

STUDIES ON THE NEUROSECRETORY SYSTEM OF *IPHITA LIMBATA* STAL.

Part II. Acid Phosphatase and Cholinesterase in the Neurosecretory Cells

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INTRODUCTION

IN the study of the finer structural details of neurosecretory cells, a determination of their enzyme content would seem desirable inasmuch as these specialised neurons perform functions which require high metabolic activity. Only few studies of this kind have been undertaken, none of them in insects. The present paper reports on the localisation of certain enzymes in the neurosecretory cells of the brain and of the metathoracic ganglion of the common Indian plant bug *Iphita limbata* Stal. (Pyrrhocoridae: Hemiptera—see Nayar, 1953, 1955).

METHODS

The neurosecretory cells of the pars intercerebralis of the brain of the adult insects were dissected out with as little additional brain tissue as possible under the dissecting microscope. Very often it was possible to pick up the cluster of 16 cells on each side with just a film of brain tissue supporting it, so that the treatment was practically confined to the neurosecretory cells. After being rapidly washed in buffer, the material was placed in a dish containing the incubating medium for the specific enzyme. The lead method of Gomori (1952) was used for acid phosphatase. Control material treated with Lugol's iodine or boiling water which inactivated the enzyme was also studied. Incubation periods of 6 to 8 hours at 37° C. gave the best results. For alkaline phosphatase, the method recommended by Gomori (1952) was employed. Koelle's method, as modified by Gomori (1952), was used for the study of cholinesterase and proved successful. The preparations were mounted in Farrant's medium. Seligman and Rutenberg's (1951) and Shelton and Schneider's (Pearse, 1953) methods were used for the study of succinic dehydrogenase. The latter proved more satisfactory. Control sections were incubated with sodium malonate added to the mixture.

OBSERVATIONS

In the neurosecretory cells of the pars intercerebralis acid phosphatase may be observed in considerable amounts. Whereas the nuclei of these cells show comparatively less enzyme than those of ordinary ganglion cells, the cytoplasm of secreting neurons may be so full of it that details of structure and localisation are obscured (Fig. 1). In cells flattened by pressure and in those where the enzyme is less abundant, the granular nature of its deposits becomes apparent. Some neurosecretory cells did not seem to contain acid phosphatase; they may have been in a different phase of secretory activity at the time of fixation. The enzyme was absent in control preparations in which it had been inactivated by Lugol's solution or boiling water.

The neurosecretory cells of the metathoracic ganglion showed the same results as those of the pars intercerebralis (Fig. 2).

No indication of alkaline phosphatase was seen in the neurosecretory cells of *Iphita*.

Cholinesterase appears in the form of brown, irregular particles in the neurosecretory cells of the brain. These particles are loosely distributed in the cytoplasm and extend into the axons. Their presence imparts an over-all brown colouration to the pars intercerebralis.

With neotetrazolium according to Shelton and Schneider's technique, it was observed that the neurosecretory cells alone remain colourless, while the rest of the brain tissue becomes deep purple. This indicates the absence of succinic dehydrogenase in the specialised cells. When blue tetrazolium was used according to Seligman and Rutenberg's method, the formation of blue diformazan particles was noted in the cytoplasm of the nerve cells with the exception of the neurosecretory neurons. Rarely the cytoplasm of the neurosecretory cells showed red coloured granules (monoformazan) indicating sites of low succinic dehydrogenase activity.

DISCUSSION

No study has so far been reported on the enzyme content of neurosecretory cells in invertebrates.

High concentrations of alkaline phosphatases have been found in various secretory cells (Danielli, 1954), including neurosecretory cells in mammalian hypothalamic centres (Scheibler: *see* Scharrer, E and B, 1954). It is of interest to note that alkaline phosphatase is absent in the neurosecretory cells of *Iphita limbata*, and that in this insect its place is taken by a phosphatase in the acid pH range. This observation is in line with the report

of Eränkö (quoted by Scharrer, E. and B., 1954) who found acid phosphatase in the hypothalamic neurosecretory cells of certain mammals. Regarding the phosphatase content of the nuclei of nervous tissue, acid phosphatase is less abundant in those of the neurosecretory cells than in those of ordinary nerve cells. By contrast, the nuclei of a variety of other cell types seem to contain alkaline phosphatase (Chevremont and Firket, 1953; Danielli, 1954).

According to Pearse (1953) acetyl cholinesterase is the predominant enzyme in the central nervous system and in sympathetic ganglia. Richards and Cutcomp (quoted by Roeder, 1953) have shown that the ganglia of *Periplaneta* and the brain of *Apis* contain proportionally more true cholinesterase than mammalian autonomic ganglia. In this connection it is of interest that the neurosecretory cells of *Iphita limbata* contain a moderate amount of cholinesterase which appears in the form of brownish particles in the cytoplasm of the perikaryon and axon, but seems absent in the nuclei.

Whereas no succinic dehydrogenase has been observed in the neurosecretory cells of *Iphita*, we have been able to demonstrate it in the A and B types of neurosecretory cells in the thoracic ganglion of the crab *Paratelphusa hydrodromous* (Nayar and Parameswaran, unpublished).

SUMMARY

1. Most of the neurosecretory cells of the brain of *Iphita limbata* have a high concentration of acid phosphatase in their cytoplasm. Some cells do not show the enzyme in the cytoplasm. The nuclei of the ordinary brain cells contain acid phosphatase, but those of the neurosecretory cells show comparatively smaller amounts. The neurosecretory cells of the meta-thoracic ganglion also contain this enzyme.

2. The cytoplasm of the neurosecretory cells of the brain contains cholinesterase.

3. Alkaline phosphatase and succinic dehydrogenase are absent in the neurosecretory cells of the brain.

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EXPLANATION OF FIGURES

FIGS. 1-2. Fig. 1. Pars intercerebralis of the brain of an adult female of *Iphita limbata* Stal., showing three neurosecretory cells completely blackened being localisations of acid phosphatase. The large nuclei are those of the neurosecretory cells and the smaller ones are those of ordinary neurons. After 6 hours of incubation, $\times 160$. Fig. 2. Part of metathoracic ganglion showing the nuclei as seats of acid phosphatase. Some of the neurosecretory cells are seen with cytoplasm containing abundant acid phosphatase, $\times 95$.



FIG. 1

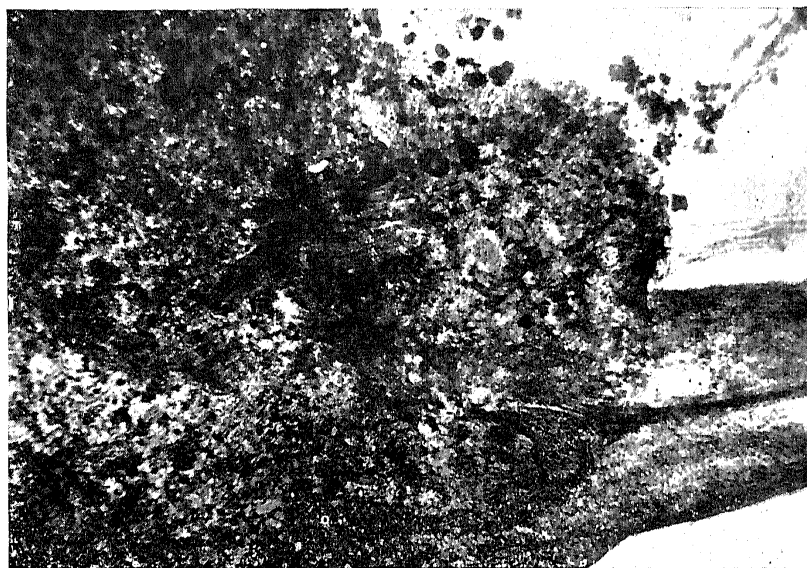


FIG. 2