YEVROSIYO PEDIATRIYA AXBOROTNOMASI/ЕВРАЗИЙСКИЙ ВЕСТНИК ПЕДИАТРИИ

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МОРФОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ РОСТВЫХ ЗОН ТРУБЧАТЫХ КОСТЕЙ У ПОТОМСТВА КРЫС С ЭКСПЕРИМЕНТАЛЬНЫМ САХАРНЫМ ДИАБЕТОМ

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Аннотация: В работе представлены результаты морфометрического анализа метаэпифизарного хряща трубчатых костей потомства крыс с экспериментальным сахарным диабетом на 14, 21, 30, 45, 60 сутки постнатального онтогенеза. У крыс с экспериментальным сахарным диабетом уменьшается длина первичных губчатых трабекул и снижается количество остеобластов. Значительные изменения выявляются в пролиферативных зонах метаэпифизарного хряща с уменьшением количества клеток. В результате замедления дифференцировки хряща в типичный костный балок происходит расширение деструктивных зон метаэпифизарного хряща трубчатых костей.

Ключевые слова: метаэпифизарный хрящ, трубчатые кости, морфометрия, экспериментальный сахарный диабет.

EXPERIMENTAL QANDLI DIABET BILAN KASALLANGAN KALAMUSHLARDA NAYSIMON SUYAKLAR O‘SISH ZONALARINING MORFOLOGIK O‘ZGARISHLARI.

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Annotatsiya: Ushbu maqolada ontogenezning 14, 21, 30, 45, 60 kunida eksperimental qandli diabet bilan og'rigan kalamushlarning nasllarida naysimon suyaklar metaepifizar tog’ayining morfometrik tahlili natijalari keltirilgan. Eksperimental qandli diabet ta‘sirida kalamushlarda birlamchi g’ovak trabekulalarning uzunligi va osteoblastlar soni kamayadi. Hujayralar sonining kamayishi bilan metaepifizar tog’ay proliferativ zonalarida sezilarli o'zgarishlar aniqlanadi. Naysimon suyaklarning metaepifizar tog’ay zonasida tog’ay to’qimasining tipik suyak balkalariga differensiatsiyasining sekinlashishi natijasida destruktiv zonalar kengayishi kuzatiladi.

Kalit so'zlar: metaepifizar tog’ay, naysimon suyaklar, morfometriya, eksperimental qandli diabet.

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MORPHOLOGICAL CHANGES OF THE GROWTH ZONES OF TUBULAR BONES IN THE OFFSPRING RATS WITH EXPERIMENTAL DIABETES MELLITUS

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Resume: This article presents the results of the morphometric analysis of the metaepiphyseal bone of the tubular bones in the offspring of rats with experimental diabetes on the 14th, 21st, 30th, 45th, and 60th days of ontogenesis. In rats with rats with experimental diabetes mellitus, the length of primary spongy trabeculae decreases and the number of osteoblasts decreases. Significant changes areidentified in the proliferative zones of metaepiphyseal cartilage with a decrease in the number of cells. As a result of slowing down the differentiation of cartilage to typical bone balck, the destructive zones of the metaepiphyseal cartilage of tubular bones are expanding.

Key words: metaepiphyseal cartilage, tubular bones, morphometry, experimental diabetes mellitus.

Relevance: Currently, diabetes mellitus is a major problem throughout the world. Diabetes mellitus is recognized by the World Health Organization as one of the diseases of global public health importance. According to the latest data from the International Diabetes Federation, more than 425 million of people worldwide suffer from this disease, and most of them are patients with 2 type of this diabetes..In Uzbekistan, the numbers of patients with diabetes mellitus is more than 245 thousand, of which more than 2,300 are children and 879teenagers . The great social significance of diabetes mellitus is that due to vascular complications micro- and macroangiopathies, it leads to early disability and mortality. The permanentincreasing costs associated with this disease place a heavy burden on healthcare. There is an obvious need to develop further measures aimed at reducing the prevalence of diabetes mellitus and its complications [3, 5,6].

It is known that diabetes mellitus of the first stage undergoes restriction of peripheral vessels.The great influence is occurred by growing organism. Therefore, it considered appropriate to study the morphological changes in bone growth zones against the background of diabetes. Metaepiphyseal cartilage ensures the growth of bones along the length, being a cartilaginous layer between the bony epiphysis and diaphysis of the proximal and distal parts of the tubular bones. The growth zone continues throughout the entire period of bone growth, and the processes of cell reproduction and differentiation, biosynthesis as well

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as mineralization of the main intercellular substance continue quite intensively [1, 2, 3, 5].

Purpose: To explorepathomorphological changes in the growth zones of long bones of rats with alloxandiabete.

Material and methods: The experimental study was carried out on 34 female rats weighing 180-200 g. After pregnancy, the animals were divided into 2 groups: 1 - control group - 9 rats; the second group of 25 pregnant animals with alloxan diabetes. Diabetes mellitus was treated by intraperitoneal administration of a solution of alloxanatetrahydrate at a rate of 15 mg per 100 g of body weight, causing a preliminary 2 days of fasting. Alloxan is a product of the formation of uric acid and is a white crystalline substance that turns pink in the air. The drug acts as a diabetogenic drug only with intraperitoneal administration of alloxan monohydrate once in the form of 0.9% normal saline solution at a dose of 150 mg/kg [2]. The experimental dose was carefully adjusted to avoid excessive damage to pancreatic tissue. The rat had a long-term form of diabetes with persistent hyperglycemia, glycosuria, polyuria, polydipsia, polyphagia and sometimes ketonuria. Alloxan solution was prepared by diluting the crystalline substrate in sterile distilled water.

Histological examination of mataepiphyseal cartilages of long bones was carried out on days 14, 21, 30, 45 and 60 of life of generation,,obtained from healthy individuals and during pregnancy, which occurred against the background's of experimental diabetes mellitus. Pieces of cartilage tissue were fixed in 10% neutral formalin and then embedded in paraffin. Histological sections were stained with hematoxylin and eosin, according to Van Gieson, and the PAS reaction was performed.

Histomorphological and morphometric studies were carried out in the IPSUM PATOLOGY laboratory.

Research results: In the studied pregnant individuals, after administration of a diabetogenic dose of alloxanatetrahydrate, gradual changes in blood glucose levels were observed: the early phase is hyperglycemic, reaching a maximum during the initial 6 hours; the middle stage - hypoglycemic, which mainly manifested itself during the first day of observation, and the further stage - the phase of persistent hyperglycemia was determined from the second day. The initial signs of diabetes manifested themselves in the form of a sharp increase in daily water consumption (more than 100 ml), polyphagia, polyuria, hyperglycemia, sudden weight loss, and hair loss. At different times of the experiment, trophic ulcers of the lower leg, gangrene with self-amputation of the tail developed. 7 animals died, in 4 animals of the main group the pregnancy was interrupted as a result of hyperglycemic or hypoglycemic

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coma at different stages of the development of alloxan diabetes and were removed from the experiment.

The negative impact of alloxan diabetes is clearly reflected in the number of offspring obtained as a result of the experiment, as well as in terminations of pregnancy and, of course, in the observed deaths (Table 1).

Table 1.

Characteristics of experimental animals (M±m)

Number of starting females, pcs	**Mass of rats, gr.**	**Died, pcs**	**Termination of pregnancy, pcs.**	**Number of rats received, pcs.**	**Mass of rats, gr.**
Control -9	186 ±17,3	—	—	**23**	18,6±0,96
Experienced -25	191,3±19,1	**7**	**4**	**51**	16,2±0,24

It is known that insulin deficiency in alloxan diabetes leads to increased breakdown of tissue proteins, increased intake of amino acids into the blood, and an increase in total blood nitrogen. A characteristic disorder of lipid metabolism is an increase in the serum content of low-density lipoproteins and triglycerides, as well as a decrease in the content of high-density lipoproteins.

15 minutes after the administration of alloxan, convulsions were observed in the animals in our experiment. In the interval of 5-7 hours, the tails turned blue, and during the next 24-48 hours there was a strong increase in appetite for water, polyuria, slight tremors and tachycardia. 48 hours after the administration of alloxan, blood was collected from between the lower teeth of the rats for laboratory examination. An increase in blood glucose to an estimated 18,5 mmol (Plus Satellit. Russia) confirmed hyperglycemia (Table 2).

Table 2

Indicators of activity phases of alloxan action in experimental animals

Groups	n	**30th minute**	**After 3 hours**	**After 4-8 hours**	**After 24 hours**	**After 48 hours**
Control	9	6,58± 1,7	6,58± 1,7	6,58± 1,7	6,58±1,7	6,58±1,7
Experience	25	4,5±1,9**	7,4±1,4**	4,2±1,8**	12,5±1,9***	18,5±1,9***

Note: *P ≤ 0.05, **P ≤ 0.001 compared to animals in the control group.

The cartilages of the growth zones of long tubular bones of the controlled generation o and

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experimental rats were subjected to morphological study during growth dynamics at the stages of early postnatal ontogenesis.

Histological results and morphometric studies have shown that alloxan diabetes mellitus of the maternal body during pregnancy and breastfeeding of a diabetic mother lead to changes in the mechanism of bone growth in the offspring. The main negative impact of diabetes mellitus is reflected in the cartilage tissue in the growth zone of long tubular bones.

As a result of the study, it was found that in rat pups whose mothers received alloxan during pregnancy, the thickness of the epiphyseal cartilage (plate) is less than in control animals in all age groups:in 14-day-old rat pups - by 24.9% ($p \leq 0.001$), in 21-day-old rat pups - by 18.9% ($p \leq 0.001$), in 30-day-old rat pups - by 14.8% ($p \leq 0.001$), in 45-day-old rat pups - by 24.5% ($p \leq 0.001$), in60-day-old rat pups - by 29.3% ($p \leq 0.001$), respectively (Table 3).Analyzing the change in the thickness of the epiphyseal cartilage in the age aspect,it can be noted that in rat pups of both groups its thickness decreases with age. However, in rat pups of the control group, the decrease in the thickness of the epiphyseal cartilage is most clearly manifested by the age of 30 days (by 28.6%), while in the pups of the experimental group - by the age of 60 days (by 26%) (Table 3). The decrease in the thickness of the epiphyseal cartilage in rat pups of the experimental group occurs not due to the thickness of the zone of definitive cartilage, but due to a decrease in the thickness of the zone of proliferating cartilage, which indicates a violation of the mechanism of formation of the epiphyseal plate.

Table 3

Comparative analysis of morphometric indicators of epiphyseal cartilage in the stages of normal growth and the effects of alloxan diabetes mellitus.

Analyzed indicators	**Day of development of control rats** (n=23)				
	14	21	30	45	60
Thickness of the epiphyseal plate, µm	219,71 ± 18,15	191,52 ±17,14	164,58 ± 12,08	121,54 ± 9,08	104,98 ± 8,12
Thickness zones of Indifference cartilage, µm	18,87 ± 1,39	17,97 ±1,28	16,96 ± 1,57	15,27 ± 1,37	14,97 ± 0,61
The thickness of the area of proliferating cartilage, µm	119,95 ± 2,67	115,07 ±3,29	95,56 ± 4,97	71,07 ± 2,51	65,14 ± 5,57
The thickness zone of definitive cartilage, µm	85,15 ± 6,71	59,11 ±6,43	56,34 ± 5,27	39,54 ± 3,48	30,03 ± 1,65

Note: * - $P \leq 0.05$, ** - $P \leq 0.001$ compared to animals in the control group.

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The first zone in the direction from the epiphysis to the diaphysis is the zone of indifferent cartilage. This zone is separated by a continuous bone plate from the bony epiphysis. Cellular elements in it, as a rule, are located mainly in intercellular substance with two or three wrong rows. Chondrocytes of rat pups in the experimental group are located chaotically, singly, in pairs, and closer to the next zone in small groups of 4-5 medium-sized cells. The shape of chondrocytes is ellipsoidal or spindle-shaped, sometimes round-shaped cells are found. In the center of the chondrocyte there is a spherical or rounded nucleus surrounded by cytoplasm.

A small number of these cells have flattened nuclei. The boundaries of the epiphyseal plate are uneven and smoothed.

Morphometricresearch on indifferentcartilage of rat from the experimental group showed that in 14- and 60-day-old rats the thickness zone of indifferent cartilage is less than in the control by 12.5 and 8.2%, respectively. At the age of 21, 30 days, its thickness differs slightly from the indicators of indifferent cartilage in the control group of animals (Table 3.).

The zone of proliferating chondrocytes is directed towards the diaphysis. Proliferative processes in this zone determine the growth of bones in length , which monitors both the number of active growth cells and the formation of the latter collagen and proteoglycans. The zone is represented by cartilaginous cells that have a wedge shape and are superimposed one on one in the form of coin columns, this forming “columns” with 12–15 cells each. Columns of chondrocytes are separated from developing columns by layers of ground substance with well-defined fibrils that run in a long direction. Mitotic figures were revealed in the cells of this area. It was noted that in the experimental group rats, mitotic figures occurred singly. Enlarged voids were identified among the columns of chondrocytes.

In animals of the experimental furnace group, the zone of proliferating cartilage decreases in age and significantly lags behind the indicated indicator in 14-, 21- and 60-day-old animals by 30.3% ($\rho \leq 0.05$), 23.8% ($\rho \leq 0$). .05) and 38.7% ($\rho \leq 0.001$) compared to animals of the control group of the corresponding ages (Table 3).

The number of proliferating cells that exhibit proliferative activity and develop at different stages of division in newborn rats of the experimental group decreased by 19.3% compared to the control. In 14-, 21-, 30-, 45- and 60-day-old animals whose mothers induce alloxan during pregnancy, the number of proliferating cells increases with age, but lags behind the control indicators by 32.2% ($p \leq 0.05$).), 29.7% ($p < 0.001$), 39.3% ($p < 0.001$) and 32.5% ($p < 0.001$),

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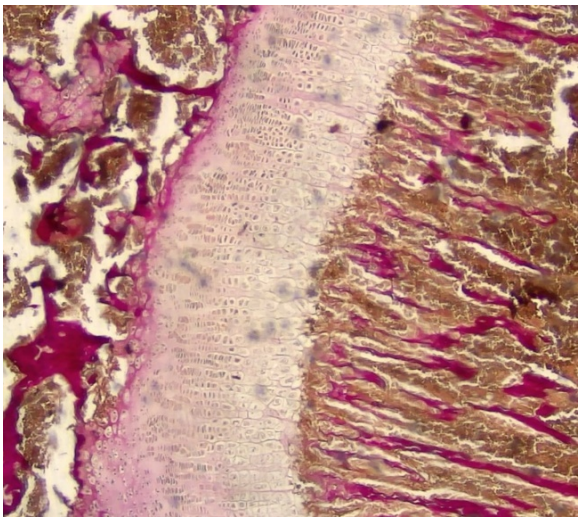
respectively, compared to the control (Table 3).

The zone of definitive cartilage contains chondrocytes of different stages of autumn, which are also organized into columns of 5–10 cells each. The cells are round in shape and larger in size than the chondrocytes of the previous zone and contain a large amount of cytoplasm. The nucleus occupies the central part of the cell. The number of binucleate cells is observed. Mitotic figures of chondrocytes are very rare, in contrast to animals in the experimental group, where mitotic figures do not occur at all.

Morphometric studies show that in experimental alloxan diabetes of the mother's body, in rat pups of the experimental group, the zones of definitive cartilage are smaller compared to animals under control. The largest difference is 20.8% ($p \leq 0.05$) and 27.4% in 14- and 60-day-old rat pups, respectively (Table 3).

The zone of definitive cartilage gradually merges with the next zone – the zone of destruction. In this zone, chondrocytes are located in 1–2 rows. They take on an irregular shape. In most cases, chondrocytes without nucleus and the cytoplasm is vacuolated in places. In the voids of this zone, red blood cells, endothelial cells and other elements are found (Page 1).

In animals whose mothers had modeled DM during pregnancy, remnants of the destruction zone are preserved only in peripheral areas that are directly adjacent to the perichondral bone cuff. It is quite difficult to measure the thickness of this zone, since it is very thin, part of it is interrupted and does not have clear boundaries. In some places, the destruction zone is represented by single anucleate cells. Around these cells, the intercellular substance becomes calcified, as a result of which the cell membranes are



Page 1. The zone of definitive cartilage gradually
merges with the thezone of destruction.
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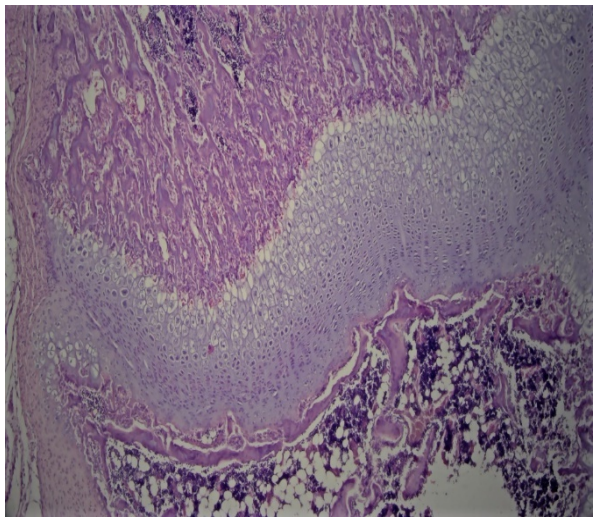

Page 2. The growth zones of tubular bones. ColoringGE. M.1:400

ISSN2. 81-1954. EISSN 2181-1962

destroyed and the chondrocytes begin to die (Page 2).

In conclusion: As a result of this study, it was established that the skeletal system experiences significant changes in the process of development and vital activity of the body in early postnatal ontogenesis. This is manifested in the restructuring of the internal structure during the development of cartilage. Changes are characterized by a certain direction and dynamics depending on exo- and endogenous influences.

Morphometric analysis showed that in rat pups born from individuals with alloxan diabetes mellitus, the general pattern of a decrease in the thickness of the structures that make up the epiphyseal cartilage and an increase in the number of proliferating cells in the proliferation zone in the age aspect is preserved. In some places, focal dystrophic changes in chondrocytes are detected, the wall of the vessels is thickened, disintegrated, their lumen is expanded, and foci of plasmorrhagia are noted around the vessels.

It has been established that the zone of proliferating cartilage experiences the greatest changes, which is significantly thinned and the number of cells in it is reduced. The process of cartilage differentiation in typical bone beams sharply worsens, and therefore the zone of destruction of the epiphyseal cartilage of the tubular bone expands.

Thus, the data obtained indicate a lag in the development of all constituent structures of the epiphysis of the tubular bones of rat pups with alloxan diabetes of the maternal body during pregnancy.

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