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LOCALIZATION OF ACETYLATED TUBULIN POSITIVE NERVE FIBRES IN THE SPLEEN OF PHEASANTS

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The distribution of the nerve fibres in the adult pheasant spleen was studied using an indirect immunohistochemical method and acetylated alfa tubulin antibody. Positive thick nerve bundles of nerve fibres were seen in the area of the hilus from where these bundles enter the spleen together with blood vessels. Here, the positive nerve fibres were found dominantly in the adventitial area. Fine nerve fibres were observed also in the fibrous capsula. Moreover in the spleen the perivascular distribution of the nerve fibres dominated over those localized in the white and red pulp. In the pheasant splenic parenchyma their presence is limited to the white pulp particularly the periarteriolar lymphatic sheath. A fine network of nerve fibres was observed in the red pulp. The interrelation of the nervous system and the immune system is discussed.

Key words: immunohistochemistry, innervation, acetylated tubulin, spleen, pheasant

INTRODUCTION

Though morphological studies of the innervation of lymphoid organs are available, they have not provided a clear morphological link between the nervous and immune systems. Such an interrelationship occurred in the central organs studied by Ader *et al.* (1990) and Kordon and Bihoreau (1989). Numerous workers have paid attention to the distribution to nervous components in the lymphatic organs using histochemical methods. Thus, Giron *et al.* (1980), Williams and Felten (1981), Walcott and MacLean (1984), Williams *et al.* (1981), Felten *et al.* (1984, 1987), Livnat *et al.* (1985), Ackerman *et al.* (1987) and others have demonstrated the presence of autonomic nerves in specific regional sites of the mammalian primary and secondary lymphoid organs. Attention has mainly been paid to the innervation of the spleen both in birds and mammals. The nerve structures in chicken and pheasant spleens, were described by Schmidtova *et al.* (1995, 1998). The presence of nerve components in the lymphatic organs was also demonstrated immunohistochemically using S-100 protein, which is considered to be a specific antibody for the glial elements of nervous tissue (Sugimura *et al.*, 1990; Marettova *et al*, 1998). The aim of this study was to localize

the nerve components in the spleen of pheasants by an indirect immunohistochemical method using acetylated tubulin antibody.

MATERIAL AND METHODS

The experiment was performed on 15 pheasants at 9 weeks old. The animals were killed by aether narcosis and their spleens were removed following laparotomy. The spleen samples were placed in 0.1 M phosphate buffered 10% formaldehyde for 24 hours at room temperature, dehydrated and embedded in paraffin. The 6 μm-thick sections were stained with haematoxylin-eosin (HE) or processed using the avidin-biotin-peroxidase complex (ABC) method (Hsu *et al.*, 1981). Following deparaffinisation, sections were hydrated, incubated for 20 min in 0.3% H_2O_2 in PBS to reduce endogenous activity and preincubated with 2% goat serum to mask nonspecific binding sites. Afterwards, the sections were incubated overnight with the first monoclonal mouse anti-acetylated tubulin antibody, clone 6-11B-1 (Sigma), dilution 1:1000. Then, the sections were washed twice in PBS and incubated with goat anti-mouse biotinylated immunoglobulin at 1:20 dilution for 1 h. Subsequently, the sections were incubated with ABC and developed with 0.05% 3'3'- diaminobenzidine (DAB) and 0.03% v / v H_2O_2. Some sections were counter stained with Mayers haematoxylin. Thereafter, the sections were dehydrated in ethanol and mounted. Negative controls were performed by omiting the primary antibody.

RESULTS

More conspicuous nerve fibres were seen accumulated as nerve bundles in the area of the hilus, where the nerves accompanied the arteria lienalis (Fig. 1). From the hilus area the nerves together with blood vessels entered the splenic parenchyma. Inside the spleen, the acetylated tubulin positive nerve fibres were exclusively found in the perivascular area of blood vessels (Fig.2). Exceptionally, the nerve fibres entered deep into the medial zone of arteries (Fig. 2). Inside the spleen the nerve fibres were seen to be concentrated around the ellipsoids, visible in both longitudinal and cross-sectional views. Some nerve fibres of these plexuses followed the longitudinal direction of the ellipsoid. Additional nerve fibres radiated away from the vasculature towards the marginal zone as small positive profiles and were also scattered within the periarterial lymphatic sheath. Nerve fibres of similar appearance also ran along the parafollicular zone, with occasional delicate fibers in the follicles (Fig. 4). Individual fibres, which were not directly associated with vessels, extended from the arteriolar branches of the central arteries into the lymphocytic zone of the white pulp itself, thus intimately making contact with lymphocytes. (Fig. 5). In the red pulp, the fine nerve fibres were freely dispersed. In the fibrous capsule (Fig. 6) nerve fibres positive for acetylated tubulin were seen in the form of short discontinuous profiles running in cross and oblique sections and localized in the lower area of the capsule.

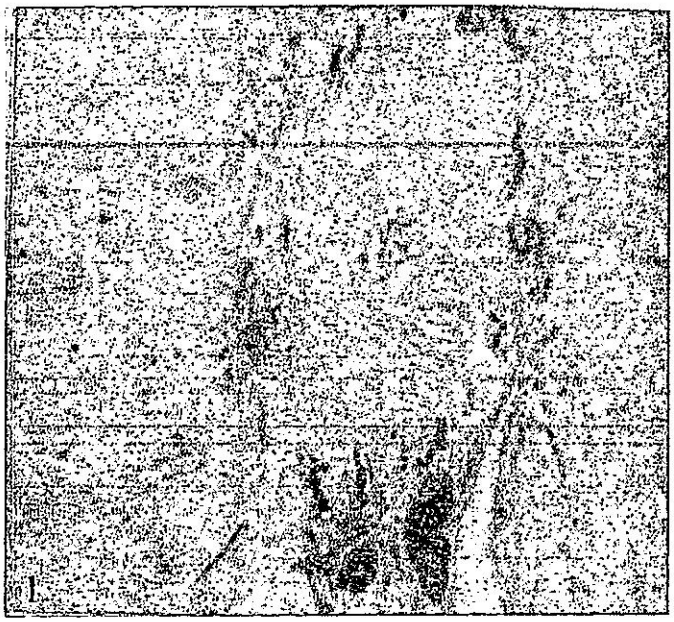

Fig. 1. A section of the spleen hilus. Large and medium sized nerves and plexus of fine nerve fibres accompany a large blood vessel. Thick and fine nerve fibres are located inside the adventitial area. x 345

Fig. 2. Acetylated tubulin-reactive nerve fibres are in the adventitia running along the blood vessel. x 345

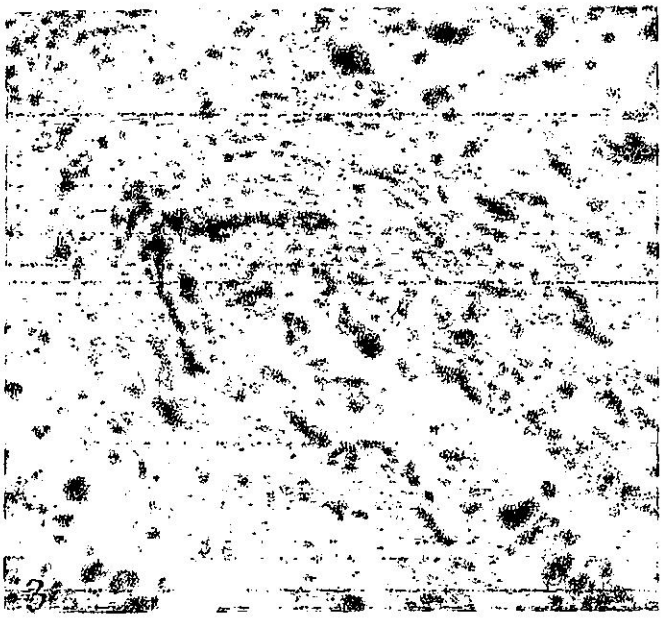

Fig. 3. Nerve fibres of different diameter are located inside the tunica media of the blood vessel. x 860

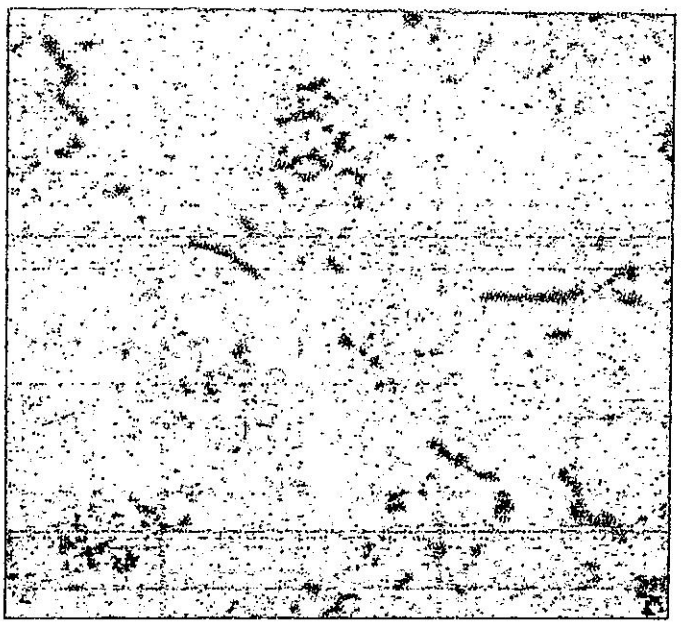

Fig. 4. Fine short nerve fibres are present in the red pulp. x 860

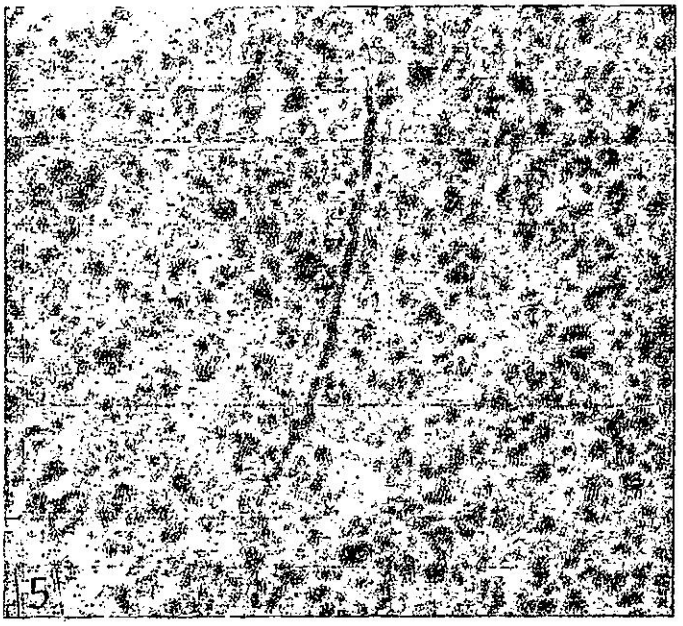

Fig. 5. A fine nerve fiber in longitudinal section is seen in the white pulp. x 860

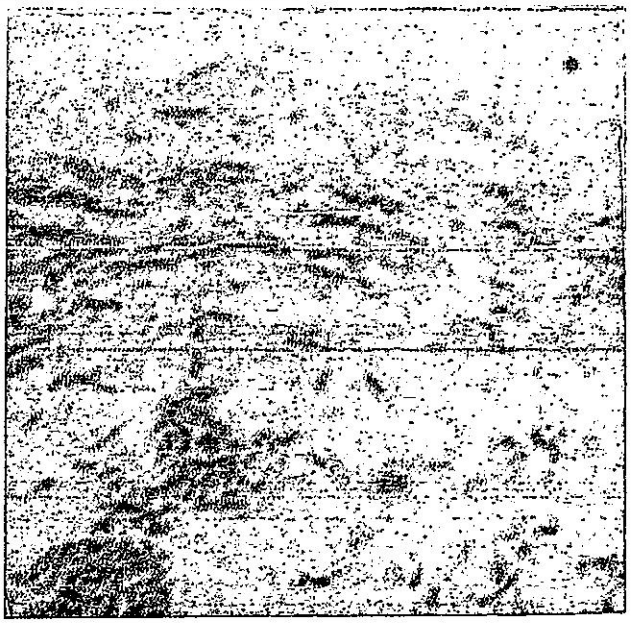

Fig. 6. Section of the spleen capsule. Fine nerve fibres are running longitudinally. x 860

DISCUSSION

In birds, the spleen structure has specific features. The most specific is that there is no framework of splenic trabeculae forming chambers as found in mammals. The white splenic pulp has reached the highest state of evolution among nonmammals. Trabecular vessels as such can hardly be said to occur in non-mammalian spleens. However, the large arteries near the hilum have strengthened the connective tissue envelopes in birds where the spleen also shows a special venous structure and an adventitial longitudinal musculature is added. In birds, the ellipsoids entirely surrounded by lymphoid tissue form a great part of the splenic parenchyma. The distribution of fibres follows this specific organization of the spleen.

This study has demonstrated the distribution of acetylated alfa tubulin in the nerve structures in the pheasant spleen. We found that the nerve fibres branched from the dense plexuses surrounding the splenic artery and as such they entered the spleen. According to Williams and Felten (1981) the spleen receives direct noradrenergic innervation to both the perivascular and parenchymal elements. As in the murine spleen, in chickens and pheasants the majority of innervation was distributed within the vasculature in the spleen. In the pheasant spleen the adventitia of the arteries supplying the white pulp was heavily innervated with nerve fibers. In man, the nerve fibres are present in the vascular smooth-muscle cells of the arterial system (Heusermenn and Stutte, l977). Schmidtova *et al.* (1998) described the white pulp to be richly innervated in rat spleen. They observed delicate nerve profiles occasionally seen both within the periarterial lymphatic sheath and follicle. In the rat spleen Felten *et al.* (1987) found sympathetic noradrenergic nerve fibres in the central artery and its branches and the parafolicullar zone with occasional delicate fibres in the follicles. Moreover, the authors recorded nerve fibres in the plexus around the central arterial system among T lymphocytes. From these reports it follows that nerve fibres richly innervate the splenic white pulp. Nerve fibres were also seen in this area in the pheasant. Thus, we can suppose that in these species nerve fibres may have close contact with lymphocytes. This contact is made by single fine often varicose fibres, which were not directly associated with the vessels, extending from the arteriolar branches of the central arteries into the lymphocytic zone of the white pulp itself.

The presence of nerve fibers in the red pulp is often controversial. Though there are differences among the animal species, data about the presence of the nerve fibres inside the red and white pulp depend on the method used in the study. Felten *et al.* (1987) stained the spleen section of the rat with antiserum for tyrosine hydroxylase and found nerve fibres to be limited to the white pulp, particularly the periarteriolar lymphatic sheath and there were only rarely positive nerve fibres in the red pulp. In this species, it was difficult to determine whether a nerve fibre was at the edge of an adjacent zone of white pulp or was actually travelling freely in the red pulp. Reilly *et al.* (1979) showed a close association between the unmyelinated nerve fibers and the blood elements in the spleen. Also Zetterstrom (1979) described varicosities within the red pulp of the dog spleen in the vicinity of the blood elements. Using NADPH - diaphorase method Schmidtova *et al.* (1995) did not observe any nerve fibres in the red pulp of the chicken spleen but did detect AchE-positive nerve fibres in the red pulp in this species. In their study the small arteries were found to be accompanied by plexuses of fine varicose

fibres which abruptly terminated at the junction with the splenic red pulp. The presence of nerve fibres in the red and white pulp in the pheasant spleen was confirmed by an immunohistochemical method.

There were no evident fibres specifically associated with the splenic capsule in the rabbit nor in the pheasant using the NADPH-diaphorase method (Schmidtová *et al*,. l998), though, after using the immunohistochemical method very fine fibres were observed running parallel predominantly in the deeper layer and in the subcapsular area in this species. In an other study Schmidtova *et al.* (1995) noted AchE-positive nerves to be regularly distributed in the chicken spleen capsule, whereas in the cat spleen they were observed in the smooth-muscle cells of trabeculae and capsule where they influenced the contraction of smooth muscle cells by diffusion innervation in this system (Saito, l990). In our study the presence of nerve fibres inside the deeper layer of the capsule was clearly demonstrated. The dense plexus just beneath the capsule suggests possible entry of the nerve fibers from the capsule into the splenic parenchyma.

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LOKALIZACIJA ACETILOVANIH TUBULIN POZITIVNIH NERVNIH VLAKANA U SLEZINI FAZANA

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SADRŽAJ

U ovom radu su izneti rezultati proučavanja zastupljenosti nervnih vlakana u slezini fazana imunohistohemijskim metodoma uz upotrebu monoklonskih mišijih antitela protiv acetilovanog tubulina izolovanog iz alfa (pozitivnih) nervnih vlakana. Snopovi nervnih vlakana su uočeni u hilusu a zatim se pored krvnih sudova pružaju u sam organ. Njihovo prisustvo je dominatno u zonama adventicije. Nervna vlakna su zapažena i u fibroznoj kapsuli a ima ih znatno više u područjima pored krvnih sudova nego u beloj i crvenoj pulpi. U parenhimu slezine fazana, prisustvo nervnih vlakana je ograničeno na belu pulpu i to posebno na periarterijski limfoidni omotač. Mre'a tankih nervnih vlakana postoji i u crvenoj pulpi a u radu se posebno razmatra veza između imunog i nervnog sistema kod ove životinjske vrste.