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## Interrelation of morphometric and biochemical changes in oral mucous membrane in animals with gastroduodenitis depending on the inflammatory response

Ihor Mysula<sup>1(A,C,D,E,F)</sup>, Valentyna Bondarchuk<sup>1(A,B,C,D,E,F)</sup>, Gustaw Wójcik<sup>2(A,D,E)</sup>,  
Walery Zukow<sup>3(A,B,E,F)</sup>, Nataliya Sydliaruk<sup>1(A,B,E,F)</sup>, Yuriy Mysula<sup>1(A,D,E,G)</sup>,  
Marianna Mysula<sup>1(A,D,E)</sup>

<sup>1</sup>I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

<sup>2</sup>Pope John II State School of Higher Education in Biała Podlaska, Poland

<sup>3</sup>Nicolaus Copernicus University in Torun, Poland

Authors' contribution

Wkład autorów:

A. Study design/planning

zaplanowanie badań

B. Data collection/entry

zebranie danych

C. Data analysis/statistics

dane – analiza i statystyki

D. Data interpretation

interpretacja danych

E. Preparation of manuscript

przygotowanie artykułu

F. Literature analysis/search

wyszukiwanie i analiza literatury

G. Funds collection

zebranie funduszy

**Address for correspondence** Ihor Mysula, Department of Medical Rehabilitation, I. Horbachevsky Ternopil National Medical University, 1 Majdan Voli str., Ternopil, Ukraine, e-mail: mysulaigor@ukr.net, phone: +38 0673387831

ORCIDiDs:

Ihor Mysula <https://orcid.org/0000-0001-5830-0186>,

Valentyna Bondarchuk <https://orcid.org/0000-0001-6906-2494>,

Gustaw Wójcik <https://orcid.org/0000-0001-5005-9711>,

Walery Zukow <https://orcid.org/0000-0002-7675-6117>,

Nataliya Sydliaruk <https://orcid.org/0000-0001-7515-8425>,

Yuriy Mysula <https://orcid.org/0000-0002-1923-8006>,

Marianna Mysula <https://orcid.org/0000-0002-0160-6683>.

### Summary

**Introduction.** There is no consensus opinion regarding the connection of morphometric and biochemical changes in oral mucous membrane of animals with gastroduodenitis depending on the type of inflammatory response. For this reason, it remains important to study the relationship between changes in the morphometric parameters of the oral mucosa, lipid peroxidation indices and the antioxidant system under the influence of various types of inflammatory reaction in the conditions of experimental gastroduodenitis.

**Material and methods.** Experiments were carried out on 42 white male rats weighing 180-200 g housed under normal conditions on a standard vivarium diet; acute gastroduodenitis was simulated for 7 days by inserting by the probe into the stomach 0.25 ml of 10% solution C<sub>2</sub>H<sub>5</sub>OH and after 5 minutes 0.5 ml of 1.25% solution of HCl. Three different types of inflammatory response were also modeled.

**Results.** The most significant changes in oral mucosa on the basis of morphometric and biochemical parameters were found in animals with hyperergic type of inflammatory reaction. Rats with hypoergic type of inflammatory reaction had a lower depth of morphometric and biochemical changes. Morphometric changes were most significant on the tenth day of the experiment, regardless of the type of inflammatory reaction.

**Conclusions.** The obtained results confirm that changes in morphometric and biochemical parameters depend on the type of inflammatory reaction.

**Key words:** cheek; mucous membrane; morphometric parameters; biochemical changes; type of inflammatory response; gastroduodenitis.

## **Introduction**

Despite high enzymatic activity, high rate of metabolic processes and the ability of oral mucous membrane epithelium to quick repair [1,2], the frequency of oral mucosa injuries is constantly increasing[3]. The mucous membrane is often affected by inflammatory processes, which depend on the state of organism. To determine the degree of damage in structural components of the oral mucosa and the state of the membrane-destroying and membrane-stabilizing processes, we used a biochemical method to determine lipid peroxidation activity and antioxidant defense [4]. Unfortunately, today there is no single point of view on the connection between morphometric and biochemical changes in oral mucosa of individuals with gastroduodenitis based on the type of inflammatory response.

## **Purpose of work**

This study focuses on correlation of changes in morphometric parameters of the buccal mucous membrane, lipid peroxidation indexes and antioxidant system in the condition of different types of inflammatory response and experimental gastroduodenitis.

## **Material and methods**

Experiments were carried out in accordance to "European Convention for Protection of Vertebrate Animals Used for Experimental and Scientific Purposes", as well as "General Ethical Principles of Animal Experiments" [5]. Forty-two white male rats weighing 180-200 g, housed under normal conditions on a standard vivarium diet, were included in this study. Acute gastroduodenitis was simulated for 7 days by inserting the probe into the stomach to deliver 0.25 ml of 10% C<sub>2</sub>H<sub>5</sub>OH solution, followed by 0.5 ml of a 1.25% solution of HCl after five minutes [6].

Hypoergic **type of inflammatory response** (TIR) was modeled with intramuscular injection of alkylating cytostatic cyclophosphane (10 mg per kg of body weight) 3 days before experimental gastroduodenitis (EG) modeling and daily for the next 7 next days [7]. Hyperergic TIR was modeled by an intramuscular introducing of 5 minimal doses of a pyrogenal in a physiological solution per animal) 1 day before EG modeling and daily for the next 7 days [8]. Normoergic TIR developed in animals with EG without additional injection of any substances.

In order to conduct the experiment, animals were divided into 4 groups: 1.untreated animals (6 rats); 2. animals with normoergic TIR (12 rats); 3. animals with hypoergic TIR (12 rats); 4. animals with hyperergic TIR (12 rats). Animals were sacrificed by rapid decapitation under Thiopental-Na anesthesia using intraperitoneal injection of 5% Thiopental-Na solution.

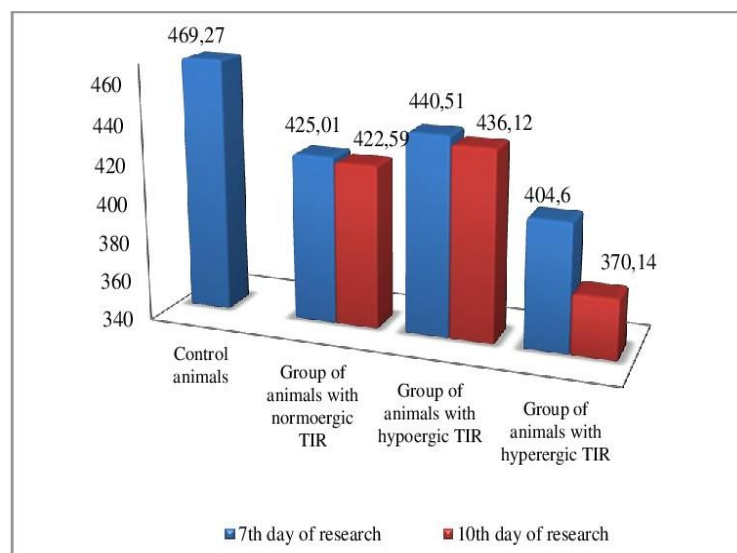
Morphometric measurements of the cheek were made using previously described methodology[9]. Morphometric evaluation was performed using an eyeglass-micrometer MOB-1-15×.

We analyzed biochemical parameters of blood serum, including the concentration of malondialdehyde(MDA), diene conjugates (DC), superoxide dismutase activity (SDA) and catalase (Cat) using published methods [10, 11, 12, 13]. Correlation analyses were performed taking into account the coefficient (r) of the pair, correlation between the morphometric indices (thickness of the mucous membrane, thickness of the epithelial layer, thickness of the own plate, number of damaged epitheliocytes of the spindle layer, relative volume of capillaries and biochemical parameters (MDA, DC, SDA, Cat). The strength of correlation was categorized as strong (r = 0.7-0.9), significant (r = 0.5-0.7), moderate (r = 0.3-0.5) or weak (r <0.3).

Calculation of results was performed using the software package Statsoft STATISTICA.

## **Results**

Average thickness of buccal mucous membrane in animals of control group was (469.27±12.31) mcm, while animals with normoergic TIR showed thinning. In particular, the thickness of buccal mucous membrane on the 7th day of the experiment animals with normoergic TIR showed a difference of 9.4% (p < 0.05), animals with hyperergic TIR demonstrated a change of 13.8% (p < 0.001). On the 10th day, the difference of the average thickness of buccal mucous membrane of the animals with normoergic TIR was 9.9% (p < 0.001), the rats with hyperergic TIR – 21.1% (p < 0.001). Thickness of buccal mucous membrane of animals with normoergic TIR was (425.01±11.34) mcm. In comparison with the group of rats with hyperergic TIR, parameters on the 10th day decreased by 15.1% (p < 0.05). Thickness of thebuccal mucous membrane of animals with hypoergic TIR on the 10th day of the time course increased compared with a group of animals with hyperergic TIR by 15.1% (p < 0.001) (Picture 1).



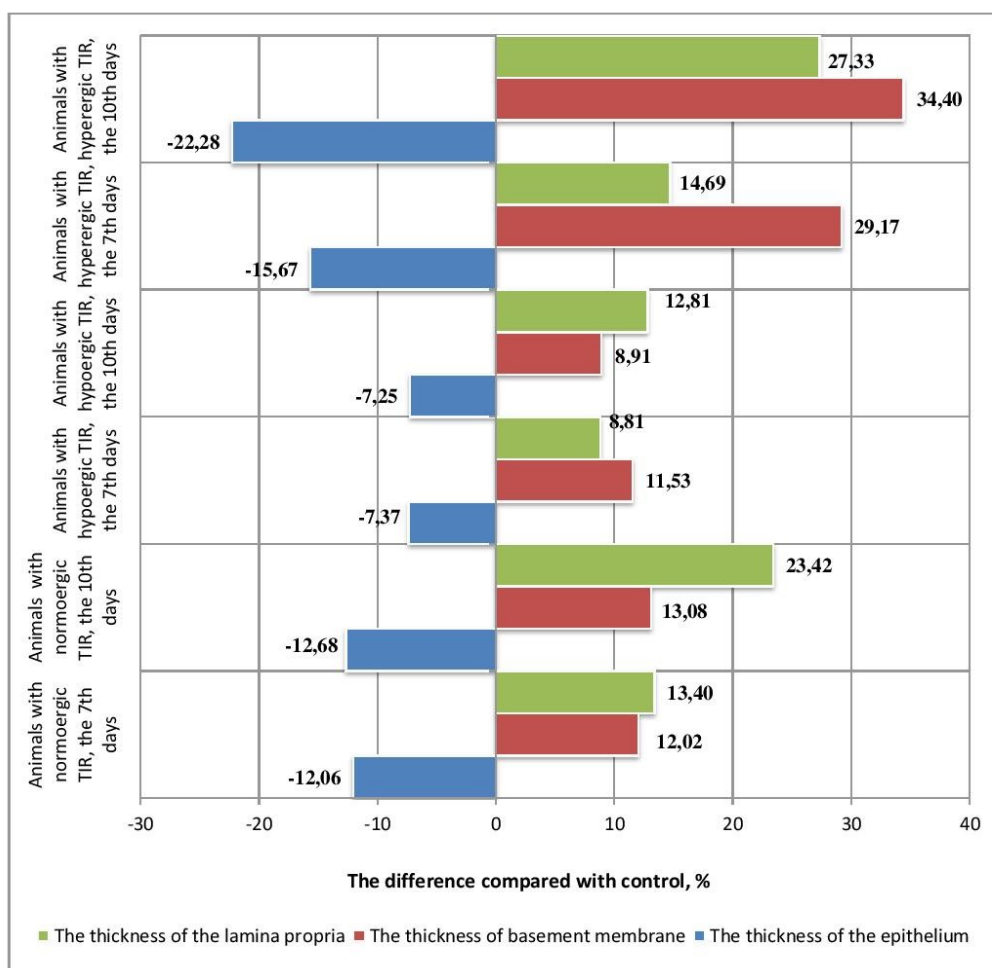
Picture 1 – Thickness of buccal mucous membrane in mcm, under the influence of various types of inflammatory reaction, the 7<sup>th</sup> and 10<sup>th</sup> days of research.

The cause of buccal mucous membrane shortening was significant thinning of the epithelial layer that can be explained by different intensity of degenerative changes. At the same time, other structural components of the buccal mucous membrane such as the basal membrane and lamina propria were enlarged. The most significant structural changes of the epithelium of the buccal mucous membrane by microscopy were found in animals with hyperergic TIR. This trend was also reflected in the morphometric study, thickness of the epithelial layer in this experimental group of animals decreased compared with the control group by  $311.08 \pm 5.05$  mcm, on the 7th day of the experiment this parameter was 15.7% lower ( $p < 0.001$ ), and on the 10th day, 22.3% lower ( $p < 0.001$ ).

Thinning of the epithelial layer in the group of animals with normoergic TIR compared with the control group was less evident and was not significant in terms of the experiment; data from the 7th day demonstrated 12.1% ( $p < 0.05$ ) regression of morphometric parameters, and on the 10th day there was a 12.7% ( $p < 0.001$ ) regression. Also on the 10th day there was a difference of 22.3% ( $p < 0.05$ ) when comparing with parameters of animals with normoergic TIR and those with hyperergic TIR. Epithelial basal membrane thickness in the buccal mucous membrane in the control group was  $(10.32 \pm 0.21)$  mcm, and enlargement of this membrane was observed in all experimental groups. On the 7th day, rats with normoergic TIR showed enlargement of epithelial basal membrane [12.1% ( $p < 0.05$ )], while in animals with hypoergic TIR the change was 11.5% ( $p < 0.05$ ) and in animals with

hyperergic TIR it was 29.2% ( $p < 0.001$ ). On the 10th day of research these parameters were also higher, 13.1% ( $p < 0.05$ ), 8.9% ( $p < 0.05$ ) and for 34.4% ( $p < 0.001$ ), respectively. The results of the morphometric study revealed that trophic violation of epithelial cells and their subsequent degeneration was the result of basal membrane enlargement. Thickness of epithelial basal membrane of the buccal area of oral cavity in animals with normoergic TIR was 15.3% lower ( $p < 0.001$ ) on the 7th day and 34.4% lower ( $p < 0.001$ ) on the 10th day, compared with animals with hyperergic TIR. Of note, this parameter was 15.8% lower ( $p < 0.001$ ) on the 7th day and 23.4% lower ( $p < 0.001$ ) on the 10th day in animals with hypoergic TIR compared to hyperergic TIR.

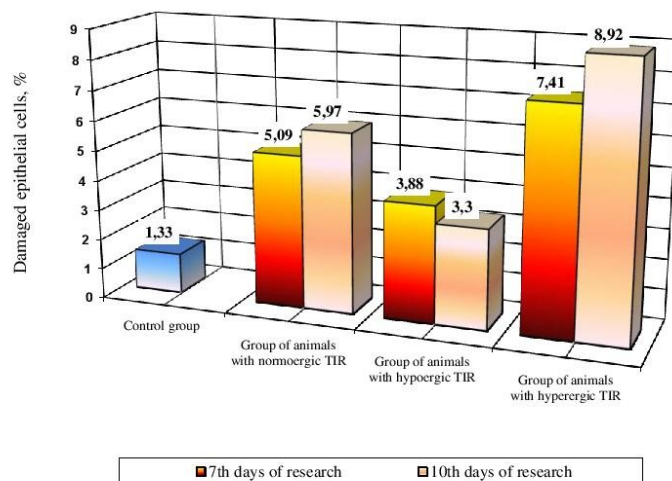
Measurement of lamina propria of buccal mucous membrane confirmed that its thickness in the control group was  $(140.62 \pm 4.18)$  mcm. Animals with normoergic TIR showed lamina propria enlargement of 13.4% ( $p < 0.05$ ), in animals with hyperergic TIR the change was 14.7% ( $p < 0.05$ ). Such changes were seen on the 7th day of investigation and in three days the thickness of lamina propria was further increased when comparing the control group with the normoergic TIR group [23.4% ( $p < 0.001$ )], similarly, in animals with hypoergic TIR the change was 12.8% ( $p < 0.05$ ) and in animals with hyperergic TIR it was 27.3% ( $p < 0.001$ ) (Picture 2).



Picture 2 – Dynamics of changes in lamina propria and basal membrane thickness and thickness of buccal epithelium of white rats under the influence of different types of inflammatory reaction, the 7<sup>th</sup> and 10<sup>th</sup> days of research comparing with the group of control

An important indicator of alteration processes in the buccal mucous membrane is the amount of damaged epitheliocytes. There were 1.33 ( $\pm 0.07$ )% morphologically changed epithelial cells in control group animals. On the 7<sup>th</sup> day, the amount of damaged epithelial cells in animals with normoergic TIR was 3.8% higher ( $p < 0.001$ ) and it was 6.1% higher ( $p < 0.001$ ) in animals with hyperergic TIR. On the 10<sup>th</sup> day of research this parameter

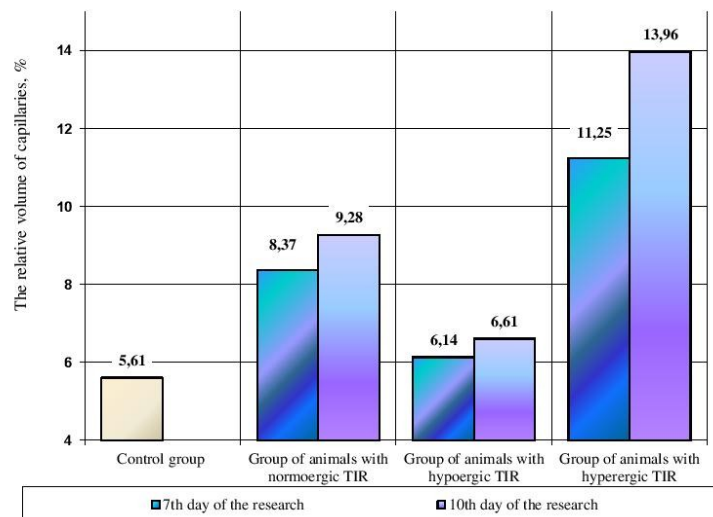
increased by 4.6%, and in 7.6%, respectively, compared with control. Statistically important differences were determined by comparison with the control group ( $p < 0.001$ ; Picture 3).



Picture 3 – Amount of damaged epitheliocytes in buccal mucous membrane of rats under the influence of different types of inflammatory reaction, the 7<sup>th</sup> and 10<sup>th</sup> days of research.

Circulatory disturbance played an important role in the pathogenesis of buccal mucous membrane changes in animals with experimental EG and with different types of inflammatory responses. The circulatory disturbance manifested as intensification of capillaries relative volume during morphometric investigation. The relative volume of capillaries of the buccal mucous membrane in animals from the control group was  $(5.61 \pm 0.18)\%$  (Picture 4).





Picture 4 – The relative volume of capillaries in buccal mucous membrane of rats under the influence of different types of inflammatory reaction, the 7<sup>th</sup> and 10<sup>th</sup> days of research.

The capillaries relative volume in animals with normoergic TIR was 2.8% higher and it was 5.6% higher in the hyperergic TIR group on the 7th day of research. On the 10th day of research, progression of morphometric parameters was 3.7% and 8.4%, ( $p < 0.001$ ) in the normoergic and hyperergic groups, respectively. On the 7th day the relative volume of the capillaries in rats with normoergic TIR, compared with animals with hyperergic TIR, was 2.9% lower ( $p < 0.001$ ), and on the 10th day it was 4.7% lower ( $p < 0.001$ ). The decrease of 2.7% ( $p < 0.001$ ) was found when comparing the measurements of animals with hypoergic TIR with the measurements from thenormoergic TIR group on the 10th experimental day. On the 7th day, the relative volume of capillaries in animals with hyperergic TIR compared with the hypoergic TIR was 5.1% higher; on the 10th day of research the measurements were 7.4% higher ( $p < 0.001$ ).

The inflammatory response is accompanied by the activation of membrane-destroying processes and at the same time membrane-stabilizing processes of the cell; the magnitude of change depends on the level of reactivity of the organism and the strength and duration of the damaging factors. The membrane-damaging factor in pathological conditions is, primarily, the lipid peroxidation processes- At the same time, there are systems to counteract the damage processes in tissues and for the lipid peroxidation activation the counteracting factor is the antioxidant system. Animals with hyperergic TIR showed a significant increase in the level of lipid peroxidation indices compared to the control group (Table 1). Comparing data from

animals with hyperergic TIR with indices of animals with normoergic TIR, it should be noted that the serum MDA level was higher on the 7th day of study by 20.6% ( $p < 0.05$ ) and on the 10th day by 17.1% ( $p < 0.05$ ); the level of DK was higher on the 10th day of the study by 5.5% ( $p < 0.05$ ). The level of MDA in animals with hyperergic TIR was higher on the 7th day by 37.0% ( $p < 0.05$ ) and on 10th day by 44.0% ( $p < 0.05$ ); the level of DK was higher on the 7th day of the study by 9.5% ( $p < 0.05$ ) and on the 10th day by 7.8% ( $p < 0.05$ ) compared to rats with the hypoergic type of inflammatory response.

**Table 1.** Changes of lipid peroxidation and antioxidant system parameters in animals with different types of inflammatory response ( $M \pm m$ )

Parameters	Control	Animals with hyperergic type of inflammatory response	Animals with normoergic type of inflammatory response	Animals with hypoergic type of inflammatory response			
		Experimental day					
		7th day of research	10th day of research	7th day of research	10th day of research	7th day of research	10th day of research
MDA (mcmol/l)	2.517±0.111	9.187±0.071 $p_1 < 0.05$ $p_2 < 0.05$	8.890±0.097 $p_1 < 0.05$ $p_2 < 0.05$	7.617±0.051 $p_1 < 0.05$ $p_4 < 0.05$	7.587±0.047 $p_1 < 0.05$ $p_4 < 0.05$	6.702±0.014 $p_1 < 0.05$ $p_7 < 0.05$	6.173±0.116 $p_1 < 0.05$ $p_7 < 0.05$
DK (Cond.units/ml)	2.973±0.208	4.082±0.062 $p_1 < 0.05$ $p_2 < 0.05$ $p_3 > 0.05$	4.212±0.037 $p_1 < 0.05$ $p_2 < 0.05$	4.028±0.038 $p_1 < 0.05$ $p_4 < 0.05$ $p_5 > 0.05$ $p_6 > 0.05$	3.992±0.048 $p_1 < 0.05$ $p_4 < 0.05$ $p_6 > 0.05$	3.727±0.032 $p_1 < 0.05$ $p_7 < 0.05$	3.905±0.029 $p_1 < 0.05$ $p_7 < 0.05$ $p_3 > 0.05$
SDA (units of activ.)	0.509±0.003	0.272±0.005 $p_1 < 0.05$ $p_2 < 0.05$	0.302±0.004 $p_1 < 0.05$ $p_2 < 0.05$ $p_3 > 0.05$	0.296±0.006 $p_1 < 0.05$ $p_4 < 0.05$	0.306±0.004 $p_1 < 0.05$ $p_4 < 0.05$ $p_5 > 0.05$	0.401±0.005 $p_1 < 0.05$ $p_7 < 0.05$	0.423±0.004 $p_1 < 0.05$ $p_7 < 0.05$
Cat (mccat/l)	0.544±0.002	0.209±0.003 $p_1 < 0.05$ $p_2 < 0.05$	0.212±0.003 $p_1 < 0.05$ $p_2 < 0.05$	0.273±0.003 $p_1 < 0.05$ $p_4 < 0.05$	0.275±0.004 $p_1 < 0.05$ $p_4 < 0.05$	0.367±0.003 $p_1 < 0.05$ $p_7 < 0.05$	0.374±0.005 $p_1 < 0.05$ $p_7 < 0.05$

Note 1.  $p_1$  - given results reliably differ from the data of control group;  $p_2$  - given results significantly differ from data in groups of animals with hypoergic and normoergic types of inflammatory response;  $p_3$  - given results are unreliable comparing with data in group of animals with normoergic type of inflammatory response;  $p_4$  - given results are significantly different from the parameters of groups of animals with hypo-allergic and hyperergic types of inflammatory response;  $p_5$  - given results are unreliable comparing with parameters of animals with hyperergic type of inflammatory response;  $p_6$  - given results are unreliable comparing with parameters of animals with hypoergic type of inflammatory response;  $p_7$  - given results significantly differ from those in groups of animals with hyperergic and normogonal types of inflammatory response.

Activity of antioxidant system was lower compared with the control group in all experimental groups (see Table 1). When comparing measurements of rats with hyperergic

TIR and measurements of those with normoergic TIR, we found that activity of SDA was lower by 8.1% ( $p < 0.05$ ) on the 7th day; Cat activity decreased on the 7th day by 23.4% ( $p < 0.05$ ) and on the 10th day by 22.9% ( $p < 0.05$ ). Compared with animals with a hypoergic type of inflammatory response, the activity of SDA in the hyperergic TIR group was lower by 32.1% ( $p < 0.05$ ) on the 7th day and by 28.6% ( $p < 0.05$ ) on the 10th day; Cat activity was lower on the 7th day by 43.0% ( $p < 0.05$ ) and by 43.3% on the 10th day ( $p < 0.05$ ).

After analyzing the changes in morphometric and biochemical parameters it can be stated that despite the decreases in the experimental parameters of mucous membrane thickness, epithelial layer and its own plate thickness, growth of number of damaged epitheliocytes of the spinous layer and the relative volume of hemocapillaries, the levels of MDA and DK increased and the SDA and Cat activity in serum decreased.

In particular, our analysis revealed a strong correlation in animals with normal TIR on the 7th day between the specific percentage of damaged epithelial cells of the spinous layer and the level of DC in the serum ( $r = + 0.779 \pm 0.134$ ). In animals with hypoergic TIR there was a correlation between thickness of the epithelial layer and the level of MDA in serum ( $r = + 0.847 \pm 0.113$ ) and between the specific portion of damaged epitheliocytes of the spinous layer and activity of SDA in serum ( $r = 0.769 \pm 0.136$ ). In animals with hyperergic TIR on the 10th day, the thickness of the mucous membrane correlated with the level of MDA in blood serum ( $r = + 0.795 \pm 0.129$ ), as well as there being a correlation between the thickness of epithelial layer and the level of MDA in serum ( $r = + 0.853 \pm 0.111$ ). The statistically significant difference was determined during comparing these groups ( $p < 0.001$ ).

## Discussion

The experiments presented here demonstrate that the thickness of the buccal mucous membrane of animals with hypoergic TIR increased compared with a group of animals with hyperergic TIR on the 10th experimental day. Buccal mucous membrane shortening was a result of significant thinning of the epithelial layer, which could be explained by different intensity of degenerative changes [14, 15]. The basal membrane of the buccal mucous membrane and lamina propria showed enlargement. The most significant structural changes of the epithelium of buccal mucous membrane at microscopy were revealed in animals with hyperergic TIR. This data was also reflected in morphometric study, thickness of the epithelial layer in this experimental group decreased compared with the control group. Epithelial thinning in animals with normoergic TIR compared with the control group was less evident and there was no significant difference in this experiment. It's worth noting that on the 10th day the difference was 22.3% ( $p < 0.05$ ) when comparing the normoergic TIR group with the hyperergic TIR group. The results of morphometric study revealed that trophic changes of epithelial cells and their subsequent degeneration was the result of basal membrane enlargement [3, 16]. Thickness of the epithelial basal membrane of the buccal area of the oral cavity in animals with normoergic TIR was lower compared to animals with hyperergic TIR. Interestingly, this parameter was lower in animals with hypoergic TIR on the 7th day of the experiment and on the 10th compared to the hyperergic group. Measurement of the lamina propria of the buccal mucous membrane confirmed that its thickness in groups with normoergic and hyperergic TIR on the 7th day of the experiment was related to lamina propria. An important indicator of alteration processes in buccal mucous membrane is the

number of damaged epitheliocytes [1, 15]. On the 7th day the amount of damaged epithelial cells in animals of both group - with normoergic TIR and in animals with hyperergic TIR was higher. Circulatory disturbance played an important role in the pathogenesis of buccal mucous membrane changes in animals with experimental EG and with different types of TIR [2]. This change manifested as intensification of capillaries relative volume during morphometric investigation.

Changes in morphometric parameters in animals with different types of inflammatory responses correlated with increased activity of membrane-destructive processes, an increase of MDA and DC in blood serum and a decrease in activity of the membrane-stabilizing factors, that was shown by inhibition of SDA and catalase activity. The results presented here confirm that the changes of morphometric and biochemical parameters depend on the type of inflammatory reaction the animal experiences.

## Conclusions

1. The morphometric parameters of structural components of the buccal mucous membrane and biochemical changes are determined based on the influence of different types of inflammatory responses.

2. The most significant morphometric and biochemical changes were found in group of animals with a hyperergic type of inflammatory response, mucous membrane thickness decreased by 21.1% ( $p < 0.001$ ) comparing with the control group and the epithelial layer decreased by 22.3% ( $p < 0.001$ ); thickness of basal membrane and lamina propria increased by 34.4% ( $p < 0.001$ ) and by 27.3% ( $p < 0.001$ ). The level of MDA increased 2.5 times ( $p < 0.05$ ) and the level of DC was higher by 41.6% ( $p < 0.05$ ), the SDA was lower by 40.6% ( $p < 0.05$ ), and catalase activity decreased by for 61.0% ( $p < 0.05$ ). Animals with a hypoergic type of inflammatory response had the least significant morphometric and biochemical changes.

3. The most significant changes in morphometric parameters of the buccal mucous membrane and biochemical changes in blood serum were detected on the 10th day of the experiment.

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