gold interference colours were taken from selected areas of the blocks, stained with uranyl acetate and lead citrate, and examined under a JEM 7-A electron microscope.

The other half of the muscles were stained by the histochemical method of myofibrillar adenosine triphosphatase (ATPase) and reduced diphosphopyridine nucleotide dehydrogenase (DPNH).

*Results.* 1. Ultrastructural observations on soleus muscles after 2 h of the single injection with a dose of 0.625 mg/kg revealed dramatic localized lesions in the areas of muscle fibres adjacent to motor end-plates (Figure 1). In the areas just beneath the end-plates, myofibrils were stretched and severely disorganized and Z-like dense material was increased in the sarcomeres. In the sarcoplasm many dense bodies, vacuoles and swollen mitochondria were also observed. Myofibrillar bundles surrounding the severed regions were strongly contracted. No significant changes, except the swollen mitochondria, were found in the terminal axoplasm. In rats given only a single injection of the drug with a dose of 0.625 mg/kg, however, it became progressively difficult to find the focal lesions in the vicinity of the motor end-plates after the 2nd week. While the same changes were observed in rats receiving lesser dosages, these changes might be proportionate to the amount administered.

2. Ultrastructural observations on the muscles of the repeatedly injected rats demonstrated that sarcoplasmic changes such as vacuoles, dense bodies and swollen mitochondria disappeared from the first week on, whereas focal lesions showing increased density of Z-like material in the disorganized myofibrils could still be recognized up to the 8th week (Figures 2 and 3). From the 6th week, however, the affected regions were gradually decreased in number.

Lesions observed in the subsarcolemmal or central parts of the muscle fibre not far from the end-plate regions were found at all times. From the first week some of nuclei found in sole plate regions showed an apparent appearance of pyknotic processes, while many others were filled with euchromatin-rich nucleoplasm. Euchromatin-rich nuclei were more frequently found in sole plates than in control. Disarranged fibrils, many membranous structures and ribosomal particles were seen near these nuclei (Figure 4). The width of the primary synaptic clefts ranged from 500 Å to 900 Å, but secondary synaptic clefts were sometimes widened. Many dense granules with membranes were found in the synaptic spaces. Processes of Schwann cells interposing between axon terminals and synaptic folds were more frequently encountered than in control. The satellite cells were occasionally located near the sole plate regions.

3. In the soleus muscles, which were previously denervated and given injections with a dose of 0.625 mg/kg every day for 21 days, no focal changes as mentioned above were found in the muscle fibres of the end-plate regions but showed the denervation atrophy.

4. Histochemical staining of the ATPase and DPNH showed predominant atrophy in the type I fibres.

5. No significant changes, however, were found in the soleus muscle fibres of the control rats injected with physiological saline solution.

*Discussion.* In the experiment described neostigmine caused marked disorganization of myofibrils in skeletal muscle fibres at end-plate regions, even after the first injection. With repeated administration of the drug for 3 weeks, the whole soleus muscle decreased the weight at 30%, indicating muscular atrophy.

Euchromatin-rich nuclei found from the first week of the daily injections, together with many membranous structures and ribosomal particles, should reflect an elevated activity in the injured muscles. These features may suggest that a process of regeneration appeared in the sarcoplasm. After the daily injections, subsarcolemmal lesions or central core-like lesions were seen at all times, whereas those of the end-plate regions were less marked as time went by. With the single injection, on the other hand, focal changes at the end-plate regions were scarcely found in the muscle fibres after 2 weeks. In either case, it might be reasonable to assume that some reconstructive changes occurred in the affected muscle fibres.

Irreversible cholinesterase inhibitors such as DFP or Paraoxan containing organophosphorous compound can produce myopathy similar to this report[5–7]. Neostigmine inhibits the cholinesterase activity at the neuromuscular junction reversibly, and resultant excess amount of acetylcholine might mechanically or chemically play a role in the formation of these changes. This report will also present many problems concerning the histopathological features and treatment of myasthenic patients as well. Further investigations of this experiment are now underway.

[5] A. Th. Ariens, E. Meeter, O. L. Wolthuis and R. M. J. van Benthem, Experientia *25*, 57 (1969).
[6] G. M. Fenichel, W. B. Kibler, W. H. Olson and W. D. Dettbarn, Neurology *22*, 1026 (1972).
[7] M. B. Laskowski, W. H. Olson and W. D. Dettbarn, Expl Neurol. *47*, 290 (1975).

## Laminar Acetylcholinesterase Localization in the Optic Tectum of Five Seawater Teleosts

A. Contestabile

*Istituto di Anatomia Comparata, Università di Bologna, Via Belmeloro 8, I-40126 Bologna (Italy), 27 October 1975.*

*Summary.* The histochemical localization of acetylcholinesterase in the optic tectum of seawater teleosts shows a characteristic laminar distribution which parallels the histological structure of the nervous centre. Significant differences have been observed between *Gobius* and the other 4 species of teleosts examined. It seems likely that cholinergic mechanisms play an important role in the function of teleost optic tectum.

The optic tectum usually reaches a noticeable degree of development in teleosts, playing a very important role in integration of visual input with other exteroceptive informations[1]. Wawrzyniak[2], using histochemical methods, observed a complex laminar distribution of acetylcholinesterase (AChE) in tectal layers of *Tinca*, while no positive reaction was achieved in *Cottus*. A recent study[3] on AChE localization in the optic tectum of 4 species of freshwater teleosts showed a similar pattern of distribution in 3 species while in the fourth, the catfish,

[1] J. J. Bernstein, in *Fish Physiology* (Eds. W. S. Hoar and D. J. Randall; Academic Press, New York 1970), vol. 4, p. 1.
[2] M. Wawrznyak, Z. Zellforsch. *58*, 234 (1962).
[3] A. Contestabile and N. Zannoni, Histochemistry *45*, 279 (1975).

a different distribution pattern was observed. In order to increase the data on AChE localization in teleost optic tectum and to verify the extent of differences in distribution patterns, I have studied the histochemical localization of AChE in 5 seawater teleosts.

*Materials and methods.* For the present research 4 species coming from Adriatic sea (*Mugil cephalus*, fam. Mugilidae; *Scomber scombrus*, fam. Scombridae; *Sardina pilchardus*, fam. Clupeidae; *Gobius paganellus*, fam. Gobiidae) and the tropical clown-fish (*Amphiprion perc ula*, fam. Pomacentridae) have been used.

The brains were fixed for 3–5 h at 4 °C in 10% formol saline, then washed for 10–20 min in 0.1 *M* acetate buffer (pH 6.2), frozen and cut in the cryostat in transverse or sagittal planes. The sections were routinely preincubated for 45 min at 20–22 °C in acetate buffer containing the selective pseudocholinesterase inhibitor iso-OMPA $3 \times 10^{-5}$ *M* and then incubated for 90 min in the media of GEREBTZOFF[4], or of KARNOVSKY and ROOTS[5], containing acetylthiocholine iodide as substrate and iso-OMPA $3 \times 10^{-5}$ *M*. Other sections were treated in preincubation and incubation bath with the selective AChE inhibitor BW 284C51 $5 \times 10^{-5}$ *M* or the inhibitor of all cholinesterases, eserine sulphate $3 \times 10^{-5}$ *M*. Other sections were directly incubated in the 2 histochemical media without inhibitors but containing butyrylthiocholine iodide as substrate, instead of acetylthiocholine.

*Results and discussion.* The two histochemical methods gave comparable results in *Mugil* and *Amphiprion*, while in the other 3 species only the KARNOVSKY and ROOTS method yielded a distinct positive reaction. The same result was previously achieved in the trout among 4 species of freshwater teleosts[3], and possibly the negative result obtained by WAWRZYNIAK[2] in *Cottus* might be due to the use of the GEREBTZOFF method alone. The sections treated with eserine or BW 284C51 showed respectively complete or almost complete disappearance of the histochemical reaction; the sections incubated in media containing butyrylthiocholine as substrate showed no appreciable reaction. The histochemical controls thus lead me to consider the reaction observed as due to the actual presence of true AChE.

For the identification of tectal layers I have integrated the denominations proposed by ARIENS KAPPERS et al.[6] and LEGHISSA[7]. AChE activity exhibits the following distribution pattern in the different tectal layers of *Mugil*, *Sardina* and *Amphiprion* (Figures 1–3), starting from the inner border of the tectum. Ependymal layer: no reaction. Periventricular gray layer: positive reaction, moderate (*Amphiprion*) to strong (*Mugil*, *Sardina*). Inner fibrous layer: no reaction. Inner gray layer: reaction uniformly strong in *Sardina*, while in *Mugil* and *Amphiprion* this layer shows an inner band with moderate reaction and an outer band with very strong reaction. Inner plexiform layer: weak reaction. Outer gray layer: the reaction is almost uniformly strong in *Sardina* and *Am-*

[4] M. A. GEREBTZOFF, Acta anat. *19*, 366 (1953).

[5] M. J. KARNOVSKY and L. ROOTS, J. Histochem. Cytochem. *12*, 219 (1964).

[6] C. U. ARIENS KAPPERS, G. C. HUBER and E. C. CROSBY, *The Comparative Anatomy of the Nervous System of Vertebrates including Man* (Hafner, New York 1960), vol. 2.

[7] S. LEGHISSA, Z. Anat. EntwGesch. *118*, 427 (1955).

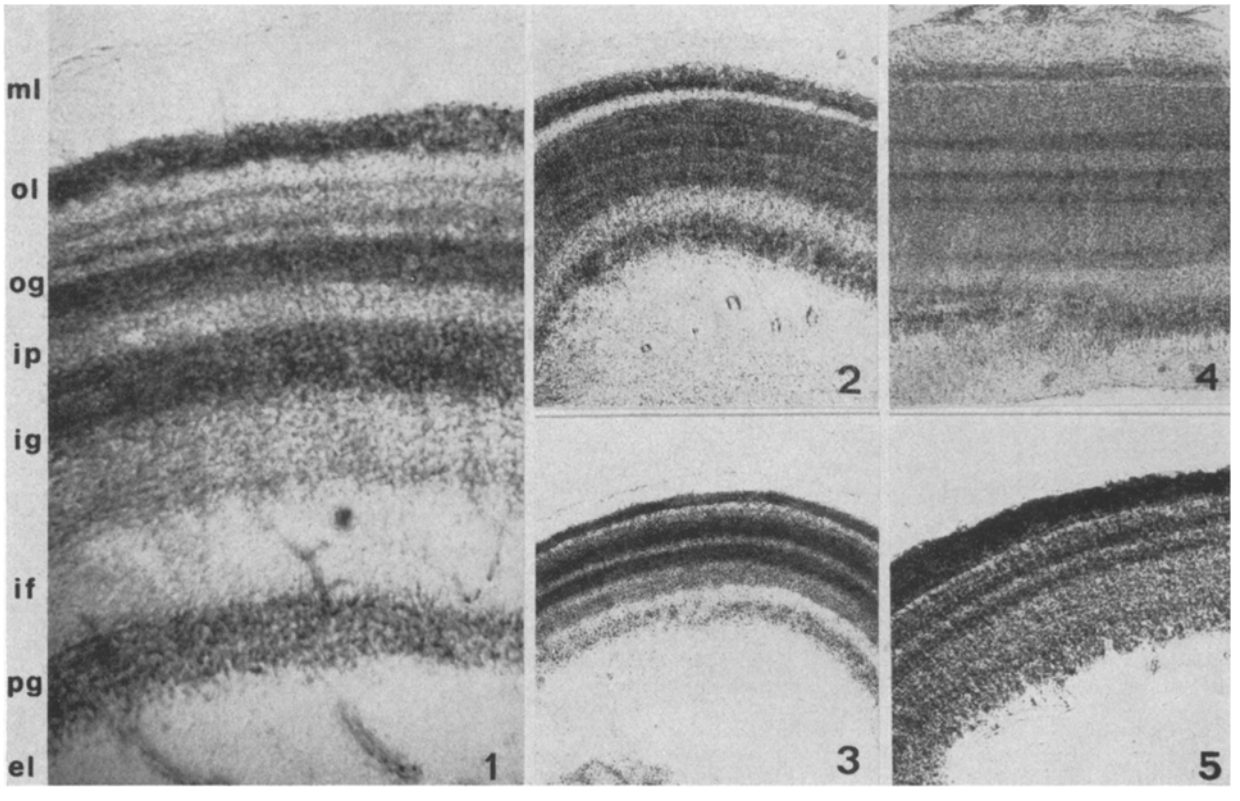


Fig. 1. AChE distribution in the optic tectum of *Mugil*. Abbreviations: el, ependymal layer; if, inner fibrous layer; ig, inner gray layer; ip, inner plexiform layer; ml, marginal layer; og, outer gray layer; ol, optic layer; pg, periventricular gray layer. GEREBTZOFF[4] method. × 180.

Figs. 2–5. AChE distribution in the optic tectum of *Sardina* (Figure 2, KARNOVSKY and ROOTS[5] method); *Amphiprion* (Figure 3, GEREBTZOFF method); *Scomber* (Figure 4, KARNOVSKY and ROOTS method); and *Gobius* (Figure 5, KARNOVSKY and ROOTS method). × 55.

*phiprion* while in *Mugil* the deep part of this layer shows very strong reaction, and the superficial part shows 4 thin bands with weak or moderate reaction. Optic layer: the inner part, corresponding to afferent retinal fibres, shows no reaction; the outer part, corresponding to the area where the marginal neurons of the optic layer are mainly arranged, exhibits very strong reaction. Marginal fibrous layer: no reaction.

In *Scomber* (Figure 4) a distribution pattern similar to that of *Mugil* is present but the intensity of the histochemical reaction appears reduced in all layers. In *Gobius* (Figure 5) AChE localization in optic tectum differs from that just described for the other species: in deep and central tectal layers AChE activity appears substantial, but the distribution pattern does not show marked variations among the different layers, while the marginal layer, which is lacking in histochemical reaction in all the species described above, exhibits very strong reaction.

Some experimental and ultrastructural works[8–17] have outlined the synaptic arrangement and the distribution pattern of fibre systems in teleost optic tectum. These results help to explain the laminar distribution of AChE and the role played by the enzyme. Previous observations[2,3] and the results of present work show a prevalent AChE distribution which suggests that most of the sensitive discharge and some important systems of stimuli propagation and modulation might be mediated by cholinergic mechanisms in teleost optic tectum. This conclusion is supported by prominent AChE activity at level of inner and outer gray layers, periventricular gray layer and marginal neuron layer, i.e. all layers which directly or indirectly receive the input of sensitive stimulation.

The above-mentioned AChE distribution pattern, however, is not the only one among the teleosts. In fact in the optic tectum of *Gobius* among seawater teleosts and *Ictalurus* among freshwater ones[3], an alternative type of AChE distribution exists. This different AChE distribution might be indicative of specific differences in synaptic patterns among teleosts or differences in chemical mediators between analogous synaptic systems. It seems likely that further experimental and ultrastructural studies on the optic tectum of teleosts with dissimilar AChE localization will be necessary in order to solve the problems arizing from histochemical analysis.


[8] M. Jacobson and R. M. Gaze, Q. Jl. exp. Physiol. *49*, 199 (1964).
[9] S. O. E. Ebesson, Brain Behav. Evol. *1*, 134 (1968).
[10] H. Ito, J. Hirnforsch. *12*, 325 (1971).
[11] H. Vanegas, E. Essayag-Millan and M. Laufer, Acta cient. venez. *22*, 82 (1971).
[12] E. Schmatolla, J. Embryol exp. Morph. *27*, 555 (1972).
[13] S. Sharma, Brain Res. *39*, 213 (1972).
[14] J. J. Anders and E. Hibbard, J. comp. Neurol. *158*, 145 (1974).
[15] M. Laufer and H. Vanegas, J. comp. Neurol. *154*, 61 (1974).
[16] M. Laufer and H. Vanegas, J. comp. Neurol. *154*, 97 (1974).
[17] F. Ciani, S. Leghissa and L. Villani, Riv. Biol. *68*, 5 (1975).


## Copper Utilization During Embryogenesis of *Palaemon lamarrei*


Katre Shakuntala

*Department of Zoology, Bangalore University, Bangalore 560001 (India), 4 August 1975.*



*Summary.* The pattern of copper utilization during the embryogenesis of the freshwater prawn, *Palaemon lamarrei* has been described. Throughout the incubation period, lasting for 14 to 16 days, the egg of *P. lamarrei* is permeable to salts and against a concentration gradient, it absorbs 11 µg of salt from the freshwater medium. Out of this total salt uptake, as much as 0.143 µg is due to the absorption of copper. Intake of copper, as a function of incubation time, exhibited a more or less similar trend to that of total salt intake and this can be attributed to the increased synthesis of haemocyanin.


Unlike in marine crustaceans[1], the required salts for the normal development of the embryo in freshwater have to be either made available along with the yolk in the egg and/or have to be absorbed against a great concentration gradient. Among the trace elements, copper is an important element present in enzyme systems as well as respiratory pigments of crustaceans[2]. There are numerous publications dealing with the utilization of energy and substance in several marine crustaceans[3–6]. Von Hentig[7] has studied the yolk utilization in *Artemia salina*. Apart from this, there is no publication which deals with the utilization of yolk and especially copper during the embryogenesis of other freshwater crustaceans. The present paper reports on the pattern of utilization of copper during the different developmental stages of the freshwater prawn, *Palaemon lamarrei*.

Changes in ash and copper content of developing eggs and zoea of *Palaemon lamarrei*

| Developmental stage | Ash content | | Copper content | |
|---|---|---|---|---|
| | µg/egg | % ash/egg | µg/egg | µg/g egg |
| I | 15.30 ± 0.82 | 3.10 ± 0.12 | 0.173 ± 0.002 | 338.7 ± 4.10 |
| II | 19.20 ± 0.44 | 3.90 ± 0.12 | 0.171 ± 0.006 | 356.3 ± 12.70 |
| III | 19.80 ± 0.38 | 4.30 ± 0.33 | 0.182 ± 0.018 | 398.3 ± 39.40 |
| IV | 22.10 ± 0.92 | 4.90 ± 0.10 | 0.245 ± 0.014 | 544.8 ± 32.50 |
| V | 22.90 ± 0.78 | 5.20 ± 0.22 | 0.286 ± 0.033 | 623.9 ± 71.60 |
| VI | 26.30 ± 0.48 | 7.07 ± 0.32 | 0.316 ± 0.019 | 736.7 ± 43.80 |

*Material and methods.* Berried female specimens of *Palaemon lamarrei* (Edwards) were collected from the Bellandur fish farm, near Bangalore. The prawns caught in several collections throughout 1969–1972 were transferred into individual glass battery jars containing aerated freshwater. The egg mass was slowly released from the plumose hairs of the pleopods onto a glass slide and the

[1] J. Needham, *Biochemistry and Morphogenesis* (The University Press, Cambridge 1950), p. 785.
[2] W. Wieser, Helgoländer wiss. Meeresunters. *15*, 282 (1967).
[3] T. J. Pandian, Helgoländer wiss. Meeresunters. *16*, 216 (1967).
[4] T. J. Pandian, Marine Biol. *5*, 153 (1970).
[5] T. J. Pandian, Marine Biol., *7*, 249 (1970).
[6] T. J. Pandian and K. H. Schumann, Helgoländer wiss. Meeresunters. *16*, 225 (1967).
[7] R. von Hentig, rer. nat. Thesis, University of Hamburg (1970).