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Temperature regimes in concurrence to Different seasonal fluctuations corroborate Alkaline Phosphatase reactions in *Lymnaea (Radix)luteola.*

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Abstract
In order to correlate the effect of temperature fluctuation [low (4°C, 10°C), near ambient (20°C, 25°C), and high (30°C, 35°C)] on their secretory dynamics, researchers have evaluated the histophysiological condition of the neurosecretory cells to one of the major ganglia. The neurosecretory material's histochemical makeup has also been noted side by side. People in both research categories who were exposed to almost room temperature are regarded as normal. In conclusion, the cerebral neurosecretory elements undergo histochemical investigations under varying temperature regimes, as reported previously, in order to determine any corresponding biochemical alterations.

Keywords: Blood Brain Barrier (BBB), Histophysiology, Histochemical Reactions, Alkline Phosphatase Reaction (ALP), Neurosecretory Cells (NSCs)

Introduction

Based on a review of the literature, it may be concluded that because molluss lack a blood- brain barrier (BBB), they lack distinct neurohaemal organs. Yes, the release sites are widely spaced and typically rest on the surface of the nerve, commissure, and connectives. They can also occasionally rest over the blood vessel wall (Gabe, 1962) [11], or even the surface of organs like the kidneys (Joosse, 1976) [15] and body wall (Raubos and Morrer-Van Delft, 1976).

It has been noted elsewhere that the conventional staining methods for identifying the neurosecretory elements are not very reliable (Berlind, 1977) [5]. The fact that structures with completely unrelated neurosecretory products can react positively to neurosecretory stains is remarkable (Bern, 1966) [6]. For this reason, WendelaarBonga (1970) [36] and Schooneveld (1974) [26, 29] demonstrated that lysosomal materials, free ribosomes, and occasionally even NSM are similar. Accordingly, it is essential to clarify how the NS substances behave histochemically in both vertebrates and invertebrates, including gastropod molluscs (Sloper, 1957; Gabe, 1962; Banerjee *et al*., 1968; Hinks, 1971; Nagabhushanam, 1974; Nanda and Goswami, 1978; Nanda and Ghosh, 1985; Bandopadhyay, 1987) [3, 11, 14, 19, 20, 30]. Overall, these results indicate that the neurosecretion is either sulphohydril (SH) and/or disulphide (SS) groups connected to proteins, or both linked with lipids. In fact, the protein component of neurosecretion is essential to function and stays constant.

Histoenzymically, presence of alkaline phosphatase renders metabolic mediation pertaining to endothelial walls of the capillaries and neural tissues. This has a bearing for the permeability of the molecules to the former (Barnett, 1954) [4]. Involvement of this enzyme for the carriage of neurosecretion into the blood stream has been attributed by Tewari and Dabholkar (1968) [32, 33]. And such mediation may be linked with "traversing the nutrient" across the NSCs for active synthesis of proteinaceous hormone. Fluctuation in the activity of this enzyme has been noted in bivalves in accordance with the seasonal changes (Lomte and Nagabhushanam, 1974) [18, 19] besides the influence of multiple physiological parameters (Tarafdar, 1988) [31]. Prior to this contention, Awasthi (1982) [1] attributed that the activity of the enzyme reaction is related with the functional state of the NSCs and both run on parallel lines.

Studies were conducted utilizing Palkovit's formula (1963) to determine the volume of the cell bodies and nuclei based on staining intensities, morphological stainable granule features, their abundance and distribution, and size. The cells under investigation were evaluated using the Hartwick nuclear plaquesamic index (Np index) calculation, which was referenced by De Roberties (1968). It should be noted that the diagnosis of their criteria takes into consideration cells with either large or medium size.

In tandem with these investigations, histochemical examinations of the neurosecretory cells were conducted, taking into account their sensitivity to varying temperature ranges as evaluated by histophysiological analyses. The following assays were used in histochemical study:

1.Calcium-Cobalt method (Gomori, 1946) [13] For alkaline phosphatase. Fixative 80% chilled alcohol, (24 hours). Optimum incubation period - 2 hours.

Observations

A. Alkaline Phosphatase Reaction (ALP)

1. Hypothermal exposure AT 4°C

Localisation of alkaline phosphomonoesterase positive granules in the cytoplasm of the neurosecretoryperikarya is demonstrable. Nuclei remain mostly conspicuous due to the deep colouration of the nucleoli. Cytoplasm normally have marginal accumulation of alkaline phosphatase reaction and their accumulation may be detected along the axonal course (Fig. 1). And accordingly, a black hue along the axonal tract may be observed. Indeed, the extent of reaction depends upon the secretory state of the cell concerned and, therefore, fluctuation in the intensity of reaction is revealing.

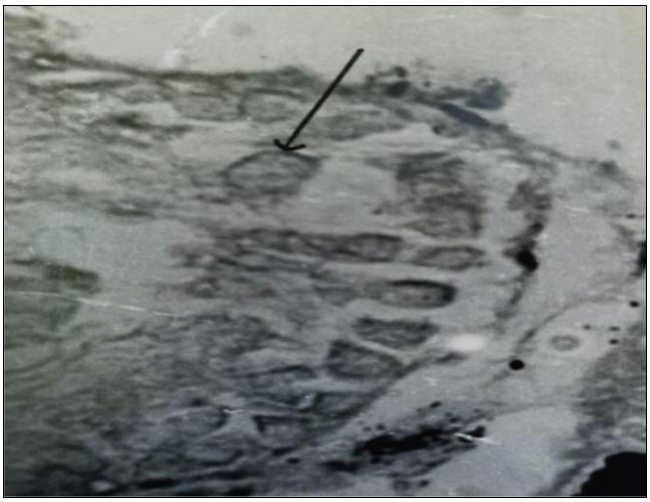


Fig 1: Section demonstrating the moderate localisation of alkaline phosphatase positive material within the cell body. Sometimes the axonal processes may show the distribution of the enzyme concentration along their courses, X 125

AT 10°C

Intensity of reaction is relatively rich and distribution of the enzyme within the perikarya is rather clear. Indeed, all that depends upon the quantum of the enzyme present within the cell at a particular point of time and accordingly various shades of reaction may be registered. The nuclei are less reactive along with the nucleoli when comparison is made with the previous hypothermic group. Detection of deep brown to black granules along the axonal course is not so difficult. Cells of larger dimension, however, do not always display the same feature. And in such cases the cytoplasm may have light brown colouration but granular consistency (Fig. 2) of the alkaline phosphatase positive particles could not be ascertained always.

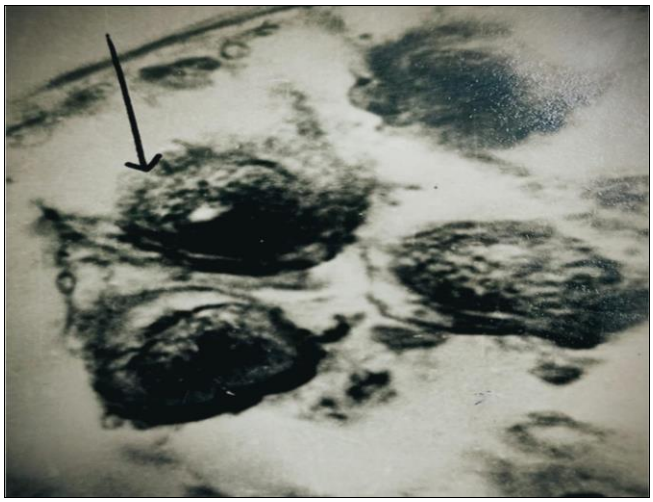


Fig 2: Section showing relatively intense reaction of the cytoplasmic material for alkaline phosphatase. Note also various shades of reaction amongst neurosecretory elements, X 532

2. Exposure to Near Ambient Temperature At 20°C

Intensity of alkaline phosphatase reaction is moderate and the cytoplasm shows possession of fine granules that are uniformly distributed throughout the perikarya. Nuclei are, in general, weakly positive. Cells of smaller dimension despite resembling the similar situation may indulge to contain alkaline phosphatase positive black particles mostly accumulated at the axon hillock regions (Fig. 3). A few of such materials may be detectable along the axonal tract.

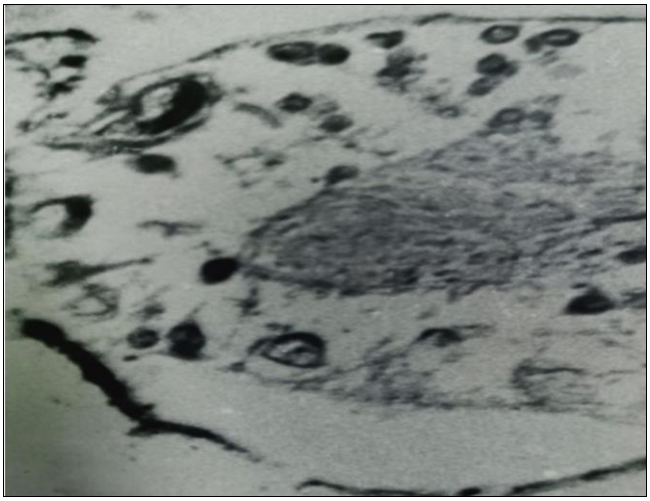


Fig 3: Section showing the distribution of alkaline phosphomonoesterase within the neurosecretoryperikarya. Note differential reactive response amongst the cell types as well as intranuclear contents. X 125

AT 25°C

Overall reaction for alkaline phosphomonoesterase turns to be moderate. Cells of larger dimension have nuclei with prominent nucleoli and intranuclear material. Cytoplasm also contain considerable quantity of enzyme positive particles but their disposition seems to be very thin. Cells of smaller dimension, too, have prominent nuclei and the cytoplasm is ladened with thin distribution of the enzyme. In some cases, strong reaction for the presence of the enzyme may be found within the perikarya but their appearance along the axonal processes is not always visible (Figs. 4).

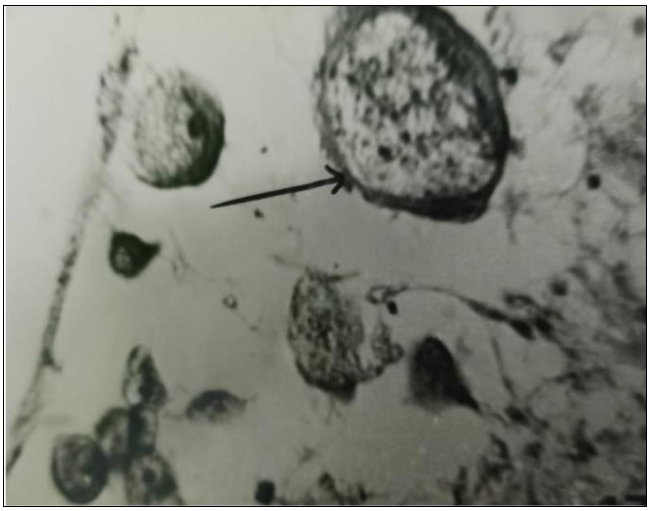


Fig 4: Section showing the extent of alkaline phosphatase reaction amongst cell types to assess distribution of the enzyme, Note moderate to thin distribution and rich reaction in large and small cells, X 532

3. Hyperthermal exposure AT 30°C

Nuclei remain prominent and the intranuclear material as well as nucleoli show intense positive reaction. These features are elicited amongst cells of larger to medium

dimension. Cytoplasm, however, remains weak positive (Fig. 5). Contrastingly, cells of smaller dimension have conspicuous nuclei; the cytoplasm is endowed with more alkaline phosphatase-positive granules which are often deep brown to black. Intensity of reaction in comparison with the preceding group to some extent is in a low gear.

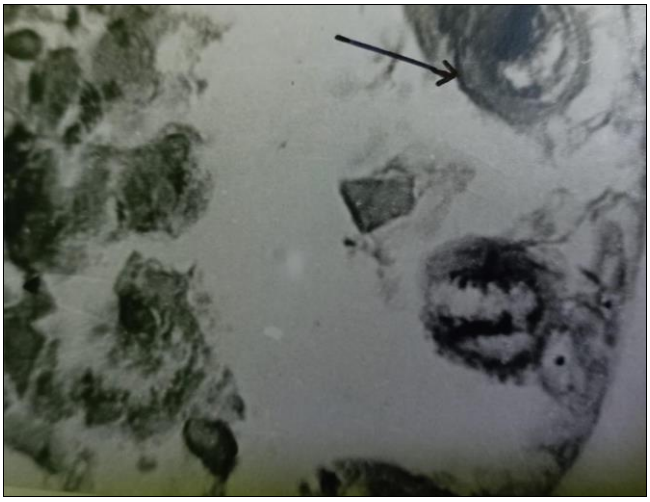


Fig 5: Section showing a few large type of neurosecretory cells possessing weak alkaline phosphatase reaction. Note intensely stained nuclei. X 532

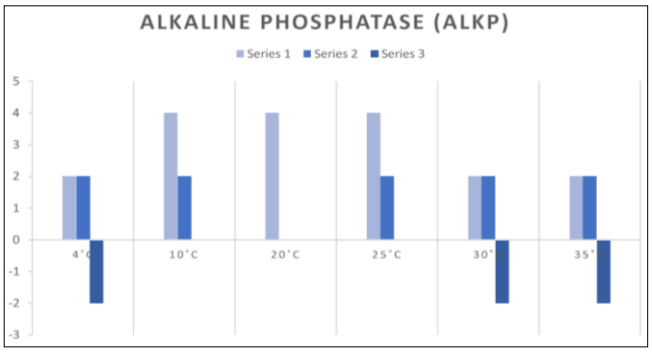
AT 35°C

Extent of reaction is not shifting from what is observed in the preceding group of individuals. But amongst cells of larger to medium dimensions, the nuclei remain near silent and sometimes display reticular appearance despite visibility of the nucleoli. Cytoplasm, in general, have brown colouration and hardly particulate consistency of alkaline phosphatase positive material is detected. Cells of smaller dimension, however, have conspicuous nuclei with beaded intranuclear chromatin material. Cytoplasm may contain thin distribution of alkaline phosphatase positive material.

Table 1: Showing alterations in the histochemical profiles of cerebral neurosecretory cells of Lymnaea (Radix) luteola under the variable temperature regimes.

Methods adopted	Reacting sites/groups	Hypothermal		Near Ambient		Hyperthermal	
Calcium Cobalt	Alkaline (Alkp)Phosphatase	4º C	10º C	20ºC	25ºC	30ºC	35ºC
		+/+-	++/+	++	++/+	+/+-	+/+-

+- Doubtful reaction
+/++/+++ Reactions of increasing intensity



Series 1: Reactions of increasing intensity

Series 2,3: Doubtful reaction intensity of Alkaline Phosphatase (AlkP) reaction is well documented in the Hypothermal and Near Ambient temperatures but in the Hyperthermal situation the same intensity dwindles.

Discussion

Histochemical techniques are frequently used to determine the biochemical properties of the neurosecretory material in vertebrates and other animals. This relates to the precise link between the secretory contents and the material that has been traditionally discolored. Numerous histochemical tests for NSM in various invertebrate groups, including pulmonates, have revealed that neurosecretory cells contain three main constituents (proteinaceous, lipidic, and glucidic) in varying amounts. As a result, the NSM may occasionally be referred to as glycoprotein, lipoprotein, or glycolipoprotein, depending on the situation (Kulkarni and Mane, 1981; Rajlakshmi Bhanu *et al*., 1983; TrinadhaBabu *et al*., 1989; and Choudhuri and Nanda, 1990) [7, 17, 27, 34].

Prior research by Rajlakshmi Bhanu *et al*. (1983) [7, 27] on the gastropod Thais bufo (Lamarck) revealed that the neurosecretory material is a lipoprotein-glycoprotein combination. Nonetheless, Boer (1965) [8] focused on the types of cerebral ganglionic cells and the conditions of their formation; as a result, Gomori-positive and Gomori-negative cells exhibit distinct affinities for glycogen and mucopolysaccharides. He claims that while lipid content is "dubious," the elaboration's proteinaceous nature is universal.

Further demonstrates the existence of reactive response amongst the neurosecretory elements under inhospitable situation and in that event certain biochemical components like free aminoacids, proteins and nucleic acids (Das and Singh, 1972) may play an important role in this regard (Nanda *et al*., 1977). Effects of both high and low thermal exposures encompass some salient changes with respect to the abundance of heterochromatin substances, electrondense granules and their disposition within the microtubular processes, appearances of the cytoplasamicfibrillar elements etc. (Nanda et. al.,2023) [23, 25]

Effects of thermal stress on the Neurosecretory complements of the species under study portray contrasting neurosecretory profiles. During hypothermic exposures the clarity of neurosecretory cells becomes obvious as and when their identity wireference to the staining intensities, number, distribution of intercellular secretoryproducts and axonal transport are taken into account. Incidentally, some of the Neurosecretory elements may reveal cytoplasmic vacuolations despite features subscribing secretory phases (Nanda et. al. 2023) [23, 25].

The fluctuating intensity is to be accounted as and when the reactive responses under variable temperature regimes are referred to. It is intriguing that hypothermic conditions demonstrate, on some occasions, doubtful reactions similar to what is being found at hyperthermal situations. In the latter case, however, PAS-silence amongst most of the neurosecretory elements is more evident. (Nanda et. al.,2023) [23, 25]

Effects of both high and low thermal exposures encompass some salient changes with respect to the abundance substances, electron dense granules and their disposition within the of heterochromatin microtubular processes, appearances of the cytoplasamicfibrillar elements etc. Such changes from one temperature regime to other provide clues to ascertain the structure- function relationship in the

context of the secretory behaviour of the cells concerned. This is specially pertinent when environmental flux in the natural habitat of the species under study is considered (Nanda et. al., 2023) [23, 25].

Conclusive Remark

Cytochemical test for the localization of alkaline phosphomono-esterase in the neurosecretoryperikarya of several invertebratespotray contradictory results. Lack of this enzyme in Iphitalimbateand Bombyx mori had been reported by Nayar (1959) [26] and Ganguly and Basu (1962) [12] respectively. In contrast, positive response for this enzyme has been claimed in the neurosecretory system of Gryllodessigillatus (Tewari and Awasthi, 1968) [32, 33], frontal ganglionic neurosecretory cells of P. americana (Nanda and Goswami,1978) [21] and cerebral neurosecretory territories of P. monodon (Nanda and Ghosh,1985) [20]; supra and suboesophageal ganglion of P. americana (Verhaert *et al*., 1990) [35]. In their studies on earthworm, Metaphirepeguana, Chaudhuri and Nanda (1990) [9] mentioned a close parallelism in the distribution of NSM and alkaline phosphatase in the ventral ganglionic neurosecretory elements. Such criteria are consistent with heightend synthesis of NSM and alkaline phosphatase activity. In the present studies on Lymnaea (Radix) luteola, moderate distribution of the enzyme in the cerebral neurosecretory elements may be correlated with the secretory potentials of the cells in question when ambient temperature is called for. retardation in the Hypothermic or hyperthermic situations render enzymic activity perhaps through interference in the permeability of may have relevance with respect to the the macromolecules. This fluctuation of the seasonal variation (Lomte and Nagabhushanam, 1974) [18, 19]. Nevertheless, implication for protein synthesis in such situation may be adhered to (Kimura and Ichihara, 1980) [16].

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