PHYSIOLOGY OF LOW TEMPERATURE ACCLIMATION IN TROPICAL POIKILOTHERMS

V. Changes in the Activity of Neurosecretory Cells in the Earthworm, *Lampito mauritii*, and Evidence for a Humoral Agent Influencing Metabolism

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INTRODUCTION

It has been shown (in earlier papers of this series) that acclimation to cold results in systemic changes and also in an increase in the protein synthetic activity of the tissues, as evidenced by increased nucleic acid phosphorus in the tissues. It is well known that in insects also similar changes occur preceding a moult and that these changes in the insect are initiated by hormonal agents arising in neurosecretory cells. The parallel between the changes preceding the moult in an insect and the changes reported earlier in cold-acclimated poikilotherms suggested to us the possibility of such hormonal agents, arising in neurosecretory cells, being involved in the events leading to cold acclimation in poikilotherms. Hence changes in the neurosecretory cells in the supra and sub-esophageal ganglia of the earthworm acclimated to low temperature were examined, and the influence of the body fluids of cold earthworms on the oxygen consumption of the tissues of normal worms was measured.

MATERIAL AND METHODS

The earthworms (*Lampito mauritii*) collected near Tirupati were used for this study. They were kept in the laboratory at the required temperature (29° and 19° C. as the case may be) in glass troughs containing wet blotter. Worms were acclimated to the required temperature for three weeks before they were used for the study. For the study of the neurosecretory cells the anterior ends of the worms were fixed in Bouin's fluid for 24 hours, dehydrated through an ascending series of alcohols, cleared in benzene and embedded in paraffin. 6 to 8 micra-serial sections were stained in Gomori's chrome-alum haematoxylin phloxin and mounted in canada balsam.
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Tissue respiration was studied using the Warburg constant volume manometric method. The body-wall muscle of normal (29°C) worms was taken in the flask in normal earthworm perfusion fluid (composition: 250 ml. of 0.54 M NaCl; 5 ml. each of 0.54 M KCl and 0.36 M CaCl₂; 1 ml. each of 0.36 M MgCl₂ and 0.44 M Na₂SO₄; and phosphate buffer 100 ml.; the mixture brought to a litre by the addition of distilled water) and to this the body fluids of cold-acclimated worms (obtained as described in Part I of this series) were added. Side by side with these flasks, controls were also used which were similar to the experimental flasks except that an equal quantity of normal worm-body fluid was added instead of the cold worm-body fluid. Of the one dozen flasks used in the Warburg bath at any given time half were controls, while the remaining half received cold worm-body fluids.

RESULTS

1. Changes in the neurosecretory cells.—The prepared slides were examined serially and the neurosecretory cells were mapped with the aid of camera lucida drawings. It was noticed that in general the same neurosecretory cells as in normal animals were also noticeable in the cold-acclimated worms. But the intensity of staining and the density of granulation in the neurosecretory cells in the brain of the cold-acclimated worms was very much greater than in the normal worms (Fig. 1). In some cases the number of cells staining was greater in the cold-acclimated worms than in normal worms.

On the other hand the neurosecretory cells exhibiting secretory activity appear to be different in position in the warm (35°C) acclimated worms (Fig. 1).

2. Effect of cold worm-body fluids on normal worm tissue respiration.—It is seen from Fig. 2 that addition of the body fluid of cold-acclimated worms to the perfusion fluid bathing the body wall muscle of normal worms results in higher oxygen consumption by the tissue. An increase of about 25% over the normal rate of oxygen consumption is noticed. A similar effect is seen to be produced even on the tissues of warm-acclimated worms (Fig. 2 B).

DISCUSSION

The results show that during acclimation to low temperature the activity of the neurosecretory cells in the brain of the earthworm increases. This presumably results in the release of secretory products into the body fluids. The fact that the body fluids of cold-acclimated worms are capable of raising the metabolic rate of the tissues of normal and warm-acclimated worms
Fig. 2. Effect of cold worm-body fluid extract on the respiration of tissues of normal and warm-acclimated earthworms.

(A) Normal worm tissues measured at 28°C.
- Closed circles: with addition of cold worm-body fluid extract.
- Open circles: Controls with an equal quantity of normal worm-body fluid extract added.

(B) Warm (35°C.) acclimated worm tissues measured at 35°C.
- Closed triangles: With addition of cold worm-body fluid extract.
- Open triangles: Controls with an equal quantity of warm worm-body fluid extract added.

might be due to the presence of an activating substance which perhaps is the product of neurosecretion.

The results of the present investigation (presented in this series of papers) indicate the following sequence of events in cold acclimation. Acclimation to low temperature results in a change in the concentration of Na, K, Ca, Mg, Cl and amino acids in the body fluids, and there is an increase in protein synthesis as evidenced by increased dry matter in the cells and increase in nucleic acid phosphorus. All these changes, both cellular and systemic, appear to be triggered by the release of a humoral agent from the neurosecretory cells in the brain. This humoral agent might play an important role not only in triggering the events that cause metabolic compensation through systemic changes and increased protein synthesis, but also in directly influencing tissue metabolism.
As had been pointed out earlier (Part I of this series) the changes in the concentration of ions in the body fluids bathing the tissues are such as to increase muscle metabolism and activity. The significance of these ionic changes lies in the increased activity recorded at low temperature.

The increased rate of protein synthesis that is noticed might result in increased concentration of metabolic enzymes within cells thus permitting higher rates of reaction. It has already been shown that such increases in concentration of enzymes occur during acclimation (Knox, 1958; Precht, Christophersen and Hensel, 1955) and that this induction of increased concentra-
tration of enzyme could be hormone-induced. The present results might therefore be interpreted to indicate that one of the important results of neurosecretory activity during cold acclimation might be the induction of increased synthesis of enzyme protein.

Figure 3 gives form to this summary, without presuming to explain the nature of the relations and the mechanisms involved in the effects produced.

The similarity between the sequence of events occurring during acclimation to low temperature and those occurring in connection with moulting in insects is noteworthy.

SUMMARY

Acclimation of the earthworm, Lampito mauritii, to low temperature (19°C) results in increased activity of the neurosecretory cells in the brain. Addition of body fluids of cold-acclimated worms to the perfusion fluid causes increased (25%) oxygen consumption of normal worm (29°C) tissues as well as warm (35°C) acclimated worm tissues.

The results are discussed and it is suggested that the physiological changes resulting in acclimation to low temperature are triggered by the neurosecretory release of one or more humoral agents which cause systemic changes and induce increased synthesis of enzyme proteins and perhaps also directly act on tissues to raise their metabolic level. Attention is drawn to the similarity of the sequence of events occurring in connection with moulting in insects.

REFERENCES


Fig. 1. Photomicrographs of the neurosecretory cells in the sub-oesophageal ganglion of normal (29°C), cold (19°C) and warm (35°C) acclimated earthworms. (A) Normal; (B) Cold-acclimated; (C) Warm-acclimated. 8 micra sections: Gomori's chrome alum haematoxylin phloxin.