ured and integrated with the densitometer Vitatron TLD-100. A standard curve was obtained by densitometric measurement of samples containing 10–40 µg of rat prolactin (NIAMD – rat prolactin RP-1; 11 IU/mg)[13].

Results and discussion. The milk spots were completely suppressed by VUFB-6683 dosed 10 and 4 mg/kg daily, and reduced by 82% at the dosage of 1 mg/kg daily. The adenohypophysial prolactin concentrations and weights are given in the Table. It is evident that after VUFB-6683 the adenohypophysial prolactin concentration sank and the adenohypophysial weight decreased. In view of these findings, an inhibitory effect of the preparation on the prolactin synthesis in adenohypophysis can be assumed to exist.

Zusammenfassung. Mit Hilfe von D-6-Methyl-8-ergolin-I-ylacetamid-Tartarat, VUFB-6683 wurde bei säugenden Ratten das Gewicht und der Prolaktinspiegel der Adenohypophyse herabgesetzt und die Laktation gehemmt.

P. Krejčí, M. Aušková, K. Režábek, J. Bílek and M. Semonský

Laboratory of Animal Physiology and Genetics of the Czechoslovak Academy of Sciences, Uhrineves near Praha, and Institute for Pharmacy and Biochemistry, Praha (Czechoslovakia), 15 March 1973.

[13] Rat prolactin RP-1 was kindly supplied by the National Institute of Arthritis, Metabolism and Digestive Diseases, Bethesda, USA.

[14] The authors are indebted to Dr. I. J. Skála, Dr. J. Hondlík, Mrs. V. Mašitová and Mrs. J. Emanuelová for technical assistance; and to Dr. I. Mikuláš for his aid in designing the study.

A New Type of 'Node' in the Myelin Sheath of an Invertebrate Nerve Fibre

The mechanisms of impulse conduction in invertebrate nerve fibres with myelin-like sheaths and their ultrastructural correlates have not yet been cleared up. In 2 cases[1,2], nodes resembling the vertebrate nodes of Ranvier have been described in crustacean nerve fibres, but their function was not analyzed. On the other hand, certain myelinated giant fibers of shrimps, for which saltatory conduction has been claimed, lack Ranvier-like nodes. The myelin sheath is interrupted only by the emerging collaterals and these sites act probably as excitable nodes[3,4]:

In the ventral cord of earthworms, only the 3 dorsal giant fibres have myelin-like sheaths, which are also interrupted by regularly spaced collaterals on their ventral side[5]. This communication describes openings in the dorsal side of the myelin sheath of the median giant fibre of the earthworm, which have been overlooked until now.

Materials and methods. The observations were made on adult specimens of *Lumbricus terrestris*. Thin sections for light microscopy (0.5 to 1 µm thick) were obtained from Araldite-embedded ventral cords after fixation with 1% glutaraldehyde followed by 2% osmium tetroxide, both in 0.1 m phosphate or cacodylate buffer and in the cold (pH 7.4). The sections were post-stained with toluidine blue.

Results. The median giant fibre of the earthworm displays 3 ventral collaterals in all segments of the cord[5]. However, examining Araldite-embedded whole mounts of the ventral cord at low magnification, 5 regularly spaced translucent spots can be discerned in its opaque myelin sheath. After cutting off the ventral part of the cord including the ventral half of the giant fibres – and therefore the 3 ventral openings of the median giant – 2 openings are still visible in the dorsal midline of the myelin sheath of the median giant fibre. No such openings could be distinguished in the lateral giant fibres (Figure 2). These dorsal openings will be henceforth called nodes. The position of the nodes is fairly constant in all regions of the cord and in all animals examined (Figure 1). They alternate with the 2 nerve bundles which leave each segment of the cord, the side nerves 1 and the paired side nerves 2 and 3. The first node is located about midway between the side nerves 1 and 2/3, some 50 to 150 µm anterior to the median giant cell[5]. The second node is found at the end of the segment slightly closer to the preceeding side nerves 2/3 than to the side nerve 1, and at about the same level as the synchronizing bridge between the lateral giant fibres[5] (Figure 1).

The distance between consecutive nodes varies between 0.5 and 0.7 mm, depending upon the length of the corresponding segment. Generally, the internodal distance within the segment appears to be somewhat smaller than the internodal distance between consecutives segments. The nodes are of approximately circular shape and about 10 to 15 µm in diameter. The myelin lamellae are arranged around the node to envelope the protruding axoplasm concentrically (Figure 3), covering it until it reaches the fibrous capsule above the giant fibre, thus forming a funnel-like structure (Figure 4).

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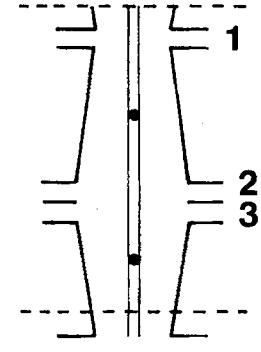
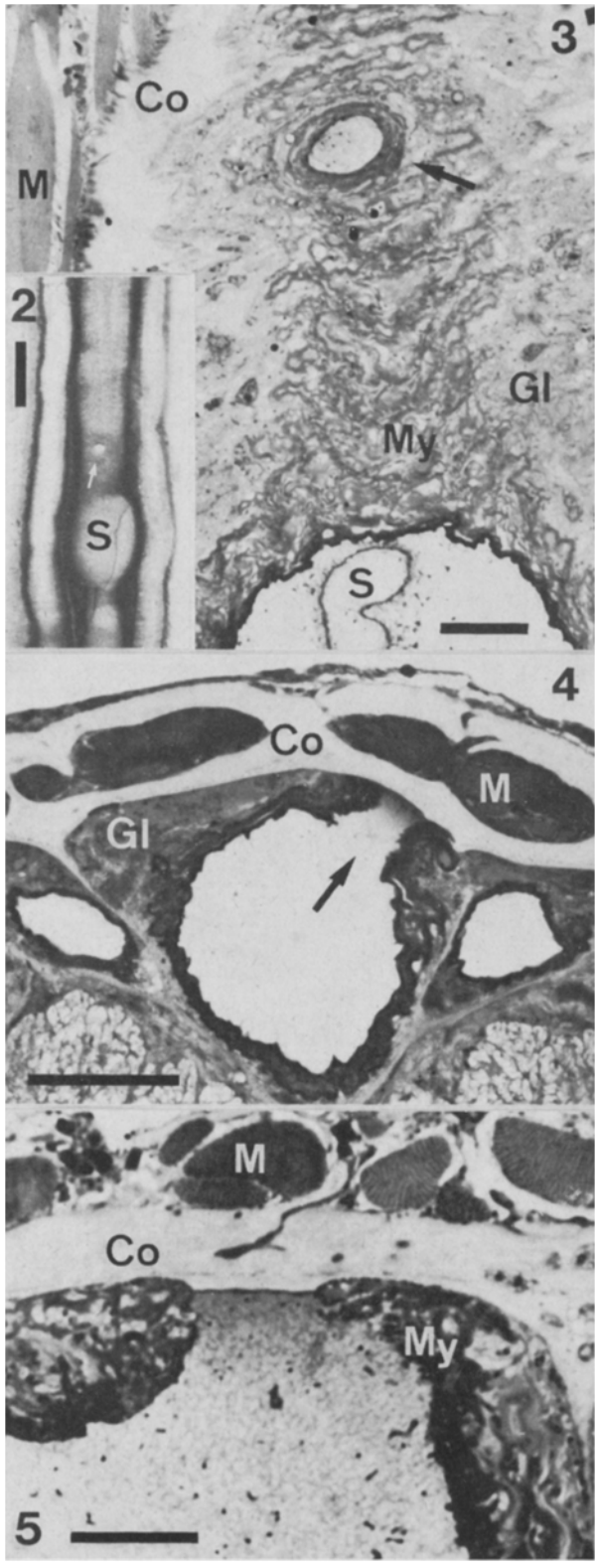


Fig. 1. Schematic drawing of a ventral cord segment showing the dosition of the dorsal nodes of the median giant fibre. Interrupted lines indicate the segmental borders of the body wall.



At the end of the axoplasm extension, the axolemma (axon membrane) of the median giant fibre faces the fibrous capsule directly, without separation by other cellular elements. This has been confirmed by preliminary electron microscopical observations. Contacts with the muscular strands which are present in the fibrous capsule were never observed. A regular peculiar cytological feature of the nodes is the enhanced density (osmiophilia) of the axoplasm, which increases towards the top of the axoplasm protrusion.

Discussion. In addition to Ranvier-like nodes and circular openings of branching collaterals, the present communication describes a new third type of 'node' in a myelinated invertebrate axon. The dorsal nodes of the median giant fibre resemble the ventral collateral openings as to shape and dimensions, but, unlike them, they are not involved in the synaptic relations of the fibre.

The extracellular fibrous capsule (collagen), which envelopes the central nervous system of the earthworm and other invertebrates has been shown to be highly permeable to ions and even larger dye molecules[6–8]. Thus it is likely to provide a low electrical resistance pathway between consecutive dorsal nodes. This should be more efficient than longitudinal current pathways between the ventral collaterals, for which only small extracellular spacings between glial and nervous elements and narrow inward extensions of the fibrous capsule are disposable[9,10]. These implications strongly suggest some specific function of the dorsal nodes in the impulse conduction of the median giant fibre, which perhaps occurs in a saltatory manner. Indeed, preliminary electrophysiological experiments recording the spike amplitude of the median giant fibre extracellularly with fine suction electrodes show 2 distinct maxima at the sites of the dorsal nodes, thus indicating an increased current density through these openings.

The peculiar density of the median giant axoplasm in the dorsal nodes recalls recent electron microscopical observations on the special osmiophilia of cellular membranes in giant fibres of crustaceans[11] and earthworms[12]. This finding has been correlated with some

Fig. 2. Low power view from ventral of the whole araldite-mounted dorsal giant fibres. The ventral part of the fibres with the ventral openings has been cut off. In the median giant fibre, the first dorsal node of the ganglion can be distinguished (arrow) ahead of the septal region (S). Segment 107, scale 100 µm.

Fig. 3. Frontal section through the dorsal part of the median giant fibre. Arrow first dorsal node with concentrically arranged myelin lamellae; S, septal membranes in the median giant fiber; My, myelin sheath of the median giant fibre. Gl, glial tissue; Co, fibrous capsule and M, muscles of the cord envelope. Segment 108, scale 20 µm.

Fig. 4. Transversal section through the giant fibres at the level of the second node (arrow) in segment 14. Gl, glial tissue; Co, fibrous capsule; M, muscles of the cord envelope. Scale 40 µm.

Fig. 5. Transversal section through the second dorsal node in segment 55, in higher magnification. My, myelin sheath of the dorsal node; Co, fibrous capsule and M, muscular elements of the envelope. Scale 10 µm.

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special biochemical reaction of the membranes due to increased current density during spike propagation in these axons[11], thus corroborating the interpretation of the physiological role of the nodes given above. Further electrophysiological and electron microscopical work is in progress to elucidate these points[13].

Zusammenfassung. Es werden neuartige, segmental angeordnete Öffnungen in der Myelinscheide der medianen Riesenfaser des Regenwurms *Lumbricus terrestris* beschrieben, durch welche die Axonmembran direkt an die extrazelluläre Kollagenhülle des Bauchmarkperineuriums heranreicht. Dieser Befund wird in Hinblick auf den Mechanismus der Erregungsleitung myelinisierter Nervenfasern von Wirbellosen diskutiert.

J. GÜNTHER

I. Zoologisches Institut der Universität, Abteilung für Zellbiologie, Berliner Strasse 28, D–34 Göttingen (Germany), 2 May 1973.

Identification of Hemocyanin in the Cyanocytes of *Carcinus maenas*

Cyanoblast and cyanocyte are the names given by FAHRENBACH[1] to the cells which synthesize hemocyanin (Hcy) in *Limulus polyphemus*. These cells have been observed, together with granulated hemocytes, in the circulatory sinusoids of the compound eye and, according to this author, they probably originate in the digestive gland. Mature cyanocytes show crystalline bodies within the cytoplasm which have been identified as Hcy on the basis of the dimensional congruence of the crystalline lattice with the size of the Hcy molecule of the same animal.

Hcy is the respiratory pigment of 2 phyla of invertebrates: Mollusca and Arthropoda, and the molecular weight of the main component present in the blood is typical not only of each phylum but also of the classes, orders and suborders of both phyla[2,3]. The Hcy of *Limulus* differs from that of other arthropod species in its high sedimentation constant: 60 S against 24 S and 16 S in Crustacea and 34 S in other Arachnomorpha[4]. Except for the observations by DILLY and MESSENGER[5] on *Octopus vulgaris* and by FAHRENBACH on *Limulus polyphemus*, both based on morphological evidence only, the problem of the biosynthesis of Hcy in Mollusca and Arthropoda is still a matter of speculation.

With the purpose of demonstrating unequivocally the endocellular presence of Hcy, the hemopoietic cells of *Carcinus maenas* have been identified and characterized by electron microscopy and the protein which is synthesized in the cyanoblast has been identified as Hcy by immunofluorescence.

Material and methods. The animals were obtained from the Hydrobiology Station of Chioggia (Venice) and kept in the aquarium until used. The dorsal carapace was removed and the tissues were hardened in situ by short prefixation (30–40 min).

For the optical microscopy, Bouin, formaldehyde or glutaraldehyde at different concentrations were employed. The sections were coloured with hematoxylin-eosin or PAS and with rubeanic acid for the detection of copper. For electron microscopy, the best fixation was obtained by using 3 or 4% gluteraldehyde in sea water and 0.05 *M* cacodylate buffer pH 7.0 (1:1 vol.) plus 0.001 *M* $CaCl_2$ and 0.025 *M* sucrose. The tissues, previously treated in situ with this solution, were removed and kept for 1 h in fresh fixative, then washed 3 times (10 min each) with the cacodylate buffer and post-fixed with 1% osmium tetroxide in 0.1 *M* cacodylate buffer pH 7.2 for 60 min. The material was dehydrated and included in DER (Dow Epoxy Resin, Carlson Co.)[6]. Fine (500 Å ca.) and 1 μm thick sections were prepared with the LKB Ultratome III. The thick sections were coloured with toluidine blue and the thin ones with uranyl acetate (saturated solution in 50° ethanol) for 25–30 min and lead citrate for 3–4 min[7]. The observations were made using a Phylips Electron Microscope Mod. 300.

Hcy was purified from the hemolymph of *Carcinus* by dialysis and ultracentrifugation according to GHIRETTI-MAGALDI et al.[8]. The rabbit Hcy antibody was prepared by injecting 5 ml saline containing 10 mg of protein once

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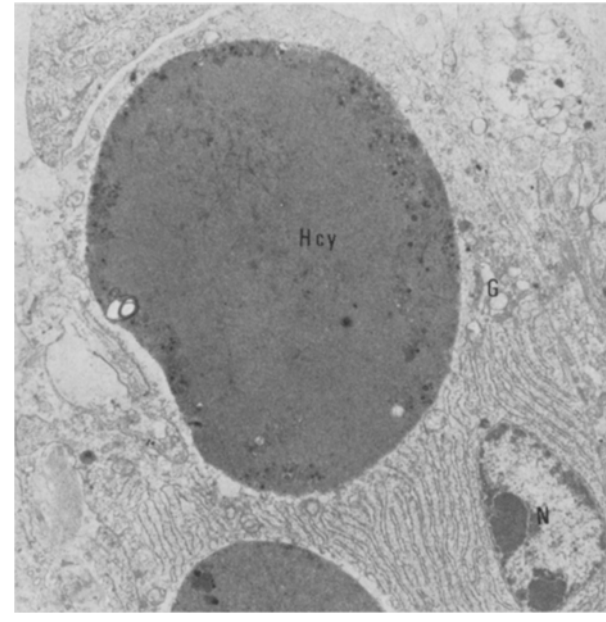

Fig. 1. Electron micrograph of a cyanocyte in the reticular connective tissue of the hepatopancreas of *Carcinus maenas* showing a large inclusion of proteic material (Hcy), the Golgi system (G) and a peripheric nucleus (N). 3% glutaraldehyde. ×9,800.