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Original Contribution

STRUCTURAL AND HISTOCHEMICAL STUDIES ON HEART VENTRICLE WALL IN THE CAT

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SUMMARY

Material from the heart ventricles of 6 sexually mature domestic cats was used in the present study. The material was processed by conventional histological and histochemical methods. Histological preparations were formalin- paraffin histoslices stained with haematoxylin (according Erlich) - eosin. Paraffin slices from material fixed in Carnua'a solution were stained with Maurice alcian blue, followed by a PAS reaction for viewing free and conjugated polysaccharides. Differentiation of glycosaminoglycans was done by histochemical tests using alcian blue at pH 1.0 and pH 2.5. The light microscopic study of the heart ventricle walls in the cat showed structural identity compared with that of other mammalian species. Histochemical reactions established that the heart ventricle myocardium contains both free polysaccharides (glycogen) and glucosaminoglycans of the non-sulphate type.

Key Words: cat, heart, structure, histochemistry.

INTRODUCTION

The heart is a particularly active organ from a functional point of view, which determines the necessity of energy provisions. In animals muscular fibres in the left heart ventricle wall contain more glycogen in comparison with these of other heart parts (1). Glucosaminoglycans of sulphate and non-sulphate type, usually found in tendons, cartilages, the eye cornea and others, can be accepted as an eventual energy reserve (2, 3). There are data about their presence in the regions of the intercellular boundaries in the normal heart muscular fibres (1), as well as in the swine mitral valve in case of endocardiosis (4).

The lack of sufficient data about the presence of polysaccharides and their localization in the valve wall in cat is the reason for the present study.

MATERIALS AND METHODS

Material from 6 sexually mature domestic cats (*Felis silvestris catus*) was used in the present study. Before the study all animals were disinfected and vaccinated. Then they were kept in a room with permanent temperature, mixed lighting and were given standard pellet food twice daily for 30 days. After euthanasia (according to *Bulgarian Animal Welfare regulations)* material from the left and right heart ventricles was taken. These organ biosamples were fixed in 10% neutral formalin (*Fluka AG- 938431, Buch, Switzerland*) and in the fixing Bouin's and Carnua's solutions. After inclusion of the material in paraffin a big number of single and series histological slices of up to 7 μm thickness were prepared (*Reichert-Jung, Wien, Austria*). Histological preparations ready for microscopic observation after their coloration with haematoxylin (according Erlich) - eosin (*Fluka AG-137840, Buch, Switzerland – LACHEMA-183941, Brno, Chehoslovakia*) were made (5). A portion of the histological slices, obtained from the material and fixed in Carnua's solution, was subjected to PAS reaction for glycogen, glycoproteids, glycolipids and others (*MERK*

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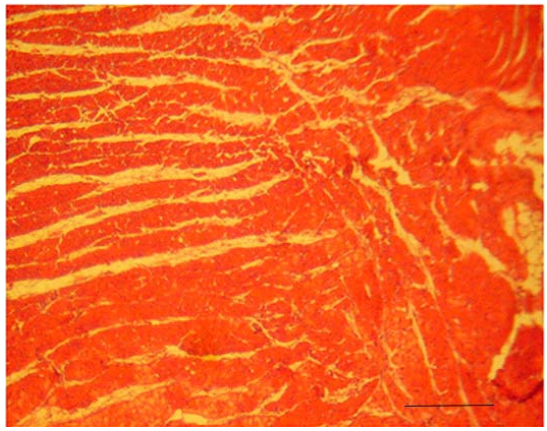
KGaA-116897, Darmstadt, Germany). The other part of the fixed slices, obtained in this way, was stained with Maurice alcian blue (MAB) (*MERK KGaA-64271, Darmstadt, Germany*), followed by a PAS reaction in order to determine simultaneously the neutral and acid mucosubstances (6, 7). Differentiation of the acid sulphate and non-sulphate glycosaminoglycans was carried out by two histochemical tests with alcian blue (AB) (*LOBA FEINCHEMIE-A2401, Duseldorf, Germany*) at pH 1.0 and pH 2.5 (8). The study of the histoslices and the micro photo documentation of the manifested histochemical reactivity were done with a universal microscope- NU2 (*Carlzeiss Jena, Germany*).

RESULTS AND DISCUSSION

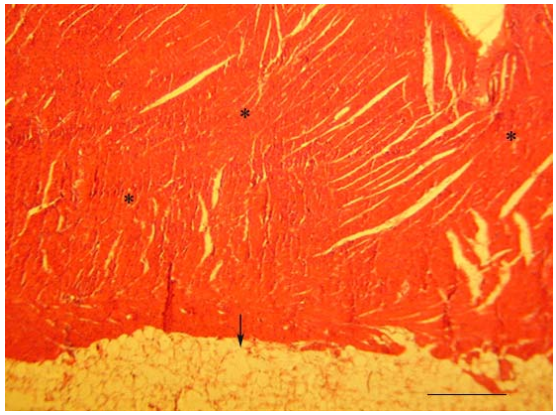
The analysis of the histological preparations, done with a light microscope, did not establish any pathological deviations in the wall microstructure in the left and right heart ventricles. We interpret this fact as evidence that the organ, used in the present study, is of full value from structural and functional point of view.

The histological study established that the heart ventricle wall in the cat consists of three layers (endocardium, myocardium and epicardium) as it does in the rest of the domesticated mammalian species, described in the specialized and school literature. The most considerable part of the heart ventricle wall is represented by the three layer myocardium where the muscular fibres are situated in different directions (an external sub layer of spiral longitudinal fibres- a middle ring-like one and an internal one with longitudinal fibres) (**Figure 1**). The endocardium, which in the left heart ventricle in the cat turned out to be thicker in some places than that of the right heart ventricle, also consists of three sub layers (endothelium, subepithelium and conjunctive tissues passing into perimisial myocardial tissue). The epicardium has grown together with the external surface of the heart ventricle myocardium. Its layer of conjunctive tissue, bordering on the myocardium, contained white fatty tissue besides the collagen fibres going into the perimisial myocardial tissue. This fact was better expressed in the histological preparations of the right heart ventricle where the quantity of fatty tissue was bigger than that of the left one (**Figure 2**). The epicardium external surface is covered with mesothelium.



bar=10μm

Figure 1. *A section from the left heart ventricle wall with a different localization of the heart muscle fibres (coloration – X/E)*

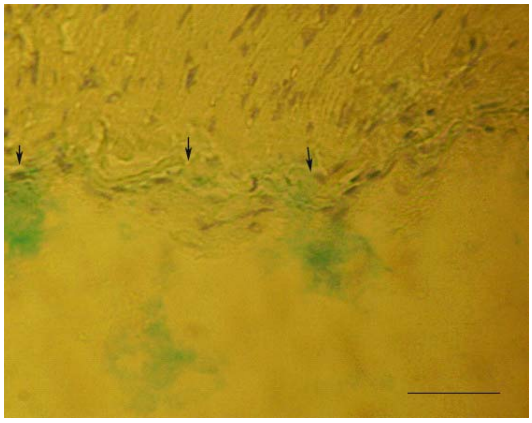


bar=-5μm

Figure 2. *A section from the right heart ventricle wall including the myocardium (*) and a part of the epicardium containing white fatty tissue (↓) (coloration – X/E)*

The light microscopic analysis, carried out after the histochemical tests, established in all histoslices from the left and the right heart ventricles expressed PAS positive reactivity of the heart muscle fibres from a slight to a medium extent. The other structural elements making up the heart ventricle wall demonstrated a lack of cytochemical reactivity after the PAS reaction. Only single, rarely found cells in the structural elements of the conjunctive tissues of the endocardium, myocardium and epicardium, showed a slight PAS positive reactivity. Cardiomyocytes in the heart ventricle heart muscle fibres showed light pink sarcoplasma after the PAS reaction. On further magnification it was found that this was due to tiny light pink micro granules filling the cardiomyocytes. The study did not establish a difference in the manifestation extent after the PAS reaction between the left and right heart ventricles in the cat. In either of the studied histoslices of the left and right heart ventricles there was no cytochemical reactivity after the test with alcian blue (pH

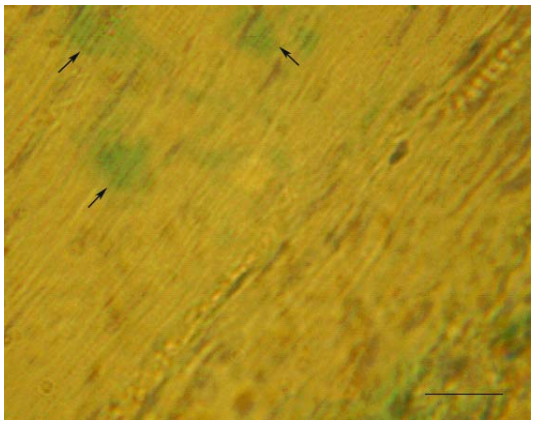
1.0). Alcianophylia, from a slight to a medium extent, was registered in the preparations from both heart ventricles after a combined coloration with Maurice alcian blue, followed by a PAS reaction. The same positive cytoreactivity was observed after the test with Alcian blue (pH 2.5). Such reactivity was observed only in some structural elements of the heart ventricle wall which were the same for the left and right heart ventricles, the manifestation extent being identical for both heart ventricles. Alcianophylia was observed as a slight to medium blue-green coloration of the heart ventricle endothelium and fibre groups in the sub endothelium sub layer of conjunctive tissue. This reactivity was well expressed in the preparations from the left heart ventricle in the sections with thickened endocardium (**Figure 3**). The epicardial mesothelium and the adjacent sub layer of conjunctive tissue in the preparations of both heart ventricles showed a similar cytochemical activity. The fibres in all structural components of conjunctive tissue in the heart ventricle wall (endocardium, myocardium and epicardium) also demonstrated alcianophylia from a slight to a medium extent. Separated cells from the row of conjunctive tissue, localized mostly in the sections with fibres coloured with alcian blue, demonstrated cytochemical reactivity after MAB/PAS and AB (pH 2.5) (**Figure 4**).



bar=12,5μm

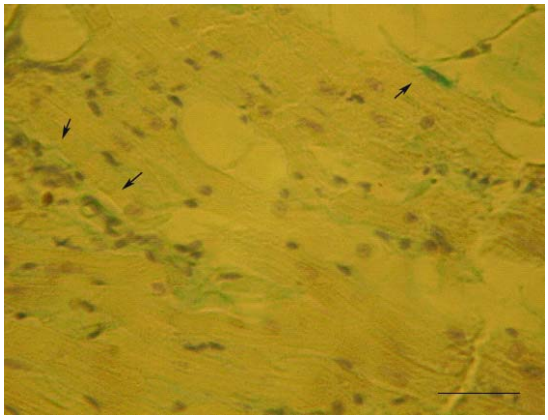
Figure 3. *Left heart ventricle endothelium and subendothelium layer of conjunctive tissue demonstrating alcianophylia from a slight to medium extent (↑) (coloration MAB/PAS).*

Separated, rarely found sections with cytochemical reactivity were observed also in the myocardium of both heart ventricles. The manifested alcianophylia varied between a slight to a medium extent of expression, localized in the places of conjunction between the cardiomyocytes in the heart muscle fibres. Because of the fact that the intercellular boundary is not always perpendicular to the longitudinal axis of the heart muscle fibres, the findings in our study looked stair-like, short, blue-green coloured bands or spots (**Figure 5**).



bar=12,5μm

Figure 4. *Epicardial section of a right heart ventricle where a part of the conjunctive tissue fibres and separate cells show alcianophilia from a slight to medium extent (↓) (coloration MAB/PAS)*



bar=12,5μm

Figure 5. *Part of the left heart ventricle myocardium containing sections with expressed cytochemical reactivity (↓) (coloration – MAB/PAS)*

According to (1) the animals' muscular cells of the left heart ventricle wall contain more glycogen in comparison with those in the other heart compartments. The results from our study did not show a visible difference in the extent of coloration reactivity in the cardiomyocytes from the left and right heart ventricles in the cat after the PAS reaction has been carried out. The alcianophylia, established in our study, in the areas of the intercellular boundaries in the heart muscle fibres confirms the opinion expressed in this earlier study in (1) that the boundaries between the cardiomyocytes contain glucosaminoglycans. The positive cytochemical reactivity shown after

MAB/PAS and at AB (pH 2.5) as well as the lack of such reactivity after the histochemical test with alcian blue (pH 1.0) gives us the reason to determine them as non-sulphate glucosamynoglycans in the cat heart. They were found in both heart ventricles, but considerably more often they could be observed in the preparations from the left heart ventricle.

CONCLUSIONS

The heart ventricle wall in the cat does not show structural differences from that of other mammalian species when observed with a light microscope.

The histochemical reactions and tests show that the heart ventricle wall contains both free polysaccharides (glycogen) in the myocardium and glucosaminoglycans of the non-sulphate type with a different localization, identical for both heart ventricles.

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