

exudate phagocytes. Resident macrophages (i.e. the iron-storing cells, perivascular and periglandular phagocytes) probably develop from endometrial stromal cells. This is supported by the presence of rudimentary cilia in endometrial stromal cells<sup>16</sup> and the phagocytes described here. The absence of proliferation in these endometrial phagocytes also suggests they are an end-stage of differentiation of endometrial stromal cells. Our morphological study reveals that exudate monocytes internalize and digest interstitial proteins. The presence of intracellular lipid droplets is in agreement with their high proteolytic activity<sup>17,18</sup>. Proteolysis reduces the colloid osmotic pressure in the interstitium<sup>19</sup>. This decrease is a prerequisite for the drainage of fluid from the tissue. Due to proteolysis a pool of free

interstitial endometrial fluid remains. We postulate that this fluid may drain via the fenestrations and transendothelial channels of the endometrial blood capillaries. Ultrastructural studies of the rat endometrial microcirculation demonstrated this pore system in large, probably venous, capillaries<sup>2,3</sup>. Such a local extravascular circulation or ultracirculation seems to exist in the hypophysis, where it prevents an edema formation after ligation of the prelymphatic pathways<sup>7</sup>. In addition it is shown that venous drainage of proteins and fluid is rather important in primitive species such as *Heterodontus portusjacksoni* (Meyer), and Elasmobranch species in which lymphatics do not yet develop but in which the blood capillaries are intensely fenestrated<sup>20</sup>.

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## The peripheral innervation of the heart of *Eledone moschata* demonstrated by histofluorescence microscopy

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**Summary.** A fluorescence histochemical investigation of the cardiac nerves of *Eledone moschata* has demonstrated that they contain catecholamines. This suggests that the cephalopod heart is supplied by a double innervation, where cholinergic and aminergic mechanisms work antagonistically. This is similar to the vertebrate cardiac innervation and therefore represents a convergent evolution.

**Key words.** Central heart; innervation; *Eledone moschata*; catecholamines; fluorescence histochemistry.

Cephalopods occupy an isolated position among the molluscan phyla with regard to their anatomy, locomotion, sense organs, behavior, embryology, and also their efficient circulatory organs<sup>1</sup>. The main pumping organ is the central heart, supported by rhythmical contractions of the paired branchial hearts and of some veins. The first anatomical and histological description of the octopod circulatory organs was given by Marceau<sup>2</sup>, Isgrove<sup>3</sup>, and Grimpe<sup>4</sup>, and the high efficiency of the octopod cardio-vascular system has been shown in many physiological experiments<sup>5-11</sup>. In the possession of these highly evolved bodily functions, cephalopods are comparable to lower vertebrates, so that it is of interest to investigate whether the cephalopod central heart, like the vertebrate heart, shows a double innervation: on the one hand by inhibitory cholinergic fibers<sup>11-14</sup>, and on the other hand by excitatory aminergic elements. It is reported that the cephalopod cerebellum contains biogenic amines<sup>15-20</sup> and peripheral aminergic nerves are also found<sup>21-27</sup>. We have investigated the central heart of *Eledone moschata* using the fluorescence histochemical method of Falck and Hillarp, as modified by Loren<sup>28</sup>, for detection of monoaminergic nerve fibers within the octopod ventricle.

**Materials and methods.** Adult *Eledone moschata* (mantle-length 7–11.5 cm, weight 380–480 g) were captured in the Mediterranean sea near Banyuls-sur-Mer (France). After anesthetization in 1% ethanol-seawater, the animals were opened from the

ventral side. The central heart was quickly dissected out and incubated for 10 min in 0.2 M phosphate buffer containing 2% glyoxylic acid, 0.5% formaldehyde and 28% MgSO<sub>4</sub>. The hearts were then deep-frozen in Freon 11, cooled by liquid nitrogen and dried for 4 days at –40°C in a Combitoron CM 30 (Leybold-Heraeus) freeze-drier. The tissue was vacuum-embedded in paraffin after treatment with p-formaldehyde vapor at 80°C for 1.5 h. 10–20-µm sections were observed with a Leitz DIALUX fluorescence microscope, fitted with a BP 390–490 excitation filter and a LP 515 barrier filter. Black and white prints were obtained from Agfachrome 50L original color slides. Additionally, histological standard methods and Bodians silver impregnation were applied on formol and Bouin-solution fixed organs.

**Results and discussion.** The central heart of *Eledone* lies in the dorsal body cavity and receives oxygenated blood from the efferent branchial vessels via enlarged veins, so-called auricles. It pumps blood through the cephalic aorta, the posterior aorta and the genital artery into the various regions of the body<sup>3,29</sup>. As in other octopods, the ventricular wall consists of a peripheral epithelium (= epicardium) which represents the residue of the pericardium, which is almost totally reduced in octopods<sup>4,30</sup>. This is followed by a solid connective tissue layer and a mass of ventricular muscle (= myocardium)<sup>2,31,32</sup>. An incomplete ventricular septum represents a relic of the paired origin

of the heart during embryonic development<sup>33</sup>. The ventricle is innervated by the cardiac nerve connecting the two fusiform ganglia and partly supporting the auricles<sup>31, 34, 35</sup>.

Smith<sup>29</sup> recently gave a detailed anatomical description of the octopod cardiac innervation showing some intra- and inter-specific variations regarding the density of the innervation. The main nerve trunks run over the ventricular surface within the connective tissue layer (fig. 1) and branch to penetrate the whole myocardium (fig. 2). Fine fibers are mainly visible within the luminal region of the heart muscle (fig. 3). In the peripheral connective tissue strongly fluorescent nerve trunks can be observed after fluorescence histochemical treatment (figs 4 and 5). It can be seen that the condensation product is localized in the nerve axons, while the Schwann-cell and the surrounding con-

nective tissue remain unstained. Furthermore, non-reacting fibers can be observed within the nerve bundles. They presumably contain other, non-aminergic (possibly inhibitory) transmitter substances. Figure 4 shows that the branches of the nerves entering the myocardium are also aminergic. Within the myocardium fine nerve fibers with fluorescent varicosities are visible (fig. 6). The color of the fluorescence varies from light green-yellow to pale green. This variability of fluorescence

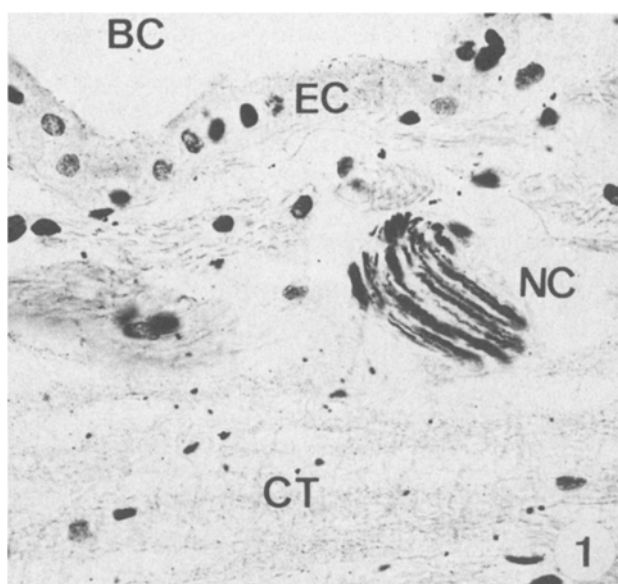


Figure 1. Bodian's staining of the outer regions of the heart; the main nerve trunks are visible within the outer connective-tissue layer; BC = body-cavity, EC = epicardium, NC = branch of the cardiac nerve, CT = connective tissue.  $\times 400$ .

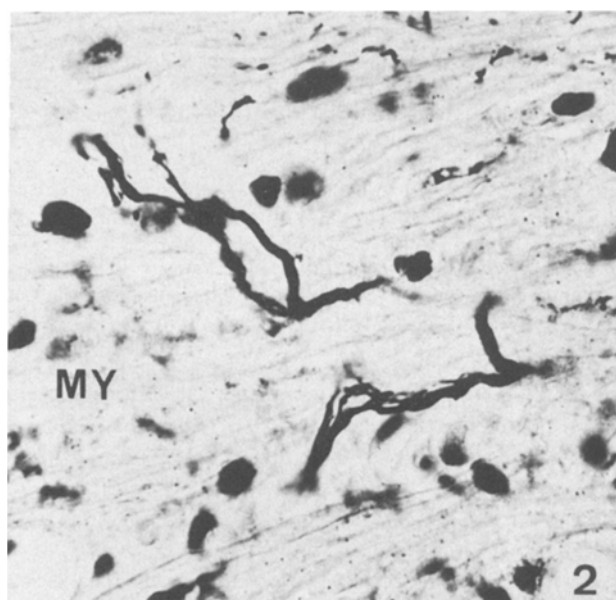


Figure 2. Major nerve fibers within the myocardium of *Eledone moschata*. Bodian's method; MY = myocardium.  $\times 630$ .

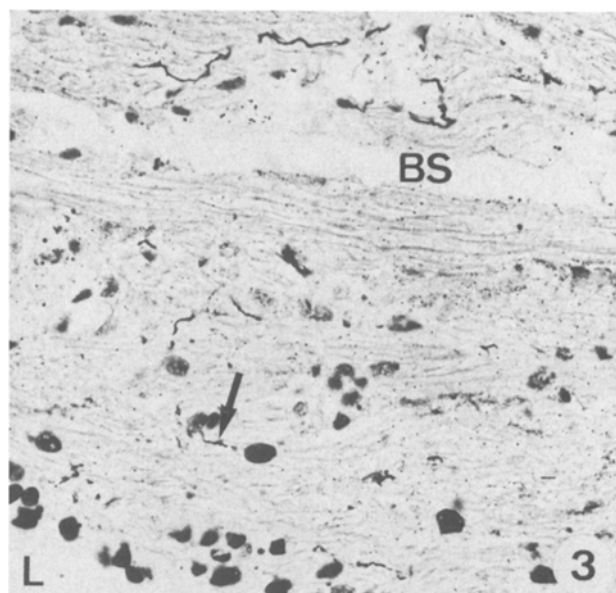


Figure 3. Finest nerve fibers in the luminal region of the central heart; L = heart lumen; BS = blood space.  $\times 400$ .

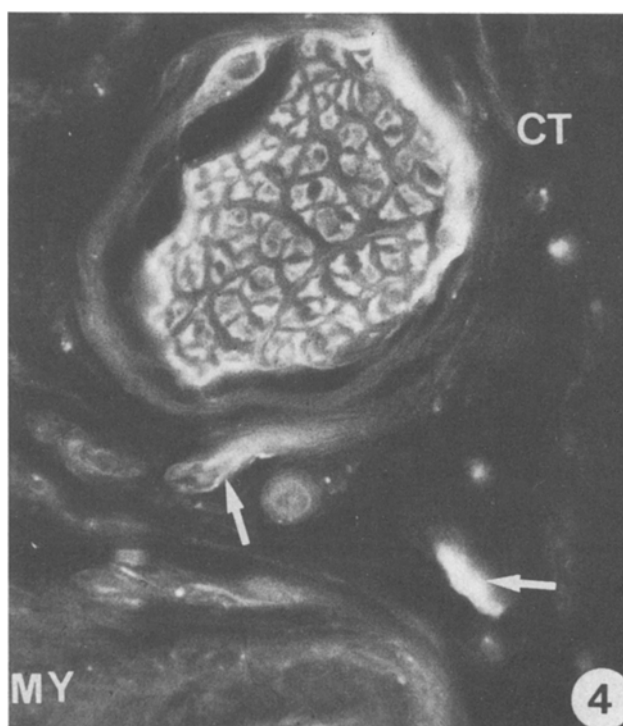


Figure 4. Aminergic histofluorescence in a peripheral fiber of the cardiac nerve. The nerves branching off and innervating the myocardium are fluorescent too (arrows); CT = connective tissues; MY = myocardium.  $\times 1200$ .

coloration, which is observed in other tissues<sup>22,36</sup>, probably depends on the amount of the reaction product within nervous structures. An aminergic innervation by noradrenalin and serotonin is probable in the octopod heart and has been demonstrated in pharmacological experiments<sup>7,11,12,37</sup>. In other molluscan hearts serotonin and dopamine are localized in the nerves by fluorescence microscopy<sup>38</sup> and are therefore possible transmitter substances.

This study demonstrates aminergic and non-aminergic innervation of the cephalopod heart, and as cholinergic effects are also seen in this tissue<sup>11,13</sup>, the cephalopod heart may show a remarkable convergence with that of the vertebrates.

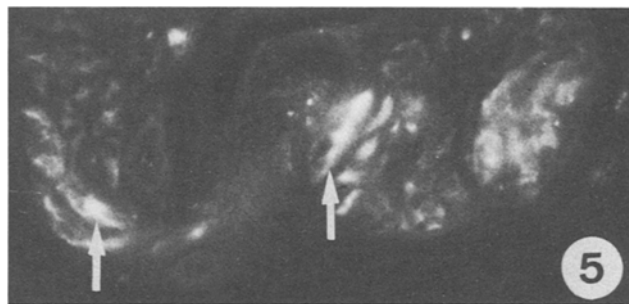


Figure 5. Tangential section through a cardiac nerve trunk. It can be seen that some fibers react negatively, whereas others show strong histofluorescence (arrows).  $\times 2175$ .

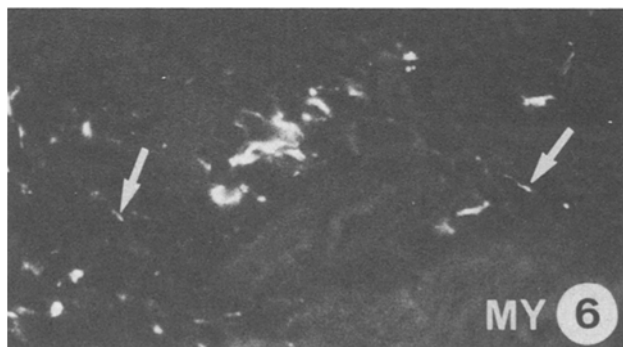


Figure 6. Aminergic fluorescent fibers within the myocardium. Singular varicosities are visible (arrows).  $\times 2175$ .

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## Ethanol diminishes the toxicity of the mushroom *Amanita phalloides*

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**Summary.** Survival of mice after lethal doses of a lyophilizate from *Amanita phalloides* ('death cap') was markedly increased by single doses of ethanol applied 30 min before or 5 min after the mushroom. Hepatic histopathological damage (confluent necrosis) was largely prevented. Acute, but not chronic, consumption of ethanol may thus influence favorably the outcome of death cap poisoning and should be taken into consideration in the evaluation of therapeutic measures.

**Key words.** Mushroom poisoning; *Amanita phalloides*; ethanol.

A variety of agents display antagonistic effects against experimental poisoning with extracts of *Amanita phalloides* ('death cap') or against individual toxins of the toadstool such as phalloidin or  $\alpha$ -amanitin<sup>1</sup>. Clinically, three treatments have been found to be associated with improved survival, namely penicillin G, silibinin and hyperbaric oxygenation<sup>2</sup>. Since hepatotoxic

agents such as carbon tetrachloride protect against the toxicity of phalloidin and the total mushroom extract<sup>1</sup>, we also investigated the effects of ethanol. Previous experiments had shown that protracted pretreatment with ethanol did not protect mice against the mushroom extract unless it was combined with small doses of mushroom<sup>3</sup>. We now report that the survival of