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Comparative ultrastructural and cytochemical analysis of the cephalopod systemic heart and its innervation

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Summary. The present knowledge of the morphology of cephalopod central hearts is presented. The cytological characteristics of the epicardial, myocardial and endothelial tissue layers are reviewed. Myocardial cells are characterized as obliquely striated myocytes with a high level of oxidative metabolism. The voluminous myocardium is intensively penetrated by nerve fibers controlling the myogenic heart rhythm by different chemical transmitter systems. Catecholaminergic fluorophores and acetylcholinesterase activity could be localized by means of histochemical and cytochemical investigations. A glio-interstitial cell system is shown to be present in connection with nerve fibers and also uncombined between heart muscle cells. Its content of large different-sized inclusions is described and their function discussed.
Key words. Cephalopods; central heart; ultrastructure; innervation; glial cells.

Introduction

The high physiological efficiency of the cephalopod cardiovascular system is well known, and it has been described in detail in other articles in this volume. But only a little has been reported about the morphological and cytological features underlying the rhythmic contraction and permanent blood flow and its structural organization. The main moving force of blood circulation is produced by the central heart supported by other contractile organs. Therefore, we intend to give a summary of earlier morphological studies regarding the central heart of cephalopods, and supplement these results by describing new morphological and histochemical findings.

1. Light-microscopical aspects

The cephalopod central heart represents a muscular hollow organ receiving blood fluid from extended branchial veins and auricles. It supplies the peripheral circulation system during the contraction phase via the aorta cephalica, the aorta posterior, and the genital artery, which can be secondarily reduced in some species. In the taxonomical order of Decapoda (Sepioidea and Teuthoidea) the organ lies in the spacious viscero-pericardial-coelomic cavity[57], covered by the coelomic epithelium (epicardium)[41,42].

In contrast, the octopod coelomic system is heavily reduced, existing only in a rudimentary way as the so-called 'Wassergefäßsystem' and a pericardium which has no connection to the coelomic cavity[16,21]. The *Nautilus* heart is also surrounded by a pericardial layer (1–13 µm thick)[20]. Only in the region of the heart septum does the epithelium lift up and cover the connective tissue ligamentum. The epicardial and myocardial layers are separated by a 14-µm-thick connective tissue sheath[20]. Epicardial cells are highly prismatic or cubical and have a round-shaped nucleus.

The greater part of the cephalopod heart consists of the muscular part, the myocardium. Also in *Sepia* the epicardium rests upon a lamina basalis, connected through a thin layer of collagenous fibers to the myocardium[35]. A peripheral blood sinus is connected to the renal appendages in the region of the heart mesentery which fixes the

organ in the coelom. These appendages, as excretory appendages of the vena cava, also absorb the venous blood of this marginal sinus[51] (fig. 1).
In the octopod heart, however, the blood fluid that has flowed through the spongy myocardium from the lumen collects in peripheral veins within the comparatively thick subepicardial connective tissue layer and then proceeds back to the central circulatory system through paired 'venae cordis'[16] (fig. 2).
A striking feature in octopods is the subepicardially located bundles of muscle fibers which are not a component of the myocardium and have a divergent enzyme pattern. Whereas the mitochondrial marker enzyme succinate dehydrogenase (E.C. 1.3.99.1.) shows a high level of activity in the myocardial musculature, the peripheral muscle bundles only show slight reaction (fig. 3). Illustrations of the monoaminoxidase (E.C. 1.4.3.4.) reveal similar results. The energy availability in these primarily tonic fibers obviously deviates from metabolism of the inner myocardial fibers.
Such peripheric scattered muscle cells were also found subepicardially in the heart of *Rossia macrosoma*. The myocardial cells of cephalopods, measuring approximately 10 µm in diameter generally branch several times, and in the light microscope picture a clear oblique striation can be seen[5,48]. In *Sepia esculenta*[24] and *Nautilus macromphalus*[20] they appear more irregular; in *Sepia officinalis*[54], *Rossia macrosoma*[23] and, to a high degree, in *Eledone moschata*[32,35] and *Octopus vulgaris*[63], well ordered circular and longitudinal layers can be distinguished, which are connected through diagonal myocytes. Towards the lumen, the musculature loosens up increasingly, forming a spongy trabecular network (fig. 4).
A special feature of the octopod heart is the almost completely muscular heart septum, which divides the ventricle into two equally large chambers. These communicate through an eccentrically situated opening. This dividing wall is a relic of the embryogenesis and shows clearly the original paired rudiments of the heart[6,16,34,35,40]. An additional indication for the original bilateral symmetry of the central circulatory system emerges from the development of the aorta cephalica in *Loligo*[40], also from the fact that all dibranchiates have an aorta cephalica on the right side, whereas *Nautilus* has this vessel on the left side[15,17,39,42].
An incomplete endocardium consisting of flattened endothelial cells forms the luminal termination of the heart muscle and they are adjacent to the myocytes with a PAS-positive lamina basalis[23,54].

2. How are cardiac nerves distributed over the myocardium?

In anatomical studies[2,46] it became clear that differences can be seen in the innervation of the central heart in octopods and sepioids; the two groups have a common feature in the nervous supply of the central heart. The origin of cardiac nerve fibers lies in the visceral nerves and thus in the pallio-visceral-ganglion of the 'central nervous system'. In *Sepia officinalis* the cardiac nerve originates in the commissura visceralis where fibers of the visceral nerves and nerve paths out of the paired ganglia cardiaca, come together and reach the ventricle, in the region of the heart septum as thick cords. They penetrate the myocardium as a compact cord (fig. 5) and only branch out in the inner loose trabecular muscle layer; from there they reach all parts of the ventricle[2,28]. Consequently the branches of the N. cardiacus which are of greater caliber can be found more luminally, whereas smaller branches appear in the compact myocardial layer. Branches of the nervus cardiacus do not extend to the auricle. Their innervation is effected by separate fibers of the ganglion cardiacum.
In contrast, the octopods have a connection through a commissure, from which the individual fibers innervate the ventricle, the auricles and the lateral venae cavae[55,61]. Due to these anatomical peculiarities, greater nerve cords are more likely to be found on the peripheric heart surface in octopods, whereas the luminal myocardium only contains fine terminal fibers. Histochemical demonstrations of the acetylcholinesterase[29] as well as fluorescence microscopical localizations of biogenic amines[32] coincide with this distribution of nerve bundles in *Octopus* heart. The nervus cardiacus of *Sepia officinalis* contains fibers with and without intraaxonal AChE-activity in direct proximity (fig. 6). However, fluorophores of the catecholamine-type, but not of the serotonin-type could be demonstrated with cytophotometrical analysis[31] (fig. 7).
Thus two antagonistically working transmitter systems have to be considered as components of the cardiac nerve. The participation of further transmitter types, especially of a peptidergic nature, cannot be excluded, and results already obtained for the neurosecretory system of the cephalic veins of coleoids suggest that it is probable.

3. Subcellular structure of heart muscle cells

In electron microscopic pictures the peripheral terminal tissue, the epicardium, is seen as a polar single row epithelium. The apical part towards the coelomic cavity is formed by a thick brush-border of microvilli, which is broken in parts by protrusions of cells, indicating exocytotic transport processes in the pericardial coelom (fig. 8). The *Nautilus* heart has a particular feature; its epicardial cells have fewer microvilli and are even, to some degree, free from them. The seam of microvilli contains rod-like structures (fig. 9), which were identified as commensal flagellate bacteria which are also found on the brush border of the pericardial appendages[53] but, however, not on the renal sacs of *Nautilus*[52]. Their possible function for the pericardial fluid has not yet been clarified; it may possibly be a question of commensals analogous to the dicyemides of the coleoid renal appendages.
Further characteristics of the epicardial cells are, in accordance with their high SDH activity (fig. 3), that they are well supplied with mitochondria, vesicular structures and vacuoles, and lateral cell interdigitations as well as deep basal infoldings. The epithelial cells rest like stilts on the lamina basalis and are interspersed in the basal part with electron-light cells, which are distinguished especially by the content of osmiophilic granules (∅ circa 250 nm) and may represent basal substitute cells. All cytomorphological features of the epicardial cells point to a high metabolic activity, although no hypotheses concern-

ing the manner and function of this transcellular transport are available. The lamina basalis is to be divided into a compact and a fibrous part whose filamentous substructure becomes clear through cytochemical demonstration of cholinesterase[50].

The connective tissue following the epicardium only appears in *Rossia macrosoma*[23] and, to a special degree, it is observed as a separate layer in octopods; especially fibrocytes, blood vessels, the above-mentioned scattered muscle fibers, and nerve fibers of large caliber whose fine structure has not yet been examined in detail, are found embedded in an amorphous base matrix.

In sepioids the connective tissue is less prominent. The myocytes of the myocardium are horizontal, have an oval cross-section and, depending on their phase of contraction, can produce of numerous lateral and terminal extensions (fig. 8). Their dimensions vary (maximal length measured: 140 µm)[54]. Each muscle cell has only one nucleus situated in the middle range; the cells form a multicellular network, but not a syncytium[23]. The predominant intracellular component is the myofibrils, which mostly run parallel in several packets but can also be arranged at angles or right angles to each other or in a network of lateral branches. A longitudinal section reveals that the myofibrils have a regular striation, consisting of a series of contrasting 'dense patches' and marking the Z-line. Kawaguti[24] calls them J-granules. The 'dense patches' do not lie vertical to the direction of the fiber, but are arranged more or less obliquely. The sarcomeres thus formed are 1.3–3.9 µm long depending on their state of contraction; their most striking component is a thick myosin filament of 220–400 Å, surrounded by less easily recognizable actin filaments of about 50–85 Å in diameter which are embedded in the Z-patches[23, 24, 54]. These Z-patches are connected laterally and terminally with the infolded sarcolemma through α-actin fibers of the marked cytoskeleton and can directly extend into the adjacent cell (fig. 11). In this way an overlapping intercellular framework is formed. Predominant terminal links of adjacent myocytes attract our attention due to special subsarcolemmal condensations with fibrillar intercellular junctions[23]. They are to be viewed as analogous to the disci intercalares of the vertebrate myocardium and form mechanical contact areas. At the level of the Z-patches the sarcolemma projects into the myocyte like a finger and extends right into the muscle cell as a fine extracellular canal system (T-system), which can be marked by tracer experiments (fig. 10). When the pictures are enlarged, narrow topical connections to the sarcotubular systems are evident, which resemble the diads or triads of the vertebrate muscle, and would be expected to have an analogous function in electro-mechanical coupling (fig. 14). The S.R. can be recognized both as a canal system running alongside the myofibrils with dilations like bubbles in the area of the Z-patches and also in the form of subsarcolemmal extensions in the area free of fibrils. The intracellular arrangement of myofibrils, the special development of Z-patches and the resulting oblique marking constitute a marked contrast to the cyto-morphology of the gastropod and bivalve hearts on the one hand and to the somatic musculature of the cephalopods on the other hand.

The somatic musculature of the cephalopods distinguishes itself by its mononucleate fusiform shape. In this cell type the myofibrils are located exclusively in the cell periphery while the central cell body contains the nucleus and sarcosome packets. The myofilaments form rhomboid sarcomeres. This results in a helicoidal arrangement of the 'Z-accumulations' and a spiroid system of T-tubules[13]. Therefore they were termed 'pseudo striated musculature', 'helicoidal striated musculature' or 'obliquely striated musculature'.

As the designation 'cross-striated musculature' should be reserved for the highly organized type of muscle of the vertebrates, and as the level of organization of cephalopod myocytes described above is different from the construction of the somatic muscle cells, we recommend the designation 'obliquely striated musculature' for the cephalopod heart muscle type. In contrast the somatic muscle cell type should be termed 'helicoidal striated musculature'[13]. This type last mentioned also covers the muscle cells of the esophagus of *Sepia officinalis*[3].

The heart muscle cells of gastropods should be included in the 'obliquely striated muscle type', even though their characteristics are less pronounced than in cephalopods. The actin filaments insert dotted Z-patches here too, but form sarcomeres which have a irregular wave-like arrangement[25 44, 45]. The heart muscle cells of the bivalves, however, resemble more the smooth muscle type of the vertebrates. Myofilaments are loosely distributed in the cell periphery, and are only parallel in the contraction phase, otherwise they run irregularly with diffuse 'dense bodies' as a point of attachment for the thin filaments[26, 27, 49, 60]. The points of attachment between myofilaments and the sarcolemma are 'attachment plaques', composed of dense bodies, connecting filaments, membrane condensations and the unit cell membrane[18].

Figure 1. Longitudinal section through the heart of *Sepia officinalis* in the region of the heart mesentery (HM) showing the connection of blood fluid collected from peripheral sinuses and the luminal blood spaces (arrows) of renal appendages (RA); visceropericardial cavity (VC) (× 50).

Figure 2. Cross section of the central heart of *Eledone moschata* demonstrating the peripheral tissue layers including the coelomic epithelium (epicardium, CE), the subepicardial muscle fibers (MF), a distinct layer of connective tissue (CT), and the peripheral myocardium (M). Arrows indicate a peripheral venous blood vessel communicating with intramural blood spaces (× 208).

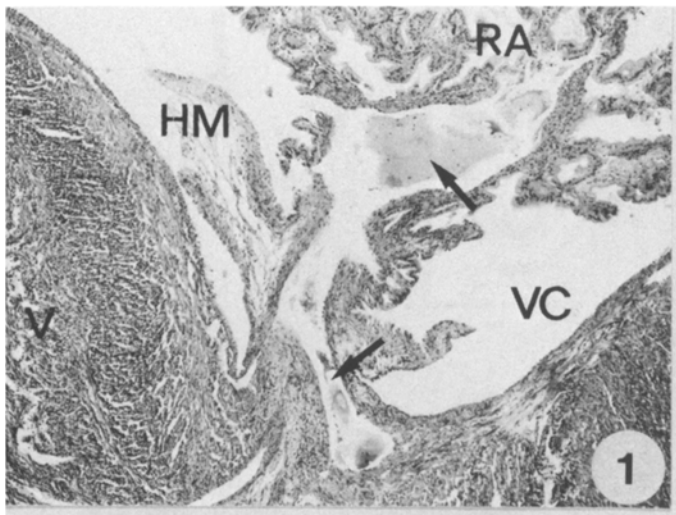
Figure 3. Cryostat section, comparable to figure 2, demonstrating succinate dehydrogenase activity (SDH) in the central heart of *Eledone cirrhosa* (× 100).

Figure 4. Inner surface of the heart of *Sepia officinalis* viewed by scanning electron microscope. Note the ramifications of the muscle trabecules (T) and the extensive intra-trabecular spaces (arrows) (× 36).

Figure 5. Light microscopical section of the central heart of *Sepia officinalis* showing cardiac nerve branches (NC) penetrating the myocardium (M) and ramifying within the inner trabecular muscle layer. Bodian's silver staining; heart lumen (LU) (× 68).

Figure 6. Cryostat section of the *Sepia* heart showing a cardiac nerve trunk (NC) after acetylcholinesterase reaction. Fibers with strong intraaxonal enzyme activity (arrows) are visible as well as unstained nerves (arrowheads) (× 157). Derived from Kling[31].

Figure 7. Longitudinal section of a cardiac nerve trunk (NC) in the heart of *Sepia officinalis* with glyoxylic acid induced fluorophores (arrows) and fibers without reaction products (arrowheads) (× 1560). Derived from Kling[31].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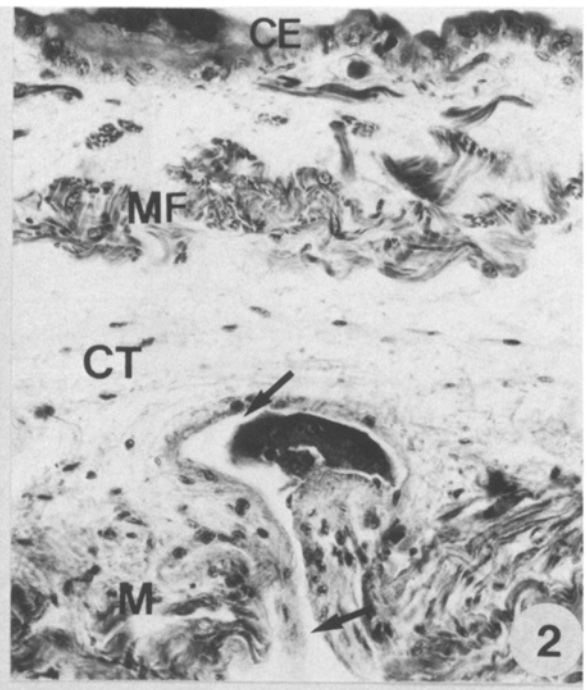
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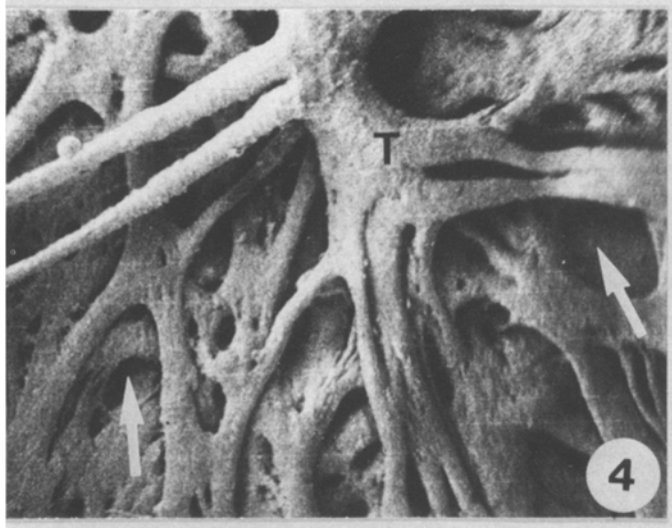
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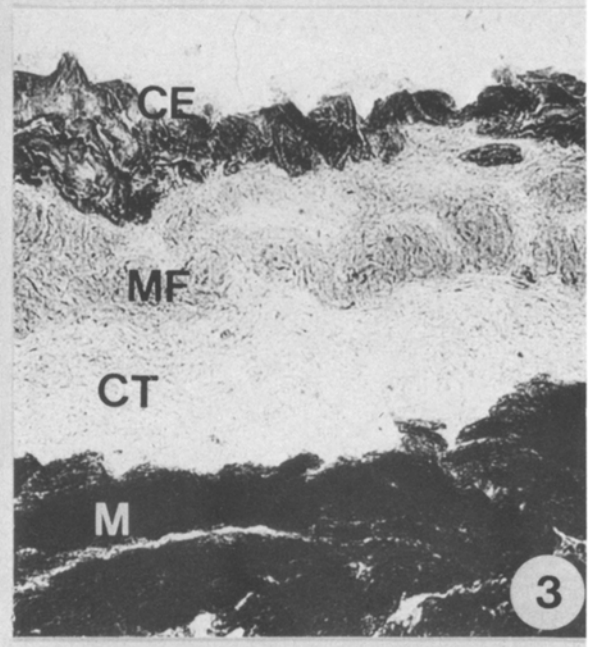
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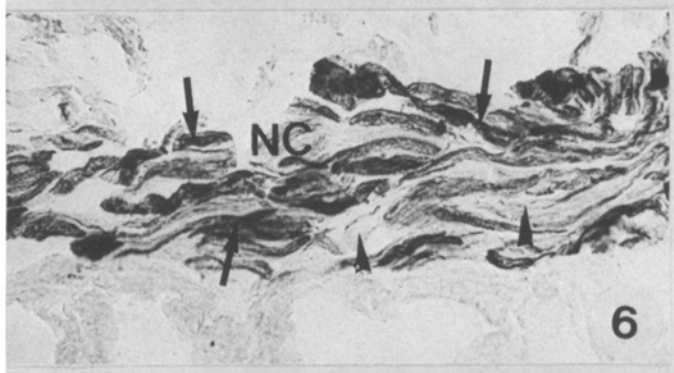
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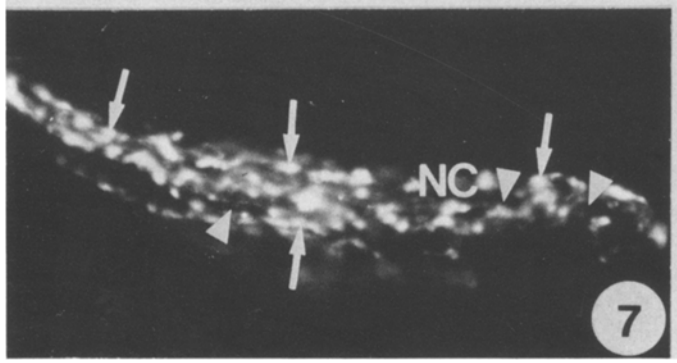
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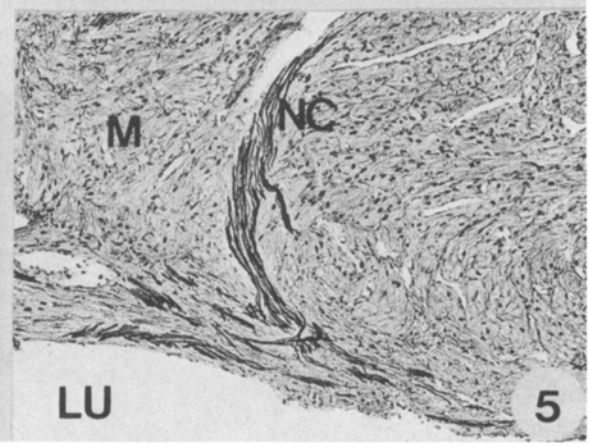
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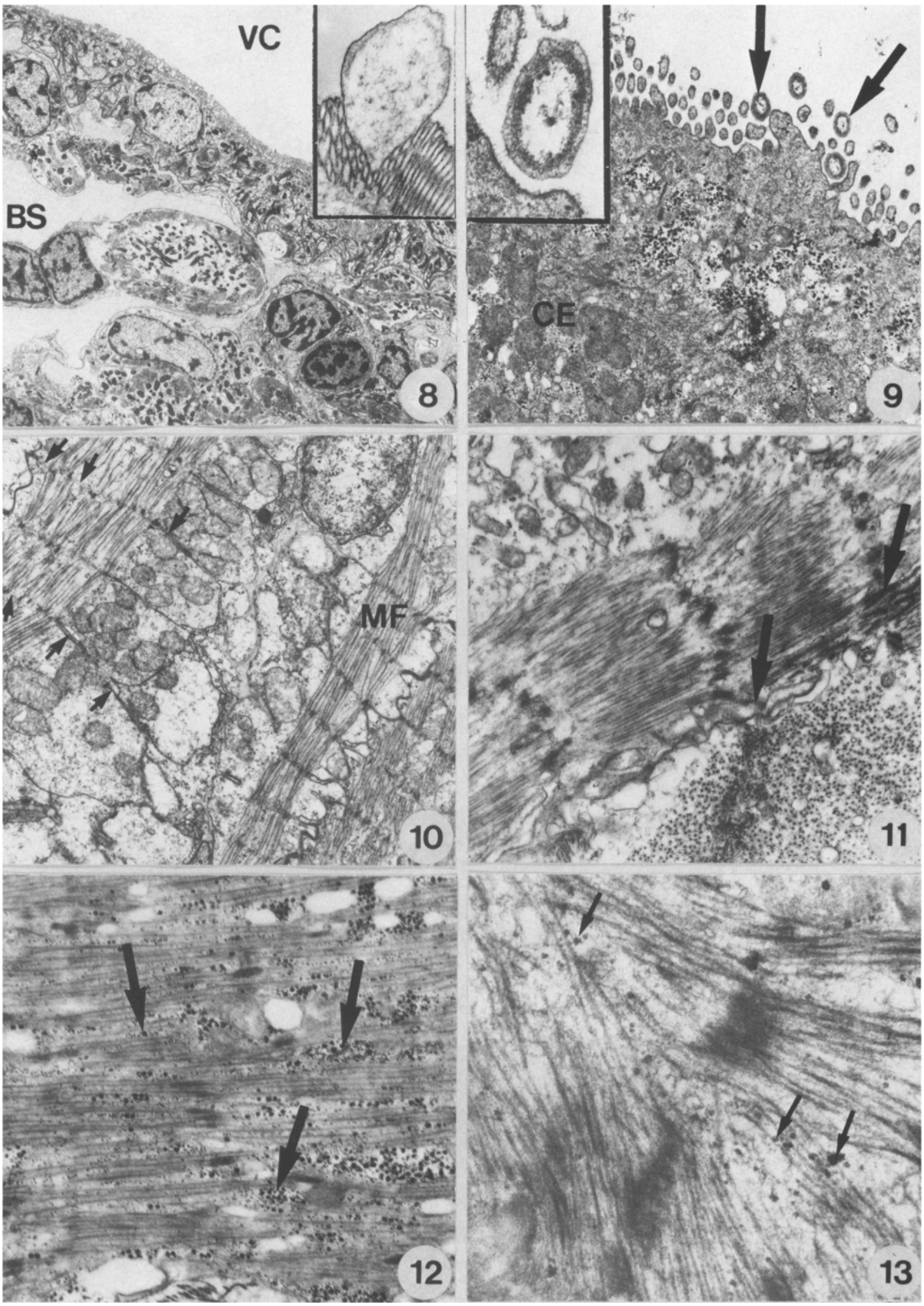

Figure 8. Electron micrograph of the heart periphery of a 20-day-old specimen of *Sepia officinalis* showing the epicardium with microvilli towards the visceropericardial coelomic cavity (VC) and peripheral blood spaces (BS) (× 2450). Inset: Magnification of a cell protrusion interpreted as apical secretion (× 11,950).

Figure 9. The epicardium of *Nautilus macromphalus*. Note the high content of mitochondria and the extracellular cross-sections of endosymbiontic/parasitic flagellates (arrows) (× 13,120). Inset: Magnification of a flagellate cross section (× 43,880).

Figure 10. Two neighboring muscle cells in the ventricular myocardium of *Octopus vulgaris* with myofibrills (MF) and lateral accumulations of mitochondria. The sarcolemma infolds finger-like into the muscle cell representing a t-system (arrows) (× 6850).

Figure 11. Electron micrograph of the ventricle of *Eledone cirrhosa* with two heart muscle cells running perpendicularly. The Z-patches (arrows) continue in the neighboring cell (× 11,620).

Figure 12. Longitudinal section of a muscle cell in the myocardium of *Nautilus macromphalus* demonstrating large amounts of glycogen besides myofilaments and vacuoles (× 13,975).

Figure 13. Myofilaments in heart muscle cells of *Nautilus macromphalus* with disseminated glycogen particles (arrows) (× 25,400).

The fact that the bivalve myocardial cells have less obvious characteristics has its physiological correlation in the irregular heart rhythm with differing levels of contraction of the heart in vivo[19] and in vitro[33, 62].

The mitochondria of the cristae-type are joined together in the myocardial cells of the cephalopods as serial packets of up to 40 single compartments and lie between the bundles of myofibrils. Single sarcosomes can also be found in the cell periphery (fig. 11). Both in the peripheral cytosol and also directly between the myofibrils, large amounts of glycogen particles are evident. The heart of *Nautilus* stands out due to a wealth of glycogen, from which one can deduce that the availability of chemical energy equivalents is effected predominantly from aerobic metabolism (figs 12 and 13). This could be documented in biochemical investigations. Differences from the vertebrate heart are that octopine instead of lactate is the oxidative end-product and the phosphagen is arginine phosphate. Additionally, *Nautilus* muscles seem to have vigorous amino acid metabolism, whereas fatty acid oxidation seems to play no important role in the myocyte energy metabolism[20]. This also applies to *Loligo*[4] and *Illex*[38] and is supported by the SDH-activity mentioned already.

Towards the lumen an incomplete endothelium forms the seal towards the blood fluid. It consists of thin-walled cells without particular features and rests on a very fine lamina basalis which is in direct contact with the blood fluid in the area of the intercellular gaps.

4. Ultrastructural indications for cardiac nerve function

Cross sections of the cardiac nerve show a poly-axonal nerve fiber with a collagenous peri- and epineurium and blood vessels (fig. 15). Neurons have the typical ultrastructure of nerve cells with neurotubuli, neurofilaments, scarce mitochondria, vacuoles and neurovesicles[30], as has also been described for giant axons of cephalopods[10, 56, 58]. Intraaxonal neurovesicles of different size and osmiophily can be observed in preterminal and terminal nerves of the *Sepia* heart[54] (figs 17–20).

Similar observations were made in the heart of *Eledone moschata*[29]. Typical synapses were rarely found, mostly the 'synapse par distance' with variable intersynaptical space occurred[22].

The above-mentioned high cholinesterase activity can be supported by electronmicroscopical results (fig. 22). Two types of nerves were distinguished; one type showed enzyme activity only along the axolemma, while in the other type the axoplasm was also marked by reaction products. Therefore, cholinergic neurons may be ascertained[31].

Two different axon-glia-relationships can be distinguished in the cardiac nerve of *Sepia*. In one type, *one* axon is surrounded and partly penetrated by *one* glial cell (fig. 15) while in the other, larger numbers of smaller axons are isolated together by a glial cell (fig. 16).

A characteristic of the more peripheral glial cells is the presence of very large (up to 1.3 µm) contrasted inclusions of different sizes; these electron-dense granules seem to be typical of peripheral glia, because they were

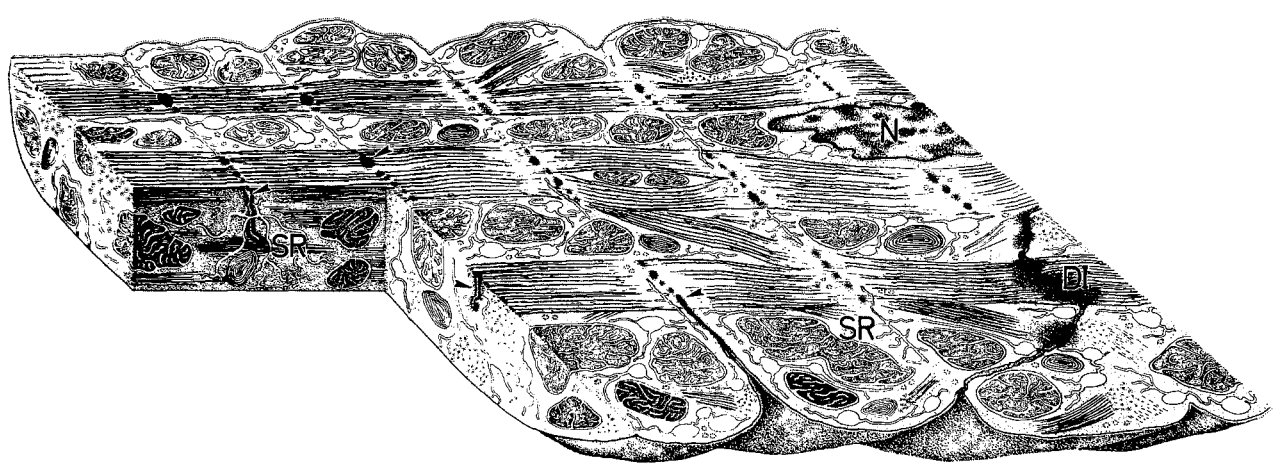

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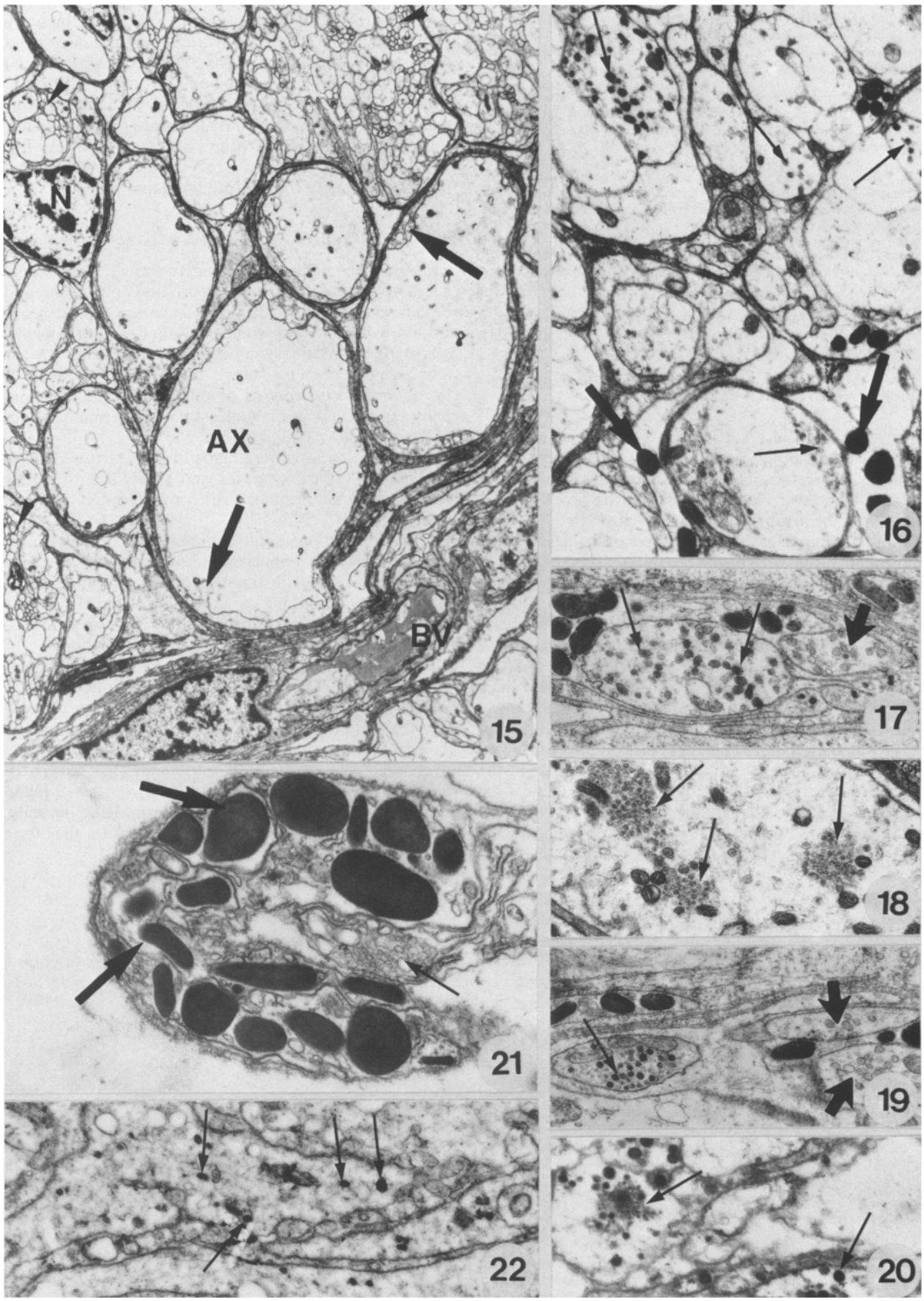
Figure 14. Semi-schematic diagram representing a myocardial muscle fiber of *Sepia officinalis*. Note the sarcomeres in contraction (left) and in dilatation (right). A t-system is formed by deep membrane invaginations (arrowheads). Nucleus (N), sarcoplasmatic reticulum (SR), intercalated disc (DI). Derived from Schipp and Schäfer[54].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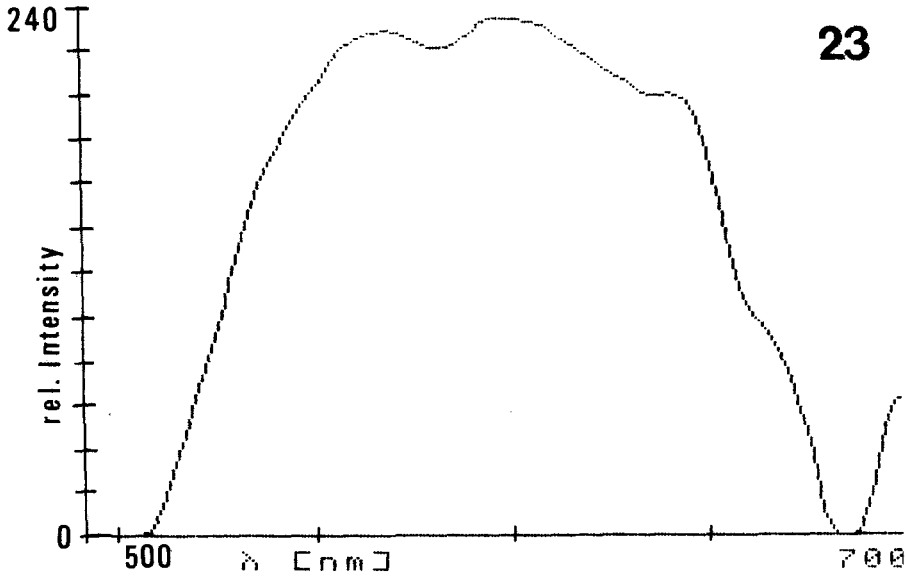

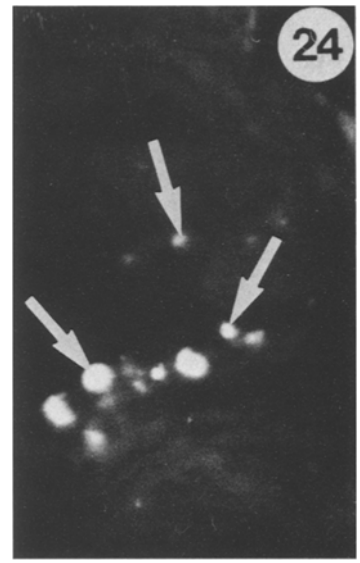

Figure 23. Cytophotometric evaluation of autofluorescent bodies within the *Sepia* myocardium. Emission curve shows three maxima (560, 600, 650 nm). Excitation wave length: 390–490 nm.

Figure 24. Micrograph of autofluorescent bodies (× 2250).

also demonstrated in the NSV-layer of the vena cava[37]. But these cells do not only occur in close juxtaposition to nerve fibers (fig. 21). They can also be distributed uncombined between muscle cells, so that we must speak of a glio-interstitial cell system as already described for other molluscs[43].

In the central nervous system of cephalopods only smaller electron-dense inclusions of about 150 Å were demonstrated in the 'protoplasmatic glial cells'[14]. Glial cells of the cephalopod 'CNS' show more intensive penetration of axons by cellular infoldings (trophospongium). They possibly have functions in electrical isolation and in the control of metabolism, because tracing with horseradish peroxidase via the blood fluid does not mark nerve cells; or the glial cells form a complete barrier to the tracer[1]. This endorses the opinion that glial cells regulate uptake and release of metabolites of neurons[45]. Besides this trophic function, a contribution to structure stabilization and cation storage has been discussed for molluscan glia[43]. Glial cells of the cephalopod 'CNS' accumulate 6-OH-dopamine but peripheral glia did not take up ^{3}H-serotonin or ^{3}H-dopamine[36]. In histochemical reactions no cholinesterases, monoaminoxidase or phosphatase were demonstrable[30], so that the content of the granules is quite unclear.

An uptake and inactivation of monoamines could be demonstrated for glial cells in *Helix*[7], and a neurosecretory function of granulated cells has also been presumed[11, 59, 65]. This contradicts the opinion of Fernandez[12] who supposes that the granular inclusions in the *Helix* heart are accumulations of lipofuscin as a metabolic endproduct of nerve cells. This interpretation is of special interest because histofluorometric investigation of the *Sepia* heart showed yellow autofluorescent granules. The emission curves measured after UV-excitation (390–490 nm) showed three maxima (560, 600 and 650 nm) (figs 23 and 24) indicating a mixture of substances or a complex organic molecule. A met-enkephaline-like substance could be demonstrated in granules of sheath-cells within the vena cava NSV-layer[37]. Therefore the question of the content and function of glial-interstitial cell granules must still remain open. Structural similarities to enterochromaffin cells and mast cells of vertebrates[8, 9, 47] could probably give further indications of nutritional and storage functions, besides those of electrical isolation and participation in immunological or endocrine processes. Summarizing we can conclude; in comparison to those of other molluscs, the cephalopod heart is a well-organized muscular chamber. It has obliquely arranged myofibrils. The specialized structure, with intensive nervous control of cardiac nerve fibers, permits a regular rhythmic myogenic contraction. So the heart rhythm can be adapted to actual physiological requirements. The reviewed ultrastructural organization of the central heart is one of the morphological preconditions for the especial physiological effectiveness of the cephalopod cardiovascular system.

Figure 15. Electron microscopical cross section of a polyaxonal cardiac nerve branch in the peripheral myocardium of *Sepia officinalis*. Some single axons (AX) are separated by one glial cell (arrows), others are embedded in groups and are in close contact with each other (arrow heads). Blood vessel (B), nucleus (N) of a glial cell (× 4658).

Figure 16. Polyaxonal nerve fibers with differently sized intraaxonal vesicles (small arrows) and large glial inclusions (large arrows) (× 16,380).

Figures 17–20. Various intraaxonal vesicles in cardiac nerves of *Sepia officinalis* (17–19) and *Nautilus macromphalus* (20). Fig. 17. Neuronal vesicles with (small arrows) and without (thick arrows) electron-dense content. Fig. 18. Dense cored vesicles associated with mitochondria and neurofilaments. Fig. 19. The presence of highly osmiophilic (small arrows) and very transparent vesicles (thick arrows) indicates at least two different transmitter systems. Fig. 20. Neurovesicles with variable diameter (arrows) (× 16,380).

Figure 21. Glio-interstitial cell of the peripheral part of the heart of *Eledone cirrhosa* with various sized intracellular dense bodies (arrows) and in close contact with a multivesicular cell process (small arrow) (× 17,160).

Figure 22. Intracellular demonstration of acetylcholinesterase activity (arrows) in cardiac nerve fibers of *Sepia officinalis* (× 16,380). Derived from Kling[31].

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Cephalopod myocardial receptors: Pharmacological studies on the isolated heart of *Sepia officinalis* (L.)

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Summary. This paper presents a synopsis of the information available about the pharmacological action of various substances on the cephalopod heart, with special emphasis on the central heart of *Sepia officinalis*.
Threshold concentrations, EC_{50} values and maximum effective concentrations have been experimentally determined. Studies with various transmitter substances, analogous compounds and antagonists have led to the following picture: Acetylcholine is the natural inhibitory transmitter substance; it acts via receptors with nicotinic properties which can be blocked by d-tubocurarine and α-bungarotoxin. The probable excitatory transmitter system is represented by a noradrenergic innervation. Noradrenaline has a positive inotropic and a positive chronotropic action on in vitro heart preparations. A positive inotropic response can also be evoked by serotonin (5-HT); this effect is not due to stimulation of the catecholamine receptor, as is shown by cross-over experiments with specific blocking agents. Furthermore, a peptidergic receptor system has been described which reacts with the 'molluscan cardioactive peptide' FMRF amide most effectively. It is assumed that cardioactive peptides may reach the central heart in the circulating blood; the sites of synthesis and release are still unknown. Possibly the NSV-layer of the vena cava is involved in hormonal cardiovascular regulation processes.
Key words. Cephalopods; central heart; pharmacology; receptors; acetylcholine; catecholamines; indolalkylamines; peptides.

General remarks

Pharmacologically active substances whose mode of action is known are often used as tools to aid research into physiological principles that are still unknown. For investigation of receptor-mediated processes it is important not only to test the effects of one drug concentration, but also to record the whole dose range from the threshold concentration up to the maximum effect, because unphysiological concentrations may cause atypical effects, depending on the sensitivity of the investigated organ preparation.
Only by means of dose-dependent effects, demonstrated by a sigmoid dose-response-curve (DRC) or concentration-response-curve (CRC) and supported by special inhibition experiments with antagonists, can a receptor stimulation be suggested.
Isolated myogenic hearts are very suitable for investigations using pharmacological methods, owing to their rhythmic activity. This provides a simple method of measurement for control and evaluation. Concentrations of active substances, and amplitude and frequency values can be recorded without much trouble.
Studies with isolated organs have various advantages compared with those on intact animals. For example, the reaction of one muscle type to the drug can be measured (this applies especially to strip preparations) without the interference of nervous regulation and counterbalance. The receptor equipment and sensitivity of an isolated muscle can thus be well established. These preparations are easily accessible for direct measurements. Furthermore, animal movements, which would otherwise the troublesome, are also excluded. The disadvantage of the in vitro technique is that such experiments only produce results for a model preparation; the conclusions cannot be transferred directly and uncritically to the intact animal, and do not represent the normal physiological relationships.
Depending on the formulation of the question, scientists must decide on the one or the other method. The two methodological possibilities do not compete, but rather complement one another in the solution of special scientific questions.
In in vitro studies on cephalopod hearts the reflow system with a Straub cannula has been most frequently used[57, 59]. This system, which was also usually applied in our investigations, has some disadvantages in comparison with continuous perfusion techniques. The flow direction of artificial blood fluid does not represent the physiological circumstances; furthermore, incubation fluid is not renewed continuously, and especially when using inhibitory substances, mixing with the incubation fluid may be inadequate[57]. On the other hand, an exact definition of pre- and after-load pressure and a better control of temperature is possible when using the perfusion technique. In spite of the above-mentioned qualitative differences,