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A Study of Regulation of Oxidative Metabolism by Substances Present in the Suprapharyngeal Ganglia and Ventral Nerve Cord in Lumbricus Terrestris

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A STUDY OF REGULATION OF OXIDATIVE METABOLISM BY SUBSTANCES PRESENT IN THE SUPRAPHARYNGEAL GANGLIA AND VENTRAL NERVE CORD IN LUMBRICUS TERRESTRIS

by

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INTRODUCTION

Neurosecretory cells have been shown to be present in the central nervous system of both invertebrates and vertebrates. Scharrer and Scharrer (1937) were the first to report the presence of these cells in Lumbricus terrestris. They have since been shown to be present throughout the nervous system of the earthworm (Gabe, 1966). It has been suggested that in the earthworm these cells may be the only source of hormone (B. Scharrer, 1959). Various studies have shown that the secretions of the neurosecretory cells of the brain of the earthworm play a role in the regulation of several physiological functions of the animal, such as control and regulation of maturation of gametes (Gabe, 1966), osmotic and ionic regulation (Kamemoto, 1964; Kamemoto et al, 1966), regeneration (Ralph, 1967), reproduction and growth (Herlant-Meewis, 1956; 1961). These secretions have been shown to play a role in carbohydrate metabolism (Lawrence, Craig, and Clough, 1972; VandenBosch, 1970). Nelson (1969) demonstrated a possible role in oxidative metabolism.

Work by various groups have demonstrated in both vertebrates and invertebrates that, in addition to polypeptide secretion of these neurosecretory cells, there is also monoamine secretion by these neurons or other neurons (Knowles and Bern, 1966). Bianchi (1967), by histochemical techniques, has demonstrated the presence

of amine secreting neurons in the brain and the ventral nerve cord ganglia of the earthworm, Octalasium complanatum. Using electron microscopic techniques Osaki (1966) demonstrated a type of amine neuron in the ganglia of the earthworm, Eisina foetida. Bianchi has suggested that based on morphological and chemical composition, the same type of cell secretes both peptides and amines. However, Myhrberg (1972) by electron microscopic technique has observed some nerve cells in Lumbricus terrestris which are different from neurosecretory cell bodies, in that they have smaller and fewer number of vesicles. These cells are thought to secrete amines.

Rude (1969), by using thin layer chromatography and spectrofluorometric techniques, has demonstrated the relative amounts of the different amines present in the ventral nerve cord of Lumbricus terrestris. Of the catecholamines present dopamine was found to be the predominant one. Adrenalin and noradrenaline were present in relatively small amounts. Serotonin, an indolealkylamine, was present in a much higher quantity amounting to about two times the amount of the catecholamines.

Very little is known about the mechanism regulating metabolic activities in the invertebrates. It has been demonstrated that there are some parallels in structure and function between invertebrates and vertebrate endocrine systems but there are some important differences (Mansour, 1967; Ralph, 1967). Keeley and Friedman (1969) and Keeley and Waddill (1971) demonstrated that

a substance present in the corpus cardiacum of the cockroach played a role in regulating its oxidative metabolism. Nelson, (1969) demonstrated a similar effect of some substance in the brains of Lumbricus terrestris on the oxidative metabolism of this earthworm. Removal of the brain of this organism depressed oxygen consumption of its intestinal and body wall tissues. Addition of brain homogenates to these in vitro systems increased their oxygen consumption.

The purpose of the present study was to investigate the chemical nature of the substance or substances present in the brain of Lumbricus terrestris which affects the oxidative metabolism of excised intestinal and body wall tissue of this earthworm. To accomplish this it was proposed to assay the ninhydrin positive fraction of the water soluble extracts of the supra-pharyngeal ganglia or brain of this earthworm from which the water soluble liquids and monoamines have been removed. The monoamines which are present in the central nervous system of Lumbricus terrestris were assayed as well as water soluble extracts of brain tissue of the rat which have been subjected to the same extraction and purification procedures as the earthworm brain. The effects of these substances on oxygen consumption of intestinal and body wall tissues were studied.

LITERATURE REVIEW

The presence of neurosecretory cells in Lumbricus terrestris was first demonstrated by Scharrer and Scharrer (1937). Since then, other investigators have shown that neurosecretory cells are present in the ventral nerve cord and the brain of Lumbricus terrestris (Le Grave, 1957; Marapao, 1959; Gabe, 1966; and Goudie, 1968).

Although different types of neurosecretory cells have been described in various species of oligochaetes, Herlant-Meewis (1956) has concluded that most of the differences seen are a function of age and secretory activity. On the basis of location, appearance and size, however, there are only two types of neurosecretory cells in Lumbricidae, and these cells have been designated as a-cells and b-cells (Goudie, 1968 and Marapao, 1959).

The secretions of neurosecretory cells in oligochaete worms have been shown to be physiologically active. McVay (1942) demonstrated that homogenates of the brain or the ventral nerve cord of Lumbricus terrestris contained substances which showed positive chromatographic results in the crayfish, Cambarus.

Osmotic and ionic regulation in the earthworms, E. foetida and L. terrestris have been shown to be under the influence of hormones produced in the brain (Kamemoto, 1964). Kamemoto et al. (1962) had previously shown ionic regulation in these forms.

Histochemical studies of the cerebral ganglia from L. terrestris subjected to hypertonic and hypotonic conditions led Goudie (1968) to suggest that secretions of the a-type cells play a role in the ionic and osmotic regulation of the animal. Orenstein (1971) described neurosecretory cells in the ventral nerve cord similar in appearance to those described by Goudie in the brain. He demonstrated a similar response by the a-type cells in this part of the central nervous system to osmotic stress. He further demonstrated that, 2-3 days after debraining, the neurosecretory cells in the ventral nerve cord apparently became secretorily active.

Studies by different research groups have shown that the brain of oligochaetes produces substances (hormones) which are essential for regeneration (Hubl, 1956; Herlant-Meewis, 1961). In his studies with Lumbricidae, Hubl found that the b-type cells of the cerebral ganglia were the source of the hormones which control regeneration in the worm.

The hormones of the neurosecretory cells in annelids have also been implicated in the regulation of growth and reproduction, (Gave, 1966; Herlant-Meewis, 1956; Durchon, 1962). From morphological observations based on histochemical studies of the supra-pharyngeal ganglia of E. foetida and Lumbricus terrestris, Herlant-Meewis (1955) noted that the neurosecretory products of the a-cells increased at the time of maturation and they decreased at the end of oviposition. Removal of the cerebral ganglion at the time of

maturation resulted in the immediate arrest of oviposition and the morphological appearance of a worm in hibernal diapause. She also found that, in E. foetida, after removal of the brain, ovulation occurred only when the cerebral ganglia and the preganglionic capillary network had both regenerated. From these observations, Herlant-Meewis concluded that secretory products of neurosecretory cells, probably a-cells in the brain of E. foetida and Lumbricus terrestris, were responsible for the regulation of reproduction in these animals.

Durchon (1962) observed that elective Ablation of the cerebral ganglia in E. foetida, resulted in the progressive disappearance of somatic sex characteristics and the arrest of the egg laying process. However, when the ganglia regenerated the disappeared characteristics returned to normal. These observations led Durchon to suggest that the secondary sex characteristics of E. foetida are controlled by hormones of the cerebral ganglia.

In general, relatively little is known about metabolic mechanisms in invertebrates and even less about their hormonal control. Most of the work done in this area has been done with insects, arthropods, trematodes and relatively few other invertebrates, (Van Der Kloot, 1962; Tombes, 1970). Lawrence et al. (1972) have shown that the cerebral ganglion of L. terrestris has an effect on blood glucose levels. Removal of the brain of the worm resulted in a decrease of glucose levels, and injection of extracts of the

cerebral ganglion raised glucose levels of both decerebrated and non-decerebrated earthworms. Vanden Bosch (1970) demonstrated that two of the four ninhydrin positive fractions of the partially purified water extract of brains of the earthworm contained hyperglycemic factors.

The brain, corpora cardiaca and corpora allata in insects have been suggested to be the source of a hormone or hormones which influence oxygen consumption and metabolism in these animals. Luscher and Leuthfold (1965) in their study of respiration of isolated fat bodies from Leucophae madare, observed that the addition of brain or corpora cardiaca homogenate to the medium elevated the respiration of the fat body significantly. Wiens and Gilbert (1965) also observed that homogenate of corpora cardiaca stimulated oxygen consumption of fat bodies from L. madare. They suggested, therefore, that the corpora cardiaca may be a source of something (a hormone) that stimulates oxygen consumption and reduces carbon dioxide evolution in vitro by fat bodies of L. madare. Nelson (1969) showed that removal of the brain from L. terrestris resulted in a decrease of oxygen consumption by the excised intestinal and body wall tissues of the worm up to the third day of decerebration. He also showed that addition of homogenates of the brain to the bathing fluid of these in vitro systems increased the oxygen consumption of tissues from both decerebrated and non-decerebrated worms.

Another evidence for the hormonal regulation of respiratory metabolism in insects comes from the work of Keeley and Waddill (1971). They have shown that a neurosecretory substance produced in the brain and stored in the corpora cardiaca, when injected into cardiectomized-allatectomized cockroaches, returned decreased QO_2 of the fat body and mitochondria to normal. However, Keeley and Friedman (1969) had demonstrated earlier that, addition of key glycolytic and Krebs cycle intermediates, disrupted cardiaca or metabolic cofactors, had no significant effect on the oxygen consumption of either the fat bodies or the mitochondria from 30-day cardiatectomized-allatectomized B. discoidalis. They had suggested that this observation may be due to a basic change in the structural or enzymatic unity of the mitochondria in in vitro studies.

Extraction, isolation and purification of neurosecretory substances (hormones) have been carried out in invertebrates. Most of the work in this area has been with insects, molluscs and crustaceans; only little has been done with annelids.

Brown (1965) isolated nine water-soluble substances from cockroach cardiacum, three of which are peptides. The peptides increased the levels of trehalose in the hemolymph of the cockroach, and also an increase in the amplitude and frequency of the cockroach heart and gut. These extracts were active even at very low concentrations. Brown suggested that their effect on the heart and the gut does not necessarily represent a physiological

function and that their participation in other homeostatic mechanisms must be suspected.

Mordue and Golsworthy (1969) observed that extracts of whole corpora cardiaca taken from mature male L. migatoria and S. gregaria produce a significant elevation of the total hemolymph carbohydrate when injected into P. americana. They separated four peptide fractions chromatographically from extracts of whole corpora cardiace from these locusts. Two of these fractions produced significant hyperglycemic effects when assayed on P. americana.

Ichikawa and Ishizaka (1961, 1963) and Ishizaka and Ichikawa (1967) extracted biologically active water-soluble substances (polypeptides) from the brain of the Bombyx pupae. Agarwal and Greeberg (1969) isolated polypeptide compounds with hormonal activity (maintainance of rhythmicity in active hearts) from fresh water molluscs. Kleinholz and Kimball (1965) extracted water-soluble polypeptide substances from the eye stalk of the crustacean P. borealices and these compounds showed cardioregulatory activity and hyperglycemic activity.

Vanden Bosch (1970) developed precedures for extracting the water soluble substances from earthworm brains. These water-soluble extracts, which were partially purified by removing monoamines and water-soluble lipids, were shown to contain four ninhydrin positive fractions. Two of these fractions were shown to produce a hyperglycemic effect when injected into earthworms.

As stated previously, Bianchi (1967), and Osaki (1966) have demonstrated the presence of amine secretory cells in the earthworms, O. complanatum and E. foetida. Myhrberg (1972) localized monoamines in the central nervous sytem of L. terrestris. Rude (1966) determined that, of the catecholamines present in the ventral nerve cord of L. terrestris, dopamine predominated and relatively small amounts of adrenaline and noradrenaline were present. About twice as much serotonin, an indoleamine, was present as the catecholamines. He also concluded that monoamines certainly play a role in neurotransmission in the earthworm. Catecholamines have been shown to act as neurotransmitters in certain invertebrates for example arthropods, molluscs, (Murdock, 1971; Bullock and Horridge, 1965). Whether they play a hormonal role over and above this role of synaptic transmission is not known. Myhrberg (1972) concluded that the amine secreting cells of the central nervous system of L. terrestris did not contain neurosecretory granules and thus were distinct from the characteristic neurosecretory cells.

Amines are observed to produce effects in many different organs and tissues of vertebrates, the effects being manifested in a number of ways. Thus, the heart will beat more forcefully and more rapidly in response to certain amines, while smooth muscle may contract or relax depending on its anatomical location or on its physiological or pharmacological status, or both. Many tissues display alterations in metabolism after exposure to amines, an especially prominent metabolic effect is the increase in glycogenolysis produced by catecholamines in the liver and skeletal

muscle of vertebrates.

With regard to invertebrate metabolism very little has been done with amines especially catecholamines. That serotonin (5-HT) has a multiple role seems clar (Page, 1958). Since the early evidence that serotonin might serve as a cardioregulator in molluscs and crustaceans (Erspamer and Ghiretti, 1951; Welsh 1963), there have been additional indications that serotonin may play other roles in certain invertebrates. Serotonin has been shown to be active on a number of invertebrate muscle prepartions (Colhoun, 1963). This evidence would point to a hormonal role of this amine in insects and possibly all invertebrates.

Evidence has been accumulating that serotonin plays a role in carbohydrate metabolism in both vertebrates and invertebrates, (Mansour, 1959a, 1959b, 1964, 1967; Mansour and Menard, 1960; Sutherland and Rall, 1962; Goodman and Gilman, 1970). Mansour and Lago (1958) showed that addition of serotonin to the parasitic trematode, Fasciola hepatica, produced a considerable increase in glucose uptake, and increased production of lactic acid along with an increased motility. These effects were not observed when catecholamines were added. Mansour (1967) subsequently found that the active phosphorylase content in these parasites was increased following addition of serotonin. The serotonin increased the formation of 3, 5-AMP in the particulate preparations of these worms while the catecholamines did not.

Work of Mansour and others has shown that the effects of

serotonin on carbohydrate metabolism in the liver fluke appear to be analogous to certain effects of catecholamines and glucagon in mammalian tissues. In the liver fluke, for example, serotonin acts and stimulates glycogynolysis directly by activating adenyl cyclase and by promoting the formation of cyclic-AMP thus increasing phosphorylase activity.

Moore and Gosselin (1962) have shown that serotonin increased the endogenous respiration, the rate of glycogen utilization and lactic acid production of the gills of the mollusc, Modiolus demissus, incubated in sea water. Both epinephrine and norepinephrine stimulated lactic acid production by the gills but only at concentrations ten fold higher than that of serotonin. Milton and Gosselin (1960) observed in Edulis, and Modiolus demissus results similar to those above. Serotonin increased the oxygen consumption of homogenates of the gill plates of these molluscs.

Bauchau et al. (1968) have shown that serotonin increases the phosphorylase in the muscle of the crab.

Increase in the amplitude and frequence of contraction of the gut of P. americana was caused by the addition of serotonin. The gut was more sensitive to serotonin than the water-soluble peptides extracted from the corpus cardiacum, (Brown, 1965). Serotonin has been shown to stimulate the contractility of the heart of Venus mercenaria at much lower concentration than the catecholamines. It is speculated that serotonin has a hormonal regulatory function in the carbohydrate metabolism in invertebrates

similar to that of epinephrine in some tissues of higher animals, (Mansour, 1967).

MATERIALS AND METHODS

General Procedures

The earthworms, Lumbricus terrestris, which were used for this investigation were obtained from a local bait shop in Kalamazoo, Michigan. The worms were kept in moist soil and maintained at a temperature range of five to seven degrees centigrade in a refrigerator. Only those worms which showed no body damages or sluggishness and had prominent clitellae, an indication of maturity, were used for the investigation. The intestinal and body wall tissues were used because of the difference in their function and structure. The intestinal tissue is the main storage organ for glycogen and has a higher metabolic activity than the body wall (Urich, 1964; Ogata and Morl, 1964). All measurements of oxygen consumption were made three days post-debraining because it had been shown that the effect of debraining was maximal at that time (Nelson, 1969). The following general procedures and methods were applied to all worms unless otherwise stated.

Tissue Preparation

All worms were anesthetised for three minutes in ten percent ethanol. After the three minutes the worms were removed from the alcohol solution and rinsed in cold tap water. Some of the worms

were debrained and some were not. The brain was excised by making a dorsal longitudinal incision from the second segment to the fith segment. The bilobed .suprapharyngeal ganglion (the brain) thus exposed was quickly and carefully cut away from the surrounding tissues and removed. Both groups of worms, experimental (debrained) and control (non-debrained) were placed in moist paper towelling in separate containers and maintained in the refrigerator for three days. All excised brains were placed either in one milliliter of concentrated methanol or in one milliliter of earthworm physiological saline and kept in the freezer compartment of a refrigerator at a temperature range of $-8^{o}C$ to $-10^{o}C$ for future use.

After the three days the worms were removed from the paper towelling and sections of their intestine and body walls were prepared for analysis of oxygen consumption. This was done by making a longitudinal incision from the fifteenth segment to the last posterior segment. The body wall was then pinned down to expose the intestine and the intersegmental partitions were cut to free the intestine from the body wall. The intestine was slit open and then removed from the body of the worm. Care was taken that no part of the ventral nerve cord adhered. The tissue was rinsed thoroughly in cold 0.8% non nutritive isotonic earthworm physiological saline, (Table 1) to remove not only all the intestinal contents but digestive enzymes as well. A 250 ± 25 milligram sample of the intestinal tissue was placed in the basin of the manometric

flask. This 250 milligram tissue sample displaced 0.25 milliliters of physiological saline (John Nelson, 1969). Three milliliters of saline was added to the tissue in the basin of the flask giving a final volume of 3.25 milliliters.

TABLE I

COMPOSITION OF THE ISOTONIC EARTHWORM PHYSIOLOGICAL SALINE USED IN THIS INVESTIGATION, (WELCH AND SMITH, 1969)

Compound	Amount in Milliliters
0.54 M NaCl	250 ml
0.54 M KCl	5 ml
0.36 M $CaCl_2$	5 ml
0.36 M $MgCl_2$	1 ml
0.44 M $NaSO_4$	1 ml
Phosphate Buffer of pH 7.4	100 ml

This was diluted to one liter with distilled water.

In the cases where additives were added to the contents in the basin of the flask during the experiment, the three milliliters of saline was reduced by the amount of additive to be added. The flask was refrigerated until the other flasks were ready.

Sections of the body wall from approximately the same area from which the intestinal tissues were removed were carefully scrapped so as to remove the blood vessels, ventral nerve cord and the remains of the intestine. These sections of body wall tissues

were then rinsed in cold 0.8% saline and, using the same procedure as outlined above, the tissues were prepared for determination of oxygen consumption. The same weight of tissue and volume of saline was used. It took approximately six to seven minutes to dissect the tissues out and set them up in each manometric flask. Total preparation time for any experiment depended on total number of flasks prepared.

Determination of Oxygen Consumption

Oxygen consumption of the intestinal and body wall tissues was measured by Warburg manometric method (Umbriet, 1968). The gas phase was air and the liquid phase was non nutritive, isotonic earthworm physiological saline. Manometric measurements were made in a constant temperature water bath at $14.5^{o}C \pm .5^{o}C$ equipped with a thermoregulator. A forty minute temperature and pressure equilibration period was allowed with the manometor stopcocks opened before any readings were taken. At the end of the forty minute equilibration period, the levels of the Brodie's solution in the manometers were set at 250 milliliters and the manometer stopcocks were closed. Readings were taken at one hour intervals for four hours.

The Warburg flasks and manometers which were used in this investigation were calibrated by using the method suggested by Umbriet, (1968). The flask constant for each flask was calculated using the formula:

$$K = \frac{Vg\left(\frac{273}{T}\right) + (Vf)a}{Po}$$

K: the flask constant.

Vg: the gas phase of the closed system which in this investigation was air.

Vf: the volume of the fluid phase. The total volume of fluid used in the flask was 3.35 milliliters at all times. This volume was made up of:

i. 250 mg of tissue.

ii. 3 ml of isotonic earthworm saline except in cases where additives were added to the flask content during the experiment.

iii. 0.1 ml of potassium hydroxide.

a: the solubility of the gas (oxygen in this experiment) in the fluid at $T^{o}C$. (a = 0.0368, Umbriet, 1968).

T^{o}: the temperature of the fluid which was $14.5 \pm 0.5^{o}C$.

P_{o}: the pressure of the Brodie's solution in milliliters of mercury which was 10,000.

At the end of the four hours each tissue was blotted dry and placed on an aluminum pan which had been previously kept in a dessicator containing dry rite for six hours to absorb any

moisture which might be present in the pans. The tissues were dried to a constant weight with an Ohaus Moisture Determination Weighing balance, with the bulb set at one inch from the pan and at a setting of six. Thus, the drying temperature was 250°F. All the experiments were run within approximately the same time period, 9am-2pm.

The results obtained for the investigation were expressed in terms of an oxygen quotient. The QO_2 (dry weight) denotes microliters of oxygen consumed, measured under standard conditions, per milligram dry weight of tissue per hour. The mean oxygen quotients for the four hours were determined for each tissue and this mean value was used for all analyses.

Analysis of variance was used to analyse the data statistically and a 90 percent confidence limit was used for significance.

Experimental Procedures

Oxygen Consumption of Tissues From Debrained, Control Non-Debrained Debrained-Decapitated and Control Non-Decapitated Earthworms

Three-day debrained and non-debrained earthworms were used to study the effect of the abscence of the brain on the oxygen consumption (betabolism) of the intestinal and body wall tissues of the earthworm. The tissues were prepared for runs using the procedure previously outlined, except in the case of the decapitated worms where the anterior ten segments of the worm were quickly decapitated before dissecting out the intestinal and body wall

tissues as previously described. The decapitation was done to eliminate the possibility of the suprapharyngeal ganglion releasing hormones or substances during dissectin out of the tissues being studied and thus influencing their oxygen consumption.

Effect of Earthworm Brain Homogenate and Ventral Nerve Cord Homogenate on the Oxygen Consumption of Intestinal and Body Wall Tissues From Control and Experimental Earthworms

The effect of homogenates of earthworm brain and ventral nerve cord on oxygen consumption of intestinal and body wall tissues from three-day debrained and non-debrained worms were homogenized in a hand glass homogenizer for five minutes in physiological saline. In all cases the volume of the homogenate was adjusted to give homogenate concentration of six brains per milliliter. One milliliter of the brain homogenate was added to the manometric flask which contained 2 ml of saline. The final concentration of the brain in the flask was therefore two brains per milliliter of solution. The effect of one brain per milliliter of solution was also studied.

1.059 grams of ventral nerve cord tissue was homogenized in saline and the volume of the homogenate was brought up to 10 ml. One milliliter of the mixture was added to 2 ml of saline in the Warburg flask. The experimental procedure as described before was followed to measure the effect of brain and ventral nerve cord homogenate on the earthworm body wall and intestinal tissues.

Effects of Monoamines on the Oxygen Consumption of Body Wall and Intestinal Tissues From Control and Experimental Earthworms

The effect of the monoamines, epinephrine (adrenaline), moradrenaline, serotonin, and dopamine on oxygen consumption of earthworm intestinal and body wall tissue were studied. Different concentrations of each amine were used and the final concentration of each amine in the manometric flasks are tabulated in Table II. The lower concentrations which were used were those which have been shown by various investigators to be present in the ventral nerve cord of Lumbricus terrestris (Rude, 1969). The second concentrations used were the same for all the amines so that their effects could be compared at the same level.

TABLE II

CONCENTRATION OF MONOAMINES ADDED TO THE INTESTINAL AND BODY WALL TISSUES OF LUMBRICUS TERRESTRIS

Amine	Concentration in ug/ml of solution	
Serotonin	8.3	16.7
Dopamine	3.3	16.7
Epinephrine	1.3	16.7
Noradrenaline	0.3	16.7

Extraction of Water Soluble Polypeptides of Earthworm Brain and Rat Brain

Adult specimens of earthworms were anesthetized in ten percent ethanol and their brains were dissected out using the same technique

used in the debraining of worms described previously. The brains were dissected out at any time of the day whenever it was convenient, even though it was realized that the amounts and types of compounds present in the brain undoubtedly vary seasonally and/or diurnally. The brains were kept in concentrated methanol (reagent grade) and stored in the freezer compartment of the refrigerator at -8° to -10°C till they were used to run experiments. The following extraction and purification procedures were developed by Vanden Bosch (1970). These brains and those which were removed during previous experiments (altogether 2,600) were homogenized in 4 ml of concentrated methyl alcohol in a glass hand homogenizer in an ice bath for fifteen minutes. The homogenate was centrifuged at 10,000 rpm in an International refrigerated centrifuge at a temperature setting of 4°C for 20 minutes. The supernatent was poured off and the homogenation and centrifugation procedures were repeated three more times on the pellet from the centrifugation, using 4 ml methanol for each homogenization. All the extraction and separation procedures were carried at 4°C. The four supernatents were pooled and the methanol was removed by vacuum evaporation using Rinco rotating evaporator. The evaporated material was then lyophilized.

Three extraction procedures were carried out on the resulting dry material to remove the water soluble lipids and the monoamines. The lyophilized material was dissolved in 3 milliliters of chloroform:methanol:water mixture (2 ml chloroform:methanol (2:1) + 1 ml water). The water phase was separated from the chloroform-methanol

phase and it was re-extracted with an equal volume of chloroform:methanol (2:1 v/v) mixture. The water phase was lyophilized.

To remove the amines the lyophilized material was dissolved in 2 ml ether:water mixture (1:1 v/v) and the pH was adjusted to two with concentrated acetic acid. The ether phase was removed and the water phase was re-extracted with 1 ml ether at pH 8.

Thin layer chromatography was used to determine the presence or absence of ninhydrin positive substances present in the water soluble extract and the chloroform and ether extracts. The chromatoplates were 8" X 8" square silica gel (Gelman) and the solvent system was n-butanol:acetic acid:distilled water (2:1:1 v:v:v).

Forty lambda of each extract and standards of the amines and mixtures of all the amines were applied in small spots separately one inch from the edge of the chromatoplates and one inch apart. The concentration of each of the substance used was 40 ug/ml of solution. The spots were allowed to dry and the plates were developed for two hours after which the plates were dried under a hood. The plates were then sprayed with ninhydrin, allowed to dry and then developed for five minutes in an oven at 100°C.

The same extraction procedure described above was used to extract the water soluble ninhydrin positive substances present in one rat brain and to remove monoamines and water soluble lipids.

Bioassay of the Purified Water Soluble Extracts of Earthworm and Rat Brain

The effect of the purified water-soluble extracts on the oxygen

consumption of intestinal and body wall tissues from non-debrained worms were studied. Two different concentrations, 16.7 ug/ml and 33.3 ug/ml. of the extract were used. The same experimental procedure described for the oxygen consumption experiments previously were followed.

RESULTS AND DISCUSSION

Examination of the results of the investigation (Table III) reveals that the body wall in both sets of worms, control (non-debrained) and experimental (debrained) had a much lower QO_2 as compared to the intestinal wall. This was in accordance with what Nelson (1969) found; viz. that the intestinal tissue in the earthworm had a much higher level of metabolic activity than the body wall. In fact, it had been demonstrated to be one of the tissues with the highest metabolic activity in the earthworm (Urich, 1964; Ogata and Morl, 1964).

Table III also shows a statistically significant decrease in QO_2 of the three-day debrained worms for both body wall and intestine. Expressed as percent change, there was a 7.9% decrease in oxygen consumption of body wall and a 43.8% decrease for intestinal tissue. The decrease is much greater in the intestinal wall than in the body wall. Nelson (1969) showed that after debraining the earthworm, the metabolic activity (oxygen consumption) of the intestinal wall tissue decreased gradually and reached a minimum level on approximately the third day after debraining.

These findings suggest that the intestinal tissue is under some control of the brain in its metabolic activity, as measured by the QO_2. This control which is most probably hormonal, is essentially stimulatory, so that removal of the brain causes the decrease in the hormone which in turn results in decreased oxygen consumption.

TABLE III

MEAN HOURLY OXYGEN QUOTIENTS OF INTESTINAL AND BODY WALL TISSUES FROM 3-DAY DEBRAINED AND NON-DEBRAINED EARTHWORMS

Experiment Condition	Intestinal Wall QO_2	Body Wall QO_2
Non-debrained worms	0.588-0.012	0.352-0.003
3-day debrained worms	0.335-0.010	0.320-0.001
Percent decrease in QO_2	43.8	7.9

QO_2 = the mean oxygen quotient in units of microliters of oxygen consumed per milligram of dry tissue weight per hour of time.

15 = runs for each tissue

In addition Nelson's results further show that the oxidative metabolism of intestinal tissue, when the brain's influence is removed, increased after three days to a level equal to or slightly above that which is maintained under the influence of the brain. Several explanations can be given to account for these findings. The writer finds the following explanation very plausible. Approximately three days after debraining, neurosecretory cells in the ventral nerve cord, similar in appearance histochemically to those in the brain, showed secretory activity (Orenstein, 1971). Thus, these cells possibly assume the functions of the brain neuroendocrine cells three days after debraining.

Table IV shows the results of another series of experiments

done to determine whether the dissection for tissue removal, which took approximately six to seven minutes, was traumatic enough to the earthworm that it was affecting the results obtained. It was felt that cutting off the anterior segments (decapitation) prior to the dissection would reveal any major effect of the dissection if these were compared to non-decapitated earthworms. This, in fact, was demonstrated by results presented in Table IV. The QO_2 of the decapitated non-debrained worms was decreased 22.0% for body wall and 25.0% for the intestinal wall. Nelson observed similar results in the earthworm but his results were not statistically significant as his observations were based on very few experiments. The magnitude of the effect of decapitation was very similar in terms of measured QO_2 changes of both the intestinal and body wall tissues. No such drop in QO_2 was observed in decapitated debrained worms. If it is assumed that this response to trauma was mediated through sensory input to the central nervous system, and subsequent release of some factor then it becomes possible to explain the speed with which the information was transmitted and the fact that the response was the same in both the body wall and the intestinal tissue cells. Decapitation of the debrained worms, in this instance, did not result in significant QO_2 change. These results supported the previous observation that the brain does indeed release some substance(s) which does influence oxidative metabolism.

TABLE IV

MEAN HOURLY OXYGEN QUOTIENTS OF THREE-DAY DEBRAINED, THREE-DAY DEBRAINED-DECAPITATED, NON-DEBRAINED, AND NON-DEBRAINED DECAPITATED EARTHWORMS

Experimental Condition	Intestinal Wall QO_2	Body Wall QO_2
Non-debrained worms	0.596±0.013	0.478±0.005
Non-debrained-decapitated worms	0.446±0.008	0.373±0.013
Percent decrease	25	22
3-day debrained worms	0.311±0.017	0.315±0.012
3-day debrained-decapitated worms	0.312±0.017	0.328±0.015
Percent increase	0.322	4.13

30 runs for each tissue.

As the above series of experiments demonstrated the possible presence of some substance(s) affecting oxidative metabolism, further experiments were run to demonstrate that the effect of brain removal was indeed due to some substance present in the brain. The effect of adding crude brain extract to tissues *in vitro* was observed. Two different concentrations were used and the results are presented in Tables Va and Vb.

This crude homogenate caused an increase in QO_2 in all tissues. Taking the normal worms (non-debrained) first we see a 22.9% increase in QO_2 for the intestinal tissue and 13.6% increase for the body wall at low concentration. However, an increase of 13.6% is quite high because if we consider the decrease of 7.9% (see

above and Table III) and also the 34.0% decrease between non-decapitated control and debrained worms (Table IV) then the homogenate influence on the body wall is impressive if not as dramatic as the intestinal tissue. The tissues from the debrained worms showed an increase but not as much as the non-debrained tissues. The increase in QO_2 observed in all the tissues was statistically significant at the 95% confidence level. It may be recalled that three days after debraining the intestinal tissue had the minimum QO_2. The thought that the cells would be responsive to the substances in the brain at this point has been borne out because the effect of brain homogenate on tissues from debrained worms was not as great as that on tissues from non-debrained (control) worms. Muller and Engelman (1968) have shown that in L. madaras, if basal metabolism was high, corpora cardiaca homogenate inhibited response and if metabolism was low, it stimulated metabolic activity. Crude homogenate of corpus cardiacum has been shown to cause both inhibition and stimulation of the gut of the cockroach (Brown, 1965; Cameron, 1953; Davey, 1962). Keeley and Friedman, (1969) demonstrated the possibility that removal of the corpora cardiaca and corpora allata resulted in a change in the structure of enzymatic unity of the mitochondria and that the depression in oxygen consumption of the fat body might be due to the malfunctioning of the mitochondria. This observation could explain the different response of the debrained and non-debrained tissues to the earthworm brain homogenate.

TABLE Va

EFFECT OF EARTHWORM BRAIN HOMOGENATE ON THE OXYGEN CONSUMPTION OF ISOLATED INTESTINAL AND BODY WALL TISSUE FROM THREE-DAY DEBRAINED AND NON-DEBRAINED EARTHWORMS. THE FINAL CONCENTRATION OF THE HOMOGENATE IN THE FLASK WAS ONE "BRAIN PAIR" IS EQUIVALENT TO 1.5 X 10^3 ug OF BRAIN TISSUE PER MILLILITER OF MIXTURE).

Experimental Condition	Intestinal Wall QO_2 No Brain Homogenate	+ Brain Homogenate	% Change	Body Wall QO_2 No Brain Homogenate	+ Brain Homogenate	% Change
Non-debrained worms	$.563 \pm 0.022$	$.692 \pm 0.006$	23.0	$.441 \pm 0.007$	$.501 \pm 0.027$	13.0
3-day debrained worms	$.465 \pm 0.006$	$.534 \pm 0.009$	15.0	$.389 \pm 0.006$	$.434 \pm 0.013$	12.0

QO_2 = the mean oxygen quotient in units of microliters of oxygen consumed per milligram of dry tissue weight per hour.

15 runs for each tissue.

TABLE Vb

EFFECT OF EARTHWORM BRAIN HOMOGENATE ON THE OXYGEN CONSUMPTION OF ISOLATED INTESTINAL AND BODY WALL TISSUE FROM THREE-DAY DEBRAINED AND NON-DEBRAINED EARTHWORMS. THE FINAL CONCENTRATION OF THE HOMOGENATE IN THE FLASK WAS EQUIVALENT TO TWO "BRAIN PAIR" PER MILLILITER OF MIXTURE

Experimental Condition	Intestinal Wall QO_2 No Brain Homogenate	+ Brain Homogenate	% Change	Body Wall QO_2 No Brain Homogenate	+ Brain Homogenate	% Change
Non-debrained worms	.551±0.003	.750±0.009	36.0	.422±0.002	.532±0.002	26.0
3-day debrained worms	.487±0.019	.603±0.021	24.0	.396±0.023	.470±0.014	19.0

QO_2 = the mean oxygen quotient in units of microliters of oxygen consumer per milligram of dry tissue weight per hour.

15 runs for each tissue.

TABLE VI

EFFECT OF VENTRAL NERVE CORD HOMOGENATE ON THE OXYGEN CONSUMPTION OF ISOLATED INTESTINAL AND BODY WALL TISSUES FROM THREE-DAY DEBRAINED AND NON-DEBRAINED EARTHWORM. THE FINAL CONCENTRATION OF THE HOMOGENATE IN FLASK WAS 1.5×10^5 mg. OF NERVE TISSUE PER MILLILITER OF SOLUTION

Experimental Condition	Intestinal Wall QO_2 No Ventral Nerve Cord	Intestinal Wall QO_2 + Ventral Nerve Cord	% Change	Body Wall QO_2 No Ventral Nerve Cord	Body Wall QO_2 + Ventral Nerve Cord	% Change
Non-debrained worms	$.602 \pm 0.003$	$.679 \pm 0.007$	12.8	$.398 \pm 0.002$	$.474 \pm 0.017$	19.1
3-day debrained worms	$.475 \pm 0.008$	$.533 \pm 0.009$	12.2	$.296 \pm 0.003$	$.333 \pm 0.004$	1.0

QO_2 = the mean oxygen quotient in units of microliters of oxygen consumed per milligram of dry tissue weight per hour.

9 runs for each tissue.

From these results it can be said that the observations made in the first series of experiments (decrease in QO_2 of the tissues from three-day debrained worms) were due to the absence of the brain or substance(s) produced by the brain and not some nervous mechanism. Further examination of the results revealed a dosage response by the tissues to the brain homogenate because doubling amount of brain homogenate resulted in increase of QO_2 (Tables Va and Vb).

The effect of the ventral nerve cord homogenate on the intestinal and body wall tissues were similar to those of the brain homogenate, that is increase in the QO_2, but the magnitude of the increase was slightly lower in all the tissues, (Table VI).

The crude homogenates which were used, contained a number of substances in addition to the substances whose presence this experiment was designed to demonstrate. It is possible that some of these compounds, most of which are probably normally not in contact with these tissues, are now in contact with them and would influence the outcome one way or another. In view of this difficulty an attempt was made to find the effect of different separable portions of the homogenate. The biogenic amines adrenaline,noradrenaline, serotonin, and dopamine have been shown to be present in the brain and ventral nerve cord of the earthworm. Two different concentrations of these amines were added to these tissues and their effect on QO_2 measured in attempt to identify the compound that has been described as the substance(s) from the brain affecting QO_2. Tables VII to XII show

the result of these runs. The lower concentrations used are those reported as the concentrations in the brain and the ventral nerve cord of Lumbricus terrestris, (see materials and methods).

In almost all these experiments the various amines caused an increase in QO_2 of tissues from both debrained and non-debrained worms. The low concentration of serotonin also caused a decrease in the QO_2 of the body wall tissues from the debrained worms. By factorial analysis of variance almost all the differences were found to be statistically significant with the exception of those changes in the non-debrained body wall tissue with 16.7 ug/ml of adrenaline, (Tables VI - IX). We see that serotonin caused the highest increase in QO_2 in all the tissues and dopamine the next. Adrenaline and noradrenaline showed the lowest. This is not due to the differences in concentrations used since one of the runs was done with the same concentrations of each of the amines. This provides an easy method of comparison.

From these results it can be speculated that perhaps either serotonin or dopamine or both play some role in the metabolic activity of the earthworm, Lumbricus terrestris. Other than a role only in neuron transmission it has been suggested by various workers that serotonin might play a role in carbohydrate metabolism similar to that played by adrenaline in mammals (Mansour, 1964: Moore and Gosselin, 1962; Goodman and Gilman, 1970).

TABLES VIIa and VIIb

EFFECT OF DOPAMINE ON THE OXYGEN CONSUMPTION OF ISOLATED INTESTINAL AND BODY WALL TISSUES FROM THREE-DAY DEBRAINED AND NON-DEBRAINED EARTHWORMS

VIIa

Experimental Condition	Intestinal Wall QO_2 No Dopamine	Intestinal Wall QO_2 +16.7 ug/ml Dopamine	% Change	Body Wall QO_2 No Dopamine	Body Wall QO_2 +16.7 ug/ml Dopamine	% Change
Non-debrained worms	$.569 \pm 0.002$	$.773 \pm 0.007$	35.8	$.321 \pm 0.003$	$.402 \pm 0.003$	25.3
3-day debrained worms	$.451 \pm 0.002$	$.577 \pm 0.003$	28.0	$.247 \pm 0.001$	$.288 \pm 0.002$	15.8

VIIb

Experimental Condition	Intestinal Wall QO_2 No Dopamine	Intestinal Wall QO_2 +3.3 ug/ml Dopamine	Change	Body Wall QO_2 No Dopamine	Body Wall QO_2 +3.3 ug/ml Dopamine	% Change
Non-debrained worms	$.491 \pm 0.004$	$.509 \pm 0.008$	3.6	$.276 \pm 0.004$	$.281 \pm 0.002$	1.8
3-day debrained worms	$.390 \pm 0.006$	$.401 \pm 0.003$	2.8	$.237 \pm 0.001$	$.243 \pm 0.001$	2.5

QO_2 = the mean oxygen quotient in units of microliters of oxygen consumer per milligram of dry tissue weight per hour.

9 runs for each tissue

TABLES VIIIa and VIIIb

EFFECT OF SEROTONIN ON THE OXYGEN CONSUMPTION OF ISOLATED INTESTINAL AND BODY WALL TISSUE FROM THREE-DAY DEBRAINED AND NON-DEBRAINED EARTHWORMS

VIIIa

Experimental Condition	Intestinal Wall QO_2 No Serotonin	Intestinal Wall QO_2 +16.7 ug/ml Serotonin	% Change	Body Wall QO_2 No Serotonin	Body Wall QO_2 +16.7 ug/ml Serotonin	% Change
Non-debrained worms	$\overset{+}{.596}\text{-}0.003$	$\overset{+}{.848}\text{-}0.006$	42.4	$\overset{+}{.387}\text{-}0.004$	$\overset{+}{.471}\text{-}0.005$	21.7
3-day debrained worms	$\overset{+}{.469}\text{-}0.003$	$\overset{+}{.642}\text{-}0.004$	37.1	$\overset{+}{.268}\text{-}0.003$	$\overset{+}{.331}\text{-}0.004$	23.5

VIIIb

Experimental Condition	Intestinal Wall QO_2 No Serotonin	Intestinal Wall QO_2 +8.3 ug/ml Serotonin	% Change	Body Wall QO_2 No Serotonin	Body Wall QO_2 +8.3 ug/ml Serotonin	% Change
Non-debrained worms	$\overset{+}{.533}\text{-}0.005$	$\overset{+}{.567}\text{-}0.004$	+6.4	$\overset{+}{.315}\text{-}0.002$	$\overset{+}{.331}\text{-}0.003$	+5.1
3-day debrained worms	$\overset{+}{.425}\text{-}0.002$	$\overset{+}{.488}\text{-}0.003$	+1.5	$\overset{+}{.297}\text{-}0.002$	$\overset{+}{.280}\text{-}0.006$	-5.5

QO_2 = the mean oxygen quotient in units of microliters of oxygen consumer per milligram of dry tissue weight per hour.

9 runs for each tissue

\+ = an increase in QO_2

\- = a decrease in QO_2

TABLES IXa and IXb

EFFECT OF ADRENALINE ON THE OXYGEN CONSUMPTION OF ISOLATED INTESTINAL AND BODY WALL TISSUE FROM THREE-DAY DEBRAINED AND NON-DEBRAINED EARTHWORMS

IXa

Experimental Condition	Intestinal Wall QO_2 No Adrenaline	Intestinal Wall QO_2 +16.7 ug/ml Adrenaline	% Change	Body Wall QO_2 No Adrenaline	Body Wall QO_2 +16.7 ug/ml Adrenaline	% Change
Non-debrained worms	$\overset{+}{.596}\text{-}0.004$	$\overset{+}{.663}\text{-}0.001$	11.2	$\overset{+}{.449}\text{-}0.003$	$\overset{+}{.493}\text{-}0.001$	18.8
3-day debrained worms	$\overset{+}{.479}\text{-}0.002$	$\overset{+}{.534}\text{-}0.002$	11.5	$\overset{+}{.381}\text{-}0.010$	$\overset{+}{.425}\text{-}0.009$	11.5

IXb

Experimental Condition	Intestinal Wall QO_2 No Adrenaline	Intestinal Wall QO_2 +1.3 ug/ml Adrenaline	% Change	Body Wall QO_2 No Adrenaline	Body Wall QO_2 +1.3 ug/ml Adrenaline	% Change
Non-debrained worms	$\overset{+}{.536}\text{-}0.004$	$\overset{+}{.554}\text{-}0.002$	+3.4	$\overset{+}{.392}\text{-}0.002$	$\overset{+}{.402}\text{-}0.003$	+2.6
3-day debrained worms	$\overset{+}{.474}\text{-}0.010$	$\overset{+}{.465}\text{-}0.005$	-1.9	$\overset{+}{.333}\text{-}0.002$	$\overset{+}{.312}\text{-}0.003$	-6.3

QO_2 = the mean oxygen quotient in units of microliters of oxygen consumed per milligram of dry tissue weight per hour.

9 runs for each tissue

\+ = an increase in QO_2

\- = a decrease in QO_2

TABLES Xa and Xb

EFFECT OF NORADRENALINE ON THE OXYGEN CONSUMPTION OF ISOLATED INTESTINAL AND BODY WALL TISSUE FROM THREE-DAY DEBRAINED AND NON-DEBRAINED EARTHWORMS

Xa

Experimental Condition	Intestinal Wall QO_2 No Noradrenaline	Intestinal Wall QO_2 +16.7 ug/ml Noradrenaline	Intestinal Wall QO_2 % Change	Body Wall QO_2 No Noradrenaline	Body Wall QO_2 +16.7 ug/ml Noradrenaline	Body Wall QO_2 % Change
Non-debrained worms	$.521 \pm 0.013$	$.541 \pm 0.010$	+3.8	$.402 \pm 0.009$	$.413 \pm 0.005$	+2.7
3-day debrained worms	$.323 \pm 0.019$	$.331 \pm 0.023$	+2.5	$.346 \pm 0.004$	$.354 \pm 0.011$	+2.3

Xb

Experimental Condition	Intestinal Wall QO_2 No Noradrenaline	Intestinal Wall QO_2 +0.3 ug/ml Noradrenaline	Intestinal Wall QO_2 % Change	Body Wall QO_2 No Noradrenaline	Body Wall QO_2 +0.3 ug/ml Noradrenaline	Body Wall QO_2 % Change
Non-debrained worms	$.597 \pm 0.003$	$.602 \pm 0.002$	+0.8	$.390 \pm 0.002$	$.398 \pm 0.001$	+2.1
3-day debrained worms	$.411 \pm 0.002$	$.404 \pm 0.003$	-1.7	$.301 \pm 0.001$	$.304 \pm 0.001$	+1.0

QO_2 = the mean oxygen quotient in units of microliters of oxygen consumed per milligram of dry tissue weight per hour.

9 runs for each tissue

\+ = an increase in QO_2

\- = a decrease in QO_2

The water soluble extract from which water soluble lipids and monoamines were removed was found to contain four ninhydrin positive compounds on thin layer chromatography with rF factors as shown, (Figure 1). These were comparable to those that Vanden-Bosch (1970) also isolated from the earthworm brain extract which were probably polypeptides. The rF factors of these compounds were different from those of the four amines used in this investigation.

The concentrations used here are arbitrary, they were arrived at by trial and error. An attempt was made to get concentrations comparable to those for catecholamines. Looking at the results one sees that the water extract produces a lesser effect than does serotonin which is still significant. The intestinal tissue in both debrained and non-debrained worms reacted in a similar manner. There is a increase of 6.9% on QO_2 for the debrained and 8.2% increase for the non-debrained tissues. At the higher concentration these values are 13.1% and 11.4% respectively. In most of the previous results there have been big differences in debrained and non-debrained tissues where the difference has been a significant one. We see further that at double the concentration the increase in QO_2 is not very different from the lower concentration that is 11.4% and 13.1% as compared with 6.9% and 8.2%.

It is quite clear from the results obtained that a compound or compounds in the partially purified water extract was causing a general increase in metabolic activity, (Table XI). All the differences recorded were statistically significant with the exception

Figure 1

Thin layer chromatography patters of water soluble extact of earthworm brain

Solvent system: BuOH:Acetic Acid:Water (2:1:1)

Solvent Front rF on TLC

0.763
0.750
0.721

0.381

Origin

TABLES XIa and XIb

EFFECT OF WATER SOLUBLE EARTHWORM BRAIN EXTRACT (POLYPEPTIDES) ON THE OXYGEN CONSUMPTION OF ISOLATED INTESTINAL AND BODY WALL TISSUES FROM THREE-DAY DEBRAINED AND NON-DEBRAINED EARTHWORMS

XIa

Experimental Condition	Intestinal Wall QO_2 No H_2O Extract	Intestinal Wall QO_2 +33.3 ug/ml H_2O Extract	% Change	Body Wall QO_2 No H_2O Extract	Body Wall QO_2 +33.3 ug/ml H_2O Extract	% Change
Non-debrained worms	$.536 \pm 0.002$	$.597 \pm 0.001$	11.4	$.392 \pm 0.001$	$.419 \pm 0.001$	0.1
3-day debrained worms	$.379 \pm 0.003$	$.434 \pm 0.002$	13.1	$.305 \pm 0.003$	$.319 \pm 0.002$	0

XIb

Experimental Condition	Intestinal Wall QO_2 No H_2O Extract	Intestinal Wall QO_2 +16.7 ug/ml H_2O Extract	% Change	Body Wall QO_2 No H_2O Extract	Body Wall QO_2 +16.7 ug/ml H_2O Extract	% Change
Non-debrained worms	$.513 \pm 0.003$	$.555 \pm 0.004$	8.2	$.421 \pm 0.003$	$.417 \pm 0.003$	0
3-day debrained worms	$.420 \pm 0.002$	$.449 \pm 0.001$	6.9	$.300 \pm 0.003$	$.297 \pm 0.002$	0

QO_2 = the mean oxygen quotient in units of microliters of oxygen consumer per milligram of dry tissue weight per hour.

9 runs for each tissue

of the low concentration run for the body wall tissue. It was probable that this substance may be a polypeptide in nature because ninhydrin positive substances were present in this partially purified water extract.

We can see from these results that the effect caused by the compounds (polypeptides) extracted from the brain could possibly be mediated through a different mechanism from that by the amines discussed above. Whereas the amines gave results that separated the debrained from non-debrained worms the polypeptides do not or at least not as definitely as the amines.

We can make the following hypothesis to explain the above observations. We can assume that the polypeptides are hormones which are released into the blood stream from the brain. These interact with effector organ cells or organelles with subsequent release of some molecule (may be an amine such as serotonin) which in turn affects certain enzymes to speed up metabolic activity. It is impossible to compare the amount of change in QO_2 obtained with the water extract with that obtained with serotonin and the other amines since one cannot compare the concentrations. However, it is possible that the effect of the polypeptides may or may not be connected with that of the amines. It is quite possible for these compounds to affect an increase in QO_2 through different mechanism. In fact, in view of the several hundred cellular processes that one can think of that will result in increased oxygen utilization, the later explanation seems the more reasonable one of what is happening.

Figure 2

Thin layer chromatography patterns of water soluble extract of rat brain

Solvent system - BuOH: Acetic Acid: Water (2:1:1)

We do see, however, in this experiment a trend that has been maintained in all previous experiments, namely that the intestinal tissue was more responsive per unit weight to the water soluble extract than the body wall tissue. This was true for both debrained and non-debrained tissues.

A similar experiment, which was to serve as another control, was done with rat brain subjected to the same extraction and purification procedure as was done for the earthworm brains. Three ninhydrin positive compounds were isolated in the purified water extract and their rF factors are shown in Figure 2. Two of the rF factors were comparable to two rF factors of the earthworm brain polypeptides. The results of the experiment runs are shown in Table XII. We see that there was no consistent pattern in the effects caused by the water extract from the rat brain. With the tissues from non-debrained worms we see a slight increase in QO_2 for the intestinal tissue. The situation was reversed for the body wall where low concentrations gave a decrease of 3.0% and the high concentration gave an increase of 3.4%. All these differences were not statistically significant. With regard to the debrained worms the picture was different because at low concentration the intestinal tissue showed a decrease in QO_2 of 4.6% and at a high concentration an increase of 6.0%. The body wall showed an increase of 5.1% with low concentration and a decrease of 7.0% with high concentration. All these effects were statistically significant. From these results it was difficult to say whether the rat brain extract did cause any

TABLES XIIa and XIIb

EFFECT OF WATER SOLUBLE RAT BRAIN EXTRACT (POLYPEPTIES) ON THE OXYGEN CONSUMPTION OF ISOLATED INTESTINAL AND BODY WALL TISSUES FROM THREE-DAY DEBRAINED AND NON-DEBRAINED EARTHWORMS

XIIa

Experimental Condition	Intestinal Wall QO_2 No H_2O Extract	Intestinal Wall QO_2 +33.3 ug/ml H_2O Extract	% Change	Body Wall QO_2 No H_2O Extract	Body Wall QO_2 +33.3 ug/ml H_2O Extract	% Change
Non-debrained worms	$.592^{+}_{-}0.002$	$.549^{+}_{-}0.001$	-7.2	$.388^{+}_{-}0.002$	$.401^{+}_{-}0.002$	+3.4
3-day debrained worms	$.431^{+}_{-}0.003$	$.457^{+}_{-}0.005$	+6.0	$.313^{+}_{-}0.003$	$.291^{+}_{-}0.001$	-7.0

XIIb

Experimental Condition	Intestinal Wall QO_2 No H_2O Extract	Intestinal Wall QO_2 +16.7 ug/ml H_2O Extract	% Change	Body Wall QO_2 No H_2O Extract	Body Wall QO_2 +16.7 ug/ml H_2O Extract	% Change
Non-debrained worms	$.580^{+}_{-}0.004$	$.592^{+}_{-}0.007$	+2.1	$.425^{+}_{-}0.001$	$.411^{+}_{-}0.001$	-3.0
3-day debrained worms	$.457^{+}_{-}0.001$	$.435^{+}_{-}0.002$	-4.6	$.324^{+}_{-}0.007$	$.342^{+}_{-}0.002$	+5.1

QO_2 = the mean oxygen quotient in units of microliters of oxygen consumed per milligram of dry tissue weight per hour.

9 runs for each tissue

\+ = an increase in QO_2

\- = a decrease in QO_2

real change in the oxygen consumption of the earthworm tissues. If any of the polypeptides isolated from the earthworm brains was a hormone then it was quite understandable why similar polypeptides isolated from the rat did not result in a similar change. An example of this is seen with regard to differences in similar proteins such as myoglobin and hemoglobin or in insulin from different species of animals. It is very likely that if these polypeptides are hormones in both sets of animals the amino acid sequence could have altered with mutation, etc. Mansour et al. (1960) have suggested that rarely do mammalian hormones affect invertebrates in the same manner. On the other hand if these isolated compounds were simple organic compounds one would expect very little (change) differences in their effect on basic metabolic process, for example, effect of adrenaline in mammals compared to that of serotonin and also adrenalin in some invertebrates. The experiment with the water extracts from both the earthworm brain and rat brain show the presence in the earthworm brain of a substance(s) probably polypeptide in nature and distinct from monoamines, which increased oxidative metabolism or oxygen utilization.

SUMMARY

1. The purpose of the present study was to investigate the chemical nature of the substance or substances present in the brain of Lumbricus terrestris which affects the oxidative metabolism of excised intestinal and body wall tissue of this earthworm.
2. The removal of the brain of the earthworm resulted in depression of oxygen consumption of intestinal and body wall tissues, within the first three days after de-braining. The decrease in the QO_2 of the intestinal tissue was higher than the decrease caused in the body wall. Decapitation of the earthworm before dissection of the intestinal and body wall tissues resulted in the lowering of QO_2 of non-debrained worms and no effect on the debrained worms. This observation support the above observation that removal of the brain depresses the QO_2 of the intestinal and body wall tissues.
3. Homogenate of the brain and the ventral nerve cord increased the oxygen consumption of the intestinal and body wall tissues, the brain homogenate producing a higher increase in the oxygen consumption.
4. Water extraction of the earthworm brain and rat brain was carried out. Following this the water extracts were partially purified by removing monoamines and water soluble

lipids. This water soluble material was tested for biological activity on the body wall and intestinal tissues of the worm.

5. Results of the assay showed that this partially purified water extract caused a general increase in metabolic activity of all the tissues especially in the intestinal tissue. It is probable that ninhydrin positive fractions present in this extract were the substances involved. The rat brain extract did not elicit any significant changes.
6. Of the amines (adrenaline, noradrenaline, serotonin and dopamine) tested serotonin and dopamine seemed to play some role in the metabolic activity of the earthworm, Lumbricus terrestris.

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