

## STUDIES ON NEUROHUMORAL INDUCTION OF COMPENSATORY MECHANISMS IN THERMAL ACCLIMATION OF POIKILOTHERMS

### I. EFFECT OF INJECTION OF CNS EXTRACT ON THE METABOLISM OF TWO SPECIES OF EARTHWORMS

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#### INTRODUCTION

Poikilothermic animals overcome the stress of the changing environmental conditions to a considerable extent by making alterations in their metabolic activities. It is now well established that animals acclimated to low temperatures show a higher metabolic rate than animals acclimated to high temperatures. This has been reviewed extensively (Bullock, 1955; Precht, Christopherson & Hensel, 1955; Prosser, 1967; Rao, 1967; Vernberg & Vernberg, 1972). It has been shown to be true in the tropical earthworms, *Megascolex mauritii* (Saroja, 1961). Likewise in several other tropical forms like some molluscs, crustacea, insects and arachnids, the rate of oxygen consumption increased on cold acclimation, and the percentage increase in the rate was 15% when measured at 20 °C and 30% when measured at 34 °C (Carlisle & Cloudsley-Thompson, 1968).

The compensatory changes in metabolic rate are also reflected in tissue respiration. However, such studies in various animals have shown that the degree of adaptability of different tissues to acclimating temperatures is variable (Bowler, 1963; Prosser, 1965, Vernberg & Vernberg, 1966). But in the earthworm it has been shown that tissue respiration shows compensatory change on acclimation to low temperature (Saroja, 1962).

The changes in various metabolic activities occurring on cold acclimation in poikilothermic animals are considered to be under the control of the neural and neuroendocrine secretions (Rao & Saroja, 1963; Rao, 1966). In earthworms *Lampito mauritii* the neurosecretory cells of the cold-acclimated worms were deeply stained and more highly granulated than in normal worms (Rao & Saroja, 1963). Increase in the neurosecretory activity on cold acclimation initiates the liberation of neurohumours into body fluids which release active principles that control the metabolic activities in these animals (Rao, 1962, 1963, 1965, 1967). The effects of the body fluids of the cold-acclimated animals on the respiratory rate of the tissues of normal worms *in vitro* in Warburg flasks showed an increase in the respiratory rate of the tissues of normal animals (Rao & Saroja, 1963; Saroja & Rao, 1965; Vijayalakshmi, 1964).

With these results in view, an attempt has been made in the present study to demonstrate the *in vivo* effects of extracts of the nervous system from cold-acclimated earthworms injected into normal worms.

#### MATERIALS AND METHODS

Two species of earthworms *Lampito mauritii* and *Perionyx excavatus* available locally were used to study the effect of low-temperature acclimation on the rate of respiration.

The worms were maintained in the laboratory in glass troughs containing a small quantity of water to keep them moist. The worms kept at room temperature ( $26 \pm 2$  °C) in the laboratory served as controls or normal worms. For acclimation purpose *Lampito* was kept at  $17 \pm 1$  °C in a cold cabinet and this was the minimum temperature that could be tolerated by this species. *Perionyx* which could tolerate a lower temperature was acclimated at  $10 \pm 1$  °C. The worms were maintained at the specified temperature for 15 days to allow them to become acclimated.

Warburg's manometric method was used to study the aerial respiration in the normal and cold-acclimated *Perionyx*. Animals of different size-groups were selected to discover the correlation, if any, between the size and the rate of metabolic activity of the species concerned.

Using Winkler's method, aquatic respiration studies were made in *Lampito*. In this case studies were made on the normal worms, on normal worms injected with the nervous extract of the normal worms and on normal worms injected with the nervous extract of cold-acclimated worms. As in the above mentioned case, animals of different size-groups were selected to discover the relationship between the size and metabolism.

In both cases the measurements were made at room temperature (25 °C).

The body-wall muscles of the normal worms, acclimated worms and normal worms injected with the nervous extract of the cold acclimated *Perionyx* were used to study tissue respiration. The tissues were weighed and well teased in Ringer before transference into Warburg's flasks along with 2 ml of Ringer. A filter paper dipped in 10% KOH was placed in the centre well of the flask to absorb the evolved carbon-dioxide. The flasks were fitted to manometers filled with Brodie's fluid. The readings were taken every 15 min for 1 h. The changes in the height of the open arm of the manometer for different time intervals were plotted (drop in height against time). From the graph, change in height of the column per hour was calculated.

The tissue respiration studies were carried out at different temperatures (20, 25, 30 and 35 °C) to check the effect of temperature on muscle tissues used in the study. Student's 't' test was applied to discover the significance of the changes observed in the respiratory rate of the tissues studied. The  $\mu$  and  $Q_{10}$  were calculated to assess the efficiency of the system in the different groups studied.

The entire nerve cords (including the brain) of the cold-acclimated worms were dissected out attached to a strip of ventral body-wall muscle. These were homogenized in cold earthworm Ringer (approximately 0.5 ml of Ringer for 1 nerve cord). The extract was centrifuged and the supernatant fluid was injected into the normal

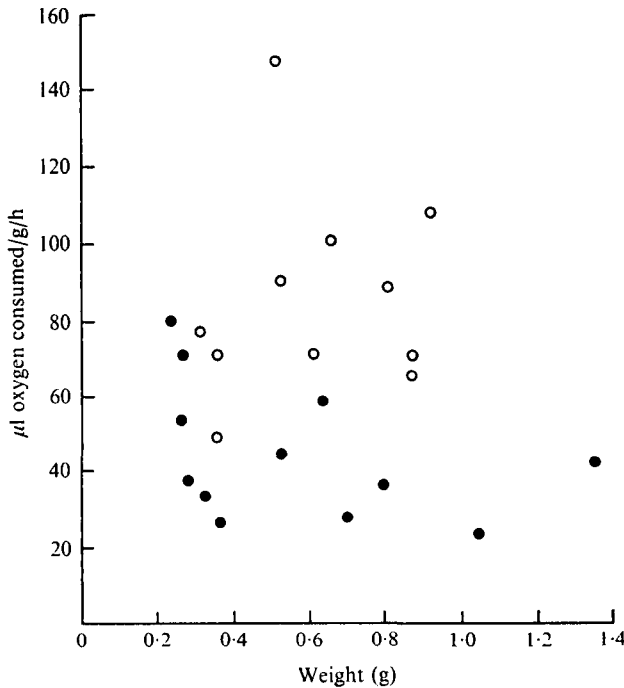


Fig. 1. Oxygen consumption of normal and cold-acclimated *Perionyx* as a function of size.  
●, Normal; ○, cold-acclimated.

worms kept at room temperature. The same procedure was used to prepare the nervous tissue extract from the normal worms. After injection with 0.1–0.2 ml of the extract, the worms were allowed to stay for 2 h in the containers. The rate of oxygen consumption was measured between 2–3 h of injection.

#### RESULTS

As in a majority of the experiments, the nerve cord extracts of the cold-acclimated *Perionyx* (which could acclimate to a very low temperature of 8–10 °C) were used to study the effect of their neuroendocrine secretions in the metabolic enzymes of normal *Lampito*. The respiratory rate of the normal and cold-acclimated *Perionyx* as well as the respiratory rate of their body-wall muscles were studied to demonstrate the compensatory response in this species. It was very well seen (Fig. 1) that the metabolic rate of the cold-acclimated (10–12 °C) *Perionyx* was higher than that of the normal animals at room temperature (23–27 °C). From Student's 't' test it was found that the change in the metabolic rate was highly significant. From these results, it is found that *Perionyx* shows good compensation on cold acclimation.

A similar compensatory response is also noticed in tissue respiration (Table 1). When the tissue respiration was measured at different temperatures, it was found that in case of normal tissues the optimum was at 30 °C; at 35 °C there was a slight decrease in the rate of oxygen consumption. In acclimated tissues the optimum shifted to 25 °C. At 35 °C the respiratory rate was greatly inhibited and a steep drop in the

Table 1. Rate of respiration of the body-wall muscle tissue of normal worms, of worms injected with 'cold' nervous extract, and of cold-acclimated *Perionyx*. (12 °C) worms, measured at 20, 25, 30 and 35 °C

	Acclimated	$t$ $P$	Normal	$t$ $P$	Injected
20 °C Mean ± s.e.	64.25 ± 8.54 (4)	4.54 <0.02	22.32 ± 3.78 (4)	2.84 <0.05	37.06 ± 3.58 (4)
25 °C Mean ± s.e.	141.83 ± 15.44 (6)	5.28 <0.005	57.94 ± 8.02 (5)	9.43 <0.001	158.25 ± 6.97 (6)
30 °C Mean ± s.e.	80.79 ± 10.12 (4)	9.11 <0.001	229.39 ± 12.8 (6)	4.64 <0.1	127.52 ± 17.84 (4)
35 °C Mean ± s.e.	45.01 ± 8.10 (5)	5.91 <0.005	164.16 ± 18.45 (5)	6.31 <0.005	39.28 ± 7.99 (5)

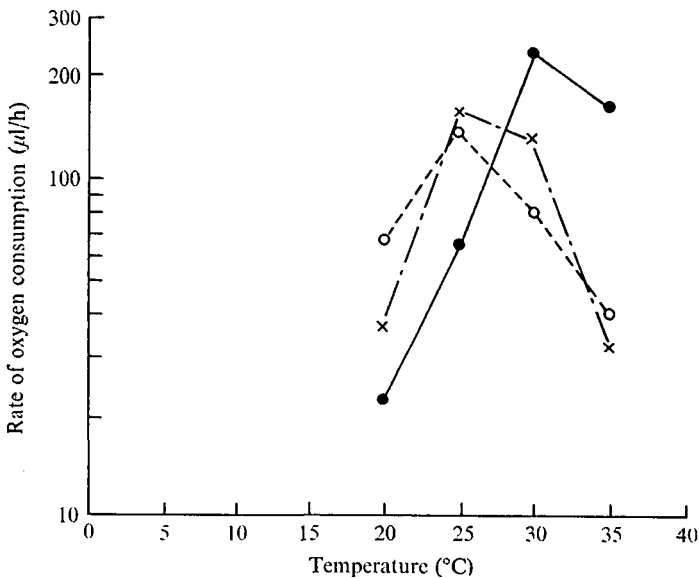


Fig. 2. Rate-temperature curve of the body-wall muscle tissue of normal, injected and cold-acclimated *Perionyx*. ●, Normal; ○, cold-acclimated; ×, injected.

rate was observed (Fig. 2). These differences observed between the normal tissues and acclimated tissues were highly significant.

To study the influence of neurosecretions on the metabolic rate of these animals the nervous tissue extract of the cold-acclimated *Perionyx* was injected into the normal animals. The tissues of the injected animals generally behaved in a way similar to those of acclimated worms (Table 1, Fig. 2). At 20 °C, although the respiratory rate of tissues from injected worms was higher than that of normal tissues, the difference was not statistically significant.

The calculations of energy of activation (20–25 °C) in case of acclimated and normal worms showed that  $\mu$  values for acclimated worms were lower (27.8 kcal) than for normal worms (34.33 kcal), showing the presence of a more efficient system at lower temperature in the acclimated animals. The same was shown by the  $Q_{10}$  values,

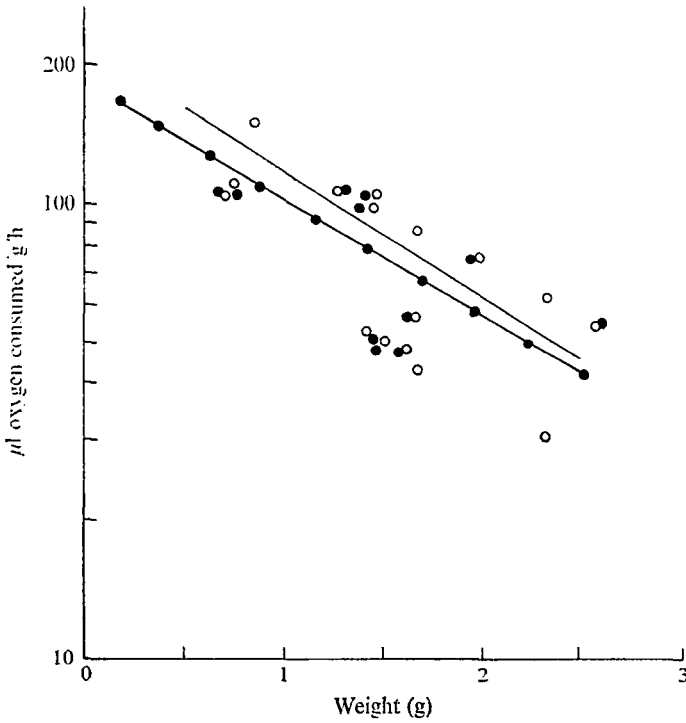


Fig. 3. Oxygen consumption of normal *Lampito* and of *Lampito* injected with the nervous extract of normal *Lampito*. ●, Normal; ○, injected.

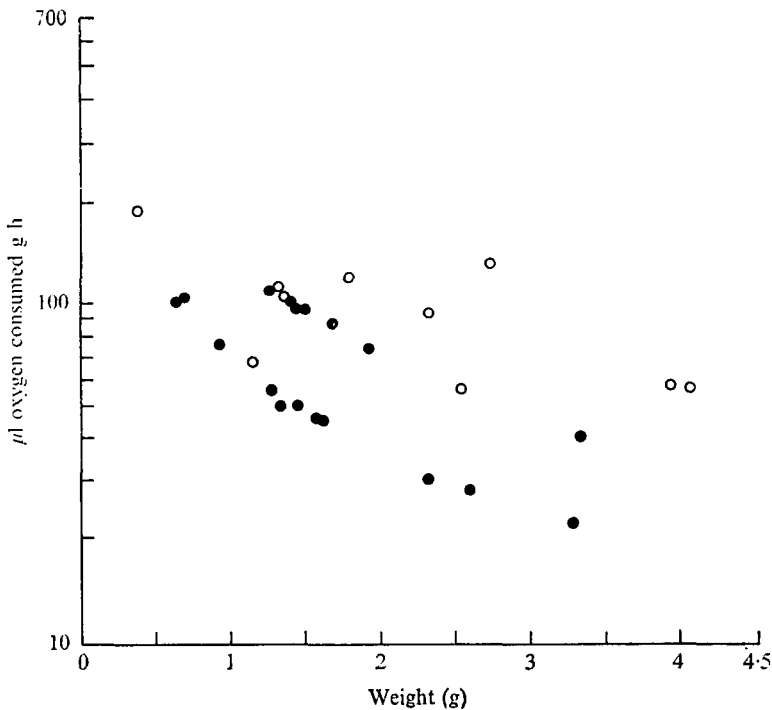


Fig. 4. Oxygen consumption of *Lampito* injected with nervous extract of normal *Lampito* and of *Lampito* injected with nervous extract of cold-acclimated *Lampito* ( $18^{\circ}\text{C}$ ), 2 h after injection. ●, Worms injected with 'normal' nervous extract; ○, worms injected with 'cold-acclimated' nervous extract.

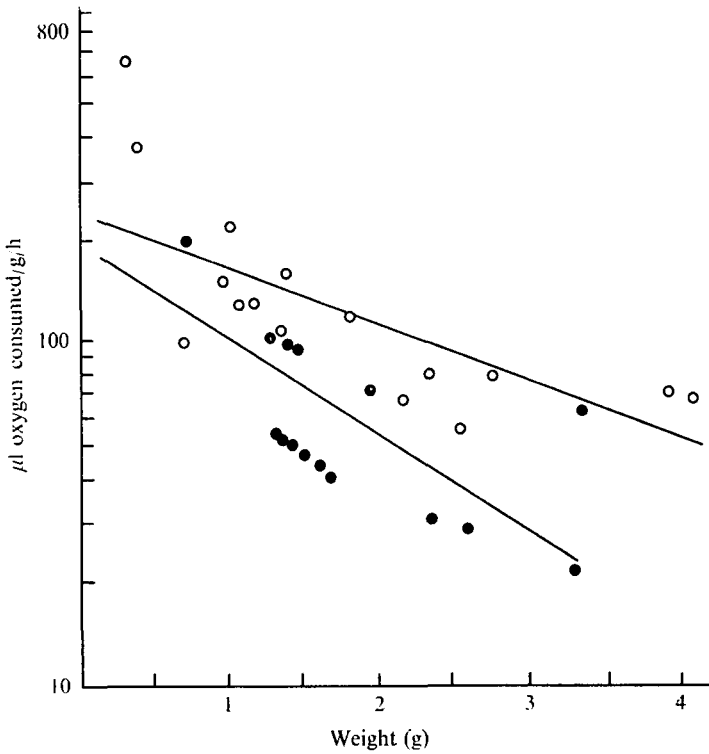


Fig. 5. Details same as for Fig. 4, but values 3 h after injection.

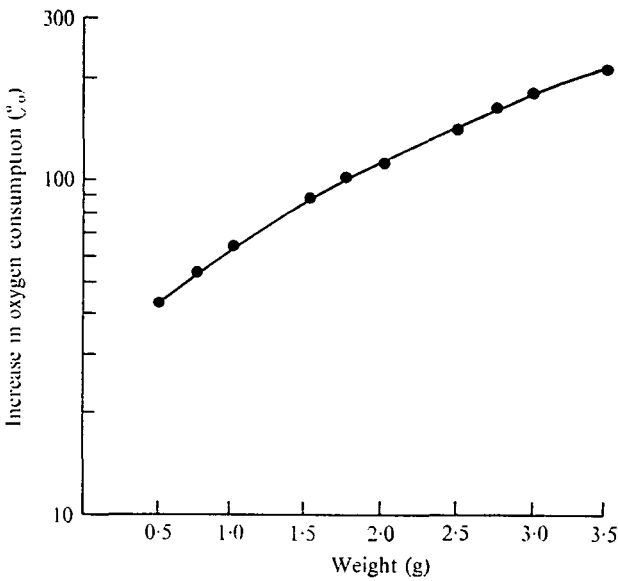


Fig. 6. Curve showing the percentage increase in oxygen consumption in relation to body size in case of worms injected with 'cold' nervous extract. Values obtained from the regression lines in Fig. 5.

where the  $Q_{10}$  for acclimated worms were lower (1.419) than those of normal worms (1.71).

Similar experiments on oxygen consumption in whole worms were performed using *Lampito*. These measurements of oxygen consumption in *Lampito* revealed that there was no significant change in the rate of oxygen consumption in the normal worms when they were injected with the nervous extract of the normal worms (Fig. 3). But when normal worms were injected with the nervous extract from the cold-acclimated worms, it was found that there was a remarkable increase in the metabolic rate after 2 h (Fig. 4, 5).

The percentage increase in case of the worms injected with 'cold' nervous extract was calculated for animals of different sizes and plotted on the graph, taking the values for the metabolic rate of worms injected with 'normal' nervous extract as 100%. This gave the increase in percentage level of consumption with increase in body size (Fig. 6).

#### DISCUSSION

##### *Oxygen consumption and temperature acclimation*

In a majority of cases it has been found that the respiratory metabolism of poikilotherms shows good compensation to temperature acclimation. A change was observed in nature where the winter animals had higher respiratory rate than the summer forms (Edwards & Irving, 1943; Saroja, 1961; Vijayalakshmi, 1964). With regard to laboratory acclimation it has been observed that usually the cold-acclimated forms have higher respiratory rate than warm-acclimated forms. Such changes were observed by Catlett (1971) in case of limpets, *Acmea scabra*. Vernberg (1959) observed that in *Uca* the oxygen consumption rate of the cold-acclimated forms was higher at low temperatures, but at high temperatures was almost similar to or less than the warm-acclimated forms.

A good acclimatory response in a terrestrial arthropod, *Heterometrus fulvipes*, was observed by Vijayalakshmi (1964) in which case the regression line for the cold-acclimated scorpions always remained above the regression line for the normal animals. The earthworms *Lampito mauritii* showed similar trends in metabolic rates when subjected to cold acclimation (Saroja, 1962). Similar studies undertaken now in *Perionyx excavatus* have shown that good compensation to cold took place in these earthworms also. The increase in respiratory rate observed in these forms is highly significant. The cold acclimation has enhanced the metabolic activities of these animals just as has been found in many other poikilotherms.

The importance of the study of tissue respiration lies in ascertaining the energy of activation of various enzyme systems operating in the cellular respiration. Such a study was carried out by Kanungo & Prosser (1959). By providing various substrates like succinate, malate and isocitrate *in vitro*, they studied the change in the respiratory rate of the tissues concerned. By adding the respiratory inhibitors *in vitro* to the samples, Ekberg (1958) and Hochachka & Hayes (1962) were able to study the different pathways that are active on cold acclimation.

In the present study there was a shift in the optimum temperature for tissue respiration towards the lower temperature range on cold acclimation. The lower  $\mu$  values obtained for cold-acclimation forms indicate the efficiencies of the various

enzymes involved in the respiratory mechanism. This is further supported by the low  $Q_{10}$  value at 20 °C and 25 °C for the cold-acclimated tissues.

*Effect of injection of extracts of nervous tissue on metabolic activity*

In highly organized animals where definite endocrine organs are found the role of various endocrine secretions on metabolic activities under changing temperature conditions could be easily studied. Metabolic change observed in the smaller mammals below thermoneutrality was found to be under the influence of hormones (Cottle & Carlson, 1960). The preference of thyroxine-treated *Rana pipiens* larvae for regions of lower temperature might be due to the effect of the hormone on the nervous system. In these forms an increase in heat production with an increase in metabolic rate might result in choosing lower temperature (Lucas & Reynolds, 1967). In young *Larus ridibundus*, the black headed gull, the oxygen consumption rate was increased on cold exposure. In these forms thyroxine and monoamines had no role to play in increasing the metabolic rate. Corticosterone enhanced the metabolic rate in 15–20 min after injection. Elevation in the corticosterone and noradrenaline content of the blood plasma appeared on cold exposure of these animals, whereas decrease in adrenaline content was found (Palokangas & Hissa, 1971).

In the invertebrates very little is known about the influence of the hormonal secretions on the metabolic activities of the organisms. What is known is mostly concerned with hormones of insects. In the case of the milkweed bugs, *Oncopeltus fasciatus*, decrease in oxygen consumption by 20% of the normal occurred on removal of neurosecretory cells (Conradi-Larsen, 1970). In the present study the effect on the rate of oxygen consumption was studied by injecting extracts from the CNS of normal and cold-acclimated *Lampito mauritii* into the normal worms kept at room temperature, and the comparison was made between the recipients of the 'normal' extract and the 'cold' extract. It was found, as shown above, that those worms receiving the 'cold' extract showed an increase in the rate of oxygen consumption compared to the worms receiving the 'normal' extract.

From earlier studies on tissue respiration in the earthworms *Lampito mauritii* (Rao & Saroja, 1963) and in the scorpion (Vijayalakshmi, 1964) conducted in his laboratory, Rao (1967) suggested that the body fluids of cold-acclimated or warm-acclimated animals could alter the respiratory rate of the normal tissue *in vitro*. He considered that hormone-like substances that have been released from the nervous system of these animals into body fluids were responsible for bringing about changes in the metabolic activity on acclimation. It is now demonstrated by *in vivo* injection that the active principle causing these changes could be obtained from extracts of the CNS in these worms. The injected worms receiving the nervous extract from cold-acclimated worms behaved more or less like the cold-acclimated worms themselves although remaining at room temperature.

From the changes noticed in the injected worms it could be suggested that the CNS extract from cold-acclimated individuals contains some hormone-like factor which on injection into normal individuals causes metabolic and enzymic changes in the direction of making such a normal recipient worm similar to a cold-acclimated worm. That this action is probably like that of a hormone is suggested by the fact that these changes are produced within a period of 2–3 h.



It is therefore suggested that some factor (or factors) which may be called 'cold-acclimation factor' is produced in the nervous system on acclimation of earthworms to low temperature.

## SUMMARY

1. The effect of cold acclimation, which brings about changes in the total metabolism, has been studied in the two species of earthworms *Perionyx excavatus* and *Lampito mauritii*.

2. Of the two species the lowest temperature that could be tolerated by *Lampito* was only  $17 \pm 1$  °C whereas *Perionyx* could survive at  $9 \pm 1$  °C.

3. The rate of oxygen consumption of the cold-acclimated *Perionyx* is higher than the rate of respiration of the normal *Perionyx* when measured at room temperature. The tissue respiration of *Perionyx* when measured at 20, 25, 30 and 35 °C shows Prosser's acclimation type IV, i.e. translation at lower temperature range and rotation at high temperature.

4. In *Lampito* the respiratory rate (measured at room temperature) of worms injected with extract of nervous tissue from cold-acclimated worms (18 °C) is higher than that of worms injected with extract of nervous tissue from normal worms.

5. In *Perionyx* the respiration of the body-wall muscle tissue from injected worms, measured at 20, 25, 30 and 35 °C, is found to be similar to that of the tissues of cold-acclimated worms.

6. It is suggested that the neurosecretions of nervous system have an important role in controlling the metabolic activities during cold-acclimation in earth-worms.

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