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SOME HISTOLOGICAL AND CYTOLOGICAL ASPECTS OF SMALL ARTERIES IN *NAUTILUS POMPILIUS* AND *N. MACROMPHALUS*

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NAUTILUS
ARTERIES
CYTOBIOLOGY
INNERVATION

ABSTRACT. – Small arteries of nautiloids are composed of a three-layered wall. Terminal nerve fibres are well established within the tunica adventitia. Only there, a high acetylcholinesterase activitiy and catecholamines could be detected. Immunohistochemical attempts to localize the neuropeptide FMRFamide yielded positive reactions within nerve fibres of the tunica adventitia. The three-layered wall of the afferent branchial vessel (ABV) shows some structural pecularities : the multilayered tunica media is also well innervated and a marginal sinus is established. Three different vesicle types are distinguished in the axons of the nerve fibres. Longitudinally arranged fibres of obliquely striated muscle cells reach from the tunica media into the collagenous network of the tunica adventitia.

NAUTILUS
ARTÈRES
CYTOBIOLOGIE
INNERVATION

RÉSUMÉ. – Les petites artères de Nautiloidés sont composées d'une paroi structurée en trois couches. Des fibres nerveuses terminales sont bien représentées dans la tunique "adventitia". C'est dans cette dernière que l'on trouve une forte activité de l'acétylcholinestérase et des catécholamines. Des essais immunohistochimiques réalisés pour localiser le neuropeptide FMRFamide démontrent des réactions positives dans les fibres nerveuses de la tunique adventitia. Le vaisseau branchial afférent (ABV) à trois couches indique des particularités structurales : la tunique "media" à multiples couches est également bien innervée et il y a un sinus marginal. On peut distinguer trois types de vésicules dans les axones des fibres nerveuses. Les fibres longitudinales des cellules musculaires obliquement striées font saillie dans le réseau des fibres de collagène de la tunique "adventitia".

INTRODUCTION

The structure of the artery wall of coleoid cephalopods with its three layers : tunica intima, tunica media and tunica adventitia (Jullien *et al.* 1957; Smith 1963; Barber and Graziadei 1967a, 1967b; Alvarado *et al.* 1969; Kawaguti 1970; Kurtscheidt 1980, unpubl.; Schipp 1987b; Mangold and Bidder 1989) resembles that of vertebrate vessels and fullfills a "Windkessel-function" (Gosline and Shadwick 1982; Shadwick and Gosline 1983; Schipp and Kleemann 1994). The cephalic aorta of *Nautilus* shows a similar functional-morphological aspect; but apart from the three afore-mentioned layers there is a further tunica, the tunica periadventitia surrounding a marginal sinus (Kleemann 1994). No details are known about the structure and function of smaller nautiloid arteries.

MATERIAL AND METHODS

Histological methods (Masson's trichrome after Goldner, Aldehydfuchsin after Gomori, PAS-reaction and toluidin blue coloration) and electron microscopical analyses were used to describe the wall structure of small arteries of Nautiloids (hepatico-columellar artery, proventricular artery, hepatic artery, afferent branchial vessel). The following technics were also used : immunohistochemical reaction against FMRFamide using the peroxidase-anti-peroxidase method as a tracer (Sternberger *et al.*, 1970; van Leeuwen, 1986), the acetylcholinesterase-reaction (AchE E.C. No. 3.1.1.7; Karnowsky and Roots, 1964), and the glyoxylicacid induced fluorescence (GIF) after de la Torre and Surgeon (1976) and Barber (1982).

Specimens of *Nautilus macromphalus*. Sowerby, 1849 (shell diameter 14-16 cm; net body weight 250-400 g) from the Coral reefs of New Caledonia and of *N. pompilius* Linné, 1758 (shell diameter 9-11 cm; net body weight 200-270 g) from Philippine costal waters were used.

RESULTS

The tunica intima is composed of a large continuous PAS-positive lamina basalis and an incomplete endothelium (Fig. 1, 2, 4). The tunica media is usually composed of 1-4 layers (depending on the vessel caliber) of circularly arranged fibres of obliquely striated muscle cells (Fig. 1, 2, 3, 4) and an elastic network (Fig. 1). The tunica adventitia is generally composed of a collagenous

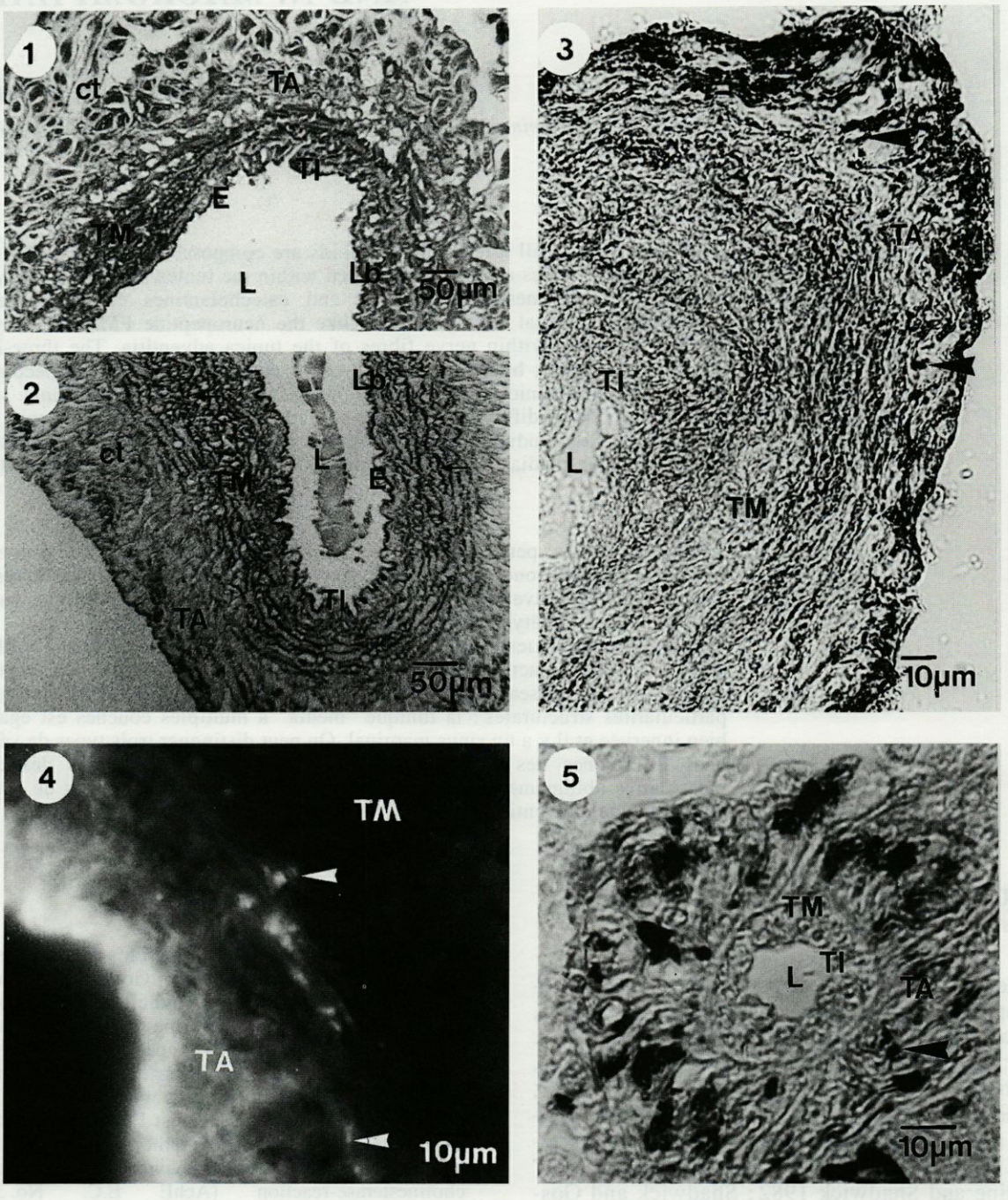

Fig. 1-5. – Cross-sections of small arteries of *Nautilus pompilius* : 1, Left proventricular artery. PAS- positive reaction of the lamina basalis and the loose connective tissue in the tunica media and tunica adventitia. 2, Elastic fibres (◄) in the tunica intima and tunica media of the hepatico-columellar artery (Aldehydfuchsin after Gomori). 3, Acetylcholinesterase activity (◄) in the tunica adventitia of an arteriole of the foregut. 4, Catecholamine fluorescence (◄) in the tunica adventitia of the hepatico-columellar artery. 5, FMRFamide reaction (◄) in nerve bundles of the tunica adventitia of the hepatic artery.

Abbreviations : Ax, axon; Ce, coelom epithelium; cf, collagen fibrils; cm, circularly arranged muscle fibres; ct, connective tissue; E, endothelial cell; G, glia cell; L, lumen; Lb, lamina basalis; lm, longitudinally arranged muscle fibres; Mi, mitochondrion; Ms, marginalsinus; N, nucleus; Nb, polyaxonal nerve fibre; SR, sarcoplasmic reticulum; TA, tunica adventitia; TI, tunica intima; TM, tunica media; tT, transverse tubule; zp, z-patch.

network with few scattered muscle cells; it contains vasa vasorum as well as a large number of polyaxonal nerve fibres. The acetylcholinesterase was demonstrated within nerve fibres of the tunica adventitia of an arteriole of the foregut (Fig. 3). In the tunica adventitia of the hepatico-columellar artery we observed fluorescent fibres, which revealed a bluish-green colour typical for catecholamines (Fig. 4). Immunohistochemical attempts to localize the neuropeptide FMRFamide yielded positive reactions within nerve fibres of the tunica adventitia in all small arteries investigated (Fig. 5).

Whereas in the other small arteries terminal nerves occurred only in the tunica adventitia, the tunica media of the afferent branchial vessel of *N. macromphalus* is densely innervated (Fig. 6). The collagenous network (periodicity of the collagen fibrils 54-64 nm) of the tunica adventitia is penetrated by longitudinally arranged fibers of obliquely striated muscle cells with few sarcosomes. Deep invaginations of the sarcolemma on the level of the z-patches are seen as a specialized t-system. The sarcoplasmatic reticulum has direct membrane contacts to the sarcolemma (Fig. 8). The axons of the peripheral nerves within the

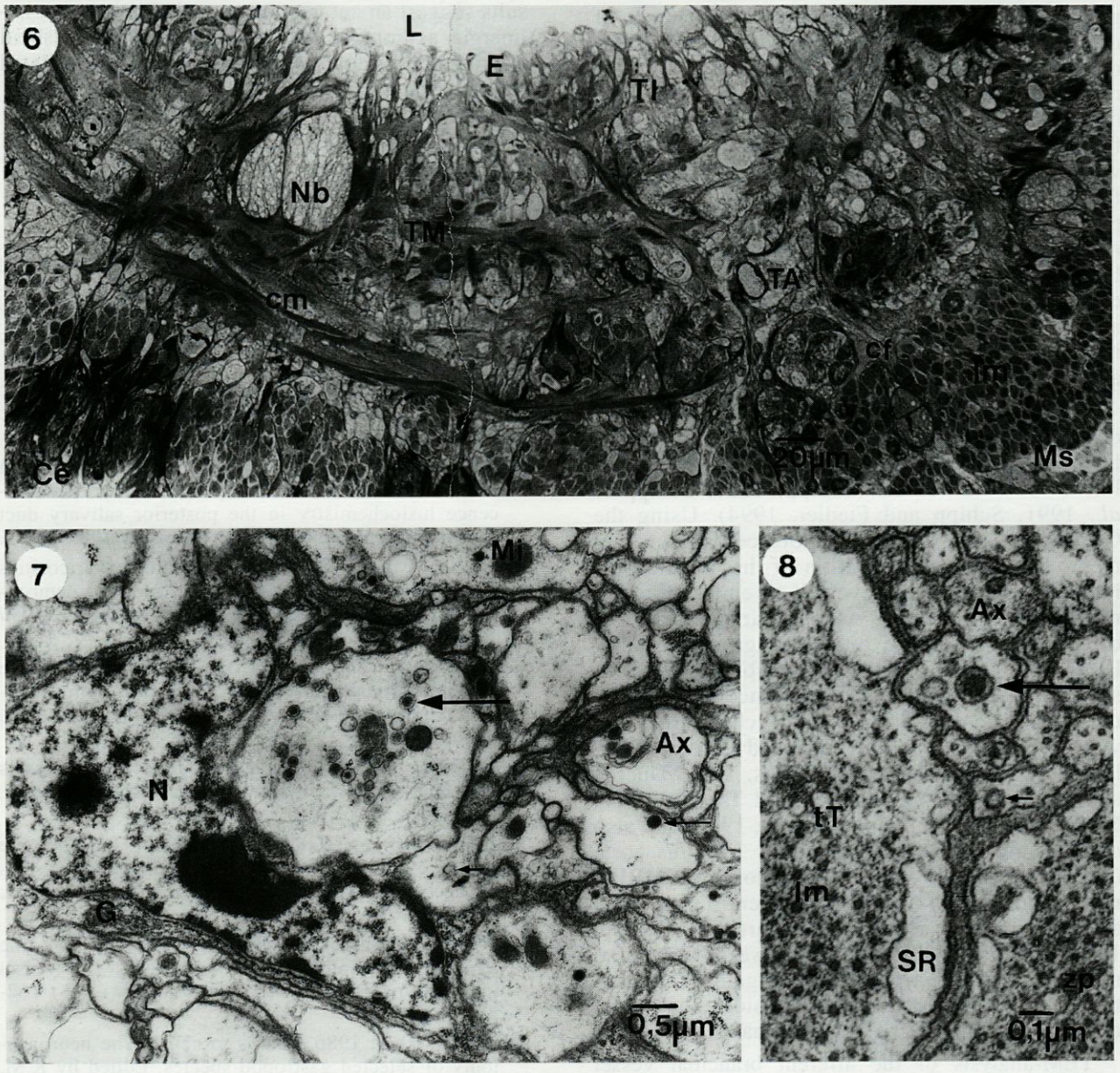

Fig. 6-8. – 6, Cross-section of the three layered vessel wall of the afferent branchial vessel (ABV) of *Nautilus macromphalus*. The obliquely striated muscle cells of the tunica media are well innervated. The longitudinally arranged muscle fibres belong to the tunica adventitia. (Semithin section, toluidin blue). Fig. 7-8 : TEM-sections of the afferent branchial vessel (ABV). 7, Polyaxonal nerve fibre in the tunica adventitia with transparent vesicles (→), dense cored vesicles (⟶) and osmiophilic vesicles (→) and dense bodies within the glia cell. 8 : Neuromuscular synapsis. The terminal axons contain dense cored (⟶) and transparent (→) vesicles (abbreviations : see Fig. 1-5).

vessel wall contain dense cored (∅ 83-98 nm), transparent (∅ 46-56 nm) and osmiophilic (∅ 72-97 nm) vesicles, neurofilaments, neurotubuli and single mitochondria (Fig. 7). The axons are surrounded by glia cells with dense bodies (∅ 180-239 nm). For the neuromuscular synapses in the vessel wall an intersynaptic cleft of 13-21 nm is typical.

DISCUSSION

Like the coleoid arteries (Smith, 1963; Barber and Graziadei, 1965, 1966, 1967a, 1967b; Kawaguti, 1970; Schipp, 1987) the small arteries of nautiloids possess a three layered wall. Considering the network of elastic fibres and the numerous circularly arranged muscle cells within the tunica media it seems probable that these vessels function as "Windkessel" too (Gosline and Shadwick, 1982; Schipp and Kleemann, 1994). These findings correspond to those on the coleoid cephalic aorta as well as on small vessels of the midgut, mantle and gills of *Sepia officinalis* (Jullien *et al.*, 1957). The occurrence of numerous elastic fibres within the tunica media is in accordance with the cephalic aorta of *Nautilus*, which is also seen as a "Windkessel"-vessel (Gosline and Shadwick, 1982; Kleemann, 1994).

The acetylcholinesterase localization in nerves of the tunica adventitia indicates that acetylcholine acts as neurotransmitter in the vessel control like in coleoid vessels (Schipp, 1987, Schipp *et al.*, 1991, Schipp and Fiedler, 1994). Using the GIP-method we observed fluorescent nerve elements in the tunica adventitia of the hepatico-columellar artery, which revealed an emission maximum ($Em_{max} = 480$ nm) characteristic of catecholamines. These results suggest a catecholaminergic component in the neuronal control of small arteries. Previous fluorescence histochemical studies have shown that catecholamines are widely distributed in the tunica adventitia of coleoid vessels (Arluison and Ducros, 1976; Ducros and Arluison, 1977; Andrews and Tansey, 1983; Kurtscheidt, 1980 unpubl.; Schipp, 1987) and also in the tunica periadventitia of the *Nautilus* aorta (Kleemann, 1994). The localization of FMRFamide in nerves of the tunica adventitia provides another similarity to the coleoid arteries (Schipp, 1987; Schipp *et al.*, 1991); but we have to note that this reaction is not specific against this peptide, but acts also against all amides that carry the sequence FM at their c-terminal side.

TEM-analysis of the afferent branchial vessel revealed a specialized muscle system in the tunica adventitia. The sarcoplasmatic reticulum has direct membrane contacts at invaginations of the sarcolemma; these diad-like junctions are probably involved in the intracellular Ca^{2+}- mediated electromechanical coupling of the muscle contraction.

According to Dorsett (1986) the ultrastructural differences of the molluscan neurovesicles are correlated with their respective, different transmitter content. The three different vesicle types detected in nerves of the afferent branchial vessels give a hint for a coexistence of acetylcholine, catecholamines and peptides. In terminal axons of the aorta of *Nautilus* (Kleemann and Schipp 1996) and *Sepia* (Schipp, 1987, Schipp *et al.*, 1991, Schipp and Fiedler, 1994; Schipp, 1995) there are also three different vesicle types. Together with our histochemical and immunohistochemical findings these results suggest an antagonistic catecholaminergic-cholinergic neuroregulation of the tonus of the small arteries of nautiloids, which is probably modulated by peptides.

REFERENCES

ALVARADO R., GONZALES-SANTANDER R. and SOCASTRO M.E. 1969. Contribution to the knowledge of the ultrastructure of the cephalopod vascular system. *Bol. R. Soc. Esp. Hist. Nat.* Secc. Biol. **67** : 175-179.

ANDREWS P.L.R. and TANSEY E.M. 1983. Aminergic innervation of the blood vessels of *Octopus vulgaris*. *Cell Tissue Res.* **230** : 229-232.

ARLUISON M. and DUCROS C. 1976. Localization of monoamine nerve fibres by formaldehyde fluorescence histochemistry in the posterior salivary duct and gland of *Octopus vulgaris*. *Tissue Cell* **8** : 61-72.

BARBER A. 1982. Monoamine-containing varicosities in the neural sheath of gastropod mollusc demonstrated by a glyoxylic acid histofluorescence. *Cell Tissue Res.* **226** : 267-273.

BARBER V.C. and GRAZIADEI P. 1965. The fine structure of cephalopod blood vessels I. Some smaller peripheral vessels. *Z. Zellforsch. Mikrosk. Anat.* **66** : 765-781.

BARBER V.C. and GRAZIADEI P. 1966. Blood vessels of cephalopods; their fine structure and innervation. *Bibl. anat.* **8** : 66-71.

BARBER V.C. and GRAZIADEI P. 1967a. The fine structure of cephalopod blood vessels II. The vessels of the nervous system. *Z. Zellforsch. Mikrosk. Anat.* **77** : 147-161.

BARBER V.C. and GRAZIADEI P. 1967b. The fine structure of cephalopod blood vessels III. Vessel innervation. *Z. Zellforsch. Mikrosk. Anat.* **77** : 162-174.

DORSETT D.A. 1986. Brains to cells : The neuroanatomy of selected gastropod species. Edited by K.M. Wilbur, The Mollusca, 9 (2), Academic Press, New York : 101-187.

DUCROS C. and ARLUISON M. 1977. Localization of monoamine nerve fibres by formaldehyde fluores-

cence histochemistry in some peripheral ganglia of Cephalopoda. *Biol. Cell.* **30** : 141-150.

GOSLINE J.M. and SHADWICK R.E. 1982. The biomechanics of the arteries of *Nautilus, Nototodarus* and *Sepia. Pac. Sci.* **36** : 283-296.

JULLIEN A., CARDOT J. and RIPPLINGER J. 1957. De l'existence de fibres élastiques dans l'appareil circulatoire des Mollusques. *Ann. Sci. Univ. Besançon*, Zool. Physiol. **9** : 25-31.

KARNOVSKY M.J. and ROOTS L. 1964. A "direct-coloring" thiocholin method for cholinesterase. *J. Histochem. Cytochem.* **12** : 219-221.

KAWAGUTI S. 1970. Electron microscopy on muscle fibers in blood vessels and capillaries of cephalopods. *Biol. J. Okayama Univ.* **16** : 19-28.

KLEEMANN S. 1994. Das Arteriensystem der Nautiliden (Cephalopoda, Tetrabranchiata) – Eine vergleichend Morphologische und cytobiologische Untersuchung. Dissertation, JLU-Giessen.

KLEEMANN S. and SCHIPP R. 1996. Innervation of the cephalic aorta of Nautiloids (Tetrabranchiata, Cephalopoda). *Zoology* **99** (4).

KURTSCHEIDT G. 1980. Vergleichende histochemische und elektronenmikroskopische Untersuchung zur Feinstruktur und Innervation von Arterien bei dibranchiaten Cephalopoden. Examensarbeit, JLU-Giessen.

LEEUWEN F. Van 1986. Pitfalls in immunocytochemistry with special reference to the specificity problems in the localization of neuropeptides. *Am. J. Anat.* **175** : 363-377.

MANGOLD K. and BIDDER A.M. 1989. Appareils respiratoire et circulatoire : respiration et circulation. Traité de zoologie, Anatomie, systématique, biologie, Edited by P. P. Grassé. Cephalopods. Masson. Paris **5** (4) : 387-434.

SCHIPP R. 1987. The blood vessels of cephalopods. A comparative morphological and functional survey. *Experientia* **43** : 525-537.

SCHIPP R. 1995. Functional morphological aspects of the cephalic aorta of *Sepia officinalis* L. (Cephalopoda). 12th Internat. Malac. Congr. Vigo : 80-81.

SCHIPP R. and FIEDLER A. 1994. Cholinergic mechanisms in the neurocontrol of the cephalic aorta of the cephalopod *Sepia officinalis. Comp. Biochem. Physiol.* C **107** : 149-157.

SCHIPP R. and KLEEMANN S. 1994. Vergleichende Untersuchungen zur Funktionsmorphologie der Aorta cephalica von Nautiliden und Coleoiden (Cephalopoda). *Verhandl. Dt. Zool. Ges.* **87**(1) : 111.

SCHIPP R., JAKOBS P.M., FIEDLER A. 1991. Monoaminergic-peptidergic interactions in neuroregulatory control of the cephalic aorta in *Sepia officinalis* L. (Cephalopoda). *Comp. Biochem. Physiol* C. **99** : 421-429.

SHADWICK R.E. and GOSLINE J.M. 1983. The structural organisation of an elastic fibre network in the aorta of the cephalopod *Octopus dofleini. Can. J. Zool.* **61** : 1866-1879.

SMITH L.S. 1963. Circulatory anatomy of the *Octopus* arm. *J. Morph.* (Philad.) **113** : 261-266.

STERNBERGER L.A., HARDY P.H., CUCULIS J.J. and MEYER H.G. 1970. The unlabeled antibody enzyme method of immunohistochemistry- preparation and properties of soluble antigen-antibody complex (Horseradish peroxidase-Antihorseradish Peroxidase) and its use in identification of Spirocetes. *J. Histochem. Cytochem.* **18**(5) : 315-333.

TORRE J.C. and SURGEON J.W. 1976. A methodical approach to rapid and sensitive monoamine histofluorescence using a modified glyoxylic acid technique : The SPG method. *Histochemistry* **49** : 81-93.

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