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***Oleksiienko V. V., Bilash S. M.***

# MORPHOLOGICAL CHANGES IN THE ARTERIOLES OF THE HAEMOMICROCIRCULATORY BED OF THE GINGIVAL MUCOSA UNDER PROLONGED EXPOSURE TO FOOD ADDITIVES

**Poltava State Medical University (Poltava, Ukraine)**
**vladoleksienko20@gmail.com**

*Nowadays, food additives are an important element of the food industry, which not only improve the taste of products, but also allow them to be stored longer. At the same time, they have negative properties that manifest themselves after long-term use. The experiment was aimed at identifying the effect of several food additives on the gums, vessels and changes in their structure. During the study, it was established that the effect of a complex of food additives (sodium glutamate, sodium nitrite, and Ponceau 4R) on the microvessels of the gums of rats leads to a violation of hemodynamic conditions, and with prolonged action leads to an irreversible violation of blood supply to the gums as a whole. Morphological changes of microvessels (first of all arterioles) are observed mainly from the fourth week of the experiment and are characterized by vasodilatation or vasoconstriction, the formation of erythrocyte aggregates, which leads to significant pathological changes in the structural organization of the gums and blood supply to the gums, as an integral organ of the oral cavity.*

***Key words:*** *gum, microcirculation, arterioles, rats, morphological changes, vessels, epithelium, nutritional supplements, morphology, restorative process.*

**Connection of the publication with planned research works.**

This article was performed as part of research work: “Patterns of morphogenesis of organs, tissues and neurovascular formations in normal condition, pathology and under the influence of exogenous factors” (state registration number 0118U004457).

**Introduction.**

In our time, when the need for the amount of food products is constantly growing, manufacturers find various ways to speed up production and increase the shelf life of products. In solving these problems, industrialists are helped by the use of various chemicals. At first glance, some food additives have useful properties, for example, they prevent spoilage of food products, improve their taste and appearance, but their usefulness or harmfulness is highly controversial and poorly studied [1-3]. Their quantity in food products is strictly regulated by both domestic and international legislation. However, even if the permissible level of chemically created food additives is observed, their regular entry into the body can cause the appearance of allergic reactions, the emergence and development of pathological changes in the organs and systems of the human body [4]. For example, widely used food additive called sodium glutamate. It’s used in cooking as a flavor enhancer. In many countries and in Ukraine also, sodium glutamate generally recognized as safe to eat. However, the systematic use of products with glutamate in humans violates the hormonal balance, problems with digestion, gastritis and gastric ulcer, obesity can develop [5].

The reaction of the human body to food additives is exclusively individual. Currently, there are literary sources in which the consequences of the separate use of various food additives are highlighted, but the changes in the body when receiving several additives at the same time are practically not investigated. Very few tests have been conducted in this area. One study, in which two preservatives were tested together, showed that they have a much stronger effect in combination than individually [6]. Therefore, the problem of studying the morphofunctional changes in the body during the daily consumption of many food additives, even in the maximum permissible amount, is an extremely urgent and important problem of ensuring the public health of the population not only of our country, but also of humanity as a whole.

**The aim of the study.**

To research the morphological changes in the structural components of the arterioles of the hemomicrocirculatory channel of the mucous membrane of the gums of rats under the influence of a complex of food additives in the experiment.

**Object and research methods.**

The study was conducted on white rats, weighing (0.350±0.15) kg, which were kept under standard conditions in the vivarium of the Poltava State Medical University. Experimental studies were carried out in compliance with biotic requirements and humane treatment of experimental animals, regulated by the Law of Ukraine “On the Protection of Animals from Cruelty Treatment” (No. 3447-IV dated 21.02.2006) and the European Convention on the Protection of Vertebrate Animals Used for Research and other scientific goals (Strasbourg, 1986).

The animals were divided into 6 groups (1 control group (n=15) and 5 experimental groups (n=75)). With the exception of the control group, each group is named according to the number of weeks the rat was removed from the experiment. Rats in the control group were given normal saline orally and drank water freely. Rats from the experimental groups orally, at the same time, were administered a mixture of a 10% solution of sodium nitrite (E 250), monosodium glutamate (E621) in a dose of 20 mg/kg in 0.5 ml of distilled water, Ponceau 4R – in a dose 5 mg/kg in 0.5 ml of distilled water once a day, also under the condition that animals have free access to drinking water. Dosages of food additives were twice less than their permissible rate in food products defined by the legislation of Ukraine “On Standardization”. Rats were removed from the experiment after 1,

4, 8, 12 and 16 weeks by overdose of thiopental anesthesia.

Then the gingival fragments fixed in a neutral formalin solution were sealed in paraffin. Histological sections made from paraffin blocks were stained with hematoxylin and eosin for examination sections. After studying immunohistochemical reactions were performed on the inspection sections, and then microscopy was performed using a Viorex light microscope with a DSM 900 digital photomicroscope. To obtain semi-thin and ultrathin sections, the studied material was fixed in glutaraldehyde on a phosphate buffer and sealed in EPON-812. Ready sections were stained with toluidine blue and polychrome dye. For electron microscopic research, epoxy blocks were used, from which ultra-thin sections were made on an ultramicrotome, which were then placed on copper support grids and contrasted with a 1% aqueous solution of uranyl acetate and lead citrate according to the Reynolds method with changes [7] and studied using an electron microscope PEM-125K.

The average total diameter, average lumen diameter and blood vessel wall thickness of arterioles in rat gingival mucosa were measured using morphometry. When performing morphological and morphometric studies, we used a visual analysis system for histological preparations. We observed the images using a microscope and a vision CCD camera. Morphometric studies were performed using VideoTest-5.0, KAAPA ImageBase, and Microsoft Excel programs. We determined the overall mean diameter, mean lumen diameter, and wall thickness of arterioles, venules, and capillaries and processed the data using Statistica 10 BiostatPro 6 software and Microsoft Excel 2019. The calculations and values in the sample were verified using the Shapiro-Wilk test. Data quantification involves determining the arithmetic mean (M) of the variation series and its standard error (m). To compare quantitative values in paired rows, we used Student's t test. Differences were considered significant at $p<0.05$.

**Research results and their discussion.**

Morphological examination showed that the gingiva of rats in the control group was covered by mucosa and had no submucosal base. The mucosa fuses directly with its own plates, and its surface layer forms tall, narrow papillae that grow into the epithelial layer of the mucosa. The epithelial layer is composed of multiple layers of flat, nonkeratinized epithelium. Elements of the blood microcirculatory channels were visualized directly in the lamina propria and its papillae and penetrated deep into the epithelial layer. We examined arterioles as resistance links of the blood microcirculatory system. An arteriole is defined as a circular structure composed of three shells: the inner shell, the middle shell, and the outer shell. The intima is represented by endothelial cells that lie on the internal elastic lamina and have a tortuous course. The mediastinum is visualized as a space composed of 1-2 layers of smooth muscle cells. The shell consists of a thin layer of loose connective tissue.

The hemomicrocirculatory channel of the mucous membrane of the gums of rats is extremely developed. Arterioles, as a resistive link of the microcirculatory channel, in the dynamics of the experiment responded to the introduction of food additives by processes of vasodilatation, vasoconstriction, thinning or thickening of the vascular wall. Thus, the average total diameter of arterioles for the 4th week of the experimental study was statistically significantly reduced at $p<0.05$ by 19.4% compared to the control indicators. During weeks 4-8 of the experiment, the average total diameter of arterioles recovered to control values, but was wider by 2.6 and 1.2%, respectively, compared to control values and was within statistical error with the group control On the 12th week of observation, this indicator, in comparison with the control indicators, increased statistically significantly at $p<0.05$ by 9.1%, and on the 16th week by 11.2%, which indicates vasodilation processes **(fig. 1).**

In accordance with changes in the indicators of the average total diameter of arterioles, there were also functional changes in the indicators of their average diameters of lumens. Thus, after 1 week of taking the food complex, the average diameter of arteriole lumens decreased by 1.7% compared to the control indicators, and already after the 4th week of the experiment, this indicator increased by 2.7% compared to the control group. After the 8th week of observation, this indicator again decreased by 1.2% compared to the control indicators. After the 12th week of the experimental study, the indicator of the mean diameter of the arteriole lumen reliably increased by 9.1% at $p<0.05$ compared to the control indicators and reached its maximum value after the 16th week of observation with a reliable increase of 11.8% at $p<0.05$ **(fig. 2).**

A detailed analysis of the structural components of the vascular wall of arterioles showed that after 1 week of taking a complex of nutritional supplements, the vascular wall significantly thins by 69.9% compared to control indicators, reliably at $p<0.05$. At the ultramicroscopic level, it was determined that the wall of arterioles underwent significant destructive changes. In the inner lining of arterioles, endothelial cells acquired a flattened appearance, and some lost their connection with the basement membrane. The internal elastic

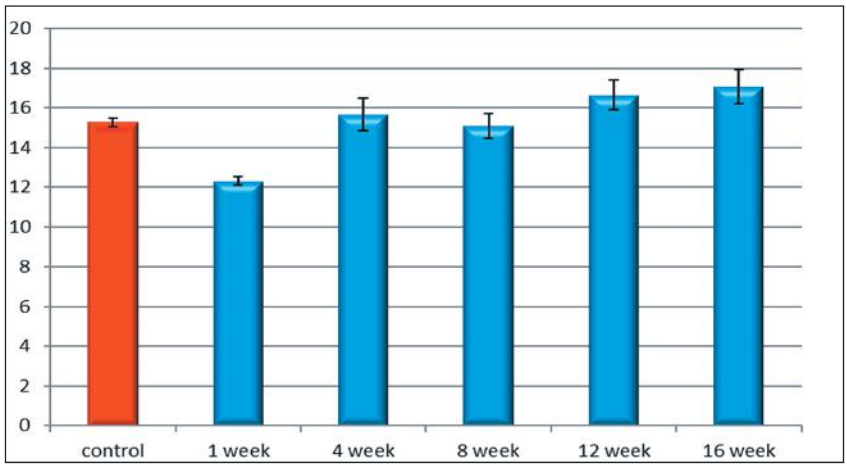


**Figure 1 – Changes of the total diameter of the arterioles of the mucous membrane of the gums of rats in the dynamics of the experiment. Firstly it reduced, on the 4th week it back to control level and after that it slightly increased to 12th week.**

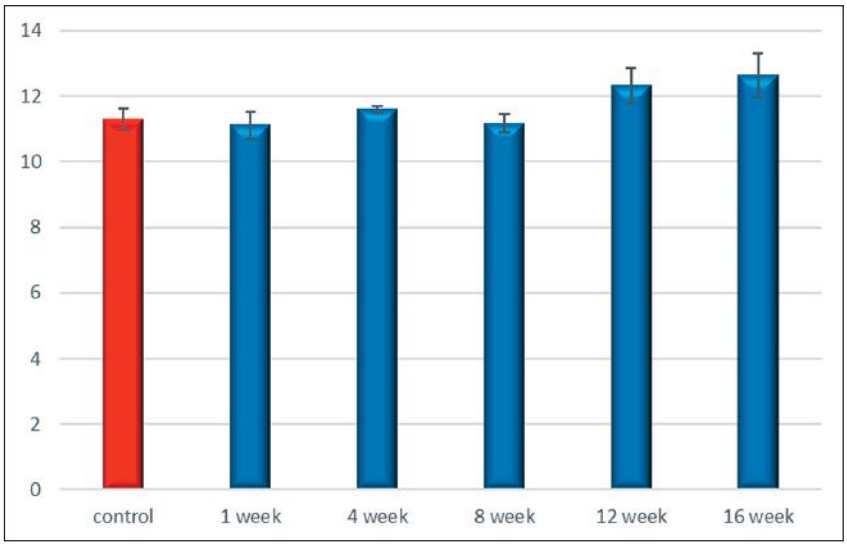


**Figure 2 – Changes in the indicators of the average diameters of the lumen of the arterioles of the mucous membrane of the gums of rats in the dynamics of the experiment. Except for the first and 8th weeks, where there is a decrease in the diameter of the lumen, in other cases there is an increase of the lumen compared to the control.**

membrane, in contrast to such structures in the control group of animals, lost its tortuous course. In the middle membrane, smooth myocytes were visualized as having a thinned and elongated shape, and in some places they had no connection with the elastic membrane at all. In the external cervical membrane, the layer of loose connective tissue was significantly thinned, compared to the arterioles of the mucous membrane of the gums of the control group of animals. In parallel with the destruction of the vascular wall in the lumens of arterioles, the wall sludge of erythrocytes is determined according to the dextran type: different sizes of erythrocyte aggregates are visualized, which are directly adjacent to the vascular wall of arterioles **(fig. 3).**

In the interval between the 4th and 8th weeks of the experimental study, it was established that there was no significant difference in the average thickness of the vascular wall between the control indicators and similar indicators in these experimental groups. A slight thickening of the arteriole wall was observed after the 4th week by 2.8%, and after the 8th week by 1.1%. Further, after the 12th week of observation, a reliable $p<0.05$ thickening of the vascular wall by 9.1% is determined. During this period, at the microscopic level, the restoration of the organizational structure of the inner membrane of arterioles is revealed due to the arrangement of endotheliocytes to the basement membrane and the restoration of the tortuosity of the latter. After the 16th week of the experimental study, there was a further reliable at $p<0.05$ thickening of the vascular wall by 11.6% due to swelling of the layer of loose connective tissue of the outer membrane of arterioles **(fig. 4).**

After experimental modeling of the effect of a complex of food additives on the mucous membrane of the gums of rats, we established the regular reactions of arterioles, their structural changes and changes in the rheological properties of blood. According to literature sources, the reaction of microvessels to the influence of external and internal environmental factors is quite specific and diverse. Therefore, during the morphological and morphometric study, we determined the changes in the arterioles of the mucous membrane of the gums of experimental animals, as a resistive link of the hemomicrocirculatory channel [8-9]. It is worth noting that after 1 week of experimental research, reliably, statistically significantly, at $p<0.05$, the average total diameter of arterioles decreases. At the same time, hyperhydration of its amorphous substance is determined on histological preparations in the connective tissue surrounding the microvessel. Due to this process, the clamping of arterioles and, accordingly, disruption of hemodynamic processes is observed. Significant thinning of the vascular wall and corresponding changes in its structural organization are also clearly defined. The fact of changing the rheological properties of blood with the formation of wall dextran slags of erythrocytes deserves special attention, which, in our opinion, is connected with the entry into the systemic bloodstream of food additives that contain glutamate and sodium nitrite in their composition and cause both allergic reactions [10] and in the future systemic inflammatory processes [11-12].

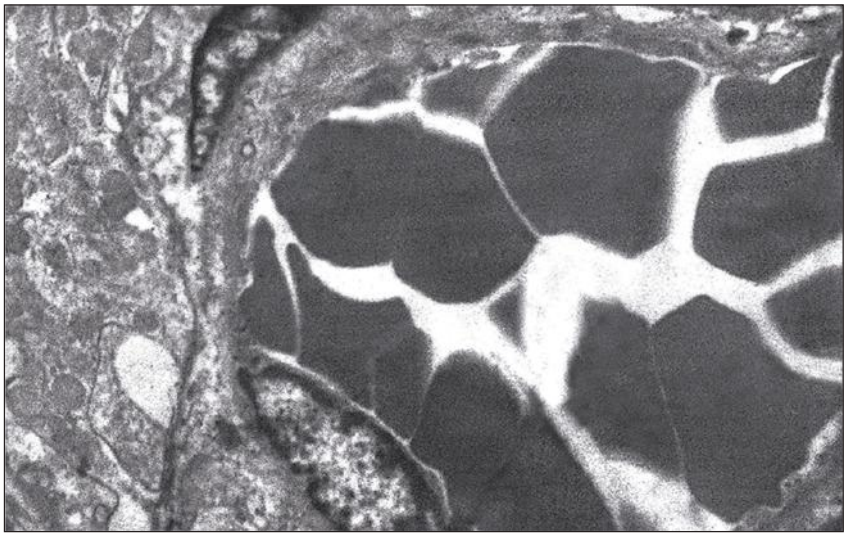

**Figure 3 – The structure of the gum and their vessels under the microscope.**

In the interval between 4 and 12 weeks of the experimental study, the average indicators of the total diameter, the diameter of the lumens, and the thickness of the vascular wall of arterioles changed in response to restorative and compensatory reactions, which were aimed at neutralizing the source of alteration and restoring the morphofunctional state of the surrounding tissue microvessels. But these processes do not fully lead to the complete restoration of normal hemodynamic indicators in comparison with similar indicators

of the control group of animals, as evidenced by the increase in the lumen of microvessels, the state of the loose connective tissue of the mucous membrane of the gums of rats, and this in turn leads to decompensation of these processes. which in turn is manifested by signs of hypoxia.

After the 16th week of observation, the morphometric indicators did not return to similar indicators of the control group of animals. At the microscopic level, numerous groups of mast cells in the stage of degranulation and in the stage of accumulation of secretory granules are visualized in the deep layers of the mucous membrane of the gums. In our opinion, mast cells play a decisive role in restoring local hemodynamics, due to the degranulation of secretory granules that contain heparin.

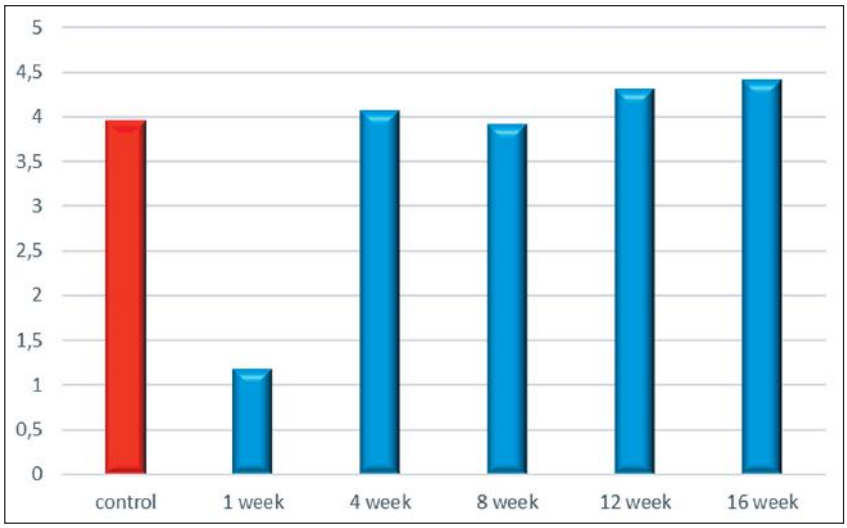


**Figure 4 – Changes in the indicators of the average thickness of the wall of the arterioles of the mucous membrane of the gums of rats in the dynamics of the experiment. After great decrease of thickness in the first week, it back to control level in the 4th week and slightly increased to 16th week.**

**Conclusions.**

1. An irreversible processes of hemodynamic changes of the mucous membrane of the gums with the development of hypoxic processes in the surrounding tissues are occurred after long-term intake of a complex of food additives into the organism of laboratory rats, which contain glutamate , sodium nitrite and Ponceau 4 R dye.

2. Increasing or decreasing of thickness of the wall of arterioles shows the destructive changes at the ultrastructural level with the formation of wall dextran deposits of erythrocytes, which leads to changes in the rheological properties of blood in all elements of the hemomicrocirculatory channel.

3. Restorative and compensatory reactions do not lead to a full restoration of normal hemodynamic parameters, which in the last weeks of the study is reflected by an increase in the average diameter of arterioles and the average diameter of the lumen of microvessels, which in turn and leads to decompensation of these processes in the mucous membrane of the gums.

4. Cells of the deep layers of the mucous membrane of the gums mostly in the stage of degranulation and accumulation of secretory granules and this is indicates the cyclic processes of restoration of local hemodynamics due to the degranulation of secretory granules that contain heparin.

**Prospects for further research.**

The results of the research could be used in the future different experiments with food additives , mostly with other types or their combinations.

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## МОРФОЛОГІЧНІ ЗМІНИ В АРТЕРІОЛАХ ГЕМОМІКРОЦИРКУЛЯТОРНОГО РУСЛА СЛИЗОВОЇ ОБОЛОНКИ ЯСЕН ПРИ ТРИВАЛІЙ ДІЇ НА НИХ ХАРЧОВИХ ДОБАВОК

**Олексієнко В. В., Білаш С. М.**

**Резюме.** Дана робота досліджує вплив комплексу харчових добавок, до яких входять глутамат натрію, нітрит натрію та Понсо 4R на ясна щурів. Дослідження спрямоване на виявлення морфологічних у стінках артеріол. Дослідження проводилось на матеріалі з ясен щурів. Підготовлені зрізи фарбували такими барвниками, як толуїдиновий синій та поліхромний. Основними методами дослідження були морфологічний, гістологічний і статистичний.

Ясна щурів у контрольній групі відзначалися наявністю слизової оболонки без підслизової основи, будучи зрощеною з власною пластинкою. Перший шар утворювали вузькі й високі сосочки, що ніби вростали в епітеліальний шар слизової оболонки. Наступний шар, епітеліальний, вистелений багатошаровим плоским незроговілим епітелієм. Артеріоли, як основні судини нашого дослідження, визначались як структури великого діаметру округлої форми з товстою стінкою, в якій наявні 3 шари.

Досліджувалися 3 основних показники: загальний діаметр, просвіт каналу та товщина стінок артеріол. Особлива увага приділялась змінам у даних значеннях та їхня інтерпретації.

На 1 тижні визначалися, в основному, зменшення показників відносно контрольної групи, які зростали або поверталися до значень контрольної групи до 4 тижня дослідження. В подальшому значення зростали до 16 тижня, але ці значення незначно перевершували показники контрольної групи. Це показує недостатню дію відновних процесів у артеріолах і внаслідок потужного вплив харчових добавок на морфологію судин, особливо при тривалій дії.

Цим дослідженням ми демонструємо, що вплив комплексу харчових добавок є значно потужнішим, ніж дії окремих добавок. Згадуючи, що всі продукти містять в собі саме комплекс добавок, цей експеримент буде цікавим працівникам харчової промисловості, а також лікарям і науковцям, що будуть вести дослідження у цьому напрямку.

**Ключові слова**: ясна, мікроциркуляція, артеріоли, щури, морфологічні зміни, судини, епітелій, харчові добавки, морфологія, відновні процеси.

## MORPHOLOGICAL CHANGES IN THE ARTERIOLES OF THE HAEMOMICROCIRCULATORY BED OF THE GINGIVAL MUCOSA UNDER PROLONGED EXPOSURE TO FOOD ADDITIVES

**Oleksiienko V. V., Bilash S. M.**

**Abstract.** This study investigates the effect of a complex of food additives, including monosodium glutamate, sodium nitrite and Ponceau 4R on the gums of rats. The study is aimed at identifying morphological changes in the walls of arterioles. The study was conducted on material from the gums of rats. Prepared sections were stained with such dyes as toluidine blue and polychrome. The main research methods were morphological, histological and static.

The gums of rats in the control group were characterised by the presence of a mucous membrane without a submucosal base, being fused to its own lamina. The first layer was formed by narrow and high papillae, which seemed to grow into the epithelial layer of the mucosa. The next layer, the epithelial layer, was lined with multilayered squamous non-cornified epithelium. Arterioles, as the main vessels of our study, were defined as large-diameter, rounded structures with a thick wall and 3 layers.

We studied 3 main parameters: total diameter, lumen of the canal and thickness of the arterioles walls. Particular attention was paid to changes in these values and their interpretation.

At week 1, there were mainly decreases in the values relative to the control group, which increased or returned to the control group values by week 4 of the study. Further, the values increased until week 16, but these values were slightly higher than those of the control group. This indicates an insufficient effect of recovery processes in the arterioles due to the powerful effect of nutritional supplements on vascular morphology, especially with prolonged exposure.

With this study, we demonstrate that the effects of a complex of food additives are much more powerful than those of individual additives. Given that all foods contain a complex of additives, this experiment will be of interest to food industry workers, as well as doctors and scientists conducting research in this area.

**Key words**: gum, microcirculation, arterioles, rats, morphological changes, vessels, epithelium, nutritional supplements, morphology, restorative process.

**ORCID and contributionship:**
Oleksiienko V. V.: 0000-0003-1616-8724 ABCDEF
Bilash S. M.: 0000-0002-8351-6090 ABCEF

Conflict of interest:
The Authors declare no conflict of interest.

Corresponding author
Oleksiienko Vladyslav Vitaliyovych
Poltava State Medical University
Ukraine, 36024, Poltava, 23 Shevchenko str.
Tel.: +380678940485
E-mail: vladoleksienko20@gmail.com

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