


**MORPHOFUNCTIONAL CHARACTERISTICS OF STRUCTURAL CHANGES IN
GASTRIC BLOOD VESSELS AFTER REMOVAL OF MANDIBULAR AND PAROTID
GLAND IN RATS**
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ABSTRACT

A significant number of scientific works are devoted to study of salivary glands role in maintaining the normal functioning of the human body and ensuring a systematic welfare, but this problem is not fully explored. As to pathogenetic connection between salivary glands affections and other diseases of the gastrointestinal tract organs, most of the studies considered it from the position of the secondary oral cavity pathology, which develops against other digestive diseases while anatomically and functionally a reversed approach would be more logical and consistent. We should take into account the fact that one of the priorities of modern morphology is to establish character and features of the circulatory system restructuring under the disturbed conditions of hemocirculation as an important link in the development of pathological processes. Removal of the parotid and mandibular glands in rats accompanied by significant remodeling of stomach blood vessels that is the progressive strengthening of blood supply with same time increasing of vascular resistance in organ during the 21th day after operation. From 21th to 28th day of the experiment, there is a partial regression of morphofunctional state of stomach bloodstream, which may be caused by compensatory hyperplasia and hyperfunction of other localization of salivary glands of other located salivary glands which are left (sublingual, buccal, gingival and lingual).

KEYWORDS: salivary glands, stomach, Vohenvort index, remodeling, blood vessels.

INTRODUCTION

A significant number of scientific works are devoted to study of salivary glands role in maintaining the normal functioning of the human body and ensuring a systematic welfare, but this problem is not fully explored.^[3] As to pathogenetic connection between salivary glands affections and other diseases of the gastrointestinal tract organs, most of the studies considered it from the position of the secondary oral cavity pathology, which develops against other digestive diseases^[6, 7, 10] while anatomically and functionally a reversed approach would be more logical and consistent. We should take into account the fact that one of the priorities of modern morphology is to establish character and features of the circulatory system restructuring under the disturbed conditions of hemocirculation as an important link in the development of pathological processes.^[8, 9]

MATERIALS AND METHODS

The experiments have been carried out on 78 non-linear white male rats, weighting 180-200 grammes. During the experiment, the animals were on a standard diet in accordance with the rules of experimental animals keeping laid down in Directive 2010/63 / EU and the

Order of the Ministry of Education, Youth and Sports of Ukraine of 01.03.2012 p. № 249.

All the animals were divided into three groups: one control group and experimental one. The control group included twelve intact animals. Another seventy-two animals underwent a simultaneous bilateral removal of the submandibular and parotid glands. Operations were carried out under general ketamine anesthesia. On 1st, 3d, 7th, 14th, 21th, and 28th days of the experiment the material in the experimental group animals were taken for morphological studies. All the experimental research was performed in accordance with "Principles of Laboratory Animal Care". The rats were taken out of the experiment by means of total bloodletting from the heart under thiopental and sodium anesthesia (60 mg per kg⁻¹ of the body internally abdominal weight).

For histological study the pieces of tissue from different parts of the stomach were fixed in 10 % neutral formalin solution, 96° spirit. Paraffin sections with 5-7 mcm were stained by hematoxylin and eosin, due to Weigert and Van Gieson Morphometric evaluation of intraorganic vessels was performed with the help of ocular

micrometer MOV-1-15CH (MOB-1-15Ч), and determine the value of external (d) and internal (d₁) diameters. The thickness of the muscle membrane (TM) was calculated by the formula^[4]

$$TM = \frac{d - d_1}{2}$$

The examination of the functional condition of the vessels was being carried out by calculating of the Index by Vohenvort (IV), that is the ratio of the area of the middle artery's layer to the area of their lumen: IV = (SM / SL)100 %, Electronic materials received due to the carried out morphologic and functional research have undergone the statistic processing with the help of Microsoft Exel. The average value (M), standard deviation (δ) and the average error (m) were determined.

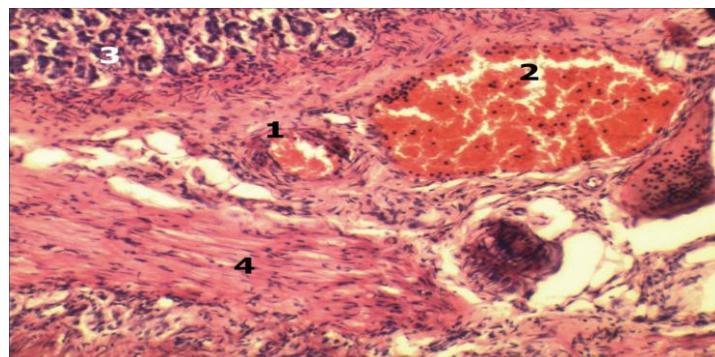
RESULTS

Simultaneous bilateral removal of the parotid and mandibular glands of rats made a significant impact on the stomach bloodstream with corresponding morphological changes in it. Besides that, found morphological changes were growing gradually, progressing in time. On alternate days after operation, histologically signs of gastric blood flow disorders

mainly lied in expressed venous and arterial plethora with the expansion of the vascular lumen "Fig. 1". Such changes quantitatively were confirmed by decreasing of Vohenvort index in all studied vascular orders with reliable differences in middle diameter arteries "Table 1".

After 3 days of experiment by the histological examination of the stomach, on the background of saved arterial and venous hyperemia, in arteries of medium and small diameter the reverse changes were observed. Compared to the previous period of observation reaction of Vohenvort index exceeded the target figures a little, which may be a sign of increasing vascular tone with reduced capacity of the arteries. At the same time in the arteries of big diameter, this index continued to decline, which may indicate a weakening of vascular walls tone with lumen area increasing, which provide a compensatory increase of vessels capacity for partial deposit of blood.

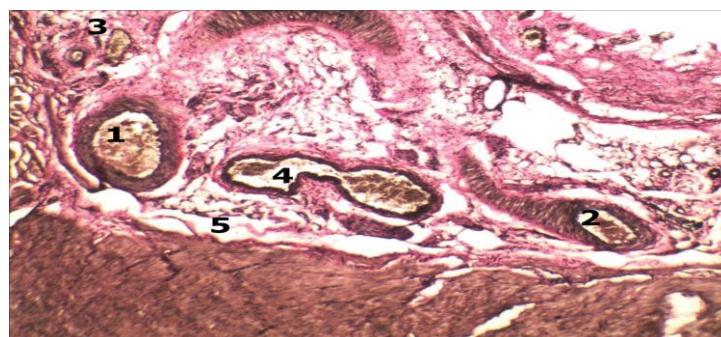
The most significant changes were observed on the 7th day of the experiment. Histologically, at this period, arteries and veins continued to be plethoric. The lumen of big diameter arteries was enlarged and filled with blood cells, their walls were thinner with.



Extended full-blooded artery – 1, extended full-blooded vein – 2, mucosa – 3, muscular layer – 4

Figure 1 – Histological section of the rats stomach wall in 1 day after bilateral remove of the parotid and mandibular glands. Hematoxylin and eosin stain x 140.

swollen endothelium. Arteries with middle and especially small diameters had comparatively thickened walls also, they contained clusters of red blood cells "Fig. 2".



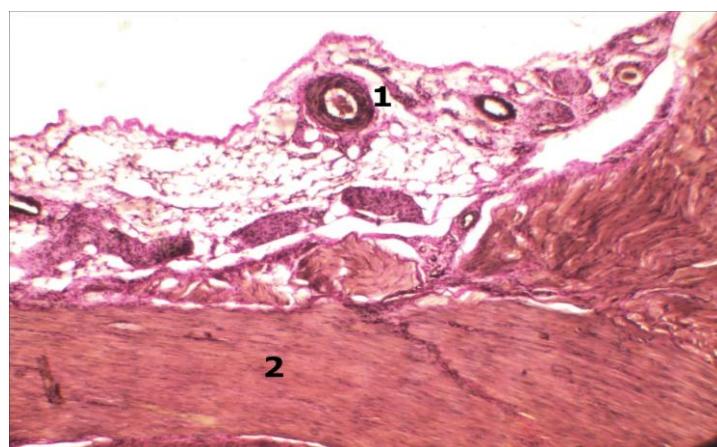
Extended full-blooded artery with bigger diameter – 1, middle diameter artery – 2, artery and vein with small diameter – 3, extended full-blooded vein – 4, paravasal space – 5.

Figure 2 – Histological section of the rats stomach wall in 7 days after bilateral remove of the parotid and mandibular glands. Weigert stain x 140.

The confirmation of functional state of different diameter arteries at this period of observation was represented with the results of morphometric analysis. Increasing capacity of arteries with external diameter of 126-150 micrometers was indicating the decreasing level of Vohenvort index that was already in 9% lower than in intact animals. In arteries with the external diameter of 126-150 micrometers, this index was close to the control level and exceed it only for a little. As for the small arteries, with the external diameter 26-50 micrometers, this indicator, unlike the previous two, was reliable exceeding the control data in 21 % "Table 1". Such dynamics of index demonstrated a total vascular wall

area increasing, compared to the vessel of lumen, which is typical for increasing vessels tone with hypertrophy of smooth muscle cells in the middle membrane.

Changes became more expressed at 14th and especially 21th days after surgery. On the 14th day of the experiment, the degree of vascular plethora slightly decreased but in the lumen of the arteries and veins, accumulations of blood cells still could be found. Walls thickening and lumen narrowing in arteries of middle and especially small diameter were established "Fig. 3". The thickening of vascular walls arose as by smooth muscle tone.

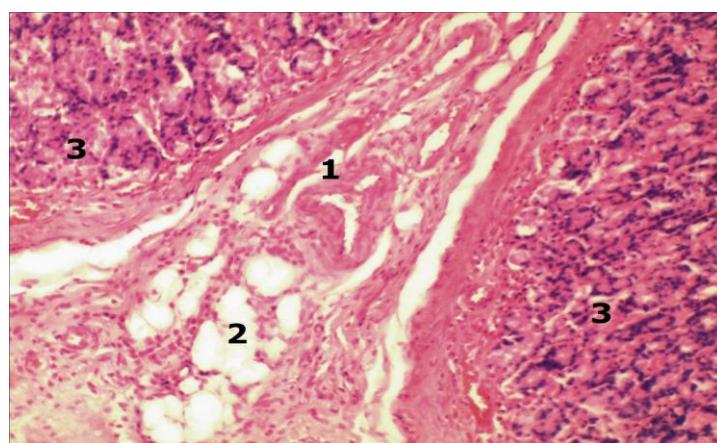


Middle diameter artery with extended wall – 1, stomach muscular layer – 2

Figure 3 – Histological section of the rats stomach wall in 14 days after bilateral remove of the parotid and mandibular glands. Hematoxylin and eosin stain x 140.

Increasing which confirmed the increased tortuosity of the internal elastic membrane. The hypertrophy of smooth muscle elements took place where the size of the nuclei increased and color of cytoplasm became more compact. Deformed arteries with irregular wall thickness were indicating on increasing vascular tone. In these arteries thickening appeared due to hyperplasia of obliquely and longitudinally oriented myocytes. On the

microscopic sections these cells could be distinguished by their oval shape nuclei unlike circular fibers with spindle-shaped cores "Fig. 4". More often than in intact animals the increased tone of smooth muscle sphincters in lateral tributaries branching in the arteries and veins orifices was detected. Increasing tone of these structures significantly affects the redistribution of blood flow, and thus on the intensity of organ blood supply.



Triangular deformation of artery submucosal layer with irregular wall thickening – 1, edema of the submucosal layer – 2, gland of mucosa – 3.

Figure 4 – Histological section of the rats stomach wall in 14 days after bilateral remove of the parotid and mandibular glands. Hematoxylin and eosin stain x 180.

On the 14th day after simultaneous bilateral removal of the parotid and mandibular glands morphological indicators, which shows the functional state of arteries with medium and small diameters were reliably growing. The capacity of big diameter arteries continued to increase through the lumen dilatation. Vohenvort index decreased in arteries with external diameter 126-150 micrometers in 12 % then in arteries with external diameter 51-125 and 26-50 micrometers it was reliably increasing in 14 % and 32 % respectively which was accompanied by thickening of their muscle layer in 9% and 12%.

On 21th day after operation, morphological changes in the bloodstream of rats stomach were the most severe. By the histological examination of organ microscopic sections thickening of arteries walls was found, especially with small and medium diameter with corresponding narrowing of the lumen. These vessels contained in their lumen different amount of red blood cells clusters "Fig. 5". Often could be observed small diameter arteries with particularly thick walls "Fig. 6". This indicates their special functional activity associated with the redistribution of blood flow and regulation of its intensity. Described above changes were confirmed by morphometric parameters.



Middle diameter artery with extended wall – 1, arteriola – 2, vein lumen filled with blood cells cluster – 3, stomach muscular layer – 4.

Figure 5 – Histological section of the rats stomach wall in 21 days after bilateral remove of the parotid and mandibular glands. Weigert stain x 140.

In this period of the experiment in arteries with external diameter 51-125 micrometers media thickness increased in 12 % and Vohenvort index also increased in 17 %. In arteries with external diameter 26-50 micrometers growth of these indicators is 18 % and 44 %. In arteries with external diameter 126-150 micrometers Vohenvort index was in 13 % lesser than the corresponding one in intact animals. The dynamics of obtained data indicates different functional orientation of arteries with different diameters. Arteries, especially small and medium diameters changed its throughput by increasing the walls

tone and narrowing of the lumen, the arteries large diameters by compensatory expands increasing its capacity.

From 21th to 28th day of the experiment there was a regression of pre-identified structural and morphological changes in the bloodstream of experimental animals with partial regression of the studied parameters, which nevertheless continued significantly predominate over the control ones.



Small diameter artery with thick walls – 1, stomach muscular layer – 2.

Figure 6 – Histological section of the rats stomach wall in 21 days after bilateral remove of the parotid and mandibular glands. Hematoxylin and eosin stain x 180.

Thus, results of the study indicate that simultaneous bilateral removal of the parotid and mandibular glands in rats significantly affects the intensity of the stomach circulation.

Discovered morphological changes in the bloodstream of the stomach are characteristic of conditions with increased blood supply in organ with intensification of vascular resistance in it. The confirmation of the increased stomach blood circulation is arterial and venous plethora and increase capacity in arteries with larger diameter. This confirmed by Vohenvort index decreasing in arteries with an external diameter 126-150 micrometers, as manifestation of compensatory reaction.^[8, 9]

With increased blood supply, including adaptive mechanisms aimed at overloading prevention of microvasculature. Histologically it is manifested by increased resistance due to walls thickening and narrowing of the lumen in arteries with medium and small diameters (from 26 to 125 micrometers) confirmed by increasing of Vohenvort index.^[8, 9] Intensity of constrictor reaction increased as the diameter of the arteries reduced. All these changes progressively grew to 21th day of the postoperative observation. Then from 21th to 28th day their partial regression was observed. This can be the proof of partial blood supply recovery of the stomach as a result of compensatory hyperplasia and hyperfunction of other located salivary glands that were not removal (sublingual, buccal, gingival and lingual), that is observed in other experimental observations.^[1, 2, 5]

Table 1 – Morphometric parameters intraorganic branching of arteries in rats stomach in norm and in different terms bilateral remove of the parotid and mandibular glands ($M \pm m$)

Parameters The duration of observation		Vessels diameter											
		Large (126-150 micrometers)				Middle (51-125 micrometers)				Small (26-50 micrometers)			
Externel diameter	Internal diameter	Media thickness	IV	Externel diameter	Internal diameter	Media thickness	IV	Externel diameter	Internal diameter	Media thickness	IV		
Control		137,83 $\pm 0,91$	96,33 $\pm 1,20$	20,75 $\pm 0,17$	104,90 $\pm 2,43$	71,00 $\pm 0,86$	46,17 $\pm 0,70$	12,42 $\pm 0,15$	136,69 $\pm 2,70$	34,50 $\pm 0,96$	20,67 $\pm 0,49$	6,92 $\pm 0,24$	178,49 $\pm 3,36$
Remove of the parotid and mandibular glands	1 th day	138,00 $\pm 0,86$	97,50 $\pm 1,26$	20,25 $\pm 0,21$	100,56 $\pm 2,76$	71,83 $\pm 0,95$	47,83 $\pm 0,75$	12,00 $\pm 0,18$	125,67 $\pm 2,59^*$	34,33 $\pm 0,92$	21,00 $\pm 0,63$	6,67 $\pm 0,17$	167,71 $\pm 4,05$
	3 th days	138,17 $\pm 0,87$	98,00 $\pm 1,18$	20,08 $\pm 0,20$	98,95 $\pm 2,48$	72,33 $\pm 0,99$	46,83 $\pm 0,87$	12,75 $\pm 0,21$	138,91 $\pm 4,12$	34,67 $\pm 0,95$	20,17 $\pm 0,40$	7,25 $\pm 0,28$	195,18 $\pm 5,07$
	7 th days	137,67 $\pm 0,88$	98,67 $\pm 1,54$	19,50 $\pm 0,34$	95,04 $\pm 3,61$	72,83 $\pm 0,83$	46,83 $\pm 0,79$	13,00 $\pm 0,13^*$	142,16 $\pm 2,42$	34,33 $\pm 0,95$	19,33 $\pm 0,49$	7,50 $\pm 0,26$	215,45 $\pm 5,83^*$
	14 th days	138,00 $\pm 0,97$	99,50 $\pm 1,69$	19,25 $\pm 0,42$	92,81 $\pm 4,16$	72,67 $\pm 1,36$	45,50 $\pm 0,99$	13,58 $\pm 0,24^*$	155,33 $\pm 3,20^*$	34,17 $\pm 0,83$	18,67 $\pm 0,42$	7,75 $\pm 0,25$	235,35 $\pm 7,74^*$
	21 th days	137,67 $\pm 0,88$	99,50 $\pm 1,69$	19,08 $\pm 0,42$	91,89 $\pm 4,14$	73,00 $\pm 1,32$	45,17 $\pm 0,95$	13,92 $\pm 0,20^*$	161,42 $\pm 2,14^*$	34,33 $\pm 0,95$	18,17 $\pm 0,40$	8,08 $\pm 0,30^*$	257,09 $\pm 7,64^*$
	28 th days	138,17 $\pm 0,87$	98,67 $\pm 1,54$	19,75 $\pm 0,40$	96,51 $\pm 4,08$	73,17 $\pm 1,17$	46,33 $\pm 1,02$	13,42 $\pm 0,15^*$	149,84 $\pm 4,17$	34,33 $\pm 0,99$	18,83 $\pm 0,79$	7,75 $\pm 0,11^*$	234,41 $\pm 8,62^*$

Note: * - $p < 0,05$

CONCLUSIONS

Remove of the parotid and mandibular glands in rats accompanied by significant remodeling of stomach blood vessels that is the progressive strengthening of blood supply with same time increasing of vascular resistance in organ during the 21th day after operation. From 21th to 28th day of the experiment, there is a partial regression of morphofunctional state of stomach bloodstream, which may be caused by compensatory hyperplasia and hyperfunction of other localization of salivary glands of other located salivary glands which are left (sublingual, buccal, gingival and lingual).

REFERENCES.

1. Evaluation of radioprotective effect of vitamin E in salivary dysfunction in irradiated rats / F.M. de Moraes Ramos, M.L. dos Anjos Pontual, S. M. de Almeida [et al.] // Arch. Oral Biol., 2006; 51(2): 96-101.
2. Licht R. Salivary gland-sparing prophylactic pilocarpine treatment has no effect on tumor regrowth after irradiation / R. Licht, H. H. Kampinga, R.P. Coppes // Radiat Res., 2002; 157(5): 596-598.
3. Afanas'ev V.V. Sljunnye zhelezy. Bolezni i travmy: rukovodstvo dlja vrachej / V.V. Afanas'ev. – M.: GJeOTAR – Media, 2012. – 296 s.
4. Avtandilov G.G. Sistemnaja stereometrija v izuchenii patologicheskogo processa / G. G. Avtandilov, N. I. Jabluchanskij, V.G Gubenko. – M.: Medicina, 1981. – 190 s.
5. Lavriv L. P. Morfohenez pryvushnoi slynnoi zalozy na dev'iatomu misiatsi vnutrishnoutrobnoho rozvystku / L.P. Lavriv, I.Iu. Oliinyk // Klinichna anatomia ta operatyvna khirurhiia., 2012; 11(3): 58-61.
6. Lukina G.I. Nekarioznye porazhenija tverdyh tkanej Zubov u gastroenterologicheskikh pacientov / G.I. Lukina, Je.A. Bazikjan // Medicinskij alfavit. – 2010; 4: 31-32.
7. Maksimovskij Ju.M. Sostojanie polosti rta u bol'nyh nespecificeskim jazvennym kolitom i bolez'ju Krona / Ju.M. Maksimovskij, V.G. Rumjancev, G.N. Mdinaridze // Stomatologija dlja vseh. – 2005; 1: 28-34.
8. Shormanov I.S. Sosudistaja sistema pochek pri stenoze legochnogo stovola s razlichnym urovnem kompensacii krovoobrashhenija / I.S. Shormanov // Bjuletent' eksperimental'noj biologii i mediciny. – 2004; 137(3): 332-335.
9. Shormanov S. V. Morfologicheskie izmenenija sosudov pecheni pri modelirovaniu stenoza legochnogo stvola i posle ego ustranenija / S.V. Shormanov, S.V. Kulikov // Bjuletent' eksperimental'noj biologii i mediciny., 2007; 144(9): 342-345.
10. Shulhai A.H. Morfometrychni osoblyvosti remodeliuvannia struktur pryvushnoi zalozy pry mekhanichnii zhovtianytsi / A.H. Shulhai, M.O. Levkiv // Shpytalna khirurhiia., 2012; 1(57): 39-42.