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CONDITION AND STRUCTURAL ORGANIZATION OF THE GLANDULAR AREA MUCOUS MEMBDANE OF ALBINO RAT HARD PALATE UNDER THE 30-DAY-LONG EFFECT OF ACRYLIC MONOMER

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Currently, the problem of the impact of removable laminar denture frames, including residual monomer, on the condition of the minor salivary glands, which play an important role in maintaining the balance in the oral cavity, remains poorly understood. Therefore, the paper was aimed at the experimental study of the effect of the monomer of the base acrylic resin "Ftoraks" on the condition and structural organization of the salivary glands of hard palate of albino rats. The findings of the study show that the monomer disrupts homeostasis of the oral cavity and causes irritation of the mucous membrane, manifested by dystrophy of the epithelial plate on day 30 of the experiment, as well as thickening of the corneal layer due to hypertrophy, which indicates the presence of hyperkeratosis. On day 30 of the experiment, thickening of the entire epithelial layer and the phenomena of hyper- and diskeratosis was detected in the experimental group; the phenomena of plethora, high lymphocyte, neutrophilic and eosinophilic leukocyte count were noted in the lamina propria of the mucous membrane and growing of relative amount of connective tissue can be regarded as the manifestation of a compensatory-adaptive reaction in response to the action of the monomer. 30-day-long experimental studies have shown insignificant effect of the monomer of the base acrylic resin "Ftoraks" on the cumulative size of the salivary glands in the submucous layer of the hard palate. Since the average recommended period of use of full removable laminar dentures is 3-5 years, it was considered necessary to continue the experiment and investigate the effect of the monomer of the base acrylic resin "Ftoraks" on the condition and structural organization of salivary glands of the hard palate of albino rats after 3, 6 and 9 months to further extrapolate the findings of the study to the human body.

Key words: salivary glands, hard palate, mucous membrane, monomer of the acrylic resin.

The work is a fragment of the research project "The effect of dental constructs and material on the prosthetic bed and adaptive abilities of the body", state registration No. 0116U004188.

The main type of masticatory efficiency restoration in edentulous patients is prosthetics with complete removable dentures, which in 90% of cases are made of acrylic resin [3, 7].

The advantages and disadvantages of the above dentures are quite convincigly stated in the publications. Particular attention is paid to the effect of residual monomer of the basic acrylic resins on the condition of the tissues of the oral cavity, prosthetic bed, homeostasis and the body as a whole [3, 4, 5]. The effect of the monomer on the condition of the salivary glands is of particular importance [9, 11]. However, the issue of the impact of removable laminar dentures, including residual monomer, on the condition of minor salivary glands, which play an important role in maintaining balance in the oral cavity, remains poorly understood to date.

The purpose of the work was to perform an experimental study of the effect of the "Ftoraks" base acrylic resin monomer on the condition and structural organization of the salivary glands in albino rat hard palate.

Materials and methods. The total of 12 laboratory Wistar rats, aged 1 to 1.5 years, were involved into the experimental morphological study. The control group involved 5 animals. Lingual mucosa of the rest 7 animals was smeared with 2% aqueous solution of the monomer of the "Ftoraks" base acrylic resin twice a day in the morning and evening to simulate the contact. The animals underwent euthanasia on day 30 of the experiment.

All studies were conducted in compliance with the rules of humane treatment of animals according to the requirements of the Tokyo Declaration of the World Medical Association and the general ethical principles of working with experimental animals, which were approved by the first National Bioethics Congress [8].

After euthanasia, each animal underwent removal of the hard palate, followed by division of the latter into two parts along the midline. One of the obtained fragments of the hard palate, together with the bone base, after fixation for one day in neutral 10% formalin was decalcified for 2-4 weeks, after which it was possible to obtain histological sections from the objects that contained bone tissue [5, 6, 10].

In the second fragment, the mucosa with the submucous layer was separated from the bone tissue using the lid scalpel under the control of a binocular magnifier. Soft tissue fragments were fixed in an upright state in 10% neutral formalin for 1-2 days.

The obtained samples, after conventional dehydration were embedded into liquid paraffin using the "Microm" station for paraffin blocks embedding. Sections of 5 μ m thick were obtained from the paraffin blocks on the "Leica" rotary microtome and stained with haematoxylin-eosin by conventional methods [5, 6, 10].

Visualization and imaging of the microspecimens was made by Olimpus BX-41 microscope equipped with digital microphoto attachment and licensed software package [1].

Results of the study and their discussion. Marked changes of the outer (covering) layer of the epithelium of the mucous membrane of the glandular area of the hard palate of laboratory animals, caused by the effect of the acrylic monomer were observed for 30 days First of all, a significant increase in the thickness of the latter, compared to the control group, was noteworthy. At the same time, no significant changes in the thickness of the basal layer were observed; the cells of the latter, similar to control group, were arranged mainly in single layer.

Notably, an increase in the number of sites with 2-3 layers of basal epitheliocytes, which indicated increased proliferative activity of the latter, was noted (fig. 1).

This fact is confirmed by the morphometric studies that showed significant increase of the mitotic index in the basal layer, compared to the control group.

In the spinous layer of the covering epithelium insignificant increase in the number of cellular layers up to 8-10 at some sites was noted. Some observations showed indistinct boundaries between cellular layers, demonstrating a certain polymorphism among the epithelial cells of this layer. In the spinous layer of the epithelium of the mucous membrane we noted the formation of keratin cysts. These structures were orbicular, almost homogeneous eosinophilic formations, which, by their tinctorial properties, resembled the squamae of the corneal layer, which on the periphery were surrounded by the granular epitheliocytes arranged in 3-4 layers, characterized by the presence of fine grains of keratohyalinum in the cytoplasm (fig. 2).

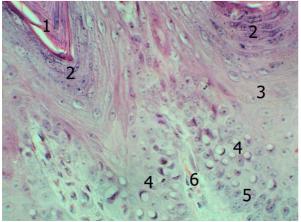


Fig. 1. Mucous membrane of the glandular area of the hard palate (experimental Group I). H&E stain. Lens: $40 \times \text{magnification}$, ocular lens: $10 \times \text{magnification}$. 1-corneal layer; 2-glandular layer; 3-spinous layer; 4-epitheliocytes with the phenomena of hydropic dystrophy in the spinous layer; 5-epitheliocytes of the basal layer; 6-plethoric vessels of the papillary layer of the lamina propria of the mucous membrane.

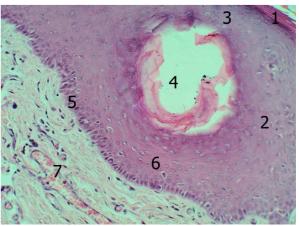


Fig. 2. Mucous membrane of the glandular area of the hard palate (experimental Group I). H&E stain. Lens: 10×magnification, ocular lens: 10×magnification. 1 – corneal layer; 2 – glandular layer; 3 – spinous layer; 4 – cavity of the keratin cyst; 5 – epitheliocytes of the basal layer; 6 – intraepithelial leukocytes; 7 – plethora of vessels of lamina propria of the mucous membrane.

The presence of keratin cysts can be regarded as manifestation of the pathological process, namely, corneal dystrophy (dyskeratosis), associated primarily with impaired differentiation of epitheliocytes and the formation of keratohyalinum.

In the basal and spinous layers, the number of intraepithelial lymphocytes markedly increased, compared to the control group, indirectly indicating the presence of inflammatory process in the underlying connective tissue. Moreover, a significant increase in the number of epitheliocytes with the phenomena of hydropic dystrophy, mainly in the lower parabasal layers of spinous cells was noted (fig. 1, 2).

However, no significant changes in the granular layer, compared to the control group, were found. Notably, insignificant increase in the number of cellular layers was noted in some areas, as well as more pronounced numerous grains of keratohyalinum, which were found in the cytoplasm of the epitheliocytes of the granular layer.

In all observations, a significant thickening of the corneal layer of the epithelium was detected. Significant hypertrophy of the corneal layer indicated the activation of the process of keratohyalinum formation, namely, hyperkeratosis (fig. 1). The latter, in this case, in conjunction with thickening of the entire epithelial layer, can be regarded as the manifestation of compensatory-adaptive (protective) reaction in response to prolonged exposure to monomer that is an external pathogen, resulting in the formation of so-called "corneal shield" along with penetration of the pathogenic chemical agent into the lamina propria of the mucous membrane.

In the granular layer sporadic small areas were detected, in which, along with horny scales (dead keratinocytes conjoined by interdegitations of their cytolemma), a large number of squamous cell with rod-shaped pyknotic nuclei were found.

Noteworthy, in such cases thinning of the granular layer of epitheliocytes, arranged in 2-3 layers, was also noted. In the publications this phenomenon has been called "parakeratosis", known as the specific type of keratinization. It is believed that the presence of such process in the epithelium of the hard palate,

in contrast to the skin epidermis, is a physiological process. In our studies, the phenomena of parakeratosis were noted more common in the mucous membrane of the hard palate of animals of the control group.

The latter circumstance may indirectly indicate stimulation of keratinization (orthokeratosis), caused by the modeled effect of the monomer, which can also be regarded as the formation of one of the links of the host defense of the mucous membrane to the effect of adverse exogenous agent.

Significant changes were detected in the lamina propria of the mucous membrane, too. Thus, in the papillary layer, a pronounced plethora of vessels, edema was noted overall; in the perivascular spaces a marked increase in lymphocytes and plasma cells was detected (fig. 1). Quite often mast cells were found at the blood vessels, in many of which the phenomena of degranulation were observed. Sporadic large areas of cellular infiltrates were found, which spread beyond the papillary layer into the depth of the lamina propria of the mucous membrane, and were represented mainly by eosinophilic leukocytes (fig. 3). This cellular composition of inflammatory infiltrates indicates the presence of an allergic component in the inflammatory process.

In addition to the areas of eosinophilic infiltrate, in the reticular layer of the lamina propria of the mucous membrane a marked plethora of small vessels, edema events were noted overall, which more were pronounced in the perivascular spaces.

In the reticular layer much more number of cellular elements of hematogenous origin, compared to the control group, were found, among which the most common were cells of monocytic-macrophage series, lymphocytes, plasma cells. Neutrophilic and eosinophilic leukocytes were sporadic. At the same time, no significant difference in the number of cellular elements of the fibroblastic series in the reticular layer, compared to the controls, was found.

Generally, no significant differences in the salivary glands were detected compared to controls. However, a more detailed study of their structural organization and morphometric studies revealed some changes that relate primarily to the stromal component.

Thus, in the stroma of the palatine salivary glands a pronounced plethora of the microvessels, the events of edema were detected. Significant increase in the number of cellular elements of hematogenous origin, which were located both diffusely and in aggregations mainly at the blood microvessels, was noted (fig. 4).

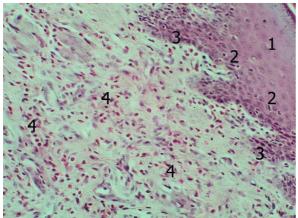


Fig.3. Mucous membrane of the glandular area of the hard palate (experimental Group I). H&E stain. Lens: 10×magnification, ocular lens: 10×magnification. 1 – spinous layer of the covering epithelium; 2 – intraepithelial leukocytes in the spinous layer; 3 – cells of the basal layer of the covering epithelium; 4 – eosinophilic infiltration in the lamina propria of the mucous membrane.

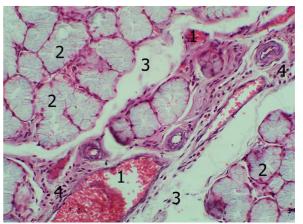


Fig. 4. Palatine salivary gland (experimental Group I) H&E stain. Lens: $10 \times \text{magnification}$, ocular lens: $10 \times \text{magnification}$. 1-blood vessels with the events of plethora; 2-secretory parts; 3-areas with the events of edema; 4-inflammatory infiltration in the connective tissue stroma.

Similar to the connective tissue of the lamina propria of the mucous membrane, phagocytes of various degrees of maturity, lymphocytes, plasma cells dominated in the cellular infiltrates. Sporadic neutrophilic and eosinophilic leukocytes were detected. Quite often in the connective tissue, stroma changes that indicated the presence of edema were noted.

However, no significant changes were noted in the acini of the salivary glands, only in isolated observations there was a slight deformation of secretory epitheliocytes and some change in their tinctorial properties. The findings of the morphometric study have shown that inside each minor palatine salivary gland, the relative amount of connective tissue increased slightly and the relative volume of the secretory parenchyma decreased accordingly, compared to the control group.

Notably, publications report that scientists are still debating the negative (toxic and allergic) effect of the basic acrylic monomer on the mucous membrane of the hard palate and minorsalivary glands in particular. The resulting data indicate that the monomer affects the homeostasis of the oral cavity and causes irritation of the mucous membrane, which on day 30 has signs of epithelial plate dystrophy, characterized by the thickening of the corneal layer due to hypertrophy, indicating hyperkeratosis. The data

are confirmed by the findings of other researchers who found that contact of the mucous membrane of the hard palate with 1% methacrylate causes irritation and disorder of the process of epithelial differentiation in the form of increased keratinization with the signs of dystrophy [12, 13].

The findings of the study revealed thickening of the entire epithelial layer, the events of hyper- and dyskeratosis on day 30 in the experimental group, as well as the events of plethora, high lymphocytes, neutrophils and eosinophils count in the lamina propria of the mucous membrane and increased amount of connective tissue, which can be regarded as a manifestation of the compensatory-adaptive response to the action of the monomer. This is fully consistent with the authors [13, 14], who proved that the structural support of the host defence of the glandular mucosa of the hard palate in the epithelial plate is represented by the intraepithelial lymphocytes and antigen-presenting Langerhans cells, and methacrylate causes changes in the number and ratio of immunocompetent cells.

Conclusion

The 30-day long experimental studies showed that the effect of the monomer of the base acrylic resin "Ftoraks" causes the events of dystrophy, hyper- and dyskeratosis in the mucous membrane of the hard palate, and the increase in the intraepithelial lymphocytes and the presence of cellular infiltrates in connective tissue indicate inflammatory process in the connective tissue. However, in the submucous layer of the hard palate, no significant difference in the cumulative size of the salivary glands, compared to similar value in the intact group, was found.

Prospects of further research are as follows. Since the average recommended period of use of full removable laminar dentures is 3-5 years, it was considered necessary to continue the experiment and investigate the effect of the monomer of the base acrylic resin "Ftoraks" on the condition and structural organization of salivary glands of the hard palate of albino rats after 3, 6 and 9 months to further extrapolate the findings of the study to the human body.

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Реферати

СТАН ТА СТРУКТУРНА ОРГАНІЗАЦІЯ ЗАЛОЗИСТОЇ ЗОНИ ТВЕРДОГО ПІДНЕБІННЯ БІЛИХ ЩУРІВ ПІД ВПЛИВОМ МОНОМЕРУ АКРИЛОВОЇ ПЛАСТМАСИ ВПРОДОВЖ 30 ДІБ

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На сьогодні залишається маловивченою проблема впливу базисів знімних пластинкових протезів, в тому числі і залишкового мономеру, на стан малих слинних залоз, які відіграють важливу роль у підтримці балансу в ротовій порожнині. Тому в даній роботі метою стало

СОСТОЯНИЕ И СТРУКТУРНАЯ ОРГАНИЗАЦИЯ ЖЕЛЕЗИСТОЙ ЗОНЫ ТВЁРДОГО НЁБА БЕЛЫХ КРЫС ПОД ВЛИЯНИЕМ МОНОМЕРА АКРИЛОВОЙ ПЛАСТМАССЫ В ТЕЧЕНИИ 30 СУТОК

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На сегодня остаётся мало изученной проблемма влияния базисов съёмных пластиночных протезов, в том числе и остаточного мономера, на состояние малых слюнных желез, которые играют важную роль в поддержании баланса в ротовой полости. Поэтому целью данной работы было

вивчення в експерименті впливу мономеру базисної акрилової пластмаси «Фторакс» на стан та структурну організацію слинних залоз твердого піднебіння білих щурів. Одержані нами в експерименті дані вказують, що мономер порушує гомеостаз порожнини рота та викликає подразнення слизової оболонки, яке на 30-ту добу має пластинки. ознаки дистрофії епітеліальної характеризується збільшенням товщини рогового шару за рахунок гіпертрофії, що свідчить про наявність гіперкератозу. Нами встановлено, експериментальній групі на 30-ту добу спостерігається збільшення товшини всього епітеліального пласта, явиша гіпер- і дискератозу; у власній пластинці слизової оболонки – явища повнокрів'я, збільшення лімфоцитів, нейтрофільних та еозинофільних лейкоцитів, зростає відносна кількість сполучної тканини, що можна розцінити як прояв компенсаторно-пристосовної реакції у відповідь на дію мономеру. Експериментальними дослідженнями впродовж 30 діб встановлено, що внаслідок дії мономера базисної акрилової пластмаси «Фторакс» в підслизовій основі твердого піднебіння сумарний об'єм слинних залоз істотно не відрізняється від аналогічного показника в інтактній групі. Оскільки середній рекомендований термін користування повними знімними пластинковими протезами становить 3-5 років, тому вважали за необхідне продовжити екперимент і дослідити вплив мономеру базисної акрилової пластмаси «Фторакс» на стан та структурну організацію слинних залоз твердого піднебіння білих щурів через 3, 6 та 9 місяців, що дасть змогу в подальшому екстраполювати отримані результати на організм людини.

Ключові слова: слинні залози, тверде піднебіння, слизова оболонка, мономер акрилової пластмаси.

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изучить в эксперименте влияние мономера базисной акриловой пластмассы «Фторакс» на состояние и структурную организацию слюнных желез твёрдого нёба белых крыс. Полученные нами в эксперименте данные указывают, что мономер нарушает гомеостаз полости рта и вызывает раздражение слизистой оболочки, которое на 30-е сутки имеет признаки дистрофии эпителиальной пластинки, характеризуется увеличением толщины рогового слоя за счет гипертрофии, что свидетельствует о наличии гиперкератоза. Нами установлено, что в экспериментальной группе на 30-е сутки наблюлается увеличение толшины эпителиального пласта, явления гипер- и дискератоза; в собственной пластинке слизистой оболочки - явления полнокровия, увеличение лимфоцитов, нейтрофильных и эозинофильных лейкоцитов, растет относительное количество соединительной ткани, что можно расценить как проявление компенсаторно-приспособительной реакции в ответ на действие мономера. Экспериментальными исследованиями в течении 30 суток встановлено, что вседствии действия мономера базисной акриловой пластмассы «Фторакс» в подслизистой основе твёрдого нёба суммарный объём слюнных желез существенно не отличается от такого же показателя в интактной группе. Поскольку средний рекомендуемый срок пользования полными съёмными пластиночными протезами составляет 3-5 лет, мы считали важным продолжить экперимент и исследовать влияние мономера базисной акриловой пластмассы «Фторакс» на состояние и структурную организацию слюнных желез твёрдого нёба белых крыс через 3, 6 та 9 месяцев, что позволит в дальнейшем экстраполировать полученные результаты на организм человека.

Ключевые слова: слюнные железы, твердое нёбо, слизистая оболочка, мономер акриловой пластмассы.

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MORPHOGENESIS FEATURES OF THE URETEROPELVIC JUNCTION IN HUMAN FETUSES AND NEWBORNS

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The purpose of the study was to find out the features of the ureteropelvic junction structure in the fetal period of ontogeny and in newborns, and to determine the critical periods of its morphogenesis. The study was performed on 67 human fetal specimens with 160.0-500.0 mm of parietococcygeal length (PCL) (4th-10th months of prenatal development). A set of morphological study methods was applied, which included anthropometry, morphometry, injection of vessels with their subsequent radiography and preparation, microscopy, graphic and three-dimensional computer reconstruction, statistical analysis. It is established that the size of the ureteropelvic junction in the early period of ontogeny increases asynchronously: during the 4th month its length increases, during the 7th month and in newborns its diameter grows. From the 4th month of intrauterine development up to the newborn period the ureteropelvic junction's diameter increases from 0.95±0.25 mm to 2.2±0.25 mm. At the beginning of the fetal period (4-5 months), the ureteropelvic junctions are determined at the level of the intervertebral interval between II and III lumbar vertebrae. During the second half of the fetal period, its asymmetrical shift occurs: to the left - to level II, and to the right - to level III of the lumbar vertebrae. In the perinatal period of ontogenesis, close anatomical correlations of the ureteropelvic junction with the renal vessels are determined - its anterior surface is crossed by 2-4 branches of the renal artery and 2-3 branches of the renal vein.

Key words: ureteropelvic junction, anatomy, fetus, newborn.

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The incidence of congenital pathology of the urinary system increases annually, which is also explained by the improvement of antenatal diagnosis [3]. In the structure of urological care, special attention is paid to the ureteropelvic segment (UPS) [15]. Pathological changes of the UPS lead to urodynamic disorders, dilatation of the pelvicalyceal system of kidney (PCSK) with the subsequent development of pyelectasia, hydronephrosis, pyelonephritis, nephrolithiasis, vasorenal hypertension. The degree of disturbance of the anatomic permeability of the UPS is determined by changes in the volume and