The role of the branchial heart complex in circulation of coleoid cephalopods

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Introduction

It was mentioned in the introductory paper that the circulatory system of coleoids is particularly efficient compared with that of nautiloids and other invertebrates. This efficiency is enhanced by the formation of auxiliary hearts. These organs are developed ontogenetically and phylogenetically from veins[12, 51, 67] and thus can be considered a part of the venous system even in adult coleoids. Apart from their primary function of overcoming the peripheral resistance of the branchial blood vessels and capillaries, their pulsatile activity is also the precondition for the ultrafiltration processes involved in the formation of primary urine, which take place in the branchial heart appendages (pericardial gland)[21, 43, 55, 64]. Furthermore, it can be assumed that a storage function of functionally important substances (eg. Fe-proteids) and catabolic excretes can also be attributed to these organs, and their significance for an immunological defense mechanism is also under discussion[48, 62, 63, 77].

Structure of the heart – morphology and function

Branchial hearts of coleoid cephalopods are paired single – chamber pump organs, which, with their appendage – the pericardial gland – form a functional unit. Situated on both sides at the base of the gills they receive venous blood from bifurcations of the vena cava and pump it into the gill lamellae via the branchial artery (= afferent branchial vessel)[78]. The increase in venous blood pressure resulting from this seems to be correlated to the body size in different species (table).

Valves are necessary for building up this pressure in order to prevent the blood from flowing back. In *Sepia officinalis,* to take one example, such muscular heart valves are located both at the entrance of the vena cava and between the branchial heart and the afferent branchial vessel. In octopods, on the other hand, this vessel is not developed and a strongly muscular bulb at the origin of the branchial vein is also missing[75]. In *Sepia officinalis* this bulb additionally contributes to increasing the pressure and overcoming the peripheral vessel resistance.

Histology and fine structure

Ransom described the branchial heart of cephalopods as a 'gland with a central lumen of irregular shape and a capsule of striated muscle'[58]. This description corresponds in principle with later findings on structure which refer first and foremost to the branchial hearts of *Sepia officinalis, Loligo vulgaris*[70, 71, 77], *Octopus joubini*[86] and *Rossia macrosoma*[24]. Despite the differences in the branchial heart complex between different species, they all have basic elements in common. The wall of the branchial heart has a 3-layered structure analogous to the wall structure of the blood vessels: an epicardium consisting of flat extended epithelial cells covered with microvilli, followed by the myocardium, a slack spongy layer containing, apart from the muscle fibres, polygonal cells rich in cytoplasm and with an ovoid nucleus. There is an incomplete endothelium towards the lumen, but sometimes it is not developed at all. The lumen itself is branched to a high degree, the heart wall being supplied with blood right up to the periphery through the lumen's sinusoidal branches[70, 71] (fig. 1).

Unlike that of the systemic heart[69] the wall of the branchial heart does not consist of several muscle layers; obliquely striated muscle cells form only a loose network of transversal and longitudinal bundles of fibers which become thicker nearer the periphery (fig. 3). Among the muscle cells with relatively few sarcosomes, individual fibers with a higher content of sarcosomes can be found, whose function may be similar to that of a pacemaker[70]. As in the myocardium of the systemic ventricle A-, I- and Z-bands can be recognized. The sarcoplasmic reticulum (SR-system) usually runs as a longitudinal network. Invaginations of the sarcolemma on the level of the Z-lines (T-system) are less frequent than in the systemic heart[24, 69, 70, 71] (fig. 2). Disci intercalares are formed at the cross-connections of the myocytes, though they are very small and can only seldom be seen. Sometimes the gap between the membranes of such contacting areas contains a central thick lamella. Nexus (gap junctions) have not yet been found[24, 73]. The relative proportion of musculature in the branchial heart wall is slight, considering the great mass of the above-mentioned polygonal cells, to which various possible functions have been attributed[70, 71, 77, 86]. Up to now the cells have been described in *Alloteuthis subulata, Sepiola, Octopus vulgaris*[48, 77], *Sepia officinalis*[70, 71] and *Octopus joubini*[86] (termed rhogocytes here). They are surrounded by a clear glycocalyx and on their surface have numerous tubular plasma invaginations (diameter 250 Å) on the basis of which coated vesicles seem to be formed (figs 4 and 5). A brisk endo- or

Branchial heart pressure of some cephalopod species

Species	Blood pressures mm H_2O Systolic	Diastolic	Reference
Octopus vulgaris	136–204	14–27	De Wilde[85]
Octopus vulgaris	40–80	2	Wells[79]
Octopus vulgaris	35–57	12–14	Schipp and Hevert[64]
Eledone cirrhosa	20–150	0–40	Smith[74]
Octopus dofleini	300–350	100–150	Johansen and Huston[25]
Octopus dofleini	250–500	150	Johansen and Martin[26]
Octopus dofleini	40–140		Potts and Todd[56]
Sepia officinalis	40–55	21–49	Schipp and Hevert[64]

pinocytotic activity seems to take place here, seemingly bearing a topological and functional relationship to large vacuoles and lysosomal dense bodies[70, 71].

There seem to be two types of polygonal cells, differing in the density of the cytoplasm and number of vesicular inclusions (vacuoles, dense bodies)[70]. It may possibly only

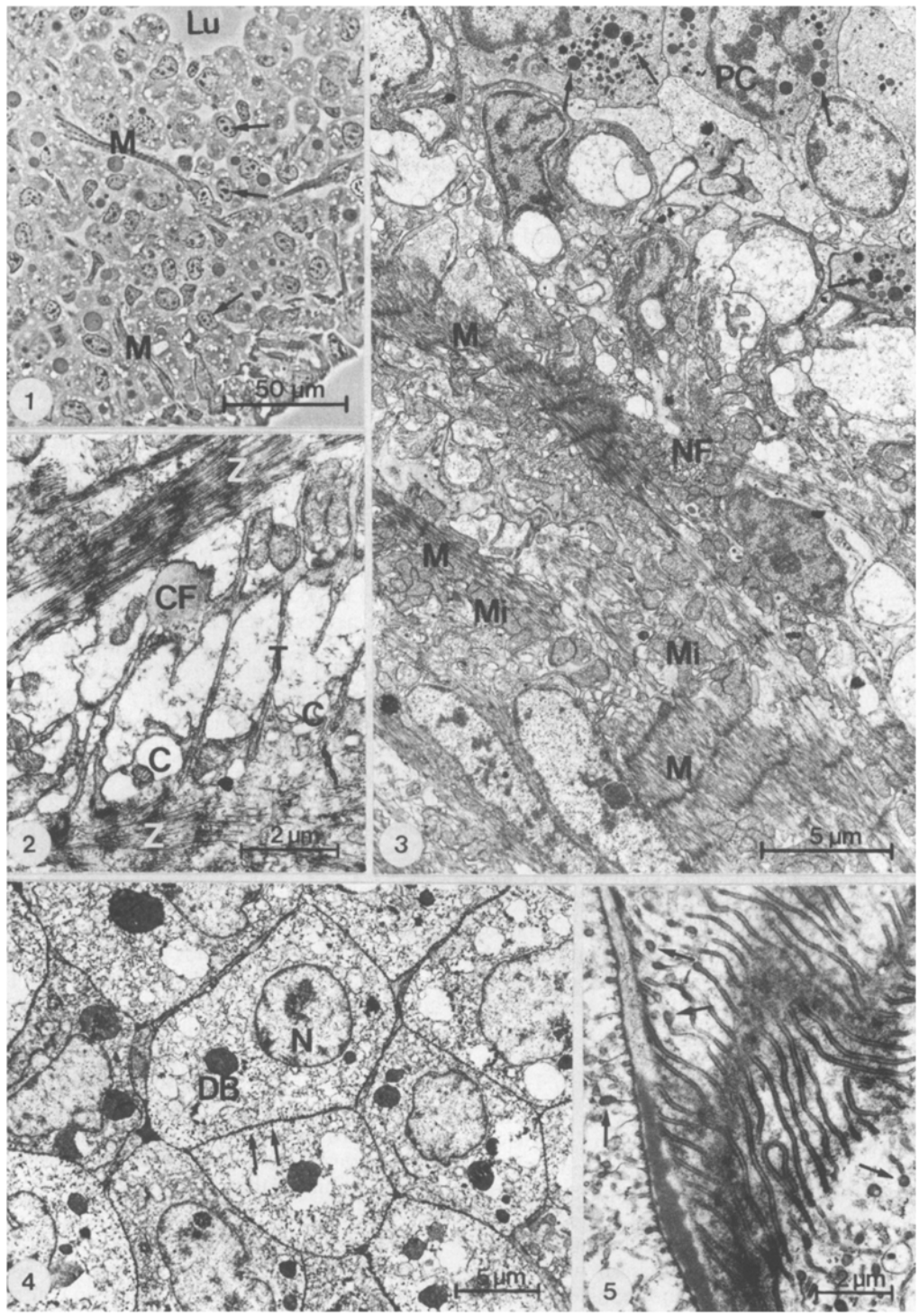

Figure 1. Section through the branchial heart wall of a juvenile *Sepia officinalis* (phase contrast). Ovoid polygonal cell with cell inclusions (arrows) more or less freely floating in the blood cavity. Central lumen (Lu), obliquely striated muscle fibers (M).

Figure 2. Sarcolemmal invaginations (T-tubuli) at the level of Z-patches (Z) in close contact with subsarcolemmal cisternae (C). *Octopus vulgaris*. Collagenous fibers (CF).

Figure 3. Section of the peripheral wall of branchial heart from *Octopus vulgaris* (juvenile). The muscle cells (M) show a high content of sarcosomes (Mi) and are in contact with nerve fibers (NF) filled with neurosecretion granules. Polygonal cells (PC) with dense bodies (arrows).

Figure 4. Polygonal interstitial cells from the branchial heart of *Eledone cirrhosa*. Cytoplasm with more or less ovoid nuclei (N) and osmiophilic inclusions (dense bodies, DB), endocytosis vesicles (arrows).

Figure 5. Tangential section of an interstitial cell from the branchial heart of *Octopus vulgaris*. The plasmalemmal invaginations seem to run as slit-like infoldings over the cell surface. In their terminal areas vesicular endocytosis (arrows).

be a question of different conditions – perhaps stages of maturity of one and the same type of cell[77].

On the basis of endocytosis processes an immunological defense system, comparable to the reticulo-endothelial system of mammals has been discussed[48], and the possibility of storage excretion[62] has been considered. By means of AAS and cytochemical methods accumulations of iron and copper were demonstrated in the polygonal cells of the branchial hearts of *Sepia officinalis*[63]. In *Octopus vulgaris,* also, accumulations of heavy metals and radio-active elements were shown after the animals had been kept for some days in a medium infused with different metals and naturally-occurring radioactive isotopes[50, 52]. Gammaspectrometrical readings revealed that in some cases more than 50% of the total absorbed amount was found in the branchial hearts. Furthermore, a subcellular localization of radioactive substances was achieved by using autoradiographic methods[50]. These substances, absorbed in the polygonal cells, were found to be associated with pigment concretions of a polyadenochrome complex – this is a coloring matter that has already been found and characterized in the cells of the branchial heart[9, 18].

This polyadenochrome is a complex-forming substance containing 3 mol glycine, 9 mol 5-mercaptohistidine, 1 Fe^{3+}-ion and 3 mol Dopa, whereby Fe^{3+} coordinates three catecholatdianions. It tends to associate compounds containing iron[53] and seems to be identical to the above-mentioned dense bodies of the polygonal cells[59, 63]. Furthermore, electron-microscopical results obtained by our team with *Sepia officinalis* revealed that myocytes in the process of autolysis were phygocytosed by adjacent polygonal cells, and moreover, metabolites of injected ^{3}H-noradrenaline were accumulated in the dense bodies of the branchial heart of *Eledone cirrhosa* (fig. 6a, b). It is clearly a case of a kind of 'detoxification process' in which harmful substances as well as any cell fragments are accumulated or metabolized and thus extracted from the body. In this connection the term 'kidneys of accumulation' crops up, as a release of the accumulated excretions or even of other cell content substances could not be traced. These results indicate that an internal-secretory activity of the ovoid-polygonal cells as has been postulated for branchial hearts[21, 62], is unlikely.

The pericardial gland

Close connections between the circulatory and excretory systems are very widespread throughout the whole animal kingdom. In dibranchiate cephalopods such a functional connection becomes clear partly through the formation of a branchial heart complex, i.e. connection between heart and excretory appendage. Nevertheless the pericardial gland is only one of the organs in which urine formation takes place in dibranchiate cephalopods. Besides this the renal appendages, the pancreatic appendages and the gills have a part to play in excretory processes[21, 62, 66, 72].

The branchial heart appendage, like the branchial heart itself, lies in the pericardial cavity and is surrounded by a transport-active epithelium, continuing at its apex in the form of invaginations towards the inside (fig. 11). At this point it makes contact with sinusoidal branches of the branchial heart lumen, penetrating the appendage[72]. Apart from blood lacunae with freely floating hemocyanin, the wall of the pericardial gland, i.e. the space between inner and outer epithelium, contains polygonal cells, similar to those of the branchial hearts, but having a less dense cytoplasm. They appear predominantly in peripheral areas and are sparsely interspersed with muscle fibers which mainly appear in combination with neurons[65, 87]. Nothing is known about whether these polygonal cells have a similar function to those in the branchial heart. The blood lacunae themselves are encompassed by a basement membrane[87]. In many places the luminal epithelium of the appendages contains cells with irregular form and continuations; their basal parts remind one of podocytes of vertebrate nephrons[40, 41, 64, 87]. Examinations of the fine structure of these cells, as well as enzymatic findings and interpretation of the relationships between hydrostatic and oncotic pressures, have revealed that this is the possible location for the above-mentioned ultrafiltration processes[21, 55, 64]. It is highly probable that a concentration of the primary urine is already effected by reabsorption and secretion in the slit-like foldings of the epithelium. This process continues in the renal sacs, and also in the ductus renopericardialis if present. A shuttle movement of the blood or hemolymph fluid is effected by alternating contractions of the branchial heart and its appendage[85], whereby a sufficient filtration pressure seems to be guaranteed in nearly every phase[64]. Furthermore, a neuronal regulation of the filtration rate seems probable, as the sparse musculature of the appendage is also supplied by nerve fibers from the cardial ganglion, just like that of the heart itself[2]. The pericardial appendages of nautiloids – consisting of a number of villi inserting directly on the 4 afferent branchial vessels – reveal homologous cell components. Therefore, these organs, though not treated in greater detail here, must also be considered with regard to similar excretory functions[67, 68].

The automatism and nervous control of the branchial heart

Under adequate filling pressure the isolated, perfused branchial heart of coleoids continues to beat spontaneously and more or less rhythmically[17, 73, 75]. Like all mollusc hearts the organ is characterized by a myogenic automatism, as no intramural ganglion cells can be found – corresponding to the ventricle but forming a contrast to the auricles[2, 22, 38, 60]. It was also noted that properly differentiated muscle cells as described by Schipp and Schäfer with regard to the branchial heart of *Sepia officinalis*[71], are obviously not concentrated in special areas of the myocardium, so that one speaks in term of a diffuse pacemaker[23].

According to our observations isolated branchial hearts of *Sepia officinalis* do however show a periodic change in amplitude and frequency or even two contraction waves like those of other species as illustrated by previously published in vivo actograms[26, 80]. These findings could give rise to the question whether perhaps two (or more) pacemaker regions might determine the rhythm of the organ by forming more or less synchronous or alternating stimulation impulses. Alternatively it is conceivable that ad hoc or with changing degrees of tension the muscle-tissue could form different and even asynchro-

nously discharging pacemakers. No research on such a relationship between latent and transitorily dominating pacemakers in molluscs seems to have been undertaken. Cardiogram readings along with ligature experiments could throw light on the possible localization of such hypothetic pacemakers. This sort of experiment on the

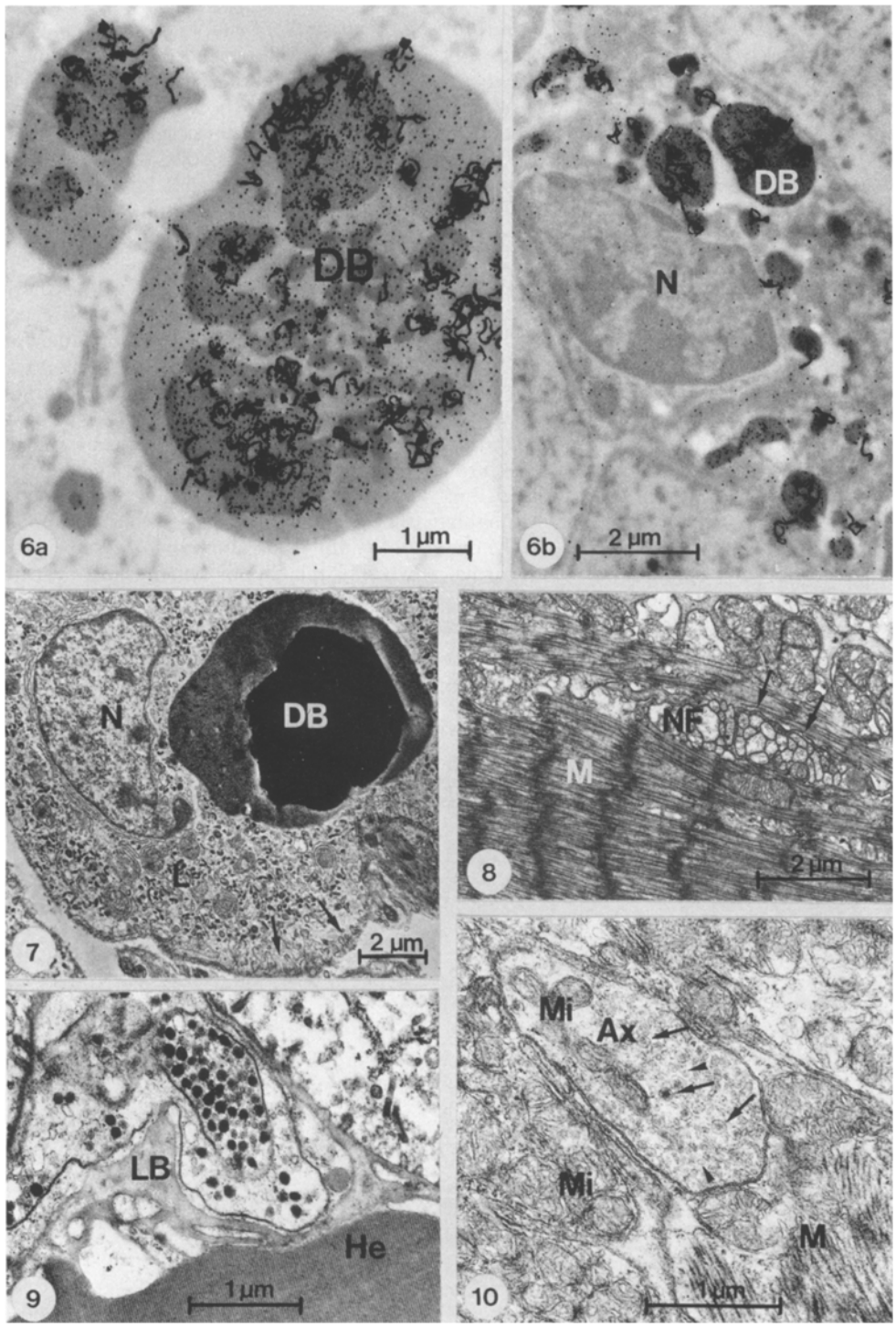

Figure 6a and b. Autoradiographs of polygonal cells of the branchial heart from *Eledone cirrhosa* after 3H-NA-injection. Note that most of the radioactive material has been accumulated in the dense bodies (DB) of the cells, especially in more distinct osmiophilic areas. Nucleus (N).

Figure 7. Polygonal cell of the branchial heart from a juvenile *Octopus vulgaris*. In this species dense bodies (DB) grow to a large size and often they nearly fill the whole cell. Compare with the size of the nucleus (N). Note the difference in density of the body. Smaller lysosomal dense bodies (L) might be related to the endocytotic activity (arrows).

Figure 8. Area of synaptic contact (arrows) between polyaxonal nerve fibers (NF) and the sarcolemma of muscle cells (M). *Octopus vulgaris*.

Figure 9. Terminal axons with dense cored granules in close contact with the hemocyanin (He) containing branchial heart lumen. Lamina basalis (LB), *Octopus vulgaris*.

Figure 10. Neuromuscular synapsis at a higher magnification. Mitochondrion (MI), dense cored vesicles (arrows), smaller transparent synaptic vesicles (arrow heads), terminal axon (Ax), muscle cell (M).

systemic heart of *Octopus vulgaris* has revealed that two so-called 'nodal areas' (see Smith[74]) are possibly located on each of the atrio-ventricular junctions.

It is a fact that the cardiac activity itself is influenced to a high degree by the filling pressure[49]. In experiments on the branchial heart of *Eledone cirrhosa*, Smith[75] found out that the isolated perfused organ only works efficiently with an afterload-pressure of less than 10 cm H_2O, whereby the difference to preload-pressure should range between 2 and 4 cm H_2O. The perfused branchial heart of *Sepia officinalis*, however, only reaches its maximum activity at a preload-pressure of about 20 cm H_2O and the afterload-pressure must be adjusted to values that correspond to the diastolic branchial heart pressure of the animals (circa 2–5 cm H_2O). We again refer to the differences in the composition of heart valves in *Eledone* and *Sepia*.

Morphology and cytological aspects of the cardiac innervation

The best morphological presentation of the innervation of the central circulatory system of decapods thus far is that of Alexandrowicz[2]. His experiments revealed that the branchial hearts of *Sepia officinalis* are probably reached by nerve branches of various origins. On the one hand these are nerves originating in the ganglion cardiacum, on the other hand they may be continuing fibers of one of the two visceral lobus nerves. Both nerve fiber types join to form the nervus cordis branchialis and this in turn forms a wide-meshed subepithelial plexus on the surface of the branchial heart. The branches of this plexus penetrate the whole branchial heart wall, thereby following the network of muscle fibrils and running in the immediate vicinity of polygonal cells (Schipp and Maier, unpublished). However, it is unclear whether they are also innervated. Terminal axons of these nerve fibers make contact with the sarcolema of individual muscle cells, the intersynaptic gap measuring less than 100 Å. The amount of neurosecretory granules in their axoplasm is very variable and large vesicles (∅ 1000–1500 Å) as well as smaller synaptic vesicles (∅ 300–650 Å) occur[70] (figs 8–10). Schipp and Schäfer[71] found primarily dense cored vesicles in the axon endings of such nerves of *Sepia officinalis*, unlike those of ventricles. This may be solely a question of monoaminergic fibers.

Branchial hearts of octopods are also innervated by peripheric cardiac ganglia; however, in the course of the two visceral nerves reaching them they are preceded by an additional pair of ganglia (ganglion fusiformis)[88]. As in *Sepia*, the ganglion cardiacum innervates the branchial heart and its appendage as well as the branchial artery and parts of the vena cava, but in this species it is marked by a pulsating sack apart from the normal ganglionic structure. The musculature of this sack, with its own vascular and nervous supply, contracts in the same rhythm as the branchial heart[76]. The postulated neurosecretory function[3] has not been confirmed further.

From experiments to cut branchial heart nerves or to stimulate them electrically[22, 75, 79, 80] one can deduce that the organ in both octopods and decapods is contacted by both inhibitory and excitatory fibers. A co-ordination which is relative, or synchronization with the rhythm of the systemic heart, seems to be secured in this way.

The localization of neurotransmitters and their catabolic enzymes

There are not many direct indications in scientific literature about the biochemical character of transmitter substances that are released at nerve endings of branchial hearts. Useful reviews of articles on this subject can be

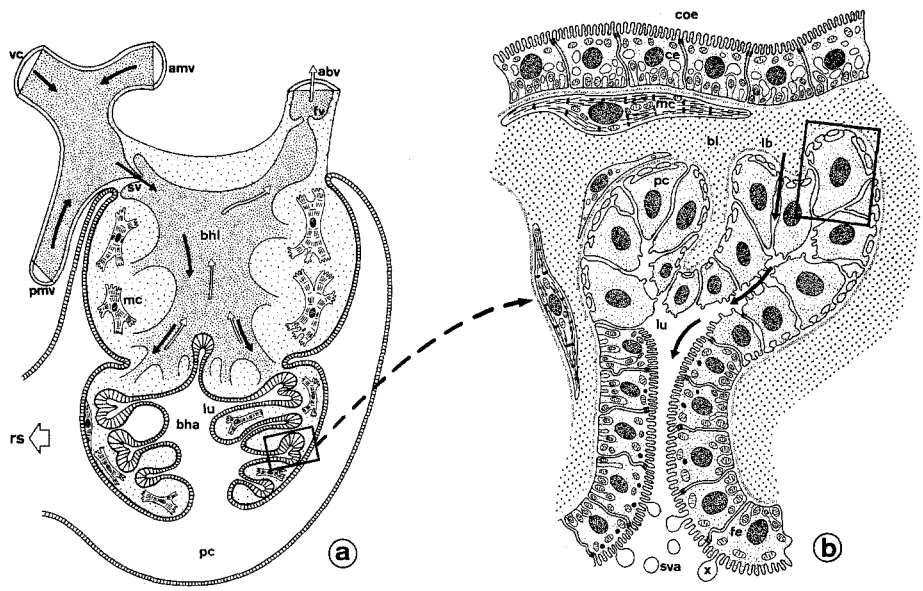

Figure 11. Diagrams of the branchial heart complex of *Sepia officinalis*. *a* The blood circulation in diastole (black arrows) and in systole (white arrows). *b* Section of the lateral wall of the branchial heart appendage (bha) shows a cluster of podocytes (pc) in close topic relation to the peripheral blood lacunae (bl) and the folded epithelium (fe); probable course of ultrafiltrate (arrows). Vena cava (vc); ant. and post. mantle vein (amv/pmv); afferent branchial vessel (abv); semilunar/fleshy valves (sv/fv); branchial heart lumen (bhl); pericardial coelom (pc/coe); lumen (lu) of the bha; to the renal sac (rs); muscle cells (mc); secreted vacuoles (sva); lamina basalis (lb) (from Schipp and Hevert[64]).

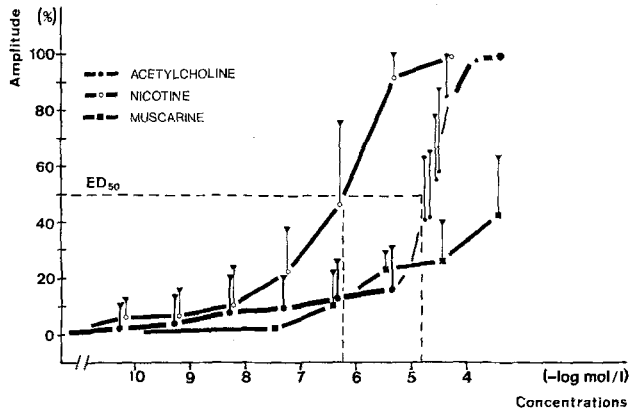

Figure 12. Concentration-response curves of the negative inotropic effect of acetylcholine compared with that of nicotine and muscarine on the isolated branchial heart of *Sepia officinalis* (from Schipp et al.[73]).

found in Östlund[54], Welsh[84] and Kerkut[33], though they tend to focus their attention on the systemic heart.

Von Euler[15] was the first to suspect the possible presence of noradrenaline in the *Octopus vulgaris* branchial heart. Extracts of organs affected the blood pressure of the cat as 0.04 µg/g NA. However, von Euler sees no indication of adrenaline. Concerning catecholamines, the general theory seems valid that cephalopods, unlike the other mollusc groups, have a highly developed NA system[28, 29]. Investigations in *Sepia officinalis* using fluorescence methods also testify to the fact that the proved monoaminergic fibers of the ventricle as well as the branchial heart tend to have an NA character, which is shown by their fluorescence spectrum[17, 36].

Moreover octopamine could be traced in *Octopus vulgaris* (0.14 – 0.36 µg/g)[31], but there are still no indications that either dopamine or 5-hydroxytryptamine (serotonin) is present in branchial hearts[1, 30].

An enzyme of the MAO type is most probably responsible for the metabolization of monoaminergic transmitters[29]; it has been proved to exist in both the ventricle[37] and in the branchial heart of *Sepia officinalis*. Moreover the latter has a significantly high activity of acetylcholinesterase (EC 3.1.1.7.) in the glycocalices of some nerve cells, the muscle cells and, to a lesser extent, the polygonal cells[61, 73]. Acetylcholine itself has already been isolated from these organ areas[8, 42].

Pharmacological aspects of the branchial heart regulation

In contrast to the numerous pharmacological in vitro experiments on central hearts of cephalopods (for review[22, 81]), branchial hearts have hitherto only been investigated to a small degree to establish whether they can be influenced pharmacologically. The reason for this may reside in the fact that the experimental techniques when working on anatomically and functionally heterogeneous objects are very difficult, especially if, as mentioned above, the object does not present a pure nerve-muscle-preparation, as does the systemic heart.

The cholinergic system

Acetylcholine has been proved to be present in the nervous tissues of many molluscs and it is generally accepted that the substance acts as a neurotransmitter to regulate circulation and heart in the whole phylum[1]. Besides the indications mentioned above, few in vivo observations[25, 83], but especially the work on the isolated organ of *Sepia officinalis*[73], prove that this is also valid for the branchial hearts of cephalopods. Acetylcholine, as in the systemic heart[34], inhibits amplitude and frequency, the registered dose-dependent effect (ED_{50} 10^{-5}M) being mimicked by nicotine (fig. 12). Muscarine, in contrast, has only slight inhibition effects. The effect of nicotine is more than 10^1 (ED_{50} 5×10^{-7}) stronger with respect to the intrinsic activity and affinity to the receptor. Curare may block the effects of ACh and nicotine, whereas atropine works more synergistically. Tetraethylammonium shows no affinity to the receptor.

In the obliquely striated heart muscle of *Sepia* a nicotinic acetylcholine receptor is obviously present which resembles those on motor endplates of vertebrates.

Nevertheless the conformity between the 2 receptor types is only relative as in this case α-Bungarotoxin does not combine irreversibly with the cholinergic receptor as it does in vertrebrates (fig. 13). Similar reversible reductions of binding capacity for cholinomimetics through an α-neurotoxin have been described for *Aplysia* neurones[32] and in addition are valid for the systemic heart of *Sepia* (see article in this multi-author review).

The possible role of 5-hydroxytryptamine

The concept hitherto accepted supposed that 5-hydroxytryptamine (serotonin) is also one of the effective transmitter substances in mollusc hearts[22, 38]. In contrast to that, and contradicting the few single findings of previous authors concerning branchial hearts of octopods in situ[25, 83], the indole derivative appears to play no role as a cardioregulative substance in the branchial heart of *Sepia*[17]. The reaction of the isolated organ after administering different concentrations of 5-HT shows great variations (fig. 14). Both acceleration and inhibition have been observed, but usually no influence on the heart-beat was registered at all, indicating that the musculature of the venous heart probably has no specific receptor for serotonin.

Both Johansen and Huston (*Octopus dofleini*)[25] as well as Wells and Mangold (*Octopus vulgaris*)[83] registered posi-

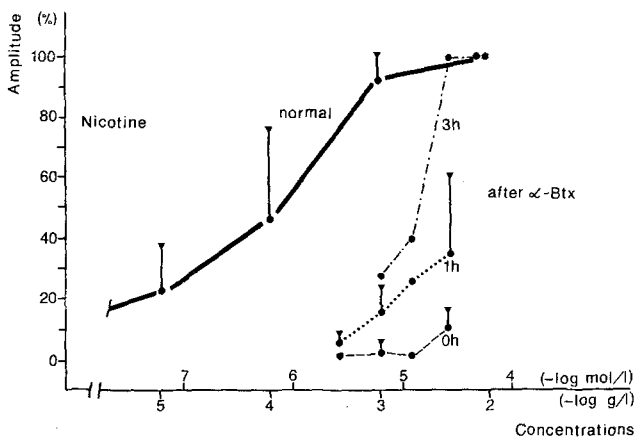

Figure 13. Reversible blocking of the negative inotropic effect of nicotine by α-bungarotoxin (from Schipp et al.[73]).

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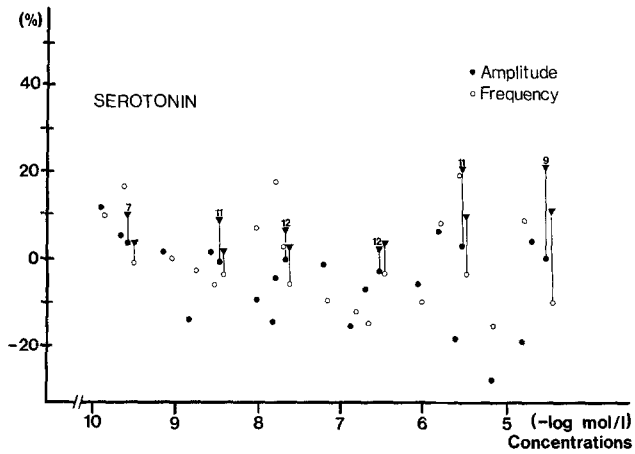

Figure 14. The effect of serotonin on the isolated branchial heart of *Sepia officinalis*.

tive inotropic and chronotropic effects after injecting the substance – the chronotropic effect in *Octopus vulgaris* is more marked. In both cases recordings of pressure and frequency were made in the afferent branchial vessels. How can the results of the in vivo experiments be reconciled with the in vitro ones or, put another way, can they be compared at all? An organ left in situ still remains subjected to the manifold influences of the adjacent organs and whole organism. Johansen and Huston recognized this dilemma, realizing that two views are possible. When measuring the organ reactions they doubt that there is a direct influence on the myocardium of the branchial heart, and suggest that central nervous information might be responsible for causing the measured changes of heart activity. This assumption is backed up by results of these authors showing that when application is performed on one side, the branchial hearts on the other side always react synchronously and with equal strength. Considering the diffusion gradient and circulating time needed by the drugs a direct humoral influence appears highly improbable.

It should now be pointed out that in such experiments on the whole organism peptidergic mechanisms of the neurosecretory system of the vena cava (NSV system)[4,5,11] might interfere. After administration of serotonin Kling (see article in this multi-author review) obtained positive effects on the isolated systemic heart of *Sepia*, and suggested that the indolealkylamine might act by stimulating a peptidergic receptor.

Effects of catecholamines

Whereas among catecholamines it is primarily dopamine which is considered a transmitter of accelerating cardiac nerves in gastropods and bivalves[27], cephalopod hearts show considerably more sensitivity to adrenaline and noradrenaline[13,14,16,37,39]. As we remarked above concerning findings for serotonin effects, there is a discrepancy between the results of in vivo and in vitro experiments which can also be observed here. Both noradrenaline and adrenaline provoke bradycardia of the systemic and also the branchial hearts in the intact animal (*Octopus dofleini*)[25]. The diastolic branchial heart pressure does not change despite variations in frequency and systolic pulse pressure. Johansen and Huston attributed this to a low level of adaptability of the short, thick-walled afferent branchial vessels to great changes in volume, among other factors. Here again the time elapsing between application and onset of the effect is so short, despite the long path of transport, that the authors consider a central nervous information as a more likely cause of the provoked bradycardia.

Isolated branchial hearts of *Sepia officinalis*, however, can be clearly stimulated with both catecholamines (fig. 15). The amplitudes of contraction are increased depending on the concentration (ED_{50} – NA = 1.2×10^{-7}M; ED_{50} – A = 7×10^{-8}M), whereas the influence on frequency is less uniform but must be described as more negative.

Concentrations surpassing this range cause a shift in tonus (incomplete dilatation), which passes over to 'tetanic' contractions with a high frequency. The length of their effect never exceeds one minute. Hitherto it was not shown conclusively which of the two substances possesses a stronger intrinsic activity, whereas the ED_{50}-values point to a higher affinity of adrenaline towards the receptor. But considering the results of fluorescence microscopic investigations[17], we realize that the indications pointing to noradrenaline as a possible cardioregulant mount up. This is obviously a case of a monoaminergic receptor system which is antagonistic to the cholinergic system revealed by Schipp et al.[17].

Analogous to other peripheral innervated cephalopod organs like the central heart[34–36] and digestive tract[6,7] a dual innervation (inhibiting – stimulating) would thus be proved in this vegetative effector organ.

According to further investigations, the β-mimetikum isoproterenol has no influence on the organ's activity (fig. 15). The adrenoceptor in the obliquely striated muscles of the myocardium of branchial hearts seems to resemble the α_1-type in smooth muscles of vertebrate blood vessels: Both noradrenaline and adrenaline can be blocked with phentolamine whereas propranolol does not reduce/block the amine effect. α_2-agonists (BHT 920) are also ineffective.

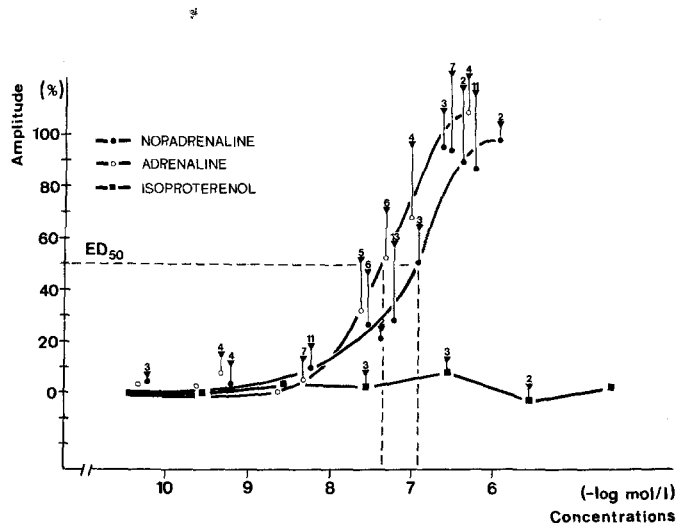

Figure 15. Concentration-response curves of the positive inotropic effect of noradrenaline in comparison with that of adrenaline and the β-mimeticum isoproterenol.

Cardioactive peptides

It has been common knowledge since the mid sixties that apart from ACh, 5HT and catecholamines, other substances with cardioactive effects in bioassays can be isolated from ganglion extracts of molluscs[19,22]. Their long lasting positive inotropic effect, especially of a fraction 'C'[1], set them distinctly apart from the other substances that had been previously examined to ascertain their effect. Further analyses showed that it was mainly a peptide (FMRF-amide[57]) that obviously interferes additionally as a neuromodulator in transmitter processes. Blanchi et al.[11], and also Berry and Cottrell[10], obtained an extract with an extremely long-lasting cardioexcitatory effect from the neurosecretory system of the vena cava (NSV)[4,5,44] using 6 different species of cephalopods. The bathing medium of an isolated vena cava with visceral nerves – electrically stimulated - caused the same effect[11]. The authors suggest that a cardioactive neurohormone is released in response to stress, in a way rather comparable with the function of adrenaline in vertrebrates. Schipp (unpublished) can confirm this conclusion for the isolated branchial heart of *Sepia officinalis* at least in some cases. After introducing the perfusate of an electrically stimulated vein he registers long lasting cardio excitation. Moreover, branchial hearts of *Octopus vulgaris,* also in vivo, reveal an increase in frequency and amplitude after injection of extracts from the anterior vena cava (AVC)[83]. It is now safe to conclude that the NSV layer of cephalopods releases one or, more usually, several hormones into the blood, whereby it has not been adequately clarified how these products act and which organs are affected. As proven by Wells[82] and Wells and Mangold[83] a participation in regulation of circulation is more than likely. The extent to which the branchial hearts are involved in this process has not yet been conclusively shown. Unlike the effect of the vena cava perfusate, the isolated branchial heart of *Sepia* could neither be stimulated by FMRF-amide nor by met-enkephaline, which corresponds to Wells' results. He was not able to obtain an effect on the intact circulatory system of *Octopus* comparable with the AVC extracts either[82]. The neurosecretion of the vena cava obviously consists of a series of cardioactive substances whose composition has not yet been clarified. Martin et al. succeeded in tracing immunocytologically, for example, FMRF-amide-like material, met-enkephaline, melanotropine-neurophysin and vasopressin-like substances in nerve endings of the neurohemal organ of *Octopus vulgaris*[45–47].

It should be added that FMRF-amide has a long-lasting positive inotropic effect on the systemic heart of *Sepia,* but deaminated structural analogues – met-enkephaline for example – do not have a cardioregulative effect (Jakobs and Kling; see article in this review). There are obviously differences between the two heart types in their equipment of receptors. Nevertheless we assume that especially the branchial hearts can be modulated peptidergically. As regards their position – immediately distal of the release point – they seem actually predestined to be the affected organs. Further pharmacological and immunocytochemical experiments should attempt to clarify the still open question of peptidergic receptors in the myocardium of branchial hearts.

Summary and conclusions

Branchial hearts play a definite role in supporting the circulation in coleoids. They are one of the features contributing to the high efficiency of the circulatory system of Dibranchiata compared with that of other mollusc groups. Certainly, the lateral parts of the vena cava are additionally responsible for the measurable increase in blood pressure in the afferent branchial vessel, which is necessary to overcome the resistance of the gills. Nevertheless, the significance of branchial hearts as accessory branchial pumps should not be denied. But it must be taken into account that branchial hearts do their work under more or less anaerobic conditions because – as mentioned above and in contrast to the central heart – they only transport venous blood.

An adaptation of the organ activity to the different conditions of circulation is obviously ensured via the nervous supply, and acetylcholine seems to be the transmitter substance of inhibiting nerve fibers. It is supposed that acceleration is caused by pouring out catecholamines from the varicosities of aminergic fibers. Hitherto it has not yet been clarified which catecholamine acts as the possible neurotransmitter. On the other hand it becomes more and more obvious that serotonin is probably not involved in heart regulation.

Further investigations should clarify whether peptidergic substances of the neurosecretory system of the vena cava might interfere with this regulation process as a humoral modulator.

Besides its hemodynamic task the branchial heart complex is also involved in excretion processes. Podocytes in the epithelium of the pericardial gland are the site of ultrafiltration i.e. urine formation which makes regulation of osmolarity possible.

Furthermore there is an additional type of excretion which takes place in the polygonal cells of the branchial heart itself. The organ accumulates functionless metabolic fragments as well as nonphysiological substances in order to detoxicate the body. It also seems to be an important storage organ for Fe-proteids.

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Reviews

Transformations of arsenic in the marine environment

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Summary. It is ten years since arsenobetaine was first isolated from the western rock lobster *Palinurus cygnus*. Subsequently this naturally-occurring arsenical has been found in many species of marine animals contributing to the human diet. The identification of arsenic-containing ribofuranosides in algae and the production of dimethylarsinoylethanol from their anaerobic decomposition has allowed speculation on arsenic metabolism in marine organisms and has suggested a possible route to arsenobetaine from oceanic arsenate.
Key words. Arsenobetaine; arsenic-containing ribofuranosides; dimethylarsinoylethanol; marine-derived foodstuffs; marine algae; arsenic metabolism.

Although it has been known since the 1920s that marine organisms contain substantial quantities of arsenic[12, 14] it is only 10 years since the first isolation and identification of an arsenic compound from this source; that of arsenobetaine (fig. 1, **13**) from the western rock lobster, *Palinurus cygnus*[20]. Since then the virtual ubiquity of arsenobetaine in marine animals (particularly those contributing to the human diet) has been demonstrated[26] and interest has developed in toxicological aspects of arsenic in marine-derived foodstuffs[27, 47] and in the biochemical transformations of arsenic in marine food chains[16, 17, 26]. It is this latter aspect that we wish to consider here.

Arsenobetaine was first prepared 50 years ago for pharmacological studies examining the possible physiological role of arsenic analogues of some simple nitrogen-containing metabolites[48, 49]. Certainly, the chemical structure of arsenobetaine suggests the possibility of close parallels to nitrogen metabolism, with the probability that arsenobetaine is biosynthesised by a pathway analogous to that for glycine betaine. This apparent similarity has been used as a basis for speculation on the metabolism of arsenic in marine organisms[39]. We consider that the biotransformations of arsenic in the marine environment show only a superficial resemblance to nitrogen metabolism (reflected in the structure of some compounds) and are fundamentally different with a detoxifying rather than central metabolic role. An overall outline of the scheme that we propose is shown in figure 1. Rigorously characterised compounds are shown in heavy type; the remainder is, to an extent, speculation. We will consider the scheme as a number of separate stages.