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HISTOCHEMICAL STUDIES OF PHOSPHOMONOESTERASES IN DIFFERENT TISSUES OF DESERT LOCUST, *SCHISTOCERCA GREGARIA* (FORSKAL)

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Acid and alkaline phosphatases were demonstrated histochemically in various tissues of the desert locust using naphthol AS-phosphates as substrates. Sharp localization of phosphatase activity as insoluble red (acid phosphatase) or blue (alkaline phosphatase) chromogens resulted. Distinct acid phosphatase activity was localized in neurons while moderate alkaline phosphatase activity was mostly localized in the neuropile mass of the brain.

The cuticle was devoid of both of these enzymes. The cardiac muscles showed weak acid and no alkaline phosphatase activity. The pericardial cells were positive for acid phosphatase and negative for alkaline phosphatase. Haemocytes and haemolymph showed no phosphatase activity.

The tracheal and muscular systems were devoid of both enzymes. Some of lobes of the adipose tissue showed weak acid and faint alkaline phosphatase activity. The functional aspect of these organs has been correlated with the physiological distribution of the phosphatase enzymes.

Introduction

The histochemical localization of phosphomonoesterases in different tissues of insects was undertaken by many workers [1–13]. Most of them localized these enzymes in holometabolous insects. Therefore, present work was undertaken to study these enzymes in a hemimetabolous insect and to correlate them with the most probable functions in different tissues. This study does not include digestive and reproductive systems, which have been dealt with separately.

Materials and Methods

(a) *Rearing of Schistocerca gregaria (Forskal).*—The locust were bred as described by Qureshi and Ashrafi[14] with certain modifications over the technique described by Jones.[15] The egg pods were incubated in an incubator running at 34°C and the nymphs of different instars were kept in different cages.

(b) *Histochemical Techniques.*—The histochemical studies were undertaken on sexually mature adult locusts starved for 48 hr. Immediately afterwards they were chilled for 40 min at a temperature of −10°C to avoid serious post-mortem distortion of phosphatases.

The dissections were done quickly in a dish containing equal volumes of alcohol and acetone, placed on crushed ice. This gave satisfactory results for the study of phosphatases. The slices (3×3 mm) of chilled tissues were placed in the fixative at −10°C for 24 hr.

Two baths of the fixative, after 8 hr interval were given for fixation and dehydration. The tissues were then washed twice for 15 min each in cold chloroform and were embedded in paraffin wax of 45–50°C melting point under reduced atmospheric pressure for 10–25 min, depending upon the thickness of the tissues. Sections cut at 9 microns were carried over microscopic slides and stretched over hot distilled water at 38–40°C. They were dewaxed in cold xylene for 30 min and passed successively through 100%, 90%, 75% and 35% alcohols for about 2 min in each grade.

The blank slides were prepared by treating them with dilute nitric acid for 10 min, and then transferred along with other slides to the incubation mixture held at the water bath temperature of 38°C from 30 min to 2 hr.

(c) *Incubation Mixture.*—The incubation mixture was prepared as described in Sigma Technical Bulletin, No. 82 (anonymous).[16] Napthol-AS-MX(A) and Naphthol AS-MX(L) phosphates were used as substrates for acid and alkaline phosphatases respectively. They hydrolysed and yield naphthols which are readily coupled with diazonium Red Violet LB and Fast Blue RR Salts to form insoluble chromogenic precipitate, red for acid and blue for alkaline phosphatase.

These compounds are capable of giving exceedingly sharp localization over a wide pH range.

The level of enzyme activity was estimated visually according to the intensity of coloration obtained. The sections were mounted in glycerol jelly.

Results

(a) *Nervous System* (Fig. 1–3).—The acid and alkaline phosphatases were not uniformly localized in the brain of *Schistocerca gregaria*. Distinct acid phosphatase activity was noticed in neurons at the periphera[1] region of the brain. Weak alkaline phosphatase activity was irregularly distributed, mostly in the neuropile mass. Moderate acid and alkaline phosphatase activity was also not uniformly localized in the ventral ganglia and nerve cord.

(b) *Dermal Tissues, Blood and Heart* (Fig. 4).—Acid and alkaline phosphatase activity in the cuticle of the desert locust was absent but the epidermal cells showed weak acid and no alkaline phosphatase activity. Cardiac muscles and dorsal diaphragm showed weak acid and no alkaline phosphatase activity. The nuclei of the cardiac muscles were strongly positive for acid phosphatase. The pericardial cells were rich in acid phosphatase activity, while no alkaline phosphatase was noticed in them. Haemolymph and haemocytes showed no phosphatase activity.

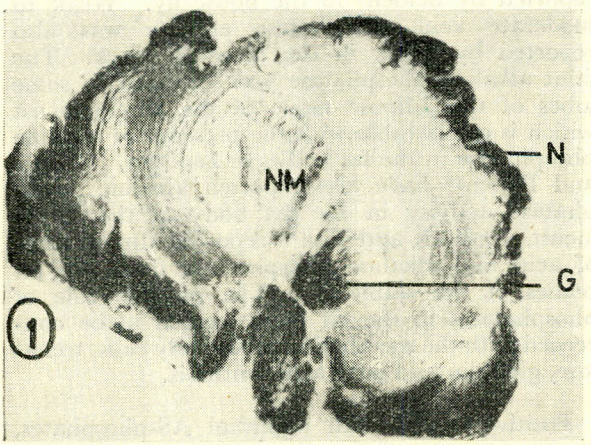

Fig. 1.—Transverse section of the deutocerebrum showing acid phosphatase activity in the neurons (N) and globulus(G). The neuropile mass (NM) is devoid of the enzyme activity. ×180.

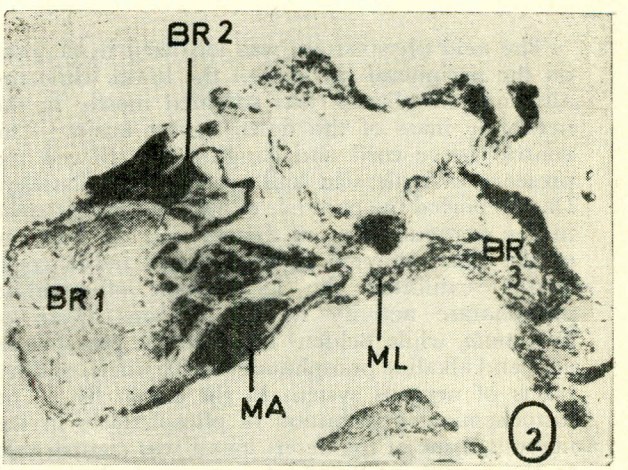

Fig. 2.— Vertical section of brain showing patchy alkaline phosphatase activity in the deutocerebrum (Br_2) and tritocerebrum (Br_3). The activity is absent in protocerebrum (Br_1), whereas the motor center for antennary nerve (MA) and labrum nerve centre (ML) are densely positive ×180.

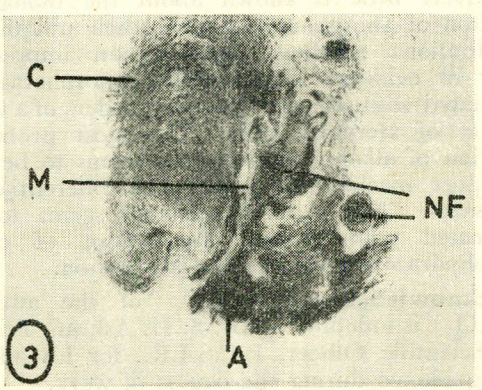

Fig. 3.—Transverse section of the ventral nerve cord showing patchy acid phosphatase activity in the medulla (M) of the nerve cord on the nerve fibres (NF). The cortex (C) is without the activity. The activity of the enzyme is dense on the axon(A). × 180.

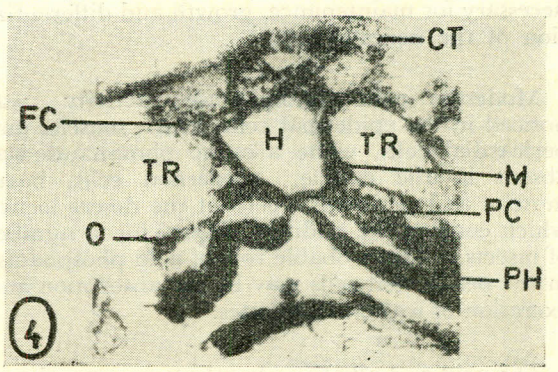

Fig. 4.—Heart (H) showing moderate acid phosphatase activity in the cardiac muscles (M). The pericardial cells (PC), Oenocytes (O) and phagocytes (PH) are also densely positive. The fat cells (FC) are moderately positive, whereas cuticle (CT), and tracheae (TR) gave no reaction for the enzyme. × 270.

(c) *Tracheal System.*—The acid and alkaline phosphatases were found to be absent in the tracheal system of the desert locust.

(d) *Muscular Tissue.*—Thoracic, abdominal and femoral muscles of the desert locust were devoid of phosphatase enzymes.

(e) *Fat Bodies.*—Some lobes of the desert locust fat bodies showed very weak acid phosphatase in the form of intracellular particles. A few lobes exhibited faint alkaline phosphatase activity.

Discussion

The acid phosphatase was localized in neurons on the peripheral margins of the brain while the alkaline phosphatase was confined mostly in the neuropile mass of the desert locust brain. The ventral nerve cord and ganglia also showed the presence of both acid and alkaline phosphatases. Day[2] reported the presence of alkaline phosphatase in the nervous tissue of *Locusta* and the absence of the same enzyme in the ventral nerve cord. Yao[3,4] exhibited the localization of alkaline phosphatase activity in the nervous tissue of *Drosophila*, while Belden[8] reported the presence of acid and alkaline phosphatases in the nuclei of fibre tracts of nervous system in the house fly. The histochemical distribution of phosphatases in the nervous tissue of the desert locust was comparable to the reports of Stay[9] who reported sharp acid phosphatase and little alkaline phosphatase activity in the nervous tissue of the blow fly larva. The possible role of the phosphomonoesterases in the nervous tissue may be correlated with the intermediary metabolism and protein synthesis necessary for maintenance, growth and differentiation of the nervous cells.

Moderate acid phosphatase activity was noticed in the epidermal cells, heart, muscles and pericardial cells, while alkaline phosphatase was absent in the cuticle, epidermal cells, heart muscles and pericardial cells of the desert locust, which confirm the findings of Day[2] for a number of insects. The probable role of acid phosphatase in the pericardial cells may be the absorption and excretion of waste metabolites.[17]

The acid and alkaline phosphatases were absent in the tracheae of the desert locust. Their absence has been reported in the tracheal system of *Blattella*, *Periplaneta*, *Ctenolepisma*, *Tenebrio* and *Lucillia* by Day,[2] house fly by Belden[8] and stable fly by Ashrafi and Fisk.[10,11]

The acid and alkaline phosphatase activity was absent in the thoracic, abdominal and femoral muscles, except the slight alkaline phosphatase activity in the circular muscles of the anterior midgut of the desert locust. Absence of acid phosphatase confirms the reports of Belden[8] for lateral tergosternal and ventral abdominal muscles of the house fly. Ashrafi and Fisk[11] reported the absence of these enzymes in the muscles of the stable fly which is similar to the present findings. The difference with Stay[9] for the presence of phosphatases in the muscles of blow fly larva may be due to the technique applied.

The acid phosphatase was found in the form of intracellular particles in the lobes of the fat bodies of the desert locust. Similar findings have been reported by Belden[8] in the house fly. Weak to moderate acid phosphatase activity was also reported by Stay[9] in the blow fly larva. The faint alkaline phosphatase was localized in some lobes of the adipose tissue of the desert locust which is comparable with the presence of alkaline phosphatase in the fat bodies of *Locusta*. George and Eapen[18] have also reported alkaline phosphatase activity in the fat body of the desert locust. Ashrafi and Fisk[11] reported the absence of acid and alkaline phosphatases from the fat bodies of the stable fly. The possible role of phosphatases in the fat body seemed to be concerned with the metabolism of fat body cells, trehalose, globulin and fatty acid synthesis.

Positive results with naphthol AS-phosphates, in the presence of the diazonium salts of higher coupling energy offer conclusive proof of acid and alkaline phosphatase activity at the site of dye deposition, as described by other workers.[11,16] Relatively little is known about the biological function of phosphatases despite their ubiquitous distribution. Anyhow, there is an impressive body of evidence that alkaline phosphatase is associated with selectively active borders of a wide variety of secretory cells. The most probable function of alkaline phosphatase seems to be the transport mechanism, growth and differentiation of tissues. The acid phosphatase seems to be connected with general metabolism of cells, carbohydrate metabolism and absorption.

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