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# Microanatomical Changes in The Bladder of Ageing Rabbits: Effect of Serotonin and Vagotomy

**Lychkova Alla Eduard[1*], Fentisov Vitaly Vladimirovich[2], Puzikov Alexander Michael[1]**

[1]Department Head, Moscow's Clinical Research Practical Center, Moscow, Russia

[2]Department of Human Anatomy, Belgorod State University, Belgorod, Russia

[*]**Corresponding author:** Lychkova Alla Eduard, Department Head, Moscow's Clinical Research Practical Center, 86 Enthusiastov sh., Moscow, 111123, Russia. Tel: +79629654923; Email: lychkova@mail.ru

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## Abstract

**Aim:** This morphological study aims to investigate the effects of serotonin on vagotomy induced aging rabbit bladder injury.

**Material and Methods:** Chinchilla rabbits of both sexes were made bilateral vagotomy and i.p. infused with serotonin on the background of vagotomy. The morphology of the urinary bladder was investigated using light microscopy. The state of vessels in the mucosa, sub/mucosal alterations, sub/epithelial layer, smooth muscles, collagen fibers, as well as nerve fibers were evaluated. Chinchilla rabbits of both sexes were i.v. infused with NaCl solution (control group).

**Results and Conclusion:** Chronic bilateral vagotomy of the bladder was accompanied by the phenomena of dystrophy of the submucosal layer and the vascular system and all wall structures enhanced with ageing. Serotonin administrated to rabbits with chronic bilateral vagotomy largely neutralizes the pathological changes associated with vagotomy.

**Keywords:** Bilateral Vagotomy; Serotonin; Urinary Bladder

## Introduction

Autonomic innervation of the bladder is mediated by sympathetic, parasympathetic, non-adrenergic and non-cholinergic (e.g. serotonergic) nerves [1-3] and sensitive nerves which are most abundant in the trigone [4,5]. A functional interaction between 5-HT and the other mediators (i.e., serotonergic and cholinergic systems) occurs in the control of low urinary tract function. The sympathetic-adrenal and parasympathetic systems with its hormones, catecholamine, and acetylcholine mediators are the body's neurohumoral systems which integrates human activity in the changing environmental conditions. A growing body of evidence suggests that 5-hydroxytryptamine (5-HT; serotonin) has both physiological and pathological functions in the lower urinary tract [6]. Have been demonstrated that preganglionic fibers and ganglionic serotoninergic neurons, expressing the 5-HT3 and 5-HT4 receptors, and the effector smooth muscle cells, expressing 5-HT1 and 5-HT2 receptors, are actively involved in the regulation of the bladder contractile activity in rabbits [7]. As is well known, an increase in the cholinergic nerve activity leads to a contraction in the muscles of the bladder. Yet, it remains unclear relative role of the cholinergic and serotoninergic systems in providing a normal function of the bladder [8,9,5].

Morphological structure of the urinary tract wall (bladder, ureters) includes mucous membrane consisting of transitional epithelium and its own plate, submucosal base, muscular and outer membranes [4]. Small blood vessels in the mucous membrane of the bladder are located near the epithelium in a collapsed or moderately stretched state; the mucous membrane of the bladder has many rugae, while the trigone, where its mucous membrane is tightly fused with the muscular membrane, has smooth walls. The muscular membrane of the bladder consists of three poorly delineated layers made of and interlacing smooth muscle bundles. Connective tissue layers divide the muscle tissue into separate large bundles. The bladder wall and its vessels is abundantly supplied with blood and lymphatic vessels, innervated by sympathetic, parasympathetic and sensitive nerves which are most abundant in

the trigone [4,5]. However, the effect of serotonin on the structure of the ageing bladder wall has not been studied enough. This morphological study aims to investigate the effects of serotonin on vagotomy and / or age induced bladder injury.

## Materials and Methods

### Animals

The morphological experiments were performed on 30 Chinchilla rabbits of both sexes. The group of rabbits of a young age included 10 animals weighing 2.4-3.3 kg, and 5-6 months of age. The group of older age rabbits included 10 ones weighing 3.4-3.5 kg, and 6.1-12 months of age. The group of senile age rabbits included 10 animals weighing 3.6-4.3 kg, and 24-38 months of age. The control group constituted of 5 animals. Serotonin in a dose of 50-100 mcg / kg was administrated to all animals. The animals were provided from the Animal Facility of the Russian National Research Medical University, Moscow, Russian Federation. Experiments were carried out in accordance with national ethical guidelines, and the animals were handled in a manner approved by the Care Committee of the Russian National Research Medical University.

### Surgery

The morphofunctional influence of the serotonergic system on the bladder was studied through chronic bilateral deparasympathization which was carried out 3 weeks prior to the main part of the experiment. Animals were placed under surgical anesthesia with Nembutal (40 mg/kg, i.p.). The peripheral segments of both cervical vagus nerves were dissected. Postsynaptic serotonin receptors were activated by i.p. injections of exogenous serotonin. Isolated bilateral vagotomy was performed for the control group rabbits.

### Drugs

The drugs used in this study were: Nembutal, used at a dose of 40 mg / kg body weight, was from Hospira, USA. Serotonin adipinate used at a dose of 50-100 mcg/kg intraperitoneally, was from Fatmzashita NPC FSUE (Russia). All drugs were dissolved in physiological 0.9% NaCl solution immediately before use.

### Morphological data

The bladder strips were fixed in 9% formalin, dehydrated in alcohols with increasing concentration, immersed in paraffin, received histological sections, stained with hematoxylin and eosin.

### Statistical analysis

Data are expressed as means ± standard error. Student's t test was used for statistical comparisons when appropriate, with the subsequent determination of the level of reliability of differences (p), the criterion $\chi 2$, and differences were considered significant at $p \leq 0.05$.

## Results

### Younger group

Morphological structure of the transitional epithelium of the bladder wall in young rabbits which underwent isolated bilateral vagotomy is not normal. Lower loose connective tissue contains a large number of dilated vessels, subepithelial layer is swollen (892 ± 163 μm, $p \leq 0.05$). Eosinophilic infiltration (42 ± 11 μm, $p \leq 0.05$) of the perivascular space of the submucosal layer and an increased number of intraepithelial lymphocytes (140 ± 24 μm, $p \leq 0.05$) of the mucosa are noted (Figure 1).

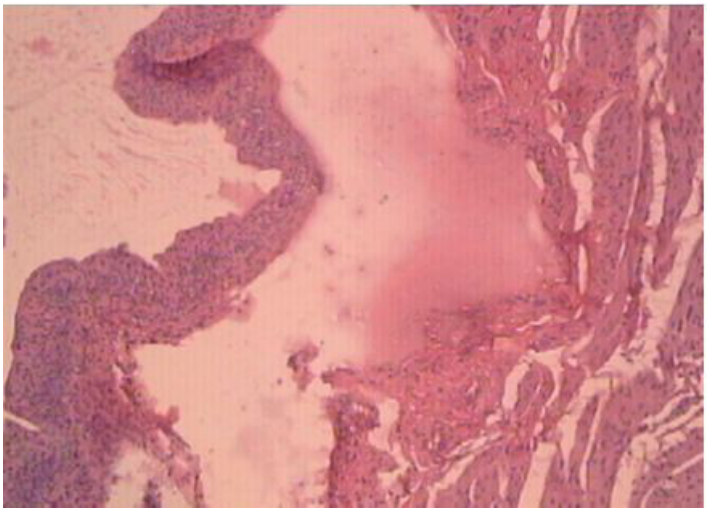

**Figure 1:** Young rabbit bladder with isolated bilateral vagotomy. Edema of the subepithelial layer. Hematoxylin and eosin. HE. x 250.

In (Figure 1) of the rabbit's bladder with isolated bilateral vagotomy it is noted an edema of the subepithelial layer. In other fields of view may be seen swelling subepithelial layer. An increased number of lymphocytes of the mucosa were marked.

The rugae of the mucous membrane of the bladder of young rabbits which underwent chronic bilateral vagotomy with the preliminary activation of the serotonergic system are of usual structure, the transitional epithelium without abnormalities. The few vessels in the subepithelial connective tissue are slightly dilated or not dilated at all. Smooth muscle tissue is preserved. A standard structured adipose tissue of the usual structure is located among the muscle bundles. There is a mild swelling of the interstitial tissue (203 ± 42 мкм, $p \geq 0.05$) and separation of muscle fibers (Figure 2).

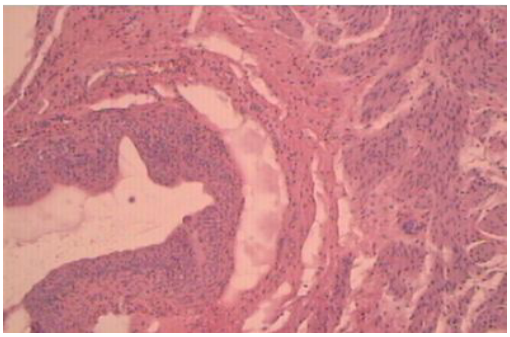

**Figure 2:** Young rabbit bladder: bilateral vagotomy with serotonin administration. Swelling subepithelial layer. Hematoxylin and eosin. HE. x 120.

In (Figure 2) the rabbit's bladder with isolated bilateral vagotomy on the background of the introduction serotonin is presented. After serotonin administration, the veins of the submucosal layer are somewhat enlarged. In the control rabbits of this age, there is a weakly expressed dilatation of the arteries and an increase in the amount of collagen fibers in their wall was noted. The bladder's epithelium contains an abundance of capillaries.

## Older group

Older rabbits with isolated bilateral vagotomy presented desquamation of the epithelium, swelling submucosa. At the apex of the bladder was marked increased blood circulation in the muscle layer. At neurovascular bundle noted advanced full-blooded vessel. Activation of the serotonergic system in rabbits of the older group under conditions of the chronic bilateral vagotomy dystrophy of the transitional epithelium (namely, vacuolar degeneration) is noted, edema of the submucosa (1087 ± 176 μm, $p \leq 0.05$), and vasodilation of this layer, dilation and enhanced blood filling of the tunica adventitia vessels. The vascular adventitia is expanded, full-blooded (Figure 3).

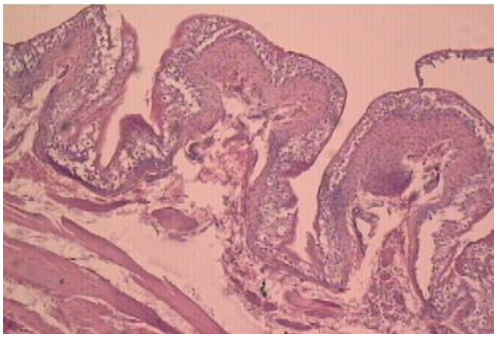

**Figure 3:** The body of the older rabbit bladder: bilateral vagotomy with serotonin administration. Swelling submucosa. Hematoxylin and eosin. HE. x 120.

Rabbit bladder in the older age (control group) contained moderately advanced vessels. Transitional epithelium bladder not changed, marked plethora capillaries. Arteries and nerve fibers of muscular layer were usual structure. Selected veins were dilated.

## Senile group

Morphological changes in the bladder wall of senile rabbits under conditions of isolated bilateral vagotomy were characterized by significant areas of atrophy of the epithelium (26 ± 9 μm, $p \leq 0.05$) of the mucous membrane of the bladder, sclerosis of the mucous membrane with proliferation of collagen fibers, venous vasodilation in the submucosa; separation of the muscular layer of the bladder wall, swelling and separation of the nerve fibers of the muscular layer, and interstitial edema. Serotonin administration at rabbit bladder in the senile age group under conditions of bilateral vagotomy. An atrophy of the epithelium of the mucosa of the bladder somewhat decreased. The area of sclerosis in the mucosa of the bladder with the growth of collagen fibers (Figure 4).

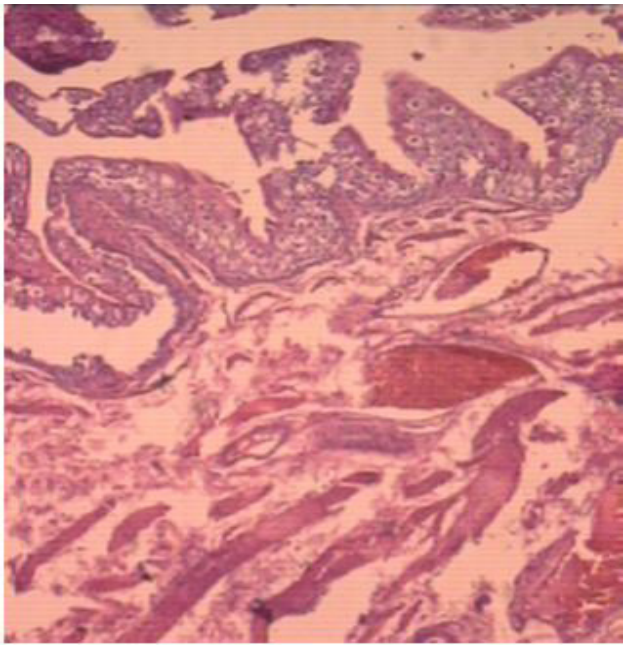

**Figure 4:** Urinary bladder of a rabbit of senile age under conditions of vagotomy and administration of serotonin. Expanded venous vessels of submucosal layer. Areas of transient epithelial dystrophy. Interstitial edema. Staining with hematoxylin and eosin. HE. x 120.

Rabbit bladder in the senile age group (control group). Dilated venous vessels of the submucosa. The dissociation of the muscular layer of the bladder wall. Swelling of the interstitial space. Muscle fiber layer swelling and dissociation (Figure 5).

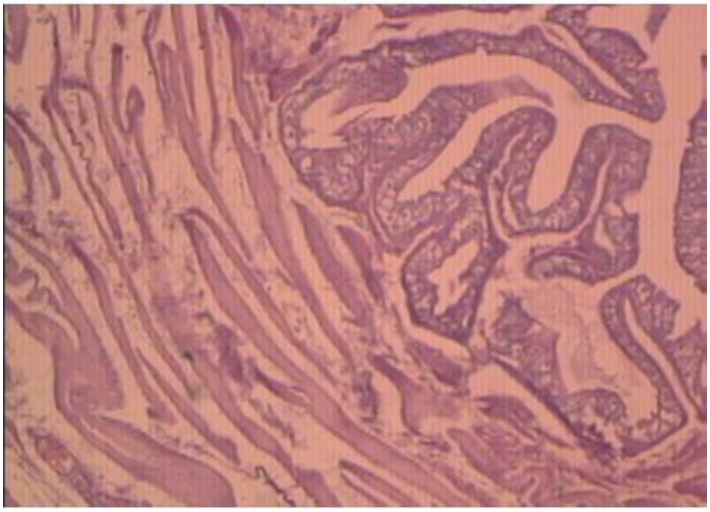

**Figure 5:** The wall of the bladder's top of a rabbit of the older age group. Staining with hematoxylin and eosin. HE. x 120.

## Discussion

We studied the balance of activity of cholinergic and serotoninergic divisions of the autonomic nervous system in the regulation of the urinary bladder function in rabbits. The storage and periodic elimination of urine depends on the contractile activity of the bladder. The cholinergic system, it bulbar and sacral parts, have a stimulatory effect on the motility of all parts of the bladder. Other neurotransmitter systems also having direct influence on contractile function or modulate respective cholinergic function. The sympathetic system has the synergistic to parasympathetic stimulatory effect on the bladder motility. Purinergic system apparently does not affect the synergistic effect of ANS on the bladder motility. The serotonergic system is one of such systems. Currently, there is the pool of data on serotonin, numbering 137 thousand articles (according the Medline). In connection with this, the problem arises of systematizing these data, which can be solved not only by writing reviews, but also experimentally. The program of the functional mapping of the effects of serotonin in the body (conducting studies on a single plan using the same method and the set of procedure) was undertaken (Lychkova A.E, Medline, 1983-2017 years).

As a marker of the effects of serotonin in a body the spontaneous and induced contractile activity of the uterus, blood vessels, the hollow muscular organs of the developing, mature and senile organisms of rabbits, guinea pigs, rats and other rodents was applied. As the method of study of serotonin contractile effects in heart cardiomyocytes, smooth muscles of uterus, blood vessels, other hollow organs of the urogenital, bronchopulmonary, digestive, endocrine, hepatobiliary, immune and other systems in norm and in pathology the electrophysiological (electromyography) method was used. The unity of approach for mapping, - the use of unified method electromyography and operational procedures, relevant animal models - allow to detect cholinergic /serotonergic systems interaction with marked contractile consequences in all studied organs, the urinary bladder including [7]. The serotonergic system has a stimulatory effect on the motility of the bladder through the activation of ganglion 5-HT3, 4 and effector 5-HT1.2 receptors [10]. A main question now in that concern is the determination of the neuroanatomical and neuropharmacological substrates where functional effects of serotonergic/cholinergic interactions originate. The present article used neuroanatomical tissue of rabbit bladder to selectively consider only one part - serotonergic - of serotonergic/cholinergic interactions. Chronic bilateral vagotomy to select own serotonin part in this interaction was used. Our findings support the hypothesis of an involvement of serotonergic system in abnormalities of lower urinary tract symptoms occurring in ageing. Serotonin administrated to rabbits largely neutralizes the vagotomy and/or age induced pathological changes in the bladder. There is still much to be done to better understand the balance of cholinergic and serotonergic effects in the bladder.

## Conclusions

The obtained results indicate that in all age groups studied the serotonin, on the background of chronic bilateral vagotomy, inhibits the vagotomy induced pathological changes of the bladder histological picture. At the same time, the dynamics of serotonin influence is age dependent. Analysis of data of 5-38-month-old animals showed that serotonin on the background of bilateral vagotomy more recovery the bladder tissues of 5-6 months old animals than that of 6.1-12 months-old rabbits, and to a lesser extent as compared with that of the bladder of 24-38-week-old rats.

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