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EARLY MORPHOLOGICAL CHANGES IN MOLAR PULP OF WISTAR RATS WITH ALLOXAN INDUCED DIABETES

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The aim of this study was to determine the period of diabetes mellitus needed for producing morphological changes in the structures of molar pulp in experimental animals. The experiment was performed on 24 male albino rats, who were initially 35 days old and weighed 89.45 + 9.95 g. The animals were separated into 4 groups. The first (T1) and third (T2) groups of animals were given a single dose of alloxan tetrahydrate, approximately 80 mg/kg body weight. Just before the application, the subsance was dissolved in physiological saline solution, and each animal was given 1 ml into the tail vein. The second (C1) and fourth (C2) groups of animals were used as appropriate control groups and received 1 ml of physiological saline solution alone. Body weights were measured and glycemia was controlled weekly. The animals in groups T1 and C1 were sacrificed on the loth day, and groups T2 and C2 on the 35th day by decapitation. The block of mandibular molars was taken for histological examination. The results showed that the animals of group T1, besides high values of glycemia, had dilated blood vessels filled with blood, which was especially marked in the capillaries of peripheral parts of the pulp. The capillaries had a perpendicular orientation all the way to predentine. In the animals of group T2 histopathological findings were different. Capillaries penetrated between the odontoblasts, but most were paralel with predentine. The line of odontoblasts was reduced in many places, somewhere lacking, and somewhere replaced with invaginations of dentine entering the pulp. Thus early changes were observed in the animals with diabetes mellitus, on the blood vessels as well as on other structures of the dental pulp.

Key words: diabetes mellitus, alloxan, dental pulp, blood vessels of the dental pulp

INTRODUCTION

Though one of the oldest and most widely spread pathological states in human medicine, diabetes mellitus is still an incurable disease. According to Krahl (1975), the first complications are chronic vascular complications, vascular diseases, macroangiopathia (premature arteriosclerosis) and microangiopathia, followed by infections of different origin and localization. The third large group of complications consists of degenerative changes which have not been clearly defined due to the etiopathogenesis, of both vascular diseases and infections.

The dental pulp is a connective vascular tissue in structure built of highly differentiated cells, fibers, blood and lymph vessels, nerves and basic substance, and is surrounded by walls of dentine. It exhibits great sensitivity and a specific response to different irritations. Karadzov (1987) states that the biggest diametar of the pulp artery is about 100 micrometers, and that between blood vessels of the pulp there is no anastomosis; i. e. they are terminal. Therefore, every change that leads to serious wall damage or to occlusion of a blood vessel(or a combination of these two elements consequently) leads to pulp damage.

It is known that the metabolic disorders of diabetes mellitus have a direct influence on small blood vessels, which include blood vessels of the pulp (Devecerski, 1982). As a consequence of metabolic disorders in diabetes, a sediment of glucoprotein appears in capillary endothelia i. e. on the basal membrane, which leads to narrowing of the blood vessel lumen. This leads to blood vessel obliteration and ischemia of certain parts of the tissue. As there is a tendency to infections (Krahl, 1975), inflammatory changes, induced by periodically present microorganisms in the blood, are also to be expected in diabetes.

Diabetes primarily has a direct influence on small blood vessels: capillaries, precapillaries and postcapillaries. Therefore, we started from the hypothesis that the arteries of the dental pulp are not spared from the above described changes during diabetes mellitus. Thus, the aim of this study was to determine the period of diabetes mellitus needed for producing morphological changes in structures of molar pulp.

MATERIAL AND METHODS

Male albino, Wistar rats - Rattus norvegicus 24 animals weight 89.45+9.95g and 35 days old were used as the experimental models. To satisfy standard conditions for alloxan diabetes, the animals were fasted for 24 hours before the beginning of the experiment, but had free access to water. During the experiment, the animals were given a totally granulated standard food for laboratory rats/mixture Pa 20g per animal, from the Veterinarian Institute of Subotica, SOUR "Agros". The animals were divided into 4 experimental groups:

-First group (T1)

animals treated with alloxan were killed on the 10th day from the beginning of the experiment (6 animals).

-Second group (C1)

animals not treated with alloxan were tilled on the 10 th day from the beginning of the experiment (6 animals).

-Third group (T2)

animals treated with alloxan were killed on the 35 th day from the beginning of the experiment (6 animals).

-Fourth group (C2)

-animals not treated with alloxan were killed on the 35th day from the beginning of the experiment (6 animals).

Animals from the treated groups (12 animals) were given alloxan tetrahydrate / C_4 H_2 N_2 O_4 4 H_2O) 4H2), Mw=214.12 g/mol / at approximately 80mg/kg body weight. The substance was dissolved in physiological saline solution just before application and each animal was injected with 1 ml of solution into the tail vein. The animals from the control groups (12 animals) received 1 ml of physiological saline solution into the tail vein.

During the experiment:

-body weight was determined at the beginning of the experimental period, and later every 7th day on a technical balance ("Mettler PS 1200"), with the precision of one decimal.

-glycemia was determined by a polygraphic method using a microhematocrit reader (MSE) at the beginning of the experimental period and afterwards every 7th day, from the moment of alloxan administration.

-Histological research was done after killing the animal by decapitation, without anesthesia. The part with the lower block of molars was separated with a diamond tiled whetstone from the rest of the mandibula. Tissues were fixed in 4% neutral buffered formalin. After fixation for 18 days, blocks of molars were rinsed and then sunkin bottles with 20% neutral solution of EDTA (ethylene diamine tetracetate. The decalcification lasted, for 57 days approximately, and the degree of decalficication was controlled radiographically. Dehydration was done in a series of alcohols, clearing in xylol and embedding in paraplast. Serial sextions of preparation for histological analysis were cut on a microtome, "Szankos" and every fourth sample 3 micrometers thick, was stained by the following methods: HE (hematoxylineosin), PAS (periodic acid Schiff), Grosman Mallory. Pathohistological analysis of the coronary part of lower molar pulp preparations was done under a microscope "Leitz Wetzlar". The results were statistically analysed and the significance of differences calculated by Student's t-test.

RESULTS

The mean values for body weight of the alloxan treated animals and control groups showed differences in value which were significant, already on 7th day of the experimental period. This statistically significant-difference increased during the whole of the experimental period. (Figure 1)

There was statistically significantly higher glycemia ($p<0.001$) in the treated groups (under the induction of alloxan) 24 hours after application compared with

the control groups. This difference persisted to read the highest value on the 35th day of the experimental period (figure 2).

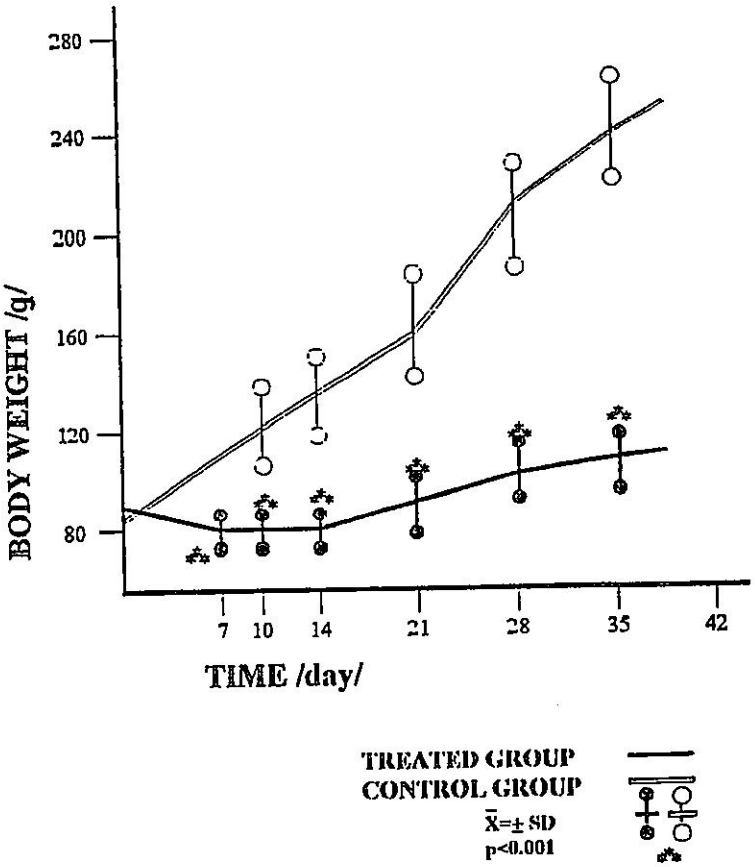

Figure 1. Body weight of control and alloxan treated animals during the experiment

Histological analysis of the first mandibular molar pulp of animals from the treated group (T1 - figure 3). showed that all blood vessels were dilated and that there was more blood than in the molar pulp of control animals (C1-figure 4a).

Blood saturation was especially visible in the capillaries of peripheral pulp, as well as on the dividing line of predentine and at the border towards predentine. PAS positive contents of the capillaries of treated animals was mostly present in the capillaries at the apical part of the odontoblasts (Figure 3). In the molar pulp of the animals from group T1 the Mallory technique showed that capillaries in the odontogenic segment spread all the way to predentine, and they were in a perpendicular direction (Figure 4b).

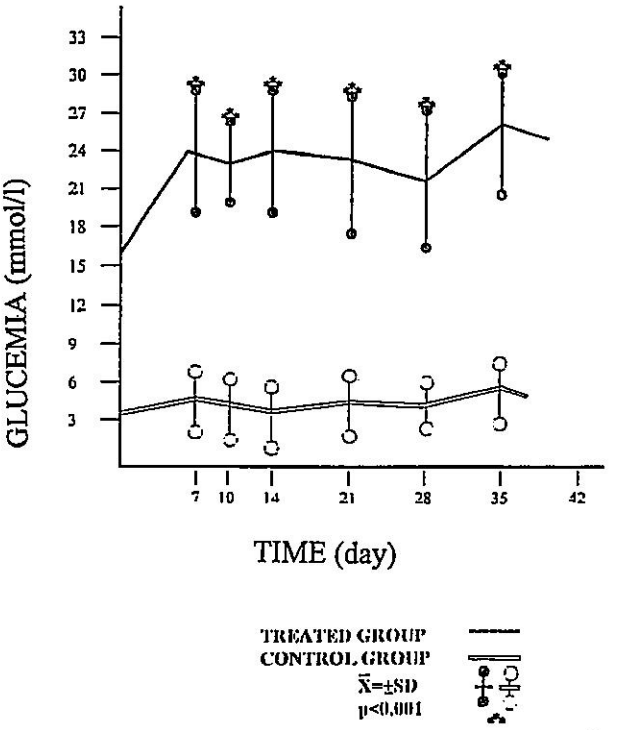

Figure 2. Glycemia in control and alloxan treated animals during the experiment

In the molar pulp of group C1 animals this was not the case (Figure 4a).

In group C2, the central blood vessels of the pulp were filled with blood. Peripheral capillaries were in parallel and perpendicular orientation in reference to predentine. The capillaries were localised on the border of the odontoblast and predentine, as well as in the dentine itself, in places where dentinogenesis was especially marked (Figure 5).

In the treated animals group T2 the pulp was obviously less vascularized and had characteristic changes of capillary orientation. The capillaries only partly penetrated between odontoblasts and mostly had a tendency of taking a parallel orientation to the predentine some capillaries were trying to spread perpendicularly, but did not reach the predentine (Figure 6a).

Further analysis of histograms of the molar pulp of group T2, showed that the continuous row of odontoblasts was damaged in many places and in some places practically nonexistent. The layer of odontoblasts was somewhere rarefied and somewhere completely replaced by invaginations of predentine into the pulp. In invaginated dentine, there were no odontoblasts nor capillaries (Figure 6b).

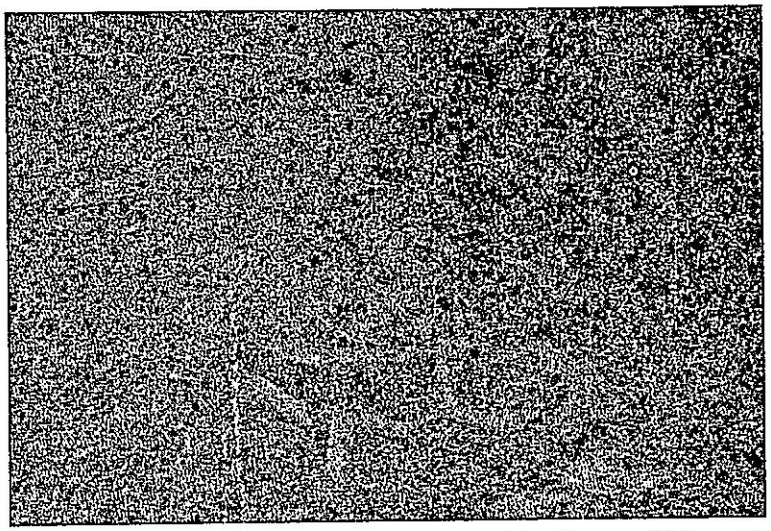

Figure 3. Pulp of the first mandibular molar (T1) group. Staining Grosman Mallory. Magnification 10x25

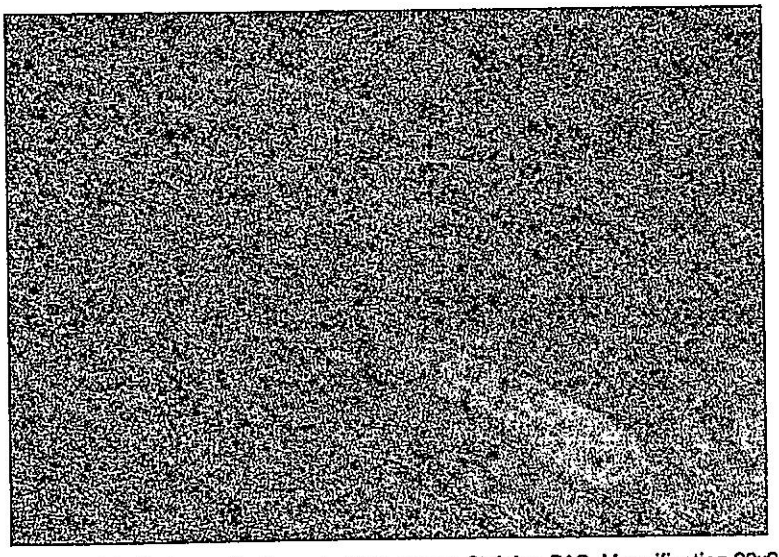

Figure 4a. Pulp of the first mandibular molar (C1) group. Staining PAS. Magnification 20x25

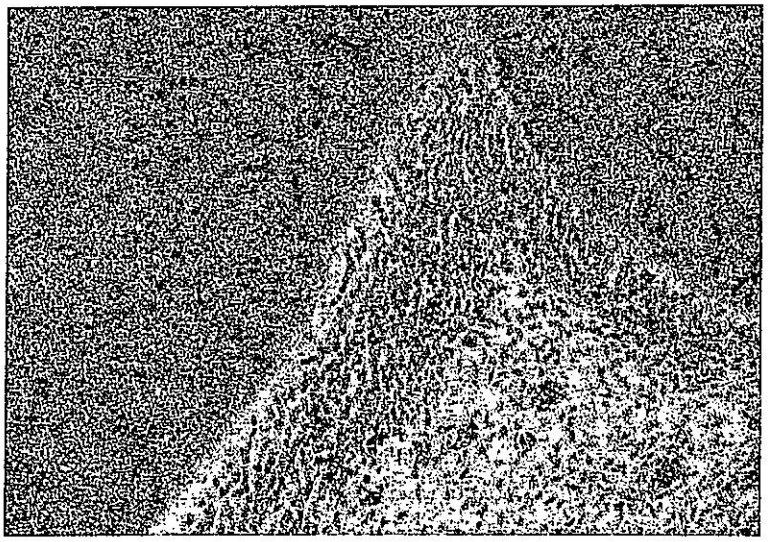

Figure 4b. Pulp of the first mandibular molar (T1) group. Staining Grosman Mallory. Magnification 10x25.

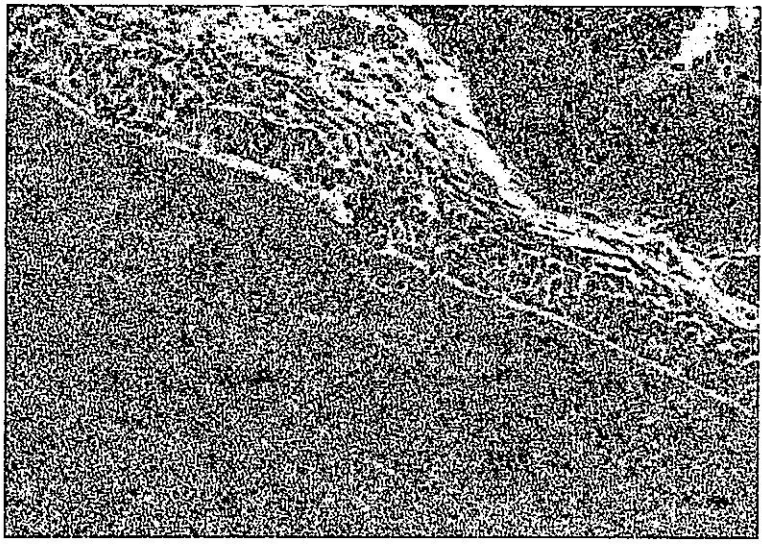

Figure 5. Pulp of the first mandibular molar (C2) group. Staining Grosman Mallory. Magnification 10x25

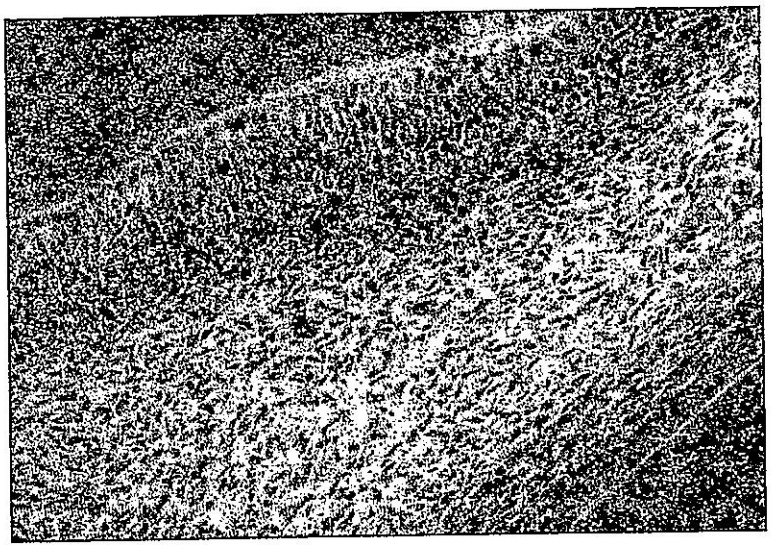

Figure 6a Pulp of the first mandibular molar (T2) group. Staining Grosman Mallory. Magnification 10x25.

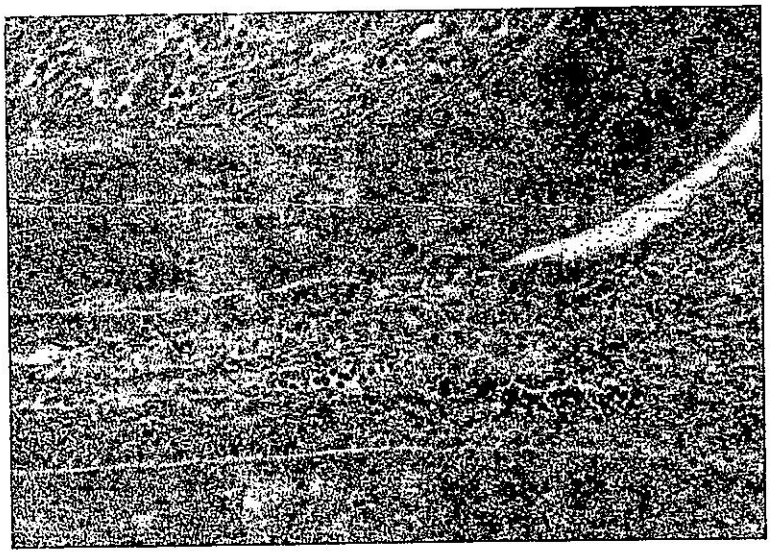

Figure 6b. Pulp of the first mandibular molar (T2) group. Staining Grosman Mallory. Magnification 10x25.

DISCUSSION

The time of sacrificing the first group of animals was selected so that their first and second mandibular molars had completed their growth on the 10 th day from application of the diabetogene alloxan.

The 35 th day from the beginning of the experiment was selected as the second time because then the animals had achieved sexual maturity (Krahl, 1975; Hill, 1967; Dunn, and Meletchie, 1943).

The presence of hyperglycemia in the experimental animals treated with alloxan (figure 2) may be interpreted as a logical consequence of the insulin deficit provoked by destruction of the endocrine pancreas beta cells, and the consecutive development of artificial diabetes. Even though the concentration of glucose in the blood is raised, in diabetes mellitus most of the cells in the organism are "hungry" because insulin is needed for normal transmembrane transport of glucose (Bondy, 1969, Devečerski, 1982). The values for body weight (Figure 1) showed that in spite of outstanding polyphagia, the alloxan treated animals succeded in establishing some calorie balance only after the third week from the beginning of the experimental period. The loss of body fluid certainly contributed to the loss of body weight in the beginning, and later on, to slowing the growth of the animals treated alloxan.

Changes in the pulp, because of the presence of diabetes mellitus in alloxan treated animals, were already noticed on the 10th day from the application of the diabetogene. The changes were characterised by the presence of a generalized hyperemia of the pulp (figure 3), while the rest of the infrastructure of the pulp was preserved. All blood vessels were dilated and had a larger quantity of blood comparing to the blood vessels of the animals from the control group (figure 4.). This blood saturation was especially noticeable in capillaries of the peripheral part of the pulp (figure 3). By means of the Grosman Mallory staining technique we could see in animals of group T1, that capillaries in odontogene sediment spread all the way to predentine, and that were in perpendicular orientation (Figure 4b). In agreement with the results of Campbell (1979), Hove and Stallard (1970), and Korec (1968), our results also showed the presence of PAS positive material in the walls of capillaries and arterioles, especially near the apical part of odontoblasts.

The presence of stronger PAS positive material (Figure 3) could be explained by the fact that subendothelial sedimentation of mucopolysaccharides had risen, while the active hyperemia was the consequence of disturbance in the arterial circulation.

On the 35 th day from the application of alloxan, we could se fewer blood vessels in the whole pulp (Figure 6a). Blood vessels in the central part of the pulp were only dilated and filled with a small number of erythocytes, while capillaries in the peripheral zone could practically not be observed (figure 6b), and were also very rare in the ontogene layer.

Besides the smaller number of blood vessels, changes of orientation of the rest of capillaries were also characteristic. In contrast to the control animals (Figure 5), with diabetic rats at 70 days old, capillaries only partly penetrated

between the odontoblasts, while most had a tendency to take parallel orientation to predentine (Figure 6a). Here, too, the capillaries were trying to spread perpendicularly, but in no case reached the predentine. Further analysis of the histogram of molar pulp in the animals of group T2 showed that the continuous line of odontoblasts was disturbed in many places and in certain places there was none to be found. The odontoblastic layer was rarefied at places, while in other places in was completely replaced by invagination in the pulp. In invaginated dentine, there were no odontoblasts nor capillaries (Figure 6b). The reasons for changed oriention of capillaries with alloxen treated animals compared to control animals probably lay in the existence of greater resistance due to the damage of blood vessels as a consequence of the blood vessel wall swelling from sedimentation of PAS positive material. Similar results were reported by Russel 1967., who found a small number of blood vessels in all parts of the peripheral third of the pulp in patents ill of diabetes mellitus while in the central part small and large vessels were present with distinctive PAS positive swelling of the wall. This small number of blood vessels (Figure 6a) is in agreement with the results of Burket 1965, too, who, describing "typical diabetical arteritis", quoted that odontalgia was often present in patients ill of diabetes, and attacked pulp vessels which led to ischemia and necrosis of pulp tissue.

Characteristic changes in the molar pulp in the animals treated with alloxan were observed in this study. After the 10th and 35 th day they were solely linked to changes on small blood vessels which are characteristic of the diabetic syndrome (Colwell, 1960; Krahl, 1975). Comparing findings in the pulp i. e. their blood vessels, reduction of the capillary net was conspicuous with the animals after 35 days of diabetes, which could be seen by comparing pictures taken after 10 and 35 days of duration of diabetes (Figure 6a, 6b).

In our opinion the observed changes in the molar pulp of the animals treated with alloxan are not linked only to duration of alloxan diabetes, but also depend on the level of hyperglycemia, because it is more marked with the animals sacrificed on the 35th day after giving alloxan (figure 2).

Based on our results we could conclude the following:

1. Pulps of rats after 10 days of diabetes mellitus showed the existence of expressed hyperemia.

2. After 35 days of diabetes mellitus a different disposition of the capillary net was found in rat pulp, as well as a reduction of the capillary net in comparison to the control group (C2) and to group T1 where advance of the process with reduction of blood vessels was noticed.

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RANE MORFOLOŠKE PROMENE U PULPI ZUBA MOLARA WISTAR PACOVA SA ALLOXAN-SKIM DIJABETESOM

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SADRŽAJ

Cilj ovoga rada bio je da utvrdi koji je najkraći period trajanja dijabetes melitus-a potreban za ispoljavanje morfoloških promena na strukturama pulpe molara eksperimentalnih životinja.

Eksperiment je izveden na 24 mužjaka, albino pacova, Wistar soja, starih 35 dana, težine 89.45 + 9.95 grama. Životinje su bile podeljene u 4 grupe. Prva (T1) i treća (T2) grupa životinja dobile su jednokratnu dozu alloxan tetrahydrat-a, u proseku 80 mg/kg težine tela. Neposredno pre aplikacije, supstanca je rastvorena u fiziološkom rastvoru i svakoj životinji je injicirano po 1 ml rastvora u repnu venu. Druga (C1) i četvrta (C2) grupa životinja služile su kao odgovarajuće kontrole i dobile su po 1 ml čistog fiziološkog rastvora. Tokom eksperimenta, svake nedelje merene su težine tela i određivana je glikemija. T1 i C1 grupe životinja žrtvovane su 10-og a T2 i C2 grupe 35-og dana od početka eksperimenta. Životinje su žrtvovane dekapitacijom a uzet je blok donjih molara u cilju histoloških istraživanja.

Rezultati pokazuju da životinje T1 grupe, pored visoke vrednosti glikemije, imaju dilatirane krvlju ispunjene krvne sudove. Kapilari se pružaju perpendikularno sve do predentina. Kod životinja T2 grupe, histopatološki nalaz je izmenjen. Kapilari se samo delimično probijaju između odontoblasta a većim delom su paralelno postavljeni u odnosu na predentin. Niz odontoblasta je razređen na mnogim mestima, a mestimično nedostaje i zamenjen je invaginacijama dentina, koje zalaze u pulpu. Na osnovu ovih rezultata uočene su rane promene, u životinja sa dijabetes melitusom, kako na krvnim sudovima tako i na drugim strukturama pulpe zuba.