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Vascular changes in pancreas in diabetes caused by abnormal metabolits of tryptophan aggravate developing of diabetes

Contrary to many models of experimental diabetes caused by chemicals, diabetes induced by endogene synthesis of xanturenic acid (XA), a metabolit of abnormal Tryptophan metabolism, approached to human diabetes. Meanwhile in these conditions of natural developing of diabetes not in result of artificial injection of diabetogenic substances, are not investigated yet state of blood vessels and blood circulation as in exocrine pancreas tissue as in pancreatic islets. Authors showed developing in experimental XA-diabetes of numerous destructive changes of blood vessels of a pancreas, fibrinoid changes of parenchyma of pancreas tissue, dystrophya and necrosis of exocrine and endocrine pancreas tissue; necrotic changes of endothelium of arteries. In pancreatic islets: necrosis of endothelium and cells in pericapillar. Authors conclude that described changes can result aggravation of developing of diabetes.

Key words: diabetes, pancreas, exocrine tissue, B-cells, vascular changes.

The main cause for mortality of patients with type 2 diabetes are cardiovascular complications [1, 2]. The leading role in development of these complications belongs to a hyperglycemia which is a cause of a number of pathological processes such as endothelial dysfunction, oxydative stress, changes of rheological properties of blood in macro- and microvessels [3]. It was reported that thickening of basal membranes is developed in capillaries as result of fixation on the endothelium of vessels of amorphous material consisting mainly of mucopolysaccharides [4]. It is known that microcirculation in diabetes accompanied by aggregation of blood cells and damage an endothelium. Sclerosis, inflammation and destruction of vessels result developing of heavy blood circulation [5].

Research objective: to study state of histostructure of blood and of stroma of tissue of pancreas in experimental Xanthurenic acid induced experimental diabetes.

Materials and methods

Diabetes in animals caused by containing of animals on diet by Y.Kotake [6] stimulated endogene synthesis of 4,8-dihydroxyquinolin-2-carboxylic acid (Xanthurenic acid, XA) which possess diabetogenic properties due to direct selective destruction of B-cells as to binding and inactivation of insulin [6]. 72 white rats Vistar 160–240 g. body wheight were used. Animals were distributed for 5 groups. Rats of Groups 1, 2 and 3 were contained 60, 90 and 120 days respectively on a diet stimulated endogene synthesis of XA. The diet components included starch, butter, sugar, casein, yeast and salt additives. Group 4 (diet+vit. B6), investigation of blood Glucose concentration and of Xanturenyria excluding histological and histochemical analysis of pancreas tissue: animals were treated within period of containing on diet by injections of water solution of vit. B6 8,7 mg/kg per day. Group 5 (control 2) — intact animals. Blood Glucose control-weekly by Glucose oxydase method. Concentration of Xanturenic acid in urine [7] was measured monthly and body weigh in the beginning and at the end of experience.

Histology. Samples of pancreas tissue fixed in Bouin liquid, carried out in alcohols 70º, 80º,90º and 100º, filled in paraffin. Leica 2125 rotation microtome used for preparing sections 4–5 mcm. For survey microscopy of tissue of a pancreas staining technology was applied using hemalaoun of Mayer and eosin [8] as hemathein of Mayer [9].

Histochemical methods: Method by Gomori, a differential staining of β-and α-cells by Aldehyde fucshine and Helmi's mix [10, 11]. Deposited form of insulin [12] revealed as violet granules in cytoplasm of B-cells. Kikui Y. and coll. method [13] using reagent Victoria 4R with floxyn, phosphorum-volfram acid and the light green was used for differential staining of β- and α-cells. Immunohistochemical method [14] staining of insulin by kits from DAKO (Denmark) was used with photometrical measuring intensity of staining of B-cells [15]. Parameter K was calculated as AB1/AB2. AB1 — light absorbtion of B-cells; AB2 — light absorbtion of exocrine tissue.

Results

Blood Glucose level. 60 days containing of rats on diet: increasing of blood Glucose level for 1,5–1,8 times in majority of number of animals excluding 6 rats have kept normal value. On average level of a glycemia is 6,91 ± 0,36 mmol/l ($p \leq 0,05$) in compared with initial 4,20 ± 0,11 mmol/l (Fig. 1). On 90th day containing on diet blood Glucose level is increased for 1,9 times comparatively with initial ($p \leq 0,001$). In some animals increasing for 2,5–3 times was observed. 4 rats have not changes of blood Glucose level. We observed till 90th day decreasing of body weight of experimental animals on the average from 216,84 ± 4,07 g to 183,20 ± 4,06 g ($p \leq 0,001$).

At 120th day of experience concentration of blood Glucose level was increased for 2,8–3 times on average, until 11,81 ± 0,56 mmol/l ($p \leq 0,001$) comparatively with initial 4,11 ± 0,19 mmol/l by 2,8–3 times; $p \leq 0,001$. The body weight was decreased for 23–25 %; ($p \leq 0,001$) comparatively with initial.

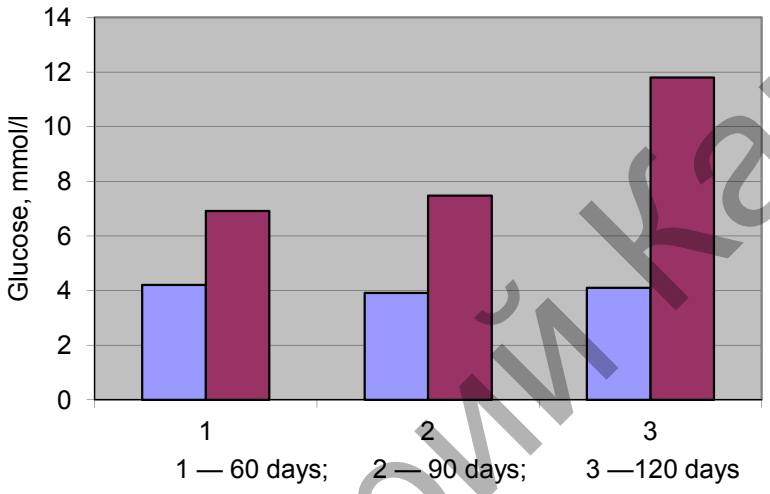
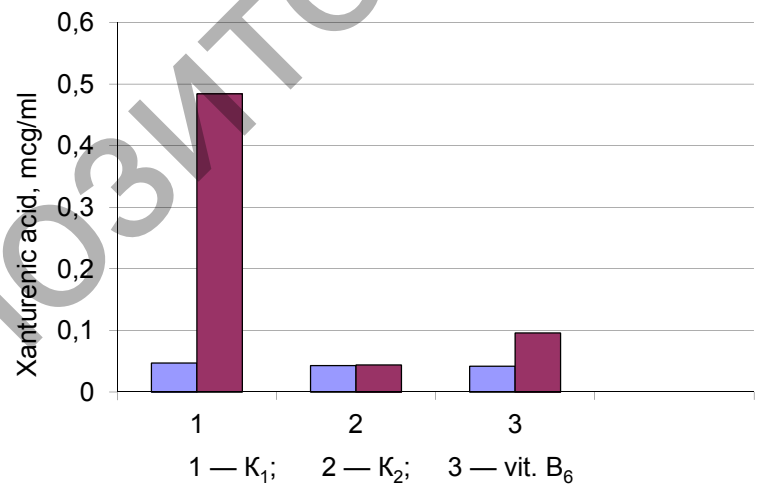


Figure 1. Blood Glucose concentration in animals contained on diet



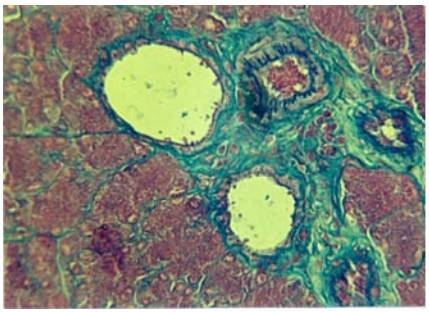
K_1 — animals contained on diet; $p \leq 0,001$; K_2 — intact animals (control);
blue column — before diet; red column — 120 days on diet

Figure 2. Concentration of Xanturenic acid in the urine of rats contained on diet and diet+vit. B6

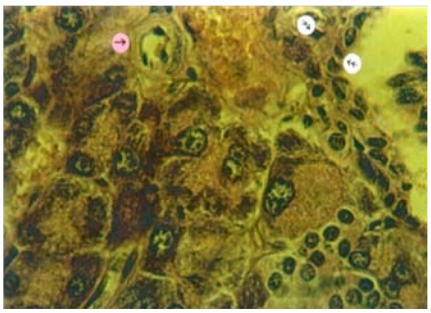
The analysis of level of xanturenuria of rats contained on a diet for 120 days showed reliable increase in compared with control for 9–10 time ($p < 0,001$) (Fig. 2). Thus, the maintenance of animals on diet accompanied by development of marked hyperglycemia reaching the maximum till 120 day.

Morphological researches

30th days containing on diet result developing of: disturbances of blood circulation; fibrinoid changes of intraglobular arteries of exocrine tissue; necrosis of veins, destroying of vessel's wall, hemostasis, lysis and infiltration of eritrocytes in tissue (Fig. 3.1); distribution of fibrinoid processes to parenchyma.



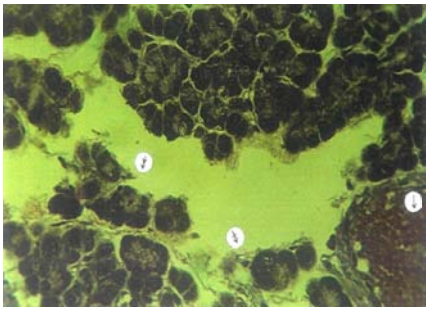
3.1



3.2

3.1 — Pancreas tissue. Stasis and hemolysis in interglobular arteries (→). Destruction and necrosis of connecting tissue of blood vessels (→→). 30 days on diet. Staining by Victoria 4R; ×280;

3.2 — Pancreas tissue. Fibrinoid changes of arteries (→). Necrosis of veins (→→) and distrophya of ductus (→→→). 60 days on diet. Staining by Hemalaoune and eosin; ×280;



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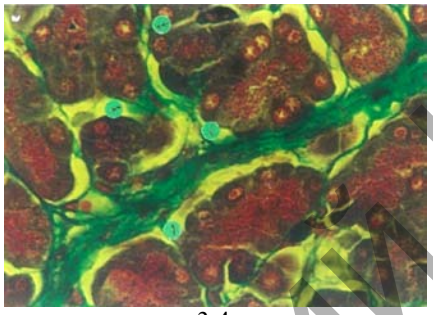
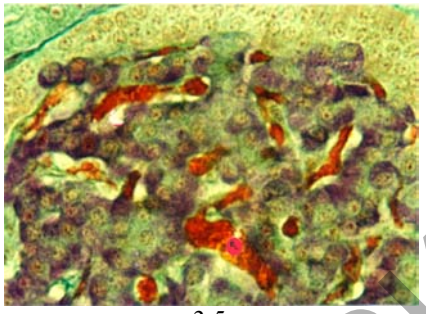


3.4

3.3 — Pancreas tissue. Stasis and hemolysis in interglobular vein (→). Fibrinoid chanhes, destruction of acinuses and atrophya of exocrine parenchyma (→→). 60 days on diet. Staining by Hemalaoune and eosin; ×280;

3.4 — Exocrine tissue of pancreas. Gwoving of collagen fibers in globules (→). Hypostasis in inter acinuses spaces and collagen fibers around acinuses (→→). 60 on diet. Staining by Victoria 4R; ×900;



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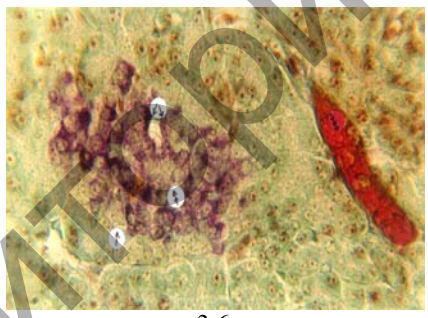
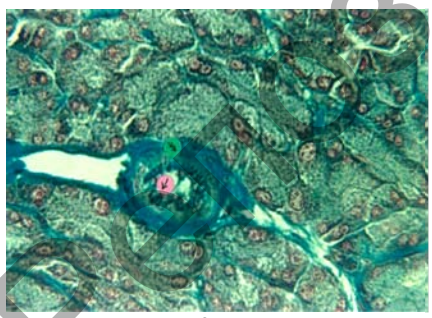


3.6

3.5 — Pancreatic islet. Marked hyperemia and lysis of eritrocytes in capillaries (→). Degranulation and destruction of B-cells (→→). 60 days on diet. Staining by Aldenyde Fucshine and Helmi; ×700;

3.6 — Pancreatic islet. Degranulation of B-cells (→). Distrophya and necrosis of B-cells (→→). Thrombosis of veins (→→→). 100days on diet. Staining by Aldenyde Fucshine and Helmi; ×280;



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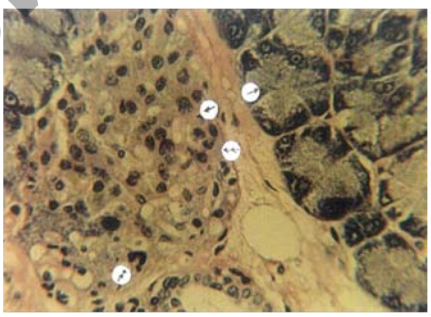

3.8

3.7 — Pancreatic islet. Destruction of internal capsule (→) and hyalinosis on middle and external capsules of arteries (→→). 120 days on diet. Staining by Victoria 4R; ×280;

3.8 — Pancreas tissue. Dissociation of acinuses (→). Destruction of islets, necrosis of B-cells (→→). 120 days on diet. Staining by Hemalaoune and eosin; ×280

Figure 3. State of pancreas tissue and vascular changes in exocrine and endocrine tissues

60th days containing on diet accompanied by: developing of destructive changes in parenchyma (Fig. 3.2); hyperemia and destruction of capillaries; growing of collagen fibers accompanied by necrosis of adventicium of vessels; necrosis of veins with infiltration of eritrocytes in tissue; fibrinoid changes in parenchyma of tissue, fat infiltration and fat necrosis of the gland's cells; intraglobular fibrillogenesis; infiltration of parenchyma by collagen fibers (Fig. 3.4); hemostasis and lysis of eritrocytes in capillaries of islets (Fig. 3.5). Arterioles: fibrinoid changes, thickening of walls. Venules: necrosis, destruction, infiltration of eritrocytes in tissue. Fibrinoid changes of stroma of pancreas, growing of fat tissue in interseptal spaces;

pressing of acinuces by fat tissue and forming of necrosis centers in gland's tissue; developing of intraglobular fibrillogenesis that accompanied by thickening of intersticium of pancreas tissue. Infiltration of parenchyma by collagen fibers, dissociation of parenchyma for little gland segments consisting of a few acinuses (Fig. 3.4).

90 days containing on diet result: hemorrhagic necrosis of exocrine tissue, sclerosis of wall of arteries; growth of fibrous structures; sclerosis of capillaries. Hyperemia in veins and in capillaries; fibrinoid changes of arterioles; thickening of basal membrane of endothelium; stagnant hyperemia in vessels of venous collector; alteration of arterial endothelium, destruction of endothelial layer in arteries and in interglobular veins, proliferation of facile muscle cells; destruction and distrophya of walls of vessels, infiltration in tissue of components of blood; sclerosis and inflammation, concentration of leucocytes in the gleam of vessels and infiltration of parenchyma of tis- sue; growing of fat tissue in islets with degranulation, distrophya and necrosis of B-cells, stasis and hemolysis in capillaries of islets (Fig. 3.6); infiltration of lymphocytes and leucocytes outside of vessels, homogenization of blood cells in vessels; thrombosis of veins and capillaries; developing of hyalinosis in arteries (Fig. 3.7); destruct- tion, distrophya and incapsulation of acinuses; agglomeration of fibroblasts, lymphocytes and collagen fibers nearest destroyed acinuses. Near vascular bunches, islands and of acinuses; the wide cavities, filled by homogeneous consistence liquid near vascular bunches as near island (Fig. 3.8).

100–120 days containing on diet. Inflammation of arteries and veins, infiltration of leukocytes outside of arteries and veins parenchyma; necrosis of parenchyma, growing of fat tissue accompanied by intraglobular lipomathosis; islets: marked hyperemia, stasis in capillaries, degranulation and necrosis of B-cells (Fig. 3.6); marked hyperemia in veins in combination with infiltration of lymphocytes into the wall of vessels; gomogenisation of collagen fibers in adventicium of arteries, concentration of fibroblasts between collagen fibers.

Thus, disturbances of metabolism in animals contained on diabetogenic diet result marked destructive changes in arteries, veins and capillaries as in islets as in exocrine tissue of pancreas that accompanied by destruction of walls of vessels and fibrinoid changes of stroma. Noted above changes accompanied by disturbances of circulation of blood in vessels and by hemostasis which is estimated as sign of acute pathological process [3.3].

Stasis is a frequent effect in disturbances of cardiovascular system and of blood circulation caused by external causes [16]. J.Andersen and coll. [17] supposed that accumulation in their wall of fibronectin, of type 4 collagen, hyaluronic acid and calcium result damage of blood vessels. Dysfunction an endotelium accompanied by angiospasm, thrombosis and tendence for developing of atherosclerosis [18, 19]. Insulin resistance is estimated as one of cause of destruction of blood vessels [20]. On 30^{th} day containing on diet we observed accumulation of fats in wall of interglobular arteries and developing of lipomathosis in globules. Diabetes accompanied by marked forms of this processes as by fibrosis and lipomathosis of intraglobular spaces [21].

Dysfuction of endothelium is shown by angiospasm, tendencies for formation of thrombs and developing of atherosclerosis [19].

Thus, at first week of experience disorders of blood circulation and destructive changes of vessels were developed and accompanied by fibrinoid changes, fibrosis and lipomathosis in intraglobular spaces. Stagnation and long time prolonged hemostasis result destruction of vessel's wall and exit of eritrocytes in exocrine tissue. Formation of blood clots is a symptom of chronic process. Vascular changes, result developing of necrosis in acinuses and of atrophya of exocrine tissue of pancreatic islets. Proliferation of epithelial tissue and periductal sclerosis of gland's ductus as hemorrhagic sclerosis of exocrine tissue cells with sclerosis of capillaries walls are estimated as structure symptoms diabetes mellitus [16, 21].

As it was observed in pancreas sections of rats contained on a diet from the 30th till 120th days, sclerotic changes underwent some stages of development: from plasmatic infiltration and fibrinoid changes of a wall of vessels to hyalinosis. Hyalinosis of small arteries and the capillaries, developing as result of plasmatic infiltration is widespread at diabetes and most expressed in a brain, a kidney, in retina and islets.

Thus, analysis of results of research of series of experience showed accruing suppression of function of the B-cells, accompanied by degenerative changes and decreasing of insulin content in cytoplasm of B-cells for 76 % caused by XA.

Developed multiple wascular changes in blood vessels as in pancreatic islets as in exocrine tissue result developing of fibrinoid changes, of sclerosis of stroma including hyalinosis of arteries and sclerosis of capillaries and veins. These changes aggravate developed diabetes in spite of the fact that are not its direct cause.

Acknowlegement

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Триптофанның диабетогенді метаболиттерінің ағзаға әсерінен қан айналымының бұзылуы және оның тәжірибелік қант диабетінің өтуін қиындатуы

Авторлар эксперименталды ксантурендік диабет кезінде ұйқыбез қантамырларының деструкциясы, оның паренхиматоздық тінінің фибриноидті өзгерістеріне себеп болғанын байқаған. Бұл өзгерістермен экзо- және эндокриндік бөлімдердің дистрофиясы мен некрозы ере жүрген. Ұйқыбез аралшықтарында капиллярлар эндотелийінің деструкциясы және капиллярлар маңындағы В-жасушалардың некроздық өзгерістері айқындалған. Авторлар ұйқыбездің инсулярлы аппаратының жетіспеушілігі, диабетогендік емдом әсерінен көмірсулар мен майлардың алмасуының бұзылыстарына, сондай-ақ қан

айналымның бұзылыстарына тәуелді екенін болжайды. Сондай-ақ зат алмасу процестердің бұзылу нәтижесінде пайда болған қантамырлардың өзгерістері В-жасушалардың жаңадан деструктивтік өзгерістеріне себеп болып диабет барысын күшейте түспек.

А.А.Кикимбаева, А.М.Тулиева, А.А.Жузжасарова, А.Е.Мораш,
Р.С.Мамитхан, Г.Д.Дилдабекова

Нарушения кровообращения при воздействии на организм диабетогенных метаболитов триптофана, усугубляющие течение экспериментального сахарного диабета

При экспериментальном ксантуреновом диабете, который по характеру развития и течения приближается к диабету у человека, авторы исследовали развивающиеся деструктивные изменения кровеносных сосудов поджелудочной железы. Они сопровождаются фибриноидными изменениями паренхиматозной ткани, те, в свою очередь, дистрофией и некрозом экзо- и эндокринной ткани. В панкреатических островках выявлены деструкция эндотелия капилляров и некротические изменения перикапиллярных В-клеток. Авторы полагают, что сосудистые изменения, не являясь прямой причиной диабета, могут значительно утяжелять его течение.

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