, which can be used as markers for evaluation of diabetic complications.

A method of noninvasive in vivo monitoring of hyperglycemic state in mice with streptozotocin diabetes based on mm-wave spectroscopy and experimentally confirmed using live animal models as objects is described in Reference [104]. The transmittance coefficient of the skin fold at the nape of mice of various lines was measured at 25 uniformly located frequencies in the range of 0.075–0.110 THz. Skin transmittance was several times higher for animals in a hyperglycemic state and depended a little on the presence of white or black hair or its absence in mice.

The development of unique optical methods is further stimulating interest in a deeper study of this formidable disease. From the point of view of diagnosis, it is important to observe the earliest changes in tissue properties caused by the initial stage of the disease. The optical methods “see” the presence of free glucose in the blood, in the interstitial fluid, and in cells through optical clearing, which is associated with the matching of the refractive indices of the scatterers and the environment, as well as with the dehydration of tissue and cells. At the same time, tissue glycation leads to a change in tissue structure and also to a change in the optical and diffusion properties of tissues, which makes it possible to assess the degree of tissue glycation.

As one can see from this chapter, many optical methods are applicable for diagnostic and monitoring of the degree of tissue and fluid glycation during the development of diabetes. Such optical techniques include fluorescence spectroscopy, refractometry, Raman spectroscopy, broad-wavelength spectrophotometry, OCT, confocal microscopy, multiphoton fluorescence microscopy, and harmonic generation, optoacoustics, laser speckle contrast imaging, and laser Doppler flowmetry. Among the many optical methods, one of the most promising is fluorescence spectroscopy, which makes it possible to register the presence of the end products of glycation in tissues, is simple to implement, and makes possible noninvasive diagnostics. Refractometric and phase methods are promising, including the use of three-dimensional diffraction tomography and OCT. Terahertz spectroscopy and imaging are also promising methods for studying glycated tissues and cells. The problem of noninvasive monitoring of early structural changes in tissues associated with diabetes is gradually losing its “unsolved” status.

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