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## Innervation of Renal Hemolymph Nodes in Rats: Fluorescence Histochemical Study

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**Abstract:** In the renal hemolymph node, adrenergic nerves supply essentially the same structural and functional parts of the organ as in the other lymph nodes. Their total number is, however, pronouncedly higher in this organ, especially locally in the areas of maximal concentration of erythrophages and siderophages. Adrenergic nerve fibres enter these nodes in a common bundle with arteries, above all, hilus, but sporadically also with penetration of fibrous capsule on the other sites of the organ surface. In conclusion, the authors discuss a working hypothesis of the potential biological importance of the renal hemolymph node as the organ, which not only eliminates erythrocytes from an affluent lymph, but at the same time, it controls a defensive immune reaction so not to arise any phenomena of autoaggression against autologous red blood cells.

**Key words:** adrenergic innervation; renal hemolymph node; rats. Innervation of renal hemolymph nodes.

### Introduction

Hemolymph nodes (HLN) in more species of mammals represent a special component of the lymphoid system, which is characterized, above all, by unusually rich representations of erythrocytes, mostly in various stages of erythrophagocytosis associating with the hemosiderosis phenomena. That all, finally causes a conspicuous brown-reddish colouring of these organs. The first report on their existence in man was published by gibbens already in 1884. In the course of time, other references have increased; partly on their occurrence in pigs (Sabin, 1905), some carnivores, ruminants and rodents (Meyer, 1913; Ezeasor *et al.*, 1989) as well as on the results of studies performed on rats under different experimental conditions. They were predominantly aimed at answering the two open questions of a key significance concerning 1) the place from where erythrocytes come and the route which erythrocytes can penetrate inside the nodes, and 2) the biological significance of a such massive invasion of erythrocytes into the organ where immediately their total decomposition, associating with accumulation of considerable amounts of hemosiderin occur.

Despite the fact that practically all the basic characteristics of HLN regarding the morphological aspect have been relatively well defined, our knowledge seems to be very surprising that in the available literature the data on their nerve supply are missing. As in a context of our complex studies concerning innervation of the lymphoid organs and tissues the above-mentioned shortage of information appeared to be a sensible hiatus, in the next stage of our work, we have set a task to explore the adrenergic innervation of so-called "renal HLN", i.e. nodes occurring in a narrow topographic relationship with kidneys, namely in rats.

### Materials and Methods

The renal hemolymph nodes (RHLNs) were examined in 30 rats of both sexes, aged 6 months. Adrenergic nerve components were visualized by the glyoxylic acid fluorescence histochemical method in modification of Schvaley and Zhuchkova (1987). In addition, semithick sections were made from histological materials embedded into synthetic resin, which were stained with thionine or methylene blue, or hematoxylin and eosin. Microscopic examinations and photo documentation were performed using a JENA LUMAR-2 (Zeiss, Jena).

### Results

The RHLNs in rats appear as tiny connective tissue - encapsulated bean-shaped organs of dirty-reddish colour, belonging to the group of para-aortic nodes lying on the level of kidneys, partially above and behind *v.renalis*. They consist, like all the lymph nodes, of tunica fibrosa, subcapsular sinus of segmented type, cortex layer of lymphoid tissue, further of medulla with medullary cords and

sinuses. On the concave side of the organ, there is a bounded segment fulfilling the role of a hilus as for the entering arterial twig as for the leaving efferent veins and lymph vessels. Entries of afferent lymph vessels are present segmentedly on the side of the organ, always in a relationship to the relevant segment of the subcapsular sinus.

Besides the excessive content of erythrocytes and hemosiderosis, there are other peculiarities for histological pictures of RHLNs. The dominance of medullary components appears to be especially conspicuous, i.e. of medullary sinuses and cords which, to a great extent, get into a direct contact with tunica fibrosa or subcapsular sinus. The amorphous precipitates of lymph proteins as well as great amounts of iron can be seen in the translucence of afferent lymph vessels and in the segments of the subcapsular sinus. Admixture of unimpaired and decomposed red cells is usually inconspicuous in this topography. Contrariwise, intermediary and medullary sinuses contain more erythrocytes, mainly in the form of rosette-like aggregations with macrophages.

The nodal cortical layer itself is represented, above all, only by irregularly scattered and mostly not very big, isolated or confluent small islands of the lymphoid tissue which form sporadically unmarked follicles of clustered lymphocytes, as a rule, without typically lighter germinal centres. So the borders among superficial cortical layer, interfollicular zone and paracortex or thicker medullary cords can be assessed only very approximately, especially due to the absence of typical lymphatic follicles. In this way, the extent of T-dependent components of cortical lymphoid tissue help to define more or less only the findings of relatively more abundant venules with high endothelium (HEV).

Specifically fluorescent adrenergic nerve components enter the RHLNs in a common bundle with arteries, mainly through the hilus, but sporadically also through other sites of the organ surface. Then behind the hilus, carrying periarterial or periarteriolar plexuses run first in thicker medullary cords. (Figs. 1, 2) During their next passing towards the nodal surface, the appearance of nerve components changes parallel to the arteriole branching. The number of brightly fluorescent nerve fibres is gradually reduced to 1-2 very thin profiles in the vicinity of the thin arterioles oriented towards the cortical layer directly or twisting in an arch shape on the cortico-medullary border. It is, however, noteworthy that a great number of very delicate and sometimes ramified nerve fibres can be seen as in the zones of relatively dense capillary plexuses within wider medullary cords as in the borderline layers of the paracortex with medulla, and moreover sporadically at the sites of direct contacts of the capsule with medulla. (Figs. 3, 4, 5) They are apparently filial generations of those perivascular nerve twigs that branch off to the parenchyma. In addition, there are

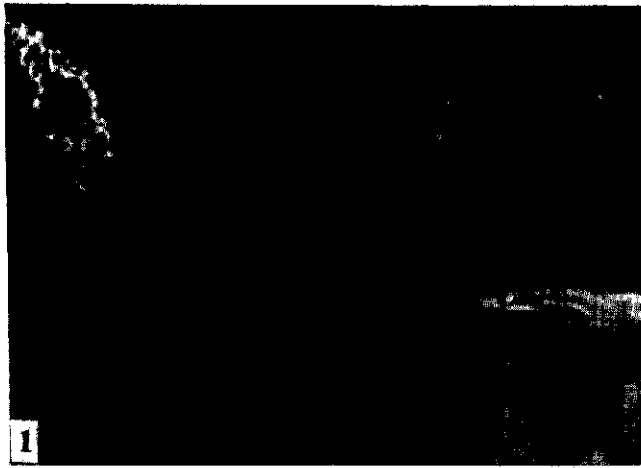


Fig. 1: Relatively rich adventitial periarteriolar plexuses lying in the fibrous capsule. Solitary nerve fibres are branching off here and there into the parenchyma of the organ. x120

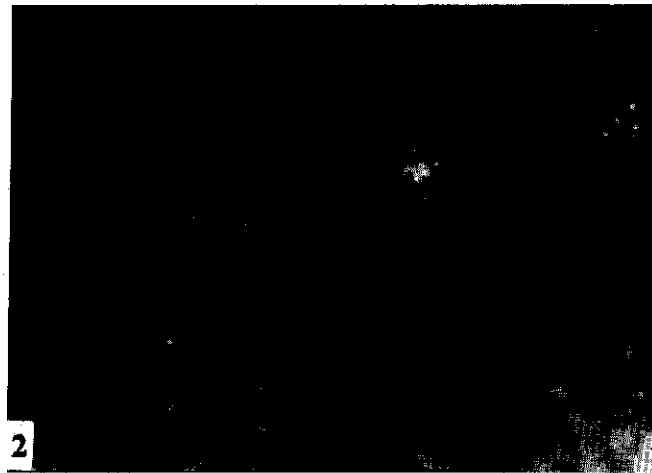


Fig. 2: Sufficiently abundant periarterial and periarteriolar adrenergic nerve plexuses can be seen around the arterial branch entering the organ from its surface and directing between non typical cortical islands of lymphoid tissue into the medulla. x120

intensively fluorescent periarteriolar plexuses lying on the outer surface of the fibrous capsule or pericapsular connective tissue as well as round the vascular twigs running among small islands of the cortical lymphoid tissue in a radial direction into the medulla. (Fig. 6) However, it is remarkable that the relatively highest density of nerve plexuses is recorded round arterioles and in the parenchyma of those parts of the cortex and medulla, where a lot of clustered erythrophages and lymphoid cells can be seen. These cellular components get also to the close contacts with adjacent nerve fibres. In the area, being poorer in free cells and not containing rosette-like aggregations with macrophages, the adrenergic innervation is usually limited only to several specifically fluorescent periarteriolar profiles.

Nerve supply of the large vein walls is only very modest and partially marked. The presence of specifically fluorescent nerve profiles in the vicinity of small veins and efferent lymph vessels could not be proved.



Fig. 3: Numerous specific fluorescent adrenergic nerve profiles situated in the medulla. x160

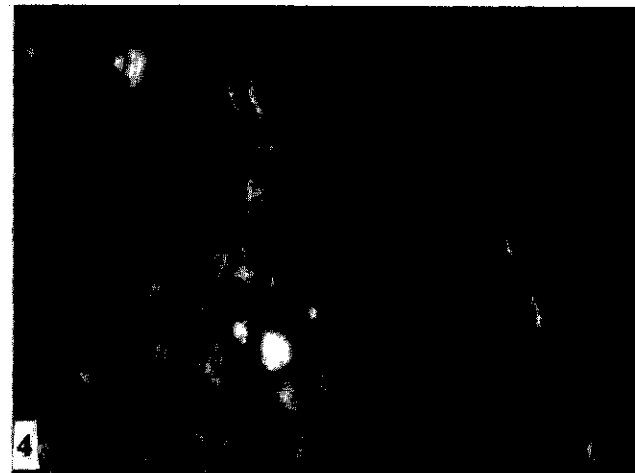


Fig. 4: The nerve profiles lying on the border of narrow stripe between the cortical lymphoid tissue and the medulla. x120

#### Discussion

Regarding purely morphological aspect, practically all the lymph nodes may be defined as the organs resembling small or bigger beans having a bounded area on the concave side fulfilling a role of the hilus as for the entering artery as for the outlet of veins and efferent lymph vessels. The nodal fibrous components form the capsule and a system of thinner and wider trabeculae running out from it inwards, which usually fuse on the hilus level. In the windows or spatial plexus of this carrying skeleton, there is "specific" reticular tissue containing numerous free and fixed cells among which different lymphocytes, plasmocytes and macrophages dominate.

Afferent lymph vessels lead into the nodal subcapsular sinus from where the node partially diffuses into the adjacent cortical layers of the parenchyma and partially flows directly in the system of medullary sinuses through the connective cortical sinuses. There are opinions that just immunogenic substances in the node are the main factor stimulating individual functional structures of the lymph nodes (Sainte-Marie *et al.*, 1982), even if potential

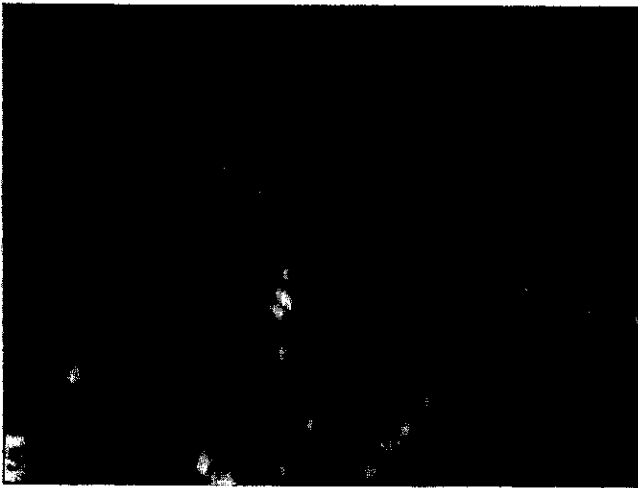


Fig. 5. In the medullary cords adjacent directly to the fibrous organ capsule the specific fluorescence findings of nerve profiles are relatively frequent. x160

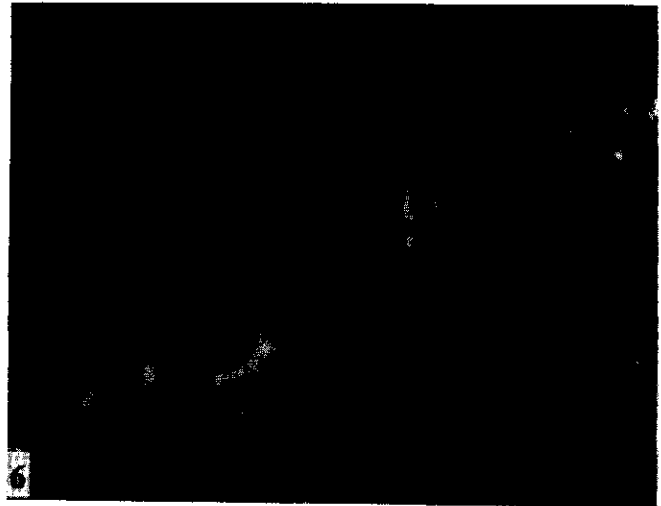


Fig. 6: The periaxillary adrenergic nerve plexuses lying in the place of direct contact between the medulla and the organ fibrous capsule. x160

modulating effects of non-immunogenic components of the node cannot be passed over.

The RHLNs, like the other lymph nodes, have the subcapsular sinus segmented so that its each separated part is, as a rule, supplied only with one afferent lymph vessels, whereas its end parts usually lead into the cortical sinus radially running to the medulla. Such bounded complex, i.e. sinus segment, adjacent cortical layers and paracortex (identified as "a deep cortex unit") represents in fact an integrated basic morphological and functional unit of the node (Bélisle and Sainte-Marie, 1981). Both the histological structure and architecture of these units sometimes can be quite different, because the content of immunogenic and non-immunogenic substances in the node supplied with individual afferent lymph vessels can significantly vary.

Concerning the massive accumulation and subsequent destruction of erythrocytes in the RHLNs, a lot of still open questions have been left. The original assumptions that erythrocytes can get into the organ sinus via a blood reflux from renal veins through efferent lymph vessels (Andreasen and Gottlieb, 1946) or by the passage of red blood cells via diapedesis through capillary walls and postcapillary venules (Nopajaroonsri *et al.*, 1974) are presently in doubt. After injections of the organ vascular bed no extravasates could be proved (Castenholz and Castenholz, 1996b). Other authors have deduced that even if penetration of a certain part of red blood cells from thin-walled blood vessels cannot be completely excluded, their main mass in the organ is supplied with afferent lymph vessels (Kazeem *et al.*, 1982). This explanation has been confirmed also by results of experiments on rats, which purport in the sense that ipsilateral kidneys are the source of erythrocytes in the afferent lymph vessels for RHLNs (Kazeem and Scothorne, 1982). Furthermore, there are opinions (Selye and Foglia, 1938) that migration of erythrocytes into the kidney interstitium can be induced as well as stimulated by stress; nephrectomy in rats was proved experimentally to cause escaping of erythrocytes from the RHLNs (Selye and Schenker, 1939).

According to the criteria for histological classification of reactive changes in lymph nodes (Van der Valk and Meijer, 1987), the functional morphology of RHLNs appears to be very complex. Within the range of not very large islands of the discontinuous cortical layer, the pictures of apparent hyporeactivity of cortical follicles come into prominence, namely in combination with the findings of relatively abundant HEV as in the interfollicular topography as in paracortex. Contrary to these findings, there is an apparent dominance of medullary components associating with extensive hyperactivity of histiocytic cellular elements.

The knowledge - that extensively mobilized macrophages besides erythrocyte decomposition also interact with lymphocytes - testifies to the fact that both the non-specific phagocytes and elements presenting antigens from autologous red blood cells to immunocompetent cells apply themselves in the organ; according to the working hypothesis of Castenholz and Castenholz (1996a), e.g. T-4 lymphocytes, which after absorbing such information in the chain of immune processes could initiate stimulation of suppressor cells and/or inhibition of antibody production by plasma cells against autologous erythrocytes. In this way, the defence system of the organism could adapt permanently to respect the principles of self-tolerance to its native red cells.

Our findings on the adrenergic innervation of RHLNs in rats are in agreement with those of other authors, who have reported that these nerves supply not only the vascular components, but also the parenchyma in the lymphoid organs and tissues (Felten *et al.*, 1987). The relatively highest density of adrenergic nerve profiles was always recorded in the periaxillary or periaxillary topography in the form of typical plexiform plexuses; whether in the medullary cords or in the vicinity of arterioles lying on the borderline of paracortex and medulla, fibrous capsule or running from the organ surface into the medulla among the small islands of the cortical lymphoid tissues. Nerve fibres, individually branching off, as in the medullary cords as in paracortex were relatively most abundantly represented in the areas of escalated decomposition of erythrocytes and accumulation of siderophages.

Even if a considerable significance is ascribed to the vasomotoric functions of adrenergic periaxillary nerves at the regulation of the blood circulation in the lymph nodes, their significance is not wholly exhausted. In these organs, there is a possibility of influencing reactions of lymphoid cells to the released neurotransmitter in the whole width of its diffusible zone into the nerve surrounding. Therefore, participation of adrenergic nerve components in the regulation of immune reactions in the RHLNs could not be minimized either.

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