Histol Histopath (1987) 2: 89-94

Histology and Histopathology

Studies on intra-arterial cushions. IV. Perivascular nerve plexuses of ramifying arteries with intraluminal cushions at the branching points

P. Böck

Department of Micromorphology and Electron Microscopy, University of Vienna, Vienna, Austria

Summary. Periarterial nerve plexuses were studied at branching points of arteries with intraluminal cushions. Serial sections were prepared from mouse kidney, pancreas and tongue, and studied by means of catecholamine fluorescence and staining for acetylcholinesterase.

The periarterial nerve plexuses did not show any peculiarities at the branching points. The intraluminal cushions, as well as the tunica media of both the parent trunk and the collateral branches, were found to be free of vegetative nerves. It is concluded that the shape of intra-arterial cushions is passively altered, following the alterations of vascular geometry, and not in response to direct nervous stimulation.

Key words: Vascular innervation - Intra-arterial cushions - Catecholamine fluorescence - Acetylcholinesterase - Mouse.

Introduction

Intra-arterial cushions or valve-like structures are frequently found in branching arteries of both the muscular and elastic type. The cushions have been described in several species of vertebrates, including *Petromyzon* (Wagenvoort, 1954), *Amphibia* (Dragendorff, 1911), and *Mammalia.* In mammals the cushions were studied in man (Märk, 1951; VeLican and VeLican, 1977), cat (Picard, 1951; Takayanagi et al., 1972; Büssow, 1981), dog (Picard, 1951; Moffat, 1952; Picard and Chambost, 1952; Nanda and Getty, 1972; Jellinger, 1974), pig (Nanda and Getty, 1972), and particularly in *Rodentia* (Picard, 1951; Shanklin and Azzam, 1963; Menschik and Dovi, 1965; Jellinger, 1966; Reale and Luciano, 1966; Rosen, 1967; Taggart and Rapp, 1969; Moffat and Creasey, 1971; Lassmann et al., 1972; Yohro and Burnstock, 1973; Böck, 1975; Gorgas and Böck, 1975, 1976a,b; Kunkelmann, 1976; Lange and Halata, 1979; Büssow, 1980; Kojimahara and Ooneda, 1980; Casellas et al., 1982; Kardon et al., 1982).

The cushions appear as independent formations which are related to both the tunica intima and media of the vascular wall. Their presence seems to depend on a particular diameter of the branching artery; the cushions are almost characteristic in *Myomorpha.* Intra-arterial cushions were shown to interfere with laminar streaming in the parent trunk and to direct axial portions of its blood stream into the branching collateral. This phenomenon was called cell skimming (Wagenvoort, 1954; Fourman and Moffat, 1961). Cell skimming is responsible for higher hemoglobin concentrations in the branching artery as compared to the hemoglobin concentration of the main trunk. Shape change of the cushions has been suggested to occur passively during pulsatile distension (Gorgas and Böck, 1975) and actively in response to alphamimetic substances (Kardon et al., 1982)., A sphincter-like function of the cushions has been proposed (Moffat, 1959) which makes possible the control over blood flow into the side branches.

Remarkably dense innervation of the arterial wall in the region of intra-luminal cushions has been mentioned by Burnstock (1975) and Hassler (1962), which possibly indicates nervous control of the cushions. However, no unusual innervation has been noted in fine structural studies on intra-arterial cushions (Reale and Luciano, 1966; Lassmann et al., 1972; Takayanagi et al., 1972; Gorgas and Böck, 1976a,b; Hesse and Böck, 1980) but electron microscopy is not suitable to investigate this question. Therefore the arterial nerve plexuses were analyzed in serial sections of cushion-bearing branching points by means of catecholamine fluorescence and staining for acetylcholinesterase.

Materials and methods

Branching arteries were studied in Swiss mice. The animals were killed by neck dislocation and tissue samples from the tongue, pancreas and kidney were

Offprint requests to: Prof. Peter Böck, Institut für Mikromorphologie und Elektronemikroskopie, SchwarzspanierstraBe 17, A-1090 widsen, Austria.

quickly frozen in precoled isopentane. Serial frozen sections were cut at 15 µm thickness and processed as followes:

1. A series of 5-10 consecutive sections was collected into a small, pre-cooled brass box and several such series in brass boxes were freeze-dried for 24 h in a Leybold-Heraeus GT 001 freeze-drying apparatus. The dried sections were treated with formaldehyde gas at 70° C for 2 h (preparation scheme of Heene, 1968). The sections were mounted with paraffin oil and studied with a Leitz fluorescence microscope equipped with a HBO 200 high pressure mercury lamp, a Schott BG 12 excitor filter and a 490 nm barrier filter.

2. An additional group of serial sections consecutive to the group collected for freeze-drying was thawed on slides and fixed in Baker's formalin-calcium (6% formalin freshly prepared from paraformaldehyde and 1% $CaCl_2$) for 20 min at 4° C. After fixation the slides were washed in distilled water and incubated at room temperature in a Karnovsky and Roots (1964) medium for acetylcholinesterase, using acetylthiocholine as substrate and 10^{-5}M iso-OMPA (Sigma, Munich) to inhibit butyrylcholinesterase.

Incubation was continuosly controlled and lasted for 30-40 min. After incubation the sections were washed in distilled water, dehydrated and mounted in Depex.

Another group of tissue samples was quenched in isopentane, cooled with liquid nitrogen, and dried in the Leybold-Haraeus freeze-drying apparatus without previous sectioning. The tissues were reacted for 2 h at 70° C with formaldehyde gas and embedded in Araldite. 1 µm thick semithin sections were cut on a Reichert OmU2 ultramicrotome and studied for catecholamine fluorescence as described above, followed by phase-constrast microscopy of the respective field (for methodological details see Böck, 1984).

Results

A. Fluorescence microscopy of semithin sections

Small arteries (100-300 µm diameter) in all the three organs studied (tongue, pancreas and kidney) are provided with a well-developed adrenergic nerve plexus. In semithin sections the axon profiles are predominantly seen in transverse or oblique sections; appearing as delicate, brightly fluorescent dots (Figs. 1,3). The adrenergic fibres are located at the medio-adventitial border. This is also the case at the branching sites of the arteries where intraluminal cushions are regularly present. Fluorescent fibres were never seen to penetrate the stromal elements of the cushions (see the phase contrast views, Figs. 2, 4). This applies both to cushions which protrude as valve-like structures (Figs. 1,2) and to cushions which merely appear as a fold produced by a few smooth muscle cells (Figs. 3, 4).

Autofluorescence of elastic material was weak in the semithin sections. Even the internal elastic lamina of the main artery is indiscernible (Figs. 1, 3).

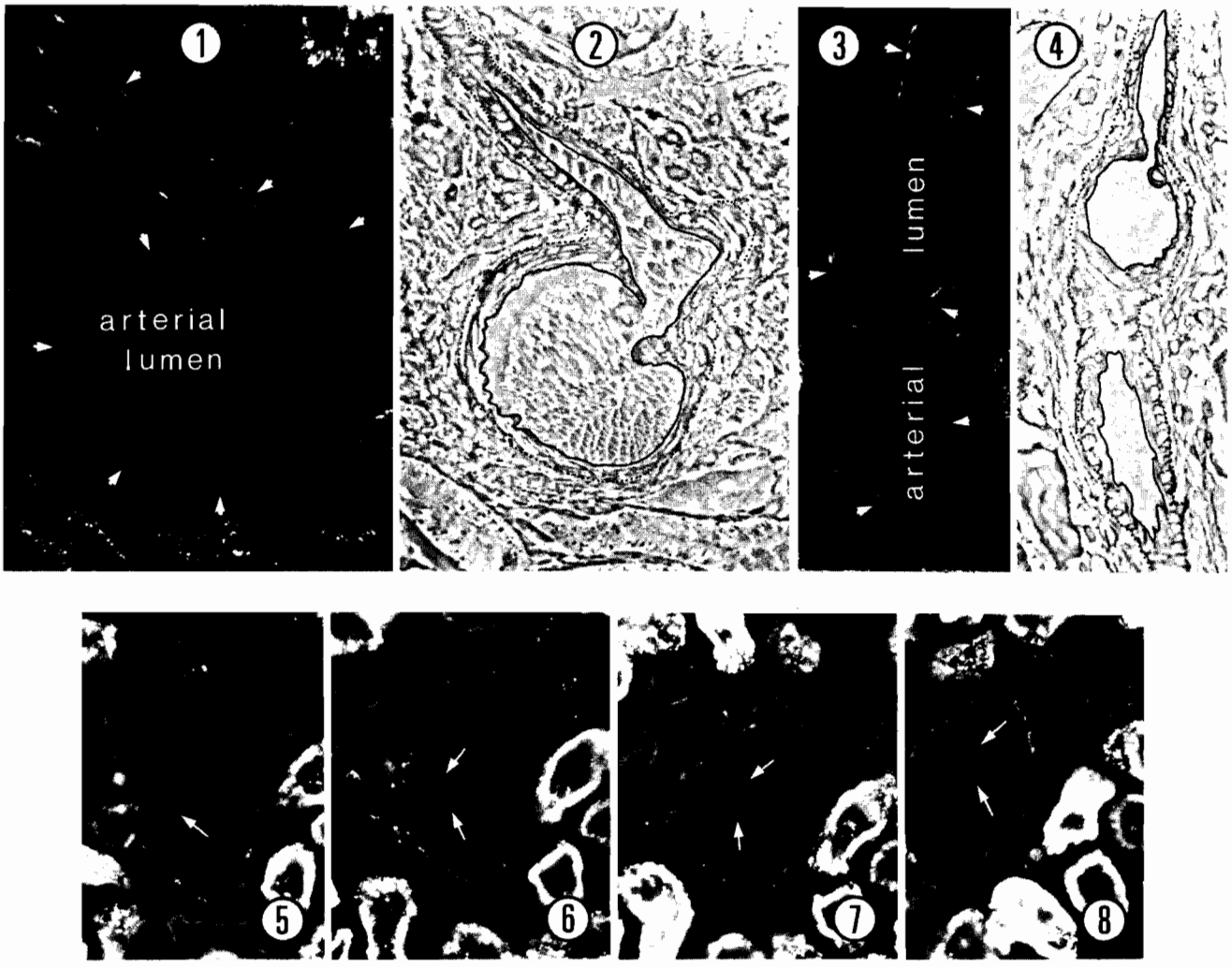
B. Fluorescence microscopy of frozen sections

Higher background fluorescence in the 15 µm thick cryostat sections enables one to discern arterial walls and intimal cushions with the fluorescence microscope. The internal elastic lamina is identified by its autofluorescence; it is interrupted where cushions encircle the orifice of a side branch (Figs. 5-8, 12-14). Because of the thickness of the sections the periarterial plexus of fluorescent fibres appears more prominent as compared to semithin sections. However, no significant differences were noted between the fluorescent plexuses around a parent trunk and the side branches, and there was no particularly dense adrenergic innervation at the branching points. No fluorescent fibres were seen within the tunica media or within the intraluminal cushions (Figs. 5-8, 12-14).

C. Localization of acetylcholinesterase activity (AChE)

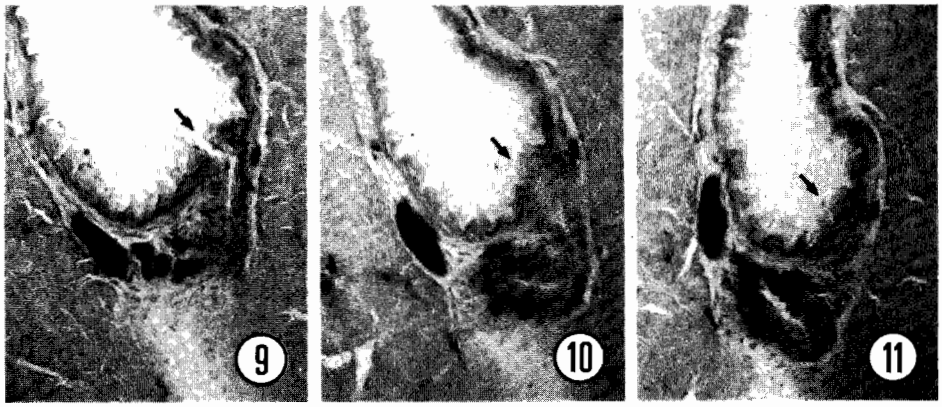
Staining for AChE reveales loose nerve plexuses in the adventitial layers of the arteries. The fine fibres correspond to the fluorescent fibres with respect to their location at the medio-adventitial border but the net of AChE-positive fibres appears to be less dense. No such fibres are seen inside the tunica media or in the stroma of the cushions. The plexus of AChE-positive fibres is inconspicuous at the branching points (Figs. 9-11).

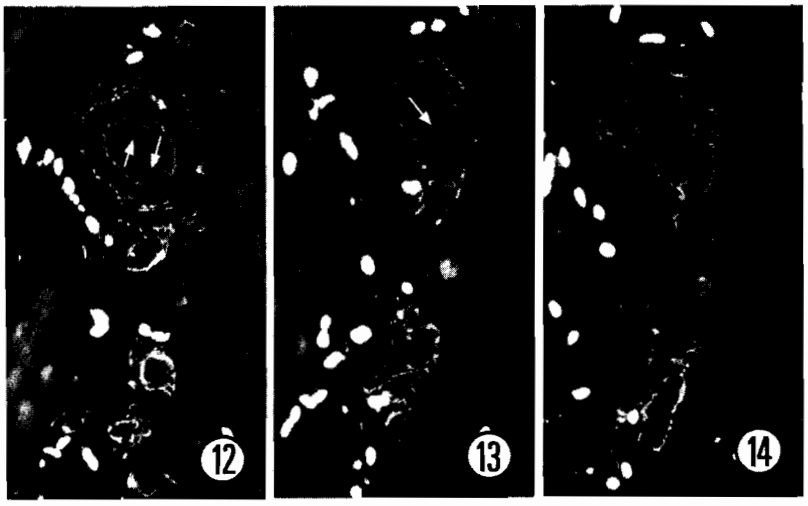
Besides the delicate periarterial plexus of AChE-positive fibres, a few thick, AChE-positive nerve bundles run along with arteries (Figs. 9-11). These bundles do not show catecholamine fluorescence.



Figs. 1-4. Cathecholamine fluorescence (Figs. 1, 3) and corresponding phase contrast microscopy (Figs. 2, 4) of branching arteries with intra-arterial cushions, semithin sections from Araldite-embedded material Figs. 1 and 2 show a branching artery from the cortical region of the mouse kidney, which is provided with well-developed valve-like cushions. Figs. 3 and 4 show a branching artery from the mouse pancreas with only flat cushions. In both these examples fluorescent axons are confined to the medio-adventitial border of the vessels (arrows). The periarterial adrenergic plexuses are inconspicuous at the branching sites. Fluorescent axons do not penetrate into the stroma of the cushions. In Figs. 2 and 4 the luminal surface of the arteries (continuous line) and the medio-adventitial border (dotted line) are accentuated with indian ink. All Figures x 300

Figs. 5-8. Catecholamine fluorescence in a series of frozen sections (15-20 μm thick) from the mouse kidney cortex, showing intra-arterial cushions (arrows). In thick sections autofluorescence of the internal elastic lamina is easily discernable. Elastic material is absent from the cushions. Fluorescent axons are restricted to the medio-adventitial border of both the trunk and branching artery. All Figures x 120





Figs. 9-11. Acetylcholinesterase staining of three consecutive frozen sections from the mouse pancreas. Arrow points to the origin of a collateral artery, which is provided with cushions. Positive staining is seen in a large nerve bundle and in delicate fibres surrounding the branching vessel. A few positive fibres are also seen in the adventitial layer of the trunk artery. The stroma of the cushions is free of nerve fibres. All Figures x 120

Figs. 12-14. Catecholamine fluorescence of three consecutive frozen sections from the mouse tongue. Periarterial nerve plexuses around small side branches are well developed. The branching point proper, however, does not show any peculiarities as to the distribution or density of adrenergic fibres. Arrows point to the intra-arterial cushions. Elastic material shows weak autofluorescence, mast cells appear as brightly fluorescent areas. All Figures x 100

Discussion

Catecholamine fluorescence and staining for AChE were used in the present study to demonstrate the main components of periarterial nerve plexuses. A third component, non-adrenergic and non-cholinergic in nature, was not considered in more detail. This particular group of axon terminals may comprise purinergic axons (Burnstock, 1972) as well as axons that display immunoreactivity for certain peptide hormones, e.g. for vasoactive intestinal polypeptide, somatostatin, subtance P, or neuropeptide Y. However, fine structural studies (Reale and Luciano, 1966; Lassmann et al., 1972; Takayanagi et al., 1972; Gorgas and Böck, 1975; Hesse and Böck, 1980), failed to show axon terminals characteristically crowded with large opaque vesicles near to the basis of intra-arterial cushions. This finding would indicate the presence of purinergic or peptidergic axons (Burnstock, 1975). The presence of purine nucleotides or one of the peptides mentioned above as a cotransmitter in adrenergic or cholinergic terminals cannot be excluded, but terminals of this kind would be shown by catecholamine fluorescence or AChE staining. Both these methods failed to show any remarkable variations of the periarterial nerve plexuses at branching arteries where the orifice of the branch is provided with intraluminal cushions. The fibres of the periarterial nerve plexuses are restricted to the medio-adventitial border and do not reach the stroma of the cushions. Because of these findings direct nervous control of the cushions seems unlikely.

Although the cushions apparently receive no direct innervation, the shape and rigidity of the cushions may be influenced indirectly:
(a) by gap junctions between ramified smooth muscle cells in the cushion stroma and smooth muscle cells in the tunica media, (b) by alpha adrenergic receptors of ramified smooth muscle cells in the cushions, which may respond to blood-borne circulating substances (Kardon et al., 1982), or (c) by changes in the vessel's geometry, which cause an altered shape of the orifice of the outbranching artery, so altering the base of the cushions and consequently the shape of the cushions (Gorgas and Böck, 1975; Kardon et al., 1982).

All these items (a) to (c) are related to slow and long-lasting variations in tonicity of arterial walls and cushions. This tonicity counteracts pulsatile distension of the arterial wall.

References

Böck P. (1975). Vergleich der Morphologie von Arterien- und Venenklappen. Verh. Anat. Ges. 69, 145-149.

Böck P. (1984). Der Semidünnschnitt. J.F. Bergmann, München.

Burnstock G. (1972). Purinergic nerves. Pharmacol. Rev. 24, 509-581.

Burnstock G. (1975). Innervation of vascular smooth muscle: histochemistry and electron microscopy. Clin. Exptl. Pharmacol. Physiol., Suppl. 2, 7-20.

Büssow H. (1981). Ultrastructure of the terminal vascular bed in the retina of mammals. Bibl. Anat. 20, 94-97.

Casellas D., Dupont M., Jover B. and Mimran A. (1982). Scanning electron microscopic study of arterial cushions in rats: A novel application of the corrosion-replication technique. Anat. Rec. 203, 419-428.

Dragendorff O. (1911). Über die Form der Abzweigungsstellen von Arterien bei den Wirbeltieren. Anat. Hefte 42, 739-808.

Fourman J. and Moffat D.B. (1968). The effect of intra-arterial cushions on plasma skimming in small arteries. J. Physiol. (Lond.) 158, 374-380.

Gorgas K. and Böck P. (1975). Studies on intra-arterial cushions. I. Morphology of the cushions at the origins of intercostal arteries in mice. Anat. Embryol. 148,59-72.

Gorgas K. and Böck P. (1976a). Studies on intra-arterial cushions. II. Distribution of horseradish peroxidase in cushions at the origins of intercostal arteries in mice. Anat. Embryol. 149, 315-321.

Gorgas K. and Böck P. (1976b). Die Struktur der GefäBwand am Ursprung der Intercostalarterien bei Mäusen. Verh. Anat. Ges. 70, 731-734.

Hassler O. (1962). Physiological intima cushions in the large cerebral arteries of young individuals. I. Morphological structure and possible significance for the circulation. Acta path. microbiol. scand. 55, 19-27.

Heene R. (1968). Histochemischer Nachweis von Katecholaminen und 5-Hydroxytryptamin am Kryostatschnitt. Histochemie 14, 324-327.

Hesse M. and Böck P. (1980). Studies on intra-arterial cushions. III. The cushions at the origin of the rat carotid body artery (CBA). Z. mikrosk.-anat. Forsch. 94, 471-478.

Jellinger K. (1966). Zur Frage der Arterienklappen im Nagerhirn. Anat. Anz. 119, 246-258.

Jellinger K. (1974). Intimal cushions in ciliary arteries of the dog. Experientia 30, 188.

Kardon R.H., Farley D.B., Heidger P.M. jr. and van Orden, D.E. (1982). Intrarterial cushions of the rat uterine artery: a scanning electron microscope evaluation utilizing vascular casts. Anat. Rec. 203, 19-29.

Karnovsky M.R. and Roots L. (1964). A direct-coloring thiocholine method for cholinesterase. J. Histochem. Cytochem. 12, 219-221.

Kojimahara M. and Ooneda G. (1980). Ultrastructural observations on bifurcations in rat cerebral arteries: 1. Young and aging rats. Virchows Arch. Cell Pathol. 34, 21-32.

Kunkelmann H. (1976). Morphologische Besonderheiten der Arterien im Glomus caroticum der Maus. Seminararbeit, Mathematisch-Naturwissenschaftliche Fakultät der Universität zu Köln.

Lassmann H., Pamperl H. and Stockinger G. (1972). Intimapolster der Arteria ophthalmica der Ratte in morphologisch-funktioneller Sicht. Z. mikrsk.-anat. Forsch. 85, 139-148.

Lange W. and Halata Z. (1979). Comparative studies on the pre- and postterminal blood vessels in the cerebellar cortex of the Rhesus monkey, cat and rat. Anat. Embryol. 158, 51-62.

Märk W. (1951). Zur Kenntnis der sg. Arterienwülste beim Menschen und bei einigen Säugern. Anat. Nachr. 1, 305-318.

Menschik Z. and Dovi S.F. (1965). Normally occuring intraluminal projections in the arterial system of the mouse. Anat. Rec. 153, 265-274.

Moffat D.B. (1952). A regulatory mechanism in the posterior ciliary arteries of the dog. Nature 169, 1015-1016.

Moffat D.B. (1959). An intra-arterial regulating mechanism in the uterine artery of the rat. Anat. Rec. 134, 107-123.

Moffat D.B. (1969). Intra-arterial cushions in the arteries of

the rat's eye. Acta Anat. 72, 1-11.

Moffat D.B. and Creasey M. (1971). The fine structure of the intra-arterial cushions at the origins of the juxtamedullary afferent arterioles in the rat kidney. J. Anat. 110, 409-419.

Nanda B. S. and Getty R. (1972). Intimal cushions of the cerebral arteries and their changes with age in the dog and pig. Anat. Anz. 132, 79-85.

Picard D. (1951). Sur la présence de valvulo-sphincters a l'origin d'arterioles glomérulaires afférentes chez cetain mammiferes. J. Urol. Med. Chir. (Paris) 57, 472-479.

Picard D. and Chambost N. (1952). Sur les artérioles aférentes des glomérules juctamédullaires, nature des dispositifs de régulation situés à leur origine. C. R. Soc. Biol. 146, 581-582.

Reale E. and Luciano L. (1966). Elektronenmikroskopische Beobachtungen an Stellen der Astabgabe der Arterien. Angiologica 3, 226-239.

Rosen W.C. (1967). the morphology of valves in cerebral arteries of the rat. Anat. Rec. 157, 481-488.

Shanklin W. M. and Azzam N.A. (1963). On the presence of valves in the rat cerebral arteries. Anat. Rec. 146, 145-148.

Taggart N.E. and Rapp J.P. (1969). The distribution of valves in rat kidney arteries. Anat. Rec. 165, 37-40.

Takayanagi T., Rennels M. L. and Nelson E. (1872). An electron microscopic study of intimal cushions in intracranial arteries of the cat. Am. J. Anat. 133, 415-430.

Velican C. and Velican D. (1977). Studies on human coronary arteries. I. Branch pads or cushions. Acta anat. 99, 377-382.

Wagenvoort C.A. (1954). Die Bedeutung der Arterienwülste für den Blutkreislauf. Acta anat. 21, 70-99.

Yohro T. and Burnstock G. (1973). Fine structure of "intimal cushions" at branching sites in coronary arteries of vertebrates. A scanning and transmission electron microscopic study. Z. Anat. Entwickl.-Gesch. 140, 187-202.

Accepted November 12, 1986