Резюмета на трудовевете след защита на докторска дисертация

№ III-14.

Bulgarian Journal of Veterinary Medicine (2012), **15**, No 1, 57–61

Short communication

MACROMORPHOMETRIC STUDY ON PARANAL SINUSES IN A BROWN BEAR (*URSUS ARCTOS*)

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Summary

Stefanov, I. S., 2012. Macromorphometric study on paranal sinuses in a brown bear (*Ursus arctos*). *Bulg. J. Vet. Med.*, **15**, No 1 , 57–61.

The aim of the study was to establish the macromorphometric parameters of paranal sinuses and their projections onto the skin in a brown bear. It is shown that the brown bear's paranal sinuses were similar to these of domestic dogs, and also located between the external and internal sphincters. Data about the colour, texture and amount of the organ' s secretion are provided. According to the present study, the brown bear possessed well developed paranal sinuses.

Key words: anatomy, brown bear, morphometry, paranal sinus

№ III -15.

Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 19
Sofia • 2012

NOS positive mast cells in the pelvic urethra of male pigs

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With regard to our thorough research on mast cells in the pelvic urethra of male pigs, the aim of the present study was to obtain more data about the histochemical features of these cells by finding out whether they were positive for nitric oxide. The incentive of the study was the key role of nitric oxide (NO) and the closely related isoenzymes of nitric oxide synthase for a number of physiological and pathological events in the animal body. The study of NOS would also contribute to obtaining more information for the innervation of the organ in this animal species.

Key words: nitric oxide (NO), mast cells, pelvic urethra, pig.

№ III -16.

Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 18
Sofia • 2012

Macromorphometrical study of the anal sac (Sinus paranalis) in dogs of different ages

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The aim of the study was to establish the macromorphometrical parameters of the paranal sinus in dogs. This study was carried out on material obtained from of 48 mongrel immature male and female dogs aged 1, 2 and 5 months, and adult males aged 2, 8 and 12 years. We were used 8 animals (4 males and 4 females)To determine weights and some morphometric parameters of the sinus in each age group of sexually immature dogs. In adult animals the same indicators were examined only in males – in 4 animals from each age group. Several macromorphometrical parameters: mass, length and diameter of paranal sinus and as well as the color and texture of the organ` s secretion were investigated.

During the period from 1 to 5 month the weight, length and perimeter of the paranal sinus as well as the diameter of its cavity increased most intensely in the second month. Sexual dimorphism in the studied parameters was not established. In adult animals the values of the studied indicators during the second year grew most intensely.

This study has shown that changes in mass, length, perimeter of the sinus, the diameter of its cavity depend on the age of animals.

Key words: sinus paranalis, morphometry, dog.

№ III -17.

Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 18
Sofia • 2012

NADPH-d expression in mast cells of porcine tube auditivae

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The aim of the present study was to determine the expression of nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) in mast cells of tuba auditiva (Eustachian tube) in domestic pigs. NADPH-d positive cells were observed in the propria of the cranial, middle and caudal parts of tube's cartilaginous portion. They were located mainly adjacently to blood vessels, mucosal glands and epithelium. The finding that mast cells of porcine tube Eustachian could synthesize nitric oxide could throw more light on the regulation of physiological events within the organ.

The presence of NADPH-d positive mast cells in the Eustachian tube wall in pigs confirms their ability to synthesized nitric oxide.

Key words: mast cells, NADPH-d, tuba auditiva, pig.

№ III -18.

Journal of Mountain Agriculture on the Balkans, vol. 15, 5, 2012, (999-1011)
Research Institute of Mountain Stockbreeding and Agriculture, Troyan

НИКОТИНАМИД АДЕНИН ДИНУКЛЕОТИД ФОСФАТ ДИАФОРАЗНА (NADPH-d) РЕАКТИВНОСТ В ТАЗОВАТА УРЕТРА НА МЪЖКИ ПРАСЕТА

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NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE DIAPHOROUS (NADPH-d) REACTIVITY IN THE PELVIC URETHRA IN MALE PIGS

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РЕЗЮМЕ

Никотинамид аденин динуклеотид диафоразата (NADPH-d) се използва за доказване биосинтезата на азотен оксид (NO) при протичане на хистохимична реакция. При проведените от нас светлинномикроскопски изследвания се установи различна степен на NADPH-d реактивност в тазовата уретра и уретралния мускул. Много добре изразена позитивна реакция се наблюдава в клетъчната мембрана (сарколемата) на мускулните клетки на m.urethralis, характеризираща се с тъмно синьо до виолетово оцветяване. Силно изразена NADPH-d реактивност се наблюдава и в епителният слой на тазовата уретра.

SUMMARY

Nicotinamide adenine dinucleotide diaphosphatase is being used as a proof of the biosynthesis of nitric oxide (NO) with each histochemical reaction.

In the conducted from our team light microscopic studies was determined that the NADPH-d has different degree of reactivity within the pelvic urethra and the supporting urethral muscle.

Very well expressed positive reaction was observed in the cell membrane (sarcolema) of the muscle cells of m.urethralis, which has the characteristical dark blue to purple color. Well expressed NADPH-d reactivity was seen also in the epitelial layer of the pelvic urethra.

№ III -19.

Bulgarian Journal of Veterinary Medicine (2012), **15**, No 4, 254–259

DISTRIBUTION AND DIMENSIONS OF NADPH-DIAPHORASE POSITIVE GANGLIONATED PLEXUSES IN PORCINE COMMON HEPATIC DUCT WALL

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Summary

Stefanov, I. S., 2012. Distribution and dimensions of NADPH-diaphorase positive ganglionated plexuses in porcine common hepatic duct. *Bulg. J. Vet. Med.*, **15**, No 4, 254–259.

The aim of the present study was to establish the expression of nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) activity in ganglionated plexuses, their distribution and dimensions in mucosal, fibromuscular and subserosal layers of porcine common hepatic duct. The material was obtained from the common hepatic duct of 6 male and 6 female pigs. Tissue pieces were taken immediately after the slaughter and fixed in 4% paraformaldehyde. The NADPH-d histochemical expression was investigated according to the method of Sherer-Singler. The data showed that NADPH-d positive neurons and nitrergic autonomic nerves in the wall of the common hepatic duct obviously produced nitric oxide. Thus they were most probably involved in the regulation of the function of epithelium, blood vessels and the organ in general.

Key words: common hepatic duct, NADPH-d autonomic ganglia and nerves, swine

№ III -20.

Доклади на Българската академия на науките
Comptes rendus de l'Académie bulgare des Sciences
Tome 65, No 5, 2012

MEDECINE
Médecine vétérinaire

NITRIC OXIDE SYNTHASE CELLS (MAST CELLS) IN THE DOG'S PARANAL SINUS

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(*Submitted by Corresponding Member O. Poljakova-Krusteva on December 14, 2011*)

Abstract

Nitric oxide synthase immunoreactivity has been detected for the first time in mast cells of canine normal paranal sinus wall in male and female dogs. Intense immunoreactivity was revealed in secretion granules of mast cells localised in the propria, around apocrine and sebaceous glands, and in the subglandular connective tissue layer; no reactivity was found in the cytoplasm of these cell types. Our results indicate that nitric oxide may be a novel modulator that determines the functional phenotype of canine paranal sinus mast cells.

Key words: nitric oxide synthase, mast cells, paranal sinus, dog

№ III -21.

ANATOMIA HISTOLOGIA EMBRYOLOGIA C
JOURNAL OF VETERINARY MEDICINE
Anatomia Histologia Embryologia

SHORT COMMUNICATION

NADPH-d-Positive Mast Cells in the Canine Paranal Sinus

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With 3 figures

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Summary

This study aimed to investigate the enzyme histochemical expression of NADPH-d in mast cells in the wall of the paranal sinus in male and female dogs. NADPH-d-positive cells with weak, medium and strong enzyme histochemical expression were observed in the stroma of the sinus near the blood vessels of the microcirculatory bed and around the apocrine and sebaceous glands. In the same areas, mast cells with similar dimensions and morphology were demonstrated by metachromasia on paraffin and cryostat cross-sections and stained with 0.1% toluidine blue in McIlvane's buffer (pH 3). These findings suggest that the mast cells that are located in the stroma near the blood vessels, the lining epithelium and the glands correspond with the cells with marked NADPH-d activity. The possibility of mast cells having nitric oxide activity could be used in the regulation of mast cells function when treating paranal sinus tumours and inflammations.

№ III -22.

TÜBİTAK

Research Article

Turk. J. Vet. Anim. Sci.
2012; 36(3): 267-274

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A study on paranal sinus micromorphometrical parameters in dogs of different ages

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Received: 11.02.2011

Abstract: This study determines the micromorphometric parameters of the main structural elements of the paranal sinus in male (n = 20) and female (n = 8) mongrel dogs of different ages. Pieces of about 1 cm3 were obtained from different parts of the organ and put in 10% neutral formalin. The tissue samples were further used for the preparation of paraffin sections that were stained with hematoxylin-eosin for light microscopy measurements. In the sinus wall, the thickness of the epithelium was measured, as was that of the subepithelial and subglandular connective tissue layers, the zona with the apocrine glands, and the entire sinus wall. The outer and inner diameters of the apocrine tubular glands and the height and number of glandular epithelial cells in the apocrine glands were also measured. Statistical data processing was done using Data Analysis tool and t-test by means of the StatMost for Windows software. For the period from the 1st month to the 2nd year, the thickness of the epithelial layer of the sinus wall remained almost identical (from 40.83 ± 9.37 µm during the 1st month to 47.79 ± 3.67 µm in the 2nd year). After the 2nd year, the thickness of the epithelial layer decreased. In the 8th year its thickness reached 35.72 ± 5.89 µm and in the 12th year it was 28.92 ± 6.53 µm. We measured the thickness of the subepithelial and subglandular connective tissue layers of the wall of the sinus, forming the organ's stroma. It was found that the thickness of these layers increased 3-fold for the period from the 1st month to the 12th year. Their thickness increased most intensively until the 5th month, followed by the period from the 5th month to the 2nd year, then from the 2nd to the 8th year, and changed the least up to the 12th year. Therefore, the increase in the thickness of the sinus wall (without its excretory duct) with age was due to the increase of the thickness of the subepithelial and subglandular connective tissue layers in particular. The results of the present study demonstrate that the period featuring the most active weight increase and growth of sinus macromorphometric parameters coincided with the period of increase in the number of cells and the active growth of the main structural elements of the organ.

Key words: Anatomy, morphometry, paranal sinus, dog

№ III -23.

NADPH-diaphorase positive cells (mast cells) around and within the autonomic nerves in the periphery of subglandular connective tissue layer of dog paranal sinus (*Sinus paranalis*)

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SUMMARY

The present study aimed to investigate the enzyme histochemical expression of nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) in mast cells and in autonomic nerves in the wall of the paranal sinus in 8 adult dogs (4 males and 4 females). The NADPH-d histochemical expression was investigated according to the method of Sherer-Singler and metachromasia evidenced by toluidine blue staining on frozen and paraffin serial sections was used for confirming the cell type. Positive NADPH-d cells (mild to strong reactivity in cytoplasmic granules) were found next and within autonomic nerves located in the periphery of subglandular connective tissue layer of paranal sinus and metachromasia of mast cells was observed in the same localisation. NADPH-d reactivity was also evidenced in autonomic nerves. These results suggest that NADPH-d positive cells (mast cells) and autonomic nitrergic nerves may produce nitric oxide and may be together involved as a structural and functional unit in the sinus function.

Keywords: Paranal sinus, dog, NADPH-diaphorase, mast

RÉSUMÉ

Cellules exprimant une NADPH diaphorase (mastocytes) associées aux nerfs autonomes en périphérie du tissu conjonctif sous-glandulaire du sinus péri-anal (*Sinus paranalis*) chez le chien

Le but de cette étude était de rechercher l'expression de la diaphorase NADPH (nicotinamide adénine dinucléotide phosphate) dépendante (NADPH-d) par histochimie dans les mastocytes et les nerfs autonomes de la paroi du sinus péri-anal chez 8 chiens adultes (4 mâles et 4 femelles). L'expression de cette enzyme a été détectée par histochimie selon la méthode de Sherer-Singler et en parallèle, la capacité de métachromasie a été mise en évidence par coloration au bleu de toluidine dans des sections sériées congelées ou incluses en paraffine en vue de confirmer le type cellulaire. Les cellules exprimant la NDAPH-d (réactivité modérée à forte des granules cytoplasmiques) étaient localisées à proximité ou au sein des nerfs autonomes situés en périphérie du tissu conjonctif sous-glandulaire du sinus péri-anal, et le phénomène de métachromasie des mastocytes a été observée dans les mêmes zones. La présence de NADPH-d a aussi été mise en évidence dans les nerfs autonomes. Ces résultats suggèrent que les cellules exprimant la

№ III -24.

Доклади на Българската академия на науките
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Tome 66, No 4, 2013

MEDECINE
Morphologie

DISTRIBUTION OF NADPH-DIAPHORASE POSITIVE GANGLIONATED PLEXUSES IN PORCINE GALL BLADDER

Ivaylo Stefanov, Angel Vodenicharov

(Submitted by Academician I. Pashev on November 21, 2012)

Abstract

The distribution and dimensions of nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) positive ganglia in the domestic pig's gall bladder and *Ductus cysticus* were studied for the first time. It was established that the number of ganglia was highest in the gall bladder's body, followed by those in the fundus and neck. The lowest number of ganglia was observed in *Ductus cysticus*. The largest ganglia were localized in the gall bladder's neck, followed by these in the body and fundus. The highest density of neurons per ganglion was detected in the neck, followed by body and fundus. Their number, however, was the lowest in the ganglia of *Ductus cysticus*. The largest neurons were established in the gall bladder's neck.

It was concluded that the NADPH-d ganglia as neuronal structures produce nitric oxide, which as a transmitter with neuronal origin most probably is involved in the control of the epithelial secretion and in the function of smooth muscle in the walls of both gall bladder and blood vessels as well.

Key words: NADPH-diaphorase, ganglionated plexus, porcine gall bladder

№ III -25.

Immunocytochemical expression of Chromogranin A in mast cells in the canine paranal sinus

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SUMMARY

The aim of the study was to establish the immunocytochemical expression of Chromogranin A in the mast cells in the paranal sinus from 8 dogs, the tryptase expression being considered as specific for mast cells. The immunocytochemical expression of Chromogranin A and tryptase was observed in the granules of mast cells. Chromogranin A positive cells were detected predominantly around the blood vessels, mainly in the subepithelial connective tissue layer, then in the zone of apocrine glands and around the sebaceous glands and poorly in the subglandular connective tissue layer of the sinus wall and its excretory duct. In addition, the Chromogranin A expression was strongly associated with the tryptase presence. These results show that mast cells in the dog paranal sinus express Chromogranin A that may play an important role in the secretory granule biogenesis.

Keywords: dog, paranal sinus, immunocytochemistry, chromogranin A, mast cells.

RESUME

Expression immunohistochimique de la chromogranine A dans les mastocytes du sinus péri-anal chez le chien

Le but de cette étude a été de détecter par immunocytochimie l'expression de la Chromogranine A dans les mastocytes du sinus péri-anal chez 8 chiens, le type cellulaire étant confirmé par l'expression spécifique de la tryptase. Ces 2 protéines ont été détectées par immunocytochimie dans les granules des mastocytes. Les cellules exprimant la Chromogranine A ont été majoritairement détectées en périphérie des vaisseaux sanguins, principalement dans la couche sous-épithéliale de tissu conjonctif, dans la zone des glandes apocrines et autour des glandes sébacées à un moindre degré et enfin dans la couche de tissu conjonctif sous-glandulaire au sein de la paroi du sinus et au voisinage du canal excréteur. De plus, l'expression de la Chromogranine A a été fortement associée à la présence de la tryptase dans les mêmes cellules. Ces résultats montrent que les mastocytes du sinus péri-anal du chien expriment la Chromogranine A qui joue sans doute un rôle important dans la biosynthèse des granules de sécrétion.

Mots-clés : chien, sinus péri-anal, immunocytochimie, Chromogranine A, mastocytes.

№ III -26.

SHORT COMMUNICATION

S-100 Protein-Positive Mast Cells in Canine Paranal Sinus (*Sinus paranalis*)

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With 2 figures

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Summary

The aim of this study was to establish the immunocytochemical expression of S-100 protein in mast cells, localized in the wall of dog's paranal sinus. Control serial sections were used for immunocytochemical detection of tryptase-positive mast cells. It was observed that S-100-positive cells have the same morphology and localization as the tryptase-positive mast cells, which indicated that S-100-positive cells are most probably mast cells with abilities as dendritic cells. In conclusion, for the first time, the current study gave evidence that mast cells in this organ possess one more function, such as dendritic cells.

№ III -27.

Bulgarian Journal of Veterinary Medicine (2013), **16**, No 1, 7–12

NADPH-DIAPHORASE POSITIVE MAST CELLS IN THE WALL OF PORCINE COMMON HEPATIC DUCT

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Summary

Stefanov, I. S., 2013. NADPH-diaphorase positive mast cells in the wall of porcine common hepatic duct. *Bulg. J. Vet. Med.*, **16**, No 1, 7–12.

There are no data about the presence of NADPH-d positive mast cells in the porcine common hepatic duct. That is why we aimed to establish the expression of NADPH-d activity in mast cells and their number in its mucosal, fibromuscular and subserosal layers. The material was obtained from the common hepatic duct of 6 male and 6 female crossbred pigs. Tissue pieces were taken immediately after the slaughter of the animals and fixed in 4% paraformaldehyde. Sections of 15–20 μm thickness were prepared by means of a freezing microtome. The NADPH-d histochemical expression was investigated according to the method of Sherer-Singler. NADPH-d positive cells were found in all layers of the organ. They were located mainly in the vicinity of blood vessels, around the peribiliary glands and near the basal lamina of the surface epithelium. The obtained data showed that NADPH-d positive cells observed in the wall of common hepatic duct are mast cells which obviously produce nitric oxide. The results gave us a reason to suggest that they are most probably involved in the regulation of the function of epithelium and blood vessels.

Key words: common hepatic duct, mast cells, NADPH-d, pig

№ III -28.

Bulgarian Journal of Veterinary Medicine, 2014, **17**, No 2, 79–87
ISSN 1311-1477; online at http://tru.uni-sz.bg/bjvm/bjvm.htm

Original article

DISTRIBUTION OF NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE DIAPHORASE POSITIVE MAST CELLS IN THE NORMAL PORCINE GALL BLADDER

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Summary

Stefanov, I. S., A. P. Vodenicharov, M. V. Gulubova & J. R. Ananiev, 2014. Distribution of nicotinamide adenine dinucleotide phosphate diaphorase positive mast cells in the normal porcine gall bladder. *Bulg. J. Vet. Med.*, **17**, No. 2, 79–87.

The aim of the current study was to determine the localisation and number of NADPH-d mast cells in the normal domestic pig gall bladder. The gall bladders of 6 male and 6 female pigs were examined by using light microscopic enzymohistochemical and immunohistochemical techniques. Enzymohistochemistry was performed to display NADPH diaphorase activity. Then, the avidin-biotin-peroxidase complex technique for detection of mast cell tryptase expression was performed in order to confirm that NADPH-d positive cells, observed in the studied organ, are actually mast cells. Light microscopy showed strong to medium NADPH-d reactivity in the mast cells granules. Mast cells number was the highest in the propria of gall bladder fundus, body and neck as well as in the same layer of the cystic duct *Ductus cysticus*, followed by fibromuscular layer. The smallest density of mast cells was observed in the subserosal layer. However, statistically significant difference between the number of these cells in the muscular layer and the tela subserosa of gall bladder fundus was not detected. The density and localisation of observed tryptase positive mast cells were the same. In conclusion, the results of this study gave us a reason to presume that NADPH-d positive cells observed in the wall of porcine gall bladder and its excretory duct possess metabolic pathway for nitric oxide synthesis. Based on obtained data it is suggested that NADPH-d positive mast cells are most probably involved in the regulation of the function of epithelium, smooth muscle layer and blood vessels of the organ.

Key words: gall bladder, mast cells, NADPH-diaphorase, pig, tryptase

№ III -29.

Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 21
Sofia • 2015

Structural Organization of Renal Medulla in Domestic Swine

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The morphological and physiological features of kidney in humans and different animals have been determined over the years. However, the detailed data about the structural organization of the porcine renal medulla are absent. In the present study it was established that in the outer stripe of outer medulla, the diameter and the number of proximal straight tubules were larger than in distal straight tubules. The number and the diameter of collecting ducts (CD) in the outer and in the inner stripe of the outer medulla were the same. The height and number of epithelial cells of CD increased towards the inner medulla. The diameter of *Ductus papillaris* was the largest. The diameter of thin limbs of Henle in the inner stripe and in the inner medulla unchanged, but in the tip of papilla it increased.

The pattern of the tubular and vascular organization of medulla that was established by the current study is evidence that porcine renal medulla belongs to the simple type of renal medulla.

Key words: renal medulla, morphometry, swine.

№ III -30

Bulgarian Anatomical Society

Acta morphologica et anthropologica, 22
Sofia • 2015

Application of Duracryl® Plus in Making of Corrosion Casts for Macro- and Microscopical Studies of the Renal Vasculature and Collecting System in Domestic Swine

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The lack of more concrete data about application of the acrylic resin Duracryl® Plus in making corrosion casts of renal blood vessels and collecting system motivated us to undergo undertake this study. We aimed to perform modified technique for making corrosion casts for 3-dimensional (3D) studies of the kidney blood vessels and collecting system and for replicating microvasculature using Duracryl. As a result, corrosion casts of the whole renal arterial network including glomeruli were obtained. The number of cortical glomeruli in kidney, which arteries were injected with casting media with dilution of 1:2, was significantly smaller than the dilution of 1:5. With regard to collecting system, the lower dilution of casting media allowed the filling of small calices while the media with a higher dilution – the collecting tubules system of the renal medulla and even of the cortex.

In conclusion, we found that the Duracryl is suitable for making three-dimensional casts of the renal arteries and collecting tubules and also microvascular corrosion casts for stereomicroscopy and scanning electron microscopy.

Key words: Duracryl® Plus, corrosion casts, kidney.

№ III -31

ORIGINAL ARTICLE

Histochemical Study of Heparin-positive Mast Cells in the Terminal Part of Porcine *Ductus Choledochus* and *Papilla Duodeni Major*

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With 3 figures and 1 table

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Summary

The study presented in detail the localization and density of mast cells (MCs) in the intramural part of the common bile duct (CBD) and in the major duodenal papilla (MDP) of domestic swine. MCs' density (number/mm^2) in different layers of both of the duct and papilla was evaluated after toluidine blue staining. Their number was higher in the *lamina propria mucosae* than in the *tunica muscularis* of the studied structures. The localization of berberine-positive, (heparin containing) MCs and the ratio between them and toluidine blue-positive MCs with γ-ma metachromasia was also established. Ratios of heparin-containing MCs in comparison with all toluidine blue-positive MCs were found as follows: *ductus choledochus* – 32% in the subglandular connective tissue of *lamina propria mucosae* in the intramural part of the duct; *m. sphincter ductus choledochus* – 31% in the circular and 0.06% in the longitudinal muscle layer; subserosa – 59%; *papilla duodeni major* – 0.03% in the subepithelial connective tissue and 34% in the subglandular connective tissue of *lamina propria mucosae*, respectively. The established large difference in heparin-positive MCs in both the subepithelial and subglandular connective tissues of CBD and MDP, respectively, is an evidence for the existence of mucosal and connective tissue MCs.