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HISTOLOGICAL STRUCTURE OF AORTAL WALL IN RATS AND BRANCHES OF ITS BLOOD CIRCULATION AT THE LATE STAGES OF STREPTOZOTOCIN-INDUCED DIABETES MELLITUS

Abstract. *Diabetes mellitus being considerably spread touches upon the interests of people of different ages and is characterized by early disability and high mortality. Till 2030 the number of patients suffering from diabetes mellitus is expected to reach 552 million (9,9% or 1 diabetic patient per 10 healthy adults), and till 2035 – to 592 million (10,1%). The most common complications of diabetes mellitus are vascular disorders known ,as "diabetic angiopathy". This notion includes lesions of small vessels – capillaries, venules and arterioles (diabetic microangiopathy), and large arteries (diabetic macroangiopathy). Investigations of many researchers deal with this pathology, although there is no a universally accepted and generalized view concerning morphogenesis of diabetic angiopathy, pathogenic factors, possibilities of prognostication and early control over the process development and its prevention. Therefore, the* ***objective*** *of our study was to perform histological investigation of the aortic wall and condition of the blood vessels in the aortic microcirculation of rats at the late terms of streptozotocin-induced insulin dependent diabetes mellitus. 26 mature albino male rats of "Wistar" line with the body weight of 100-160 g were used as the material of the study. Experimental diabetes mellitus was simulated by means of a single intraperitoneal injection of streptozotocin produced by "Sigma" in the dose of 7 mg per 100 g of the body weight (prepared on 0,1 mole of citrate buffer, pH = 4,5). The control group included intact albino rats of similar weight, sex and age. The development of diabetes mellitus during 2 weeks was controlled by the increasing glucose level in the blood measured by means of glucose oxidase method. The samples for histological examination were pieces of the ascending portion, arch and descending portion of the aorta. The specimens were stained with azan according to Heidenhain, and the nuclei – with Weigert's iron hematoxylin. The specimens were examined and photos were made under the microscope МБИ–1 with magnification (ocular 7, objective lens 8), (ocular 10, objective lens 8), (ocular 7, objective lens 20), (ocular 10, objective lens 20). The results of the study were indicative of the fact that in the majority of animals after 6 weeks of streptozotocin-induced diabetes mellitus the aorta preserved its macroscopic structure. Histological examinations of the sections of the aortic wall of rats enables to find the signs of development of diabetic macroangiopathy, that is, atherosclerotic disorders of the endothelial layer of the vascular wall in the form of fatty consolidation (fatty stripes) occupying 30-50% of the internal aortic surface. The end of the sixth week of the experiment was characterized by the signs of arterial hyalinosis and fine inflammatory infiltrations represented by macrophagocytes and lymphocytes. After 8 weeks of streptozotocin-induced diabetes mellitus the internal surface of the aortic wall became dark brown in colour and tuberous, the intima was irregular, whitish in colour, and all its surface containing tubercles and depressions. Elastic fibers of the median membrane are not well-organized, inter-laminar spaces are wide with loss of inter-laminar relations, especially expressed in the external layers of the median membrane. The links of blood circulation are dilated, arterioles with irregular outlines, dilated venules, and swelling round the vessels are found in certain areas.*

Key words*: aorta, atherosclerosis, microstructure, blood circulation, streptozotocin, diabetes mellitus, albino rat.*

Introduction. Diabetes mellitus (DM) is one of the most serious medical-social issues of nowadays and it gets priority in the national public health systems practically in all the countries of

the world.

In recent years DM has occupied the third position among direct causes of death after cardiovascular and oncological diseases. Social consequences of DM are unfavourable as well, since the control and therapy of the disease require heavy expenses from the health care systems.

According to the data of the World Health Organization (WHO) and International Diabetic Federation (IDF) the number of diabetic patients in the world in 1985 was 30 million among the adult population aged from 20 to 79; in 1995 it was 135 million of the population; in 2000 it numbered 150,9 million (4,6%), in 2003 –194 million (5,1%),in 2010 – 285 million (6,4%), in 2011– 366 million of the population (8,3%), 2012– 371 million (8,3%), and in 2013 – 382 million (8,3%) of diabetic patients [3, 4, 7, 11, 15, 18]. Till 2030 the number of diabetic patients is suggested to become 552 million (9,9% or 1 diabetic patient per 10 healthy adults), and till 2035 – to 592 million (10,1%) [19].

The most common complications of diabetes mellitus are vascular disorders known as “diabetic angiopathy”. This notion includes lesions of small vessels – capillaries, venules and arterioles (diabetic microangiopathy), and large arteries (diabetic macroangiopathy). Diabetic angiopathy remains the main causes of inability to work of diabetic patients and in the majority of cases it determines the life prognosis for a patient.

Diabetic macroangiopathy are atherosclerotic by its origin.

Damage of the major vessels in diabetic patients occurs in the following forms: a) atherosclerosis – fatty plaques on the intima, b) calcifying Monckeberg’s sclerosis, c) diffuse fibrosis of the intima. These forms of lesions are found in the coronary, cerebral, renal arteries, arteries of the upper and lower limbs. Atherosclerotic lesions of the large and small vessels do not practically differ from atherosclerotic lesions of those individuals who do not suffer from DM [1, 8].

The risk of macroagiopathy is caused by such factors as hypertension, excessive body weight, hyperinsulinemia, smoking, metabolic disorders of cholesterol, lipids and lipoproteids, hemorheological disorders and hyperglycemia [1, 12].

Diabetic microangiopathy is characterized by thickening of the basal membrane, proliferation of the endothelium and accumulation of an excessive amount of PAS-positive substances. Microangiopathy may be of two types: a) hyalinic thickening of the arteriole walls, b) dilation of venules and thickening of the capillary walls [9, 10, 20].

Thickening of the basal membrane does not depend on age before the onset of the disease, hyperglycemia degree, residual ability to insulin secretion, frequency of ketoacidosis conditions, hypoglycemia and the type of therapy administered.

Pathogenesis of diabetic microangiopathy is characterized by the following factors: deterioration of the blood circulation resulting in hypoxia and decreased supply of the endothelium; carbohydrate metabolic disorders or a complex of polysaccharides (glycosaminoglycan) in the basal membrane of capillaries and connective tissue; glycosylation of proteins and accumulation of the final glycosylation junctions; decreased ability of the erythrocytes to deformity resulting in increased pressure in the capillaries and thickening of the basal membrane; accumulation of the immune complexes in the basal membrane and extracellular matrix followed by disorders of phagocytic activity of the basal membrane cells and humoral-mediated gene expression of different proteins; increased permeability of the vascular wall for plasma proteins or other macromolecules; microcirculation disorders [16].

In addition, other mechanisms participate in pathogenesis of diabetic microangiopathy: hormonal – elevation and fluctuation of somatogenic hormone, ACTH, cortisol, aldosterone and catecholamine levels in the blood. The role of “local” hormones is not excluded: bradykinin, serotonin and prostaglandins.

Genetic factors play a certain role in the development of diabetic angiopathy. Generalized character of diabetic microangiopathy and its combinations cause different forms of lesions in the clinics.

The problem of macro- and microvascular complications in case of DM is topical and

considerably determines the course and development of the disease. Investigations of many researchers deal with this pathology, although there is no a universally accepted and generalized view concerning morphogenesis of diabetic angiopathy, pathogenic factors, possibilities of prognostication and early control over the process development and its prevention. Therefore, the task of our study was to investigate the aortic wall and condition of the blood vessels in the aortic microcirculation of rats at the late terms of experimental diabetes mellitus. The article is a fragment of the scientific-research work of Danylo Halytskyi Lviv National Medical University "Morphological peculiarities of the blood circulation in the aortic wall of a rat within the norm and in case of experimental diabetes mellitus".

Objective of the study: to perform histological investigation of the aortic wall and condition of the blood vessels in the aortic microcirculation of rats at the late terms of streptozotocin-induced insulin dependent diabetes mellitus.

Materials and methods. 26 mature albino male rats of "Wistar" line with the body weight of 100-160 g were used as the material of the study. All the animals were kept in the vivarium, and the study was performed according to the "Regulations of Conducting Studies with the Use of Experimental Animals". Experimental diabetes mellitus was simulated by means of a single intraperitoneal injection of streptozotocin produced by "Sigma" in the dose of 7 mg per 100 g of the body weight (prepared on 0,1 mole of citrate buffer, pH = 4,5). The control group included intact albino rats of similar weight, sex and age. The development of diabetes mellitus during 2 weeks was controlled by the increasing glucose level in the blood measured by means of glucose oxidase method. The study was carried out since the second week of the experiment on animals with glucose level over 13,48 millimole per 1 liter. Three groups of animals were used in the study: 1) 10 intact rats; 2) 8 rats (5+3 control) with DM developed (6 weeks after streptozotocin injection); 3) 8 rats (5+3 control) with DM developed (8 weeks after streptozotocin injection). The material for histological examinations was taken after euthanasia of rats by means of intraperitoneal injection of sodium thiopental in the dose of 25 mg per 1 kg of the body weight. The samples for histological examination were pieces of the ascending portion, arch and descending portion of the aorta. Before fixation the material was washed in warm physiological solution. The material was fixed in 10% formalin solution during 24 hours prepared directly before its use. After fixation the material was washed under running water.

Numbered and stitched into gauze sacs pieces of the tissue were washed under running water during 24 hours. The material was dehydrated in ethyl alcohol of an increasing concentration during 20 hours: 73 ˚ ethyl alcohol; 80 ˚ ethyl alcohol; 86 ˚ ethyl alcohol; 86 ˚ ethyl alcohol; 96 ˚ ethyl alcohol; 96˚ ethyl alcohol.

Blooming and removing ethyl alcohol was made in organic solutions (xylene or chloroform – 2 portions per 1 hour each).

Paraffin infiltration of the samples was made in 2 dishes-thermostats at the temperature of 56˚ during 2 hours. Then the material was filled in paraffin blocks. The material filled in blocks was fixed and cut on the sliding microtome – МС–1 5–7 mcm thick. The specimens were stained with azan according to Heidenhain, and the nuclei – with Weigert's iron hematoxylin [9]. Then the stained specimens were placed into Canadian balsam (dissolved in toluene and xylene) and dried in the dry box. The specimens were examined and photos were made under the microscope МБИ–1 with magnification (ocular 7, objective lens 8), (ocular 10, objective lens 8), (ocular 7, objective lens 20), (ocular 10, objective lens 20).

Results and discussion. In the majority of animals after 6 weeks of streptozotocin-induced diabetes mellitus the aorta preserved its macroscopic structure. Histological examinations of the sections of the aortic wall of rats enables to find the signs of development of diabetic macroangiopathy, that is, atherosclerotic disorders of the endothelial layer of the vascular wall in the form of fatty consolidation (fatty stripes) occupying 30-50% of the internal aortic surface. These are massive fatty deposits of pale yellow colour mostly consisting of macrophages containing a number of lipids with a minimal amount of smooth muscular cells (Fig. 1).

According to the data presented in the professional literature [1] such kinds of changes

do not result in vascular occlusion and therefore do not stipulate pronounced clinical manifestation. The end of the sixth week of the experimental DM was characterized by the signs of arteriole hyalinosis and fine inflammatory infiltrations in the form of macrophagocytes and lymphocytes. The walls of the blood circulatory bed are thickened and eosinophilic (Fig. 2).

Microaneurysms of the arterioles are found. The internal membrane of the arterioles is presented by the endothelial cells located on the basal membrane. The loose fibrous connective

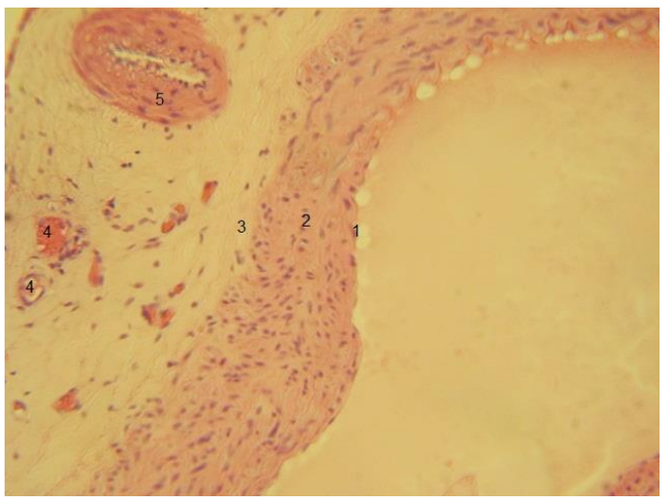

Fig.1. The aorta of an albino rat 6 weeks after streptozotocin-induced diabetes mellitus. Micrograph. Staining with hematoxylin and eosin. Magnification: 200. 1 – internal membrane of the aortic wall; 2 – median membrane of the aortic wall; 3 – external membrane of the aortic wall; 4 – capillary; 5 – arteriole wall.

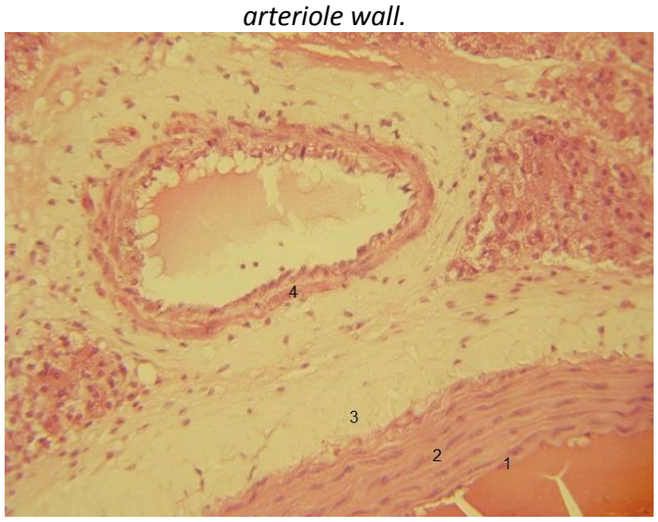

Fig. 2. The aorta of an albino rat 6 weeks after streptozotocin-induced diabetes mellitus. Micrograph. Staining with hematoxylin and eosin. Magnification: 400. 1 – internal membrane of the aortic wall; 2 – median membrane of the aortic wall; 3 – external membrane of the aortic wall; 4 – arteriole wall.

tissue is located in the subendothelial area with its penetration into the lumen of vessels. The loose fibrous connective tissue is swollen. Collagen fibers are pale stained with eosin and swollen without clear outline. The venules are full of blood with erythrostasis with their agglutination into fine conglomerates usually located in the center of the lumen (Fig. 3).

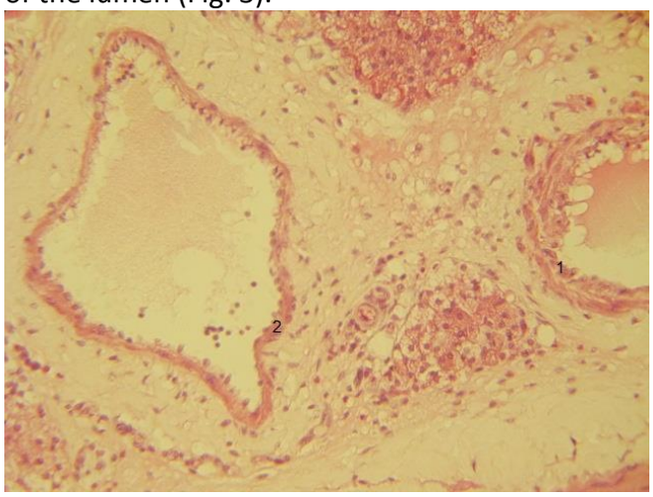

Fig. 3. The aorta of an albino rat 6 weeks after streptozotocin-induced diabetes mellitus. Micrograph. Staining with hematoxylin and eosin. Magnification: 400. 1 – arteriole wall; 2 – venule wall.

Endothelial cells with flat nuclei form their internal membrane. Endothelial cells are absent on certain intervals where they adhere to the basal membrane. Smooth myocytes form the middle and external membranes, a part of them is located longitudinally, and another part – circulatory concerning the vascular wall. Loose fibrous connective tissue form adventitial membrane where clear outlines of thin eosinophilic collagen fibers are visualized. The latter penetrate into the interstitial tissue with considerable swelling in the perivascular areas.

After 8 weeks of streptozotocin-induced diabetes mellitus deep destructive aortic changes are found on histological sections. The internal surface of the aortic wall became dark brown in colour and tuberous, the intima was irregular, whitish in colour, and all its surface containing tubercles and depressions (Fig. 4).

The areas of an orange colour are seen on the tubers with white margins, and yellow spots. According to the data of professional literature [5, 6, 8], whitish tubers are fibrous plaques formed due to the growing of the connective tissue into the detritus thickness. Orange spots with white margins are intramural hematomas as the results

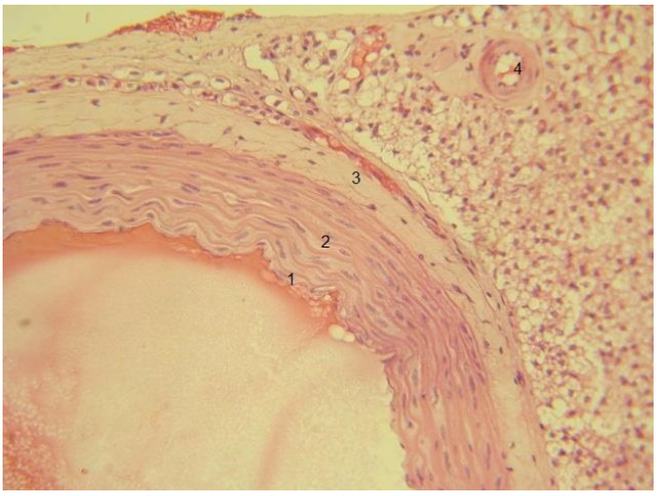

Fig. 4. The aorta of an albino rat 6 weeks after streptozotocin-induced diabetes mellitus. Micrograph. Staining with hematoxylin and eosin. Magnification: 200. 1 – internal membrane of the aortic wall; 2 – median membrane of the aortic wall; 3 – external membrane of the aortic wall; 4 – pre-capillary arteriole.

of plaque destruction or its calcification in case of atheromatosis. The white margin is the area of calcification, and the plaques available are indicative of progressing of atherosclerotic changes and stratification of a new "wave" of lipoidosis on the previous old changes, exfoliation of a part of the endothelial aortic layer is indicative of th formation of exfoliating aneurism [6].

Elastic fibers of the median membrane are not well-organized, inter-laminar spaces are wide with loss of inter-laminar relations, especially expressed in the external layers of the median membrane.

After 8 weeks of streptozotocin-induced diabetes mellitus the links of the blood circulation are dilated, in certain areas arterioles with irregular outlines are found together with dilated venules, swelling is seen round the vessels. The lumen of arterioles is filled with homogenic oxyphilic stained fluid with characteristic signs of hemolysis and absence of cellular elements. Due to plasmatic infiltration of the median and external membranes of the arteriole walls are thickened, they are of homogenous rosy-pink colour with the signs of fibers separation (Fig. 5). One layer of the endothelial cells is visualized in the microstructure of the venules located on the basal membrane. In venules with longitudinal section endothelial cells are located at a distance one from another with acellular areas –

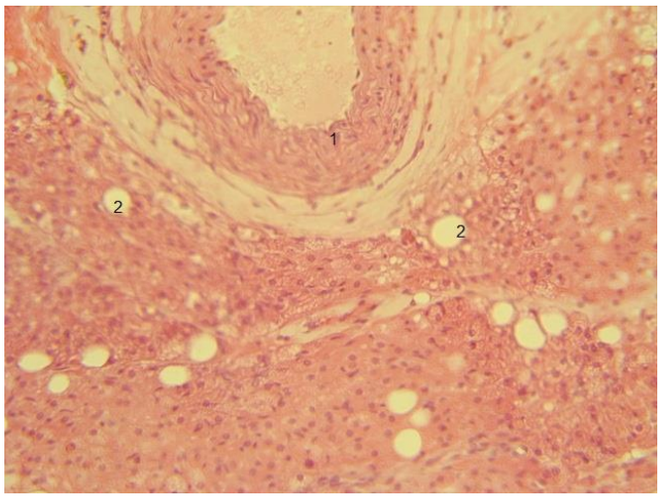

Fig. 5. External aortic wall of an albino rat 8 weeks after streptozotocin-induced diabetes mellitus. Micrograph. Staining with hematoxylin and eosin. Magnification: 400. 1 – arteriole wall; 2 – vacuole.

perforations. The shape of endothelial cells is flat and elongated. Erythrocytes and plasmatic fluid are found in the lumen of vessels. The plasmatic fluid has parietal location on a large interval and it penetrates into the perivascular intersticium through the intervals between endothelial cells. In addition, stasis and sludge are found with marginal localization of erythrocytes and formation of agglutination clots, which partially or completely block the lumen of vessels (Fig. 6).

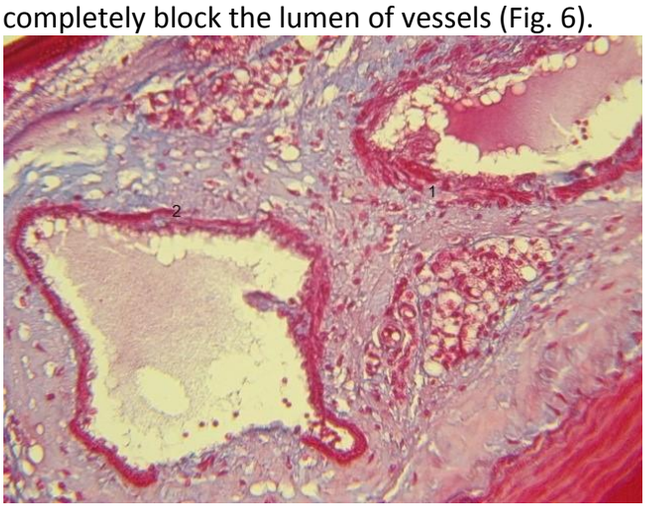

Fig. 6. External aortic membrane of an albino rat 8 weeks after streptozotocin-induced diabetes mellitus. Micrograph. Staining with hematoxylin and eosin. Magnification: 400. 1 – arteriole wall; 2 – venule wall.

Conclusions. Our study resulted in determination of morphological peculiarities of the aortic wall on histological level 6 and 8 weeks after streptozotocin-induced diabetes mellitus. After 8 weeks of streptozotocin-induced diabetes mellitus deep changes are found in the aortic wall and vessels of its circulation bed which is

indicative of the development of diabetic arteriosclerosis.

Prospects of further studies. New data obtained concerning microstructural changes of the aortic wall and links of its circulation bed investigated on the experimental DM at the late stages can be of a certain practical value in future investigations, elaboration of new diagnostic and preventive measures concerning this pathology.

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