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Histochemical Study of the Localization of Cholinesterases in the Central Nervous System of *Sepia officinalis*

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Summary. Koelle-Friedenwald's technique was used in the histochemical localization of cholinesterases in the central nervous system of *Sepia officinalis*. This first study is only concerned with sagittal sections. The reaction is exclusively neuropilar. Acetylcholinesterases and pseudo-cholinesterases show the same distribution in almost all the lobes except for the superior frontal and vertical lobes. The intensity of the reaction is different in the various lobes studied, especially at the level of the supra-oesophageal mass.

Key words: Brain — *Sepia officinalis* — Cholinesterases — Synapse.

Résumé. La localisation histochimique des cholinestérases a été faite par la méthode de Koelle-Friedenwald. Cette première étude porte uniquement sur les coupes sagittales. La réaction est exclusivement neuropilaire. Mises à part quelques exceptions (lobes vertical et supérieur frontal) les acétyl-cholinestérases et les pseudo-cholinestérases présentent la même répartition. L'intensité de la réaction est différente selon les lobes, surtout au niveau de la masse supra-oesophagienne.

Introduction

Since Bacq and Mazza (1937 demonstrated for the first time the quantity of acetylcholine in the nervous lobes of *Octopus*, the problem of a possible cholinergic synaptic transmission in Cephalopods has interested many authors. Cholinesterases in Cephalopods have been mainly studied with biochemical techniques, the main studies in this field being those of Loe and Florey (1966) and of Welsch and Dettbarn (1972). By studying extracts of different parts of the brain of *Octopus*, Loe and Florey were able to draw a map of the distribution of cholinesterases and acetylcholine in the central nervous system. Apart from these studies, little research has been done using histochemical methods. Only the studies of Arvy (1960), Ducros (1971) and Martin and Barlow (1972) on the posterior salivary glands of *Octopus* can be mentioned as well as Barlow's (1971) on the vertical lobe of *Octopus*. It therefore seemed interesting to use histochemical methods in order to localize cholinesterases in the central nervous system of the cuttlefish.

Material and Methods

Decapod Cephalopods (*Sepia officinalis*) were used throughout this study. The animals came from the laboratory of Luc-sur-Mer where cuttlefishes are bred permanently according

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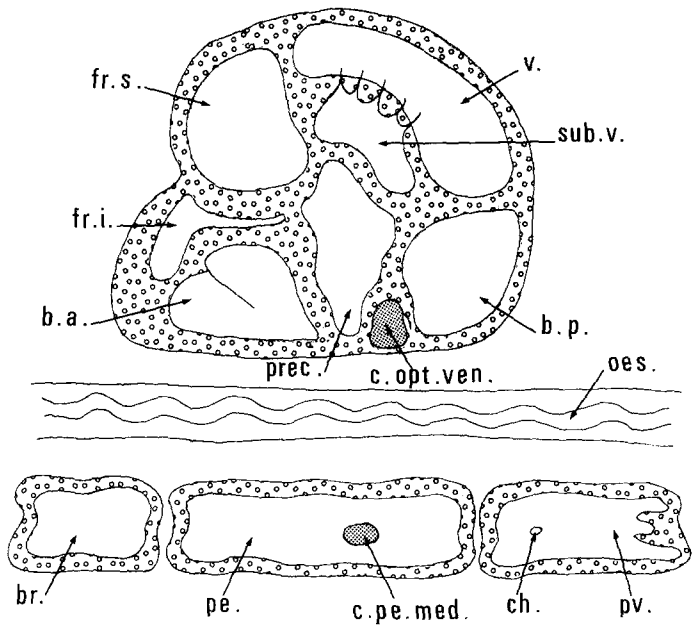


Fig. 1. Schematic sagittal section of the Central Nervous System of *Sepia officinalis*. *b.a.* anterior basal lobe; *b.p.* posterior basal lobe; *br.* brachial lobe; *c opt. ven.* ventral optic commissure; *c.pe.med.* medial pedal commissure; *ch.* giant fiber chiasma; *fr.i.* inferior frontal lobe; *fr.s.* superior frontal lobe; *oes.* oesophagus; *pe.* pedal lobe; *prec.* precommissural lobe; *pv.* palliovisceral lobe; *sub.v.* subvertical lobe; *v.* vertical lobe

to Richard's technique (1971). Their weight varied from 10 to 50 grams. The histochemical technique of Koelle and Friedenwald (1949) was used, for it allows good general localization when applied to the nervous system of Vertebrates.

Sections of fresh tissues were cut at 16 μm on a cryostat. They were incubated, at room temperature, for 60 minutes, with either acetylthiocholine iodide or butyrylthiocholine iodide. The pH of the buffer (Walpole) used was 5.9. Some sections were incubated with either cholinesterase inhibitor: eserin sulphate (at 10^{-5}M) or with iso-OMPA (at 4×10^{-5}M) for 30 minutes prior to the addition of the substrates.

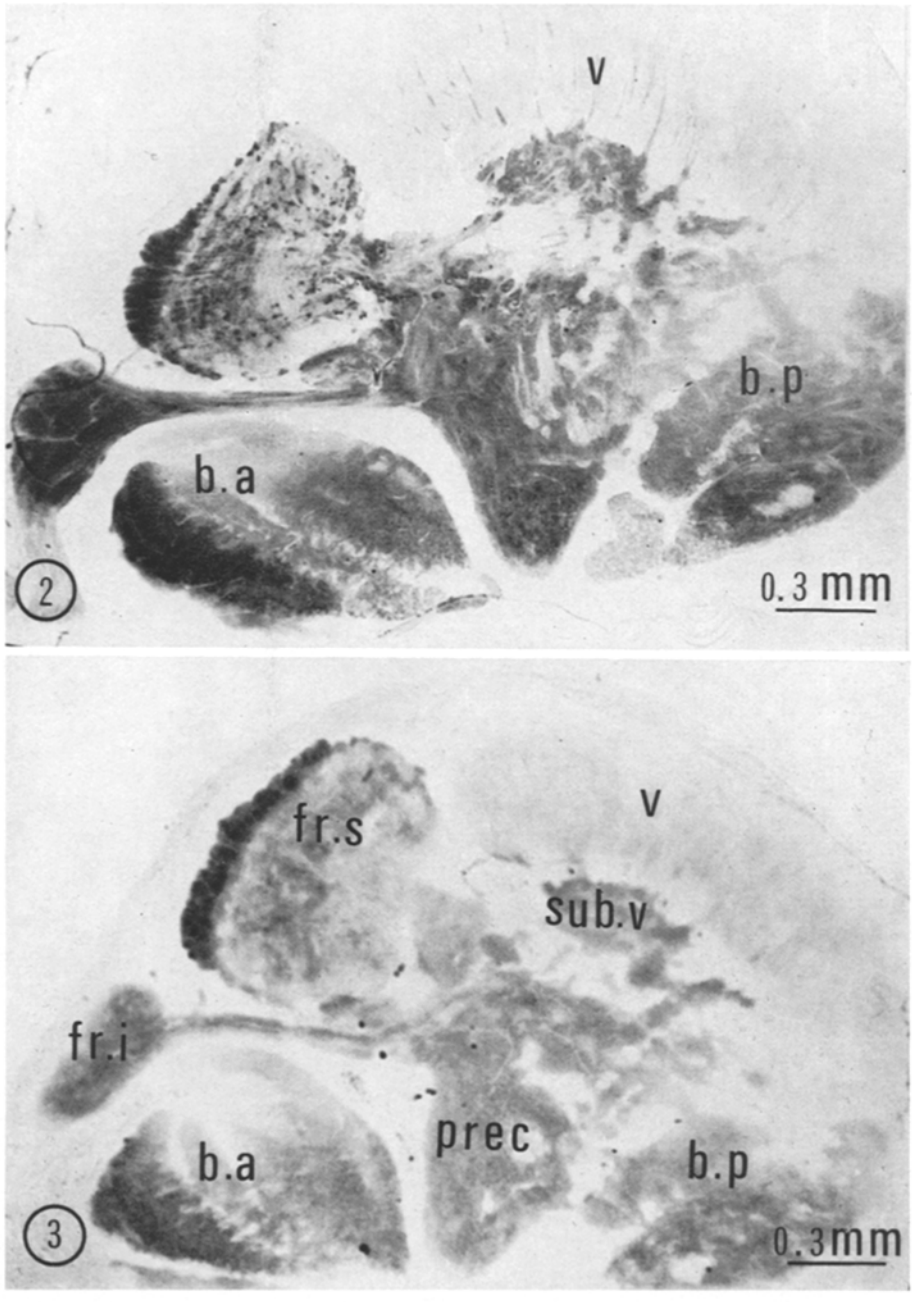
Results

In this first paper only sagittal sections will be taken into consideration (Fig. 1). The sections treated with eserin were practically totally inhibited.

A. Supraoesophageal Mass

1. Incubation with Acetylthiocholine Iodide

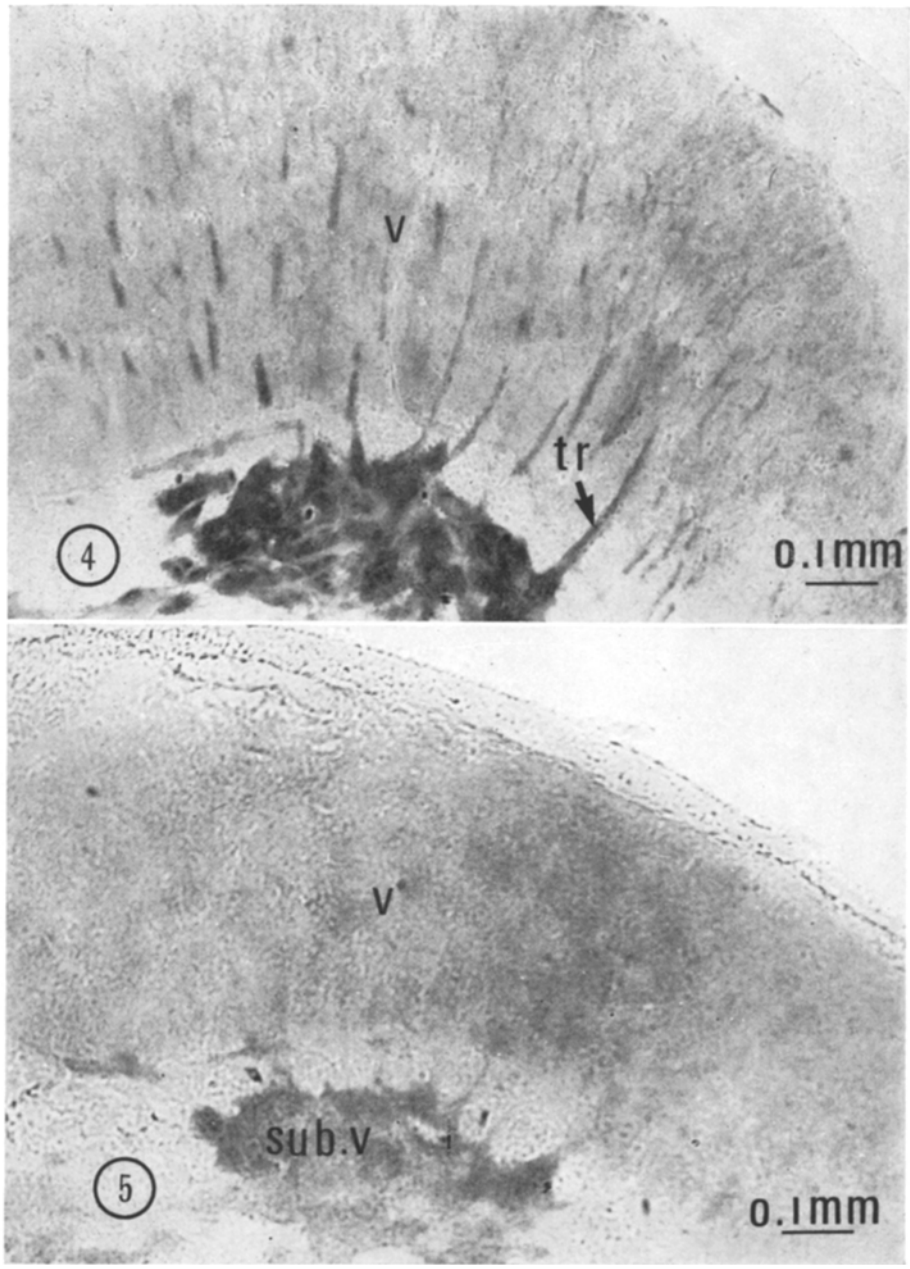
The supraoesophageal mass of the cuttlefish, in sagittal sections, shows a very precise distribution of cholinesterases (Fig. 2). The reaction is localized in the neuropils of the lobes only. The cell layers always show a negative reaction. However this reaction reveals a different intensity depending on the lobes.



Figs. 2 and 3. Supraoesophageal mass

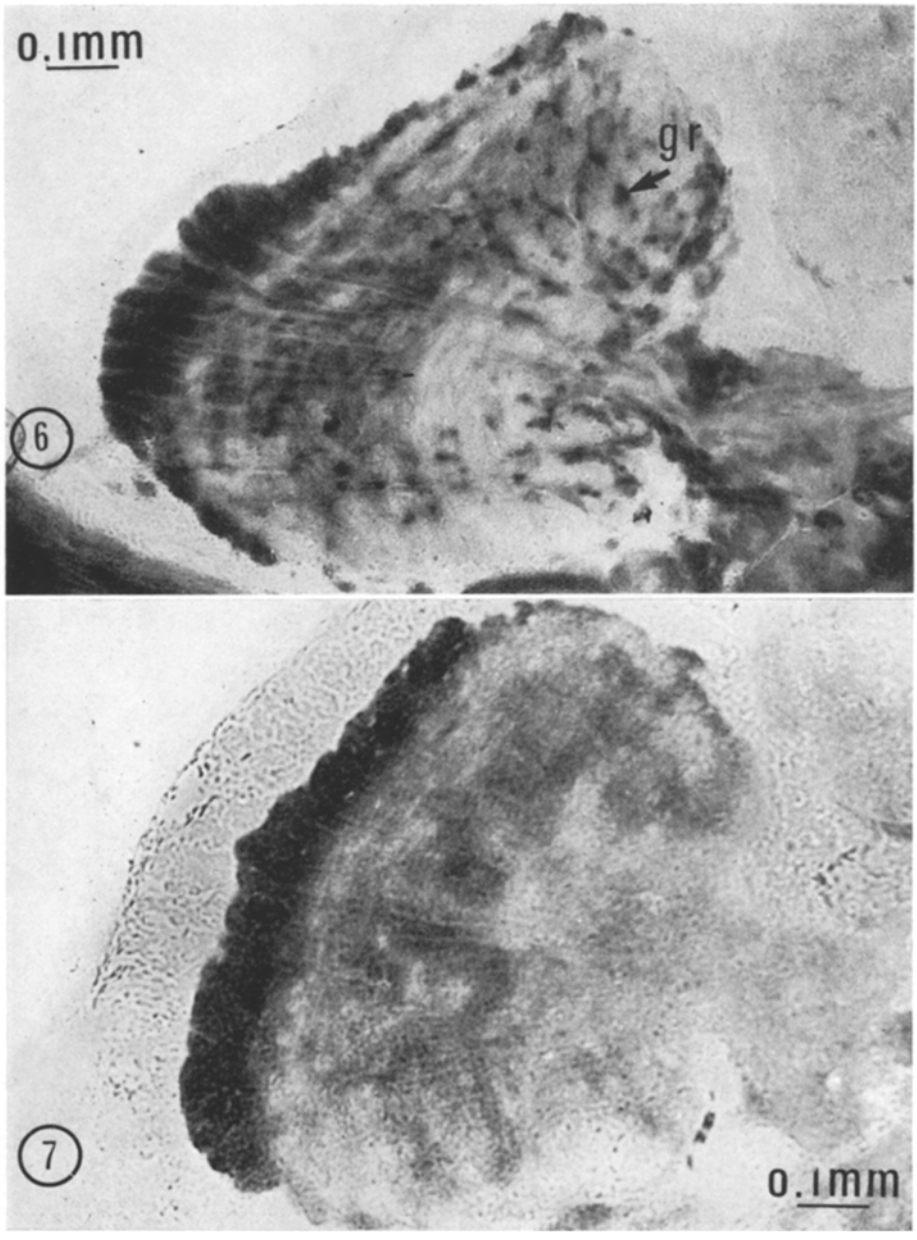
Fig. 2. Incubation with acetylthiocholine iodide

Fig. 3. Incubation with butyrylthiocholine iodide



Figs. 4 and 5. Vertical and subvertical lobes
Fig. 4. Incubation with acetylthiocholine showing the "tracts" (*tr.*)
Fig. 5. Incubation with butyrylthiocholine

Vertical Lobe. It presents a general reaction which is very slightly positive. However "tracts" which connect this lobe to the subvertical lobe can be found. They present a very definite positive reaction (Fig. 4).



Figs. 6 and 7. Superior frontal lobe

Fig. 6. Incubation with acetylthiocholine showing the "granules" (*gr.*)

Fig. 7. Incubation with butyrylthiocholine

Subvertical Lobe and *Precommissural Lobe*. They both show a very definite positive reaction.

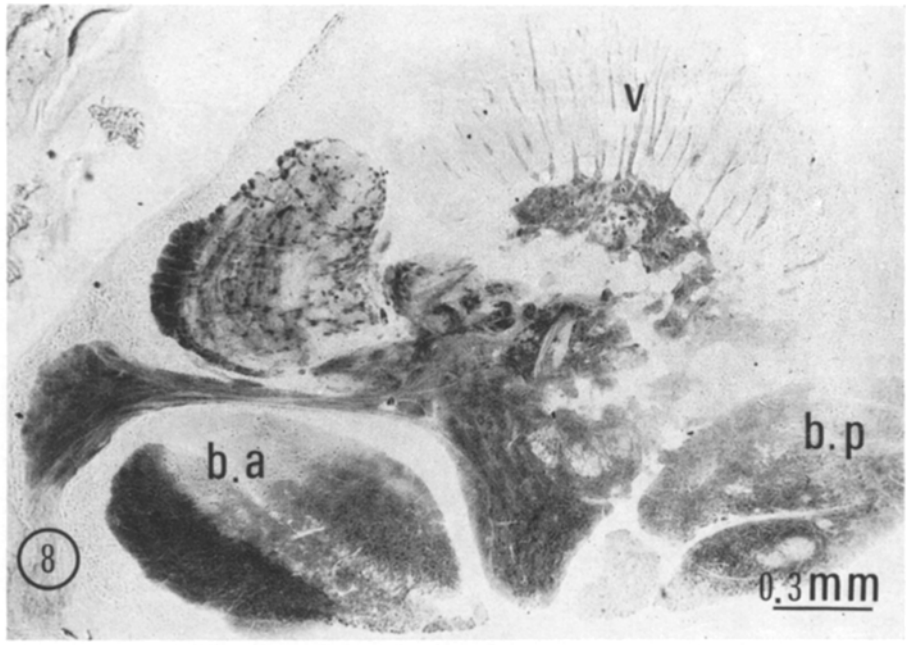


Fig. 8. Supraoesophageal mass. Incubation with acetylthiocholine iodide after a preincubation with iso-OMPA

Basal Lobes. The anterior basal lobe shows a highly positive reaction in its anterior portion. The rest of the lobe presents a moderate reaction. The posterior basal lobe presents a slightly positive reaction.

Frontal Lobes. The inferior frontal lobe shows an homogeneous and highly positive reaction. The superior frontal lobe, in addition to a moderate general positive reaction, shows a highly reactive area in the external and anterior portion of the neuropil as well as "fine granules" which are very reactive and dispersed over the entire neuropil (Fig. 6).

Ventral Optic Commissure. At this level the reaction is very slight.

2. Incubation with Butyrylthiocholine Iodide

After incubation with butyrylthiocholine the following changes can be noticed: The intensity of the reaction is less pronounced for all the lobes (Fig. 3). In the *vertical lobe* which is then very slightly reactive, the "tracts" disappear (Fig. 5). In the *superior frontal lobe*, the "granules" disappear (Fig. 7). There is no reaction at all on the level of the *ventral optic commissure*.

3. Incubation with Acetylthiocholine after a Preincubation with Iso-OMPA

The general aspect of the sections is identical with that of the sections which were incubated with acetylthiocholine iodide without preincubation. However the iso-OMPA causes a general diminution of the intensity of the reaction and the localization seems sometimes more clearly defined (Fig. 8).

B. Sub-oesophageal Mass

1. Incubation with Acetylthiocholine Iodide (Fig 9)

The sub-oesophageal mass shows a fairly homogeneous and positive reaction in the three main lobes of which it is composed: the brachial lobe, the pedal lobe and the pallio-visceral lobe. Here again one must notice that only the neuropils show a positive reaction. The middle pedal commissure shows an almost negative reaction as does the giant fiber chiasma which is situated in the neuropil of the "palliovisceral complex".

2. Incubation with Butyrylthiocholine Iodide (Fig. 10)

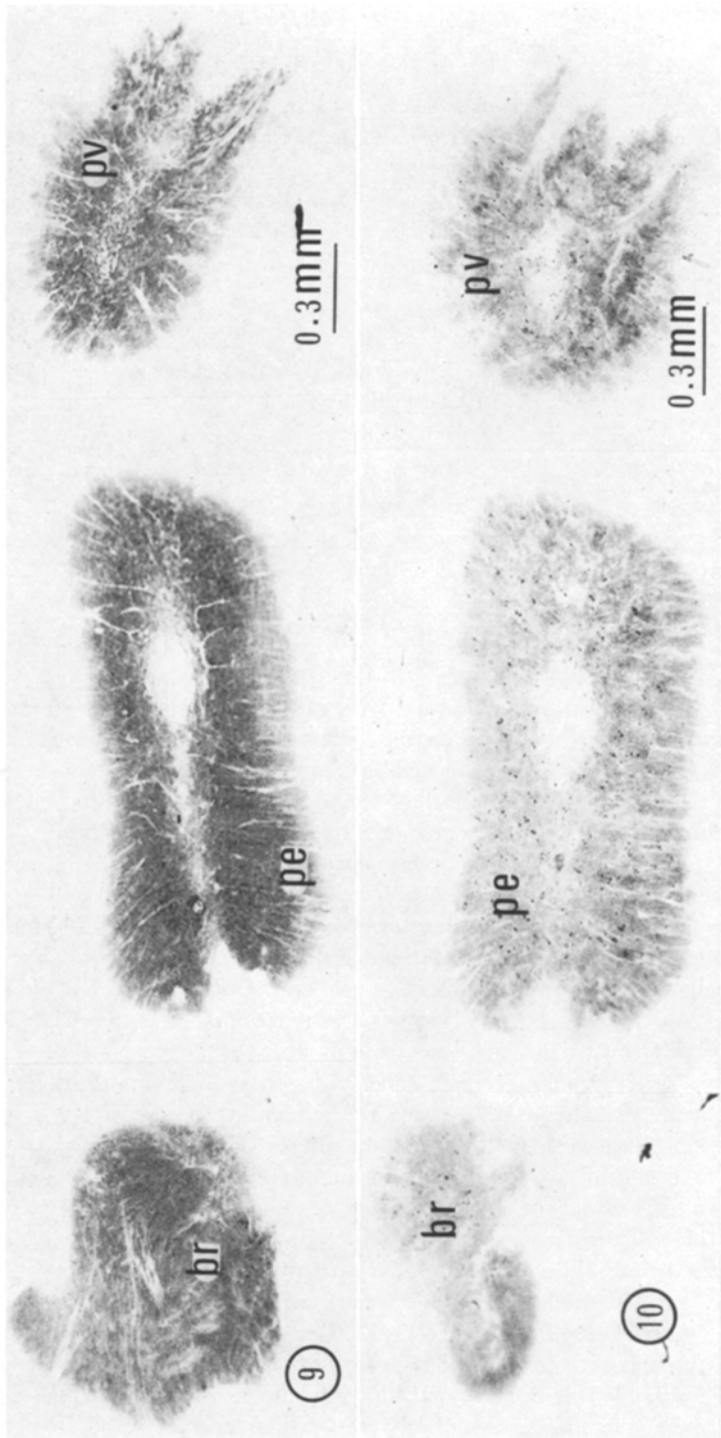
The general aspect of the sections is identical to that of the preceeding sections. Only a certain decrease in the intensity of the reaction can be noticed.

3. Incubation with Acetylthiocholine Iodide after Preincubation with iso-OMPA (Fig. 11)

The same remark as for the supraoesophageal mass can be made: decrease in the intensity of the reaction.

Discussion

This study allows us to demonstrate with greater precision a few points raised in the former biochemical studies on the localization of cholinesterases in central nervous system of Cephalopods, especially if we take into consideration Loe and Florey's studies. The localization they found with their technique could have only been approximate. On the contrary, the histochemical technique we used allowed us to determine a very precise localization at the level of each lobe. Sections incubated with acetylthiocholine iodide show very clearly a totally heterogeneous distribution of cholinesterases: some lobes like the inferior frontal lobe, the anterior portion of the anterior basal lobe and a portion of the superior frontal lobe show a very strong positive reaction. On the contrary the vertical lobe, for instance, shows a very slight positive reaction. The study of the sections incubated in different solutions allows us to assume that, except for a few specific points, the "true" cholinesterases and the "pseudo-cholinesterases" might occupy the same undifferentiated space at the level of the neuropilar structures. In the cuttlefish, we noticed the constant lack of positive reaction at the level of the cell layers of all the lobes; it is an already established fact that in Vertebrates the cellular bodies of cholinergic neurons show a positive reaction (Koelle 1954). On the contrary our results can be compared with those shown in other Invertebrates where the cell bodies of the neurons which are supposed to be cholinergic, do not present a cholinesterase reaction (Wigglesworth, 1958 and Smith and Treherne, 1965; with Insects; Zs. Nagy and Salanki, 1965 with Gastropods). This essentially neuropilar localization of the reaction of cholinesterases is in favor of a possible role in the synaptic transmission in Cephalopods, especially since the synapses could be essentially axo-axonal and localized in neuropilar zones.



Figs. 9 and 10. Suboesophageal mass

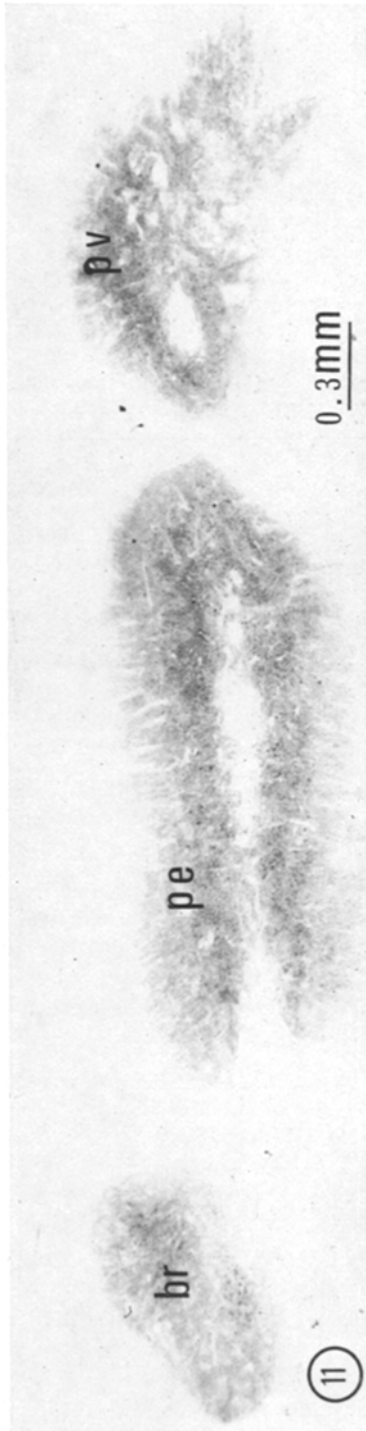


Fig. 9. Incubation with acetylthiocholine

Fig. 10. Incubation with butyrylthiocholine

Fig. 11. Subeosophageal mass. Incubation with acetylthiocholine iodide after a preincubation with iso-OMPA

Further research, associated with electrophysiological and pharmacological techniques might allow us to demonstrate the existence of cholinergic structures in the nervous system of this particularly interesting Invertebrate because of the numerous converging aspects it presents with Vertebrates.

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