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The Role of the Suprapharyngeal Ganglia of Lumbricus Terrestris in Respiratory Metabolism

John D. Nelson

THE ROLE OF THE SUPRAPHARYNGEAL GANGLIA OF LUMBRICUS TERRESTRIS IN RESPIRATORY METABOLISM

by

John D. Nelson

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INTRODUCTION

Although neurosecretory cells have been identified since the late 1930's in invertebrates, it has not been until the last fifteen years that regulatory functions have been attributed to their secretions. It has been reported that in oligochaetes neurosecretory cells are present in the suprapharyngeal ganglia, in the subpharyngeal ganglia, and in the ventral nerve cord ganglia. Regulatory roles for substances which are present in these structures and which are presumably secretions of neurosecretory cells have been suggested for growth, reproduction, nervous tissue regeneration, somatic tissue regeneration, osmotic and ionic balance, and probably carbohydrate metabolism.

The corpora cardiaca and possibly the allata of insects are structures which serve as neuroheamal organs (Carlisle and Knowles, 1953) for neurosecretory cells located in the central nervous system. In addition secretory cells have been described in these organs, also. Numerous investigations on insects have reported an influence by substances present in the corpora cardiaca and to a lesser extent, in the corpora allata on both whole body respiration, and on respiration of fat body tissue and muscle tissue. Elzinga (1963) studied the influence of the suprapharyngeal ganglion of Lumbricus terrestris on whole body respiration, but not on isolated non-homogenized tissues. This present study was designed to determine if the oxidative metabolism of isolated tissues of earthworms, L. terrestris,

are influenced by the substances present in the suprapharyngeal ganglion. To demonstrate this the effect of removing the brain on the oxygen consumption of intestinal wall and body wall tissue was studied. Following this, the effect of adding brain homogenates on oxygen consumption was studied on these tissues in vitro.

The two types of tissues selected for study in this investigation were the intestinal wall and the body wall. This selection was made because of the distinct difference in function and in structure between the two types of tissues. The intestinal wall of earthworms is the major storage organ for glycogen. In this respect the intestine of earthworms is analogous to fat body of insects, to liver of mammals, and to heptopancreas of crustaceans. The glycogen in the intestine of earthworms is stored in specialized cells, chlorogogen cells. The intestinal wall has a higher level of metabolic activity than other tissues of earthworms which suggests other metabolic functions besides digestion, storage, and absorption (Urich, 1964). Body wall tissue has lower metabolic activity as demonstrated by the enzymatic activity (Ogata and Morl, 1964). It is made up of different muscle tissue with no apparent chlorogogen cells as in the intestinal tissue.

LITERATURE REVIEW

Neurosecretory cells were first reported in the cerebral ganglion of L. terrestris by B. Scharrer (1937). These neurosecretory cells have been found not only in the brains of earthworms, but in the subpharyngeal ganglia, in the ventral nerve cord ganglia (Gabe,

1966). Amine secreting neurons have been identified in the brain, subpharyngeal ganglia, and ventral nerve cord ganglia of Octolasium complanatum by histochemical procedures (Bianchi, 1967). Since there is such a fine line of distinction between the structure of amine secreting neurons and neurosecretory cells, possibly they could be one and the same in some instances.

Several investigators have suggested that numerous types of neurosecretory cells are present in the suprapharyngeal ganglia of earthworms (Hubl, 1956b; Brandenburg, 1956; Ortemba, 1961; Santhakumari, 1963). They based their identifications mainly on the properties of the neurosecretory cells and on the presence of granules and/or vacuoles. However, the general contention has been that only two types of cells exist, the a-cells and the b-cells (Scharrer, 1937; Herlant-Meewis, 1955, 1956a, 1956b; Bianchi, 1967; Marapao, 1959; Goudie, 1968). The two cell hypothesis was further supported by electronmicroscopy which revealed considerable intrastructual differences from cell to cell suggesting that the described differences may be merely different functional stages of the two single lines of cells (Scharrer and Brown, 1961). Herlant-Meewis (1955, 1956a, 1956b) suggested a classification of neurosecretory cells using well defined anatomical features and histophysiological observations for identifications instead of completely relying on stain affinities of the particular cells. She identified the two types of neurosecretory cells in the cerebral ganglion of Eisenia foetida by location and the presence of neurosecretory contents of different staining capacities. Herlant-Meewis's a-cells are situated

in the posterior and the superior portions of the ganglion while the b-cells are in a zone ventral to the a-cells. Marapao (1959) identified two groups of cells present in the brain of L. terrestris. Of these two groups, one group of cells could be sub-divided into three stages. Although the actual number of distinguishable kinds of cells were greater than two, from his study, he concluded that only two major groups existed with the different stages showing some alterations within the group. Goudie (1968) also was able to distinguish the same two types of neurosecretory cells in the cerebral ganglion of L. terrestris as a result of a histochemical study.

Several functions have been ascribed to secretions of these neurosecretory cells on the basis of observed changes occurring in these cells associated with changes in some function or structure. Also, still other regulatory roles have been shown by substances present in the brain, which perhaps resulted from secretions from these cells.

Herlant-Meewis (1956b) suggested that they played a role in growth because of morphological changes in these cells that occurred during postembryonic development in Eisenia foetida. Also when Eisenia foetida were taken from winter environments, they showed a rich content of neurosecretory products in their a-cell axons (Herlant-Meewis, 1956a, 1956/57). A considerable amount of neurosecretory products was observed in the axons of the a-cells when sexual maturation was reached. However, these observable neurosecretory products disappeared at the end of the oviposition period. If

the cerebral ganglion was removed at the time of sexual maturation, oviposition came to immediate arrest with all morphological appearances being that of a worm in hibernal diapause. As a result of this, she concluded that substance(s) present in the cerebral ganglion apparently regulated the sexual maturation of the earthworm. Regeneration of the cerebral ganglion was far advanced in Eisenia foetida three weeks after debraining, but no periganglionic capillary network restoration was found (Gabe, 1966). It was only when regeneration of both the cerebral ganglion and the periganglionic capillary network occurred that ovulation began again. Sham operation affected ovulation for only a short time. However, if the circulatory system about the ganglion was damaged, ovulation was retarded for a longer span of time. Herlant-Meewis (1956/57) concluded that the a-cells in this earthworm elaborated a substance which seemed essential for normal functioning of the genital apparatus and the substance(s) which affected the reproductive process apparently were transported by a vascular route.

Hubl (1953, 1956a) found, in four species of Lumbricidae, when the posterior segments were amputated that some definite morphological changes occurred in the b-cells. It was mentioned above that there was no agreement in regards to kinds and numbers of neurosecretory cells present in oligochaetes and in this respect, it should be noted, that Hubl's b-cells were not the same as Herlant-Meewis's b-cells, but probably were identical to Herlant-Meewis's a-cells (Gabe, 1966). Regeneration in these species of Lumbricidae

appeared to depend on the brain being present for at least 48 hours after posterior segment amputation. After this time the brain's necessity was reduced (Hubl, 1956a). He found the ventral nerve cord ganglia above the clitellum and the subpharyngeal ganglion inhibited regeneration if removed during posterior segmental amputation.

Substances present in the brain of earthworms have recently been associated with osmotic and ionic regulation (Kamemoto, 1964). With removal of the brain, the worm gained weight when placed in tap water and the sodium ion concentration in the blood and coelomic fluid decreased. Kamemoto counteracted this osmotic and ionic change by brain transplants which displayed the osmotic and ionic balance of the earthworms dependency on factor(s) present in the brain. Goudie (1968) found that the a-cells in the cerebral ganglion of the earthworm apparently contained the neurosecretory substances responsible for the osmotic and ionic regulatory activity. This was done by making histochemical observations of the cerebral ganglia of earthworms after they had been subjected to hypertonic and hypotonic environments. He also concluded that the b-cells of the cerebral ganglion were involved in a generalized stress response which was first described by Maropao (1959).

Craig (1966) found in L. terrestris that the glucose level of the blood dropped to zero in 24 hours following debraining. Elzenga (1963) found when the brain was removed from L. terrestris, whole body respiration decreased drastically within the first 36

hours. This was followed by a slight rise in oxygen consumption, to a steady level which was less than that of the normal worms used for controls. Their work indicated the brains influence on carbohydrate metabolism and oxidative metabolism.

Substance(s) present in the corpora cardiaca and the corpora allata have been suggested to have an influence on oxidative metabolism in insects. Wiens and Gilbert (1965) found that adult male Leucophaea maderae fat bodies, when incubated with corpora cardiaca, were stimulated as indicated by an increased oxygen consumption. The fat body is the main storage organ of reserve food supplies for insects. From this increased oxygen consumption, which they concluded resulted from an increased glucose availability, they postulated that the substance(s) present in the corpora cardiaca may affect carbohydrate metabolism in two ways. In the first place, it could increase the rate of glycogen degradation, and secondly, it may divert the mobilization of hexose residues from the glycolytic pathway into the blood as trehalose. The increased metabolic rate reflected by an observable increase in oxygen consumption would provide greater amounts of ATP for regeneration of UTP. The UTP is necessary for the endogonic process of trehalose synthesis from glucose resulting from glycogenolysis in insects. Steele (1969) reviewed the work which demonstrated the presence of a hyperglycemic factor in the corpora cardiaca and the corpora allata of Periplaneta americana, and supported the earlier postulate that this factor increased the degradation of glycogen and converted the residues into

blood trehalose. By dilution bioassay techniques, he determined that the corpora allata had only 10% the active hyperglycemic factor(s) that was contained in the corpora cardiaca.

Within ten days following removal of the corpora cardiaca from the adult male Blaberus discoidalis, the respiratory metabolism of both whole body and fat body tissue was lower than that of the controls (Keeley and Friedman, 1967). The addition of glucose substrate did not restore the depressed rate of oxygen consumption in cardiatectomized cockroaches which supported their theory that substrate availability was not the answer to the observed depression in oxygen consumption. Allatectomized animals did not display a drop in oxygen consumption, and they concluded that the affectors of respiratory metabolism were present in the corpora cardiaca rather than in the corpora allata. Although cardiatectomy was found to affect whole body and fat body respiration, it had no effect on respiratory rates of muscles.

Mordue and Goldsworthy (1969) demonstrated the presence of four ninhydrin positive fractions in water extracts of corpus cardiacum in Periplaneta. Of these four fractions, two were effective in increasing active phosphorylase levels of fat body and in elevating the hemolymph sugars in Periplaneta. It was interesting to note that the four fractions they obtained by chromatography from the corpora cardiaca were also found in the nervous tissue and the fat body tissue. However, the fractions from the latter two sources showed little if any biological activity indicating that the possibility

existed of similar chemical substance(s) which are perhaps identical, being present outside the brain without the same biological activities.

When corpus cardiacum homogenates were administered, in vitro, to the fat bodies of female Leucophaea maderae which had a high tissue respiration rate, the homogenate seemed to inhibit respiratory metabolism (Muller and Engelmann, 1968). When homogenates were added to fat bodies from L. maderae with low basal metabolic rates, the homogenates seemed to stimulate respiratory metabolism. Thus, the lower the basal rate, the higher the degree of stimulation by the homogenate and the higher the basal rate, the lower the degree of stimulation. An enormous variability in the response of fat body tissues to corpus cardiacum extracts was also seen in Leucophaea (Luscher and Leuthold, 1965) and in Blaberus craniifer (Ralph and Matta, 1965). Muller and Engelmann (1968) found it reasonable to assume that these large degrees of variations in responses were due to the amounts of active agents present in the extracts, or variations in the target tissues. A graded combination of the two variables could also determine the response.

Blaberus discoidalis, following cardiatectomy-allatectomy, displayed not only a lower oxygen consumption rate of fat body tissues as compared to that of the controls, but also of isolated fat body mitochondria (Keeley and Friedman, 1969). They also demonstrated that adding substrates such as alpha-ketoglutarate and pyruvate, and co-factors such as NADP, NAD, and CO-A, had no effect on oxygen consumption. From this they suggested that the absence of the corpora cardiaca and the corpora allata resulted in a change

in the structure or enzymatic unity of the mitochondria. Thus the total depression in oxygen consumption of the fat body might be due to a mitochondrial malfunctioning. Their conclusion was that the corpora cardiaca was important in regulating fat body oxidative metabolism. Although the corpora allata was not the source of the metabolic substance(s), its integrity can effect the neurosecretory activity of the brain-corpora cardiaca complex (Steele, 1969). Its removal may disrupt the synthesis of regulatory substances released from the brain or the corpora cardiaca (Keeley and Friedman, 1969).

METHODS AND MATERIALS

The earthworms that were used for this study were obtained throughout the year from a local bait shop in Kalamazoo, Michigan. The earthworms were identified as Lumbricus terrestris and only those animals that had prominent clitellae (indicating maturity) were selected for experimental use. The stock supply of earthworms was kept in moist soil in a refrigerator at a temperature range of 2-7 degrees centigrade.

Oxygen consumption of tissues studied was determined manometrically by means of the Warburg apparatus (Umbreit et. al., 1964). In all cases the temperature was maintained at 14$\pm$ 0.5 degrees centigrade by means of a water bath in which the reaction flasks were shaken at 60 revolutions per minute. The temperature was selected on the basis of the need for a cool temperature and because 14$\pm$ 0.5 degrees centigrade was the coolest that could be consistently maintained

with the equipment available. Unless otherwise indicated, the solution in the reaction flasks was a non-nutritive, isotonic saline solution of 0.8% concentration (Table 1).

Table 1. Composition for the isotonic saline for earthworms (Welsh and Smith 1964)

Compound	Amount of
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	100 ml
pH 7.4	dilute to 1 liter with distilled water

A volume of 3.0 milliliters was selected for the bathing media because this volume covered the tissues adequately but was not enough to flood the center well upon shaking and thus dilute the potassium hydroxide solution present. For carbon dioxide absorption, a 0.1 milliliters of a 20% potassium hydroxide solution was deposited on a 2 x 4 centimeter piece of number 500 Sargent filter paper which was fluted and placed in the center well of the reaction flask. It was established, by means of water displacement, that a section of intestinal wall and body wall from the earthworm weighing 250 milligrams displaced a volume of water that was equivalent to 0.25

milliliters. This was needed in determining the total volume of tissue and fluid for calculating the flask constant of the reaction flasks. Therefore the total volume in the reaction flask for any experiment was 3.35 milliliters, consisting of 3.0 milliliters of saline, 0.1 milliliters of potassium hydroxide, and 0.25 milliliters of tissue volume. In the case of some additive being administered to the tissue during an experiment, the total volume was still maintained at 3.35 milliliters. This was accomplished by reducing the bathing media volume by that amount which was to be added from the side arm of the reaction flask.

A flask constant, K, had to be calculated for each flask before a volume of oxygen consumed could be determined. This value was determined from the following formula:

$$K = \frac{V_g\ (273^{o} + T^{o}\ C.) + V_f\ (alpha)}{Po}$$

Where V_g is the volume of the gas phase of the closed system, V_f is the volume of the fluid phase, alpha is the solubility of the gas in the fluid at 14 ± 0.5 degrees centigrade, and Po is the pressure of Brodie's solution in millimeters of mercury. The alpha of 0.0368 was found in Umbriet et. al. (1964) for a saline solution.

With the exception of decapitation of one group of worms described later, all the earthworms were handled identically. The worms were anesthetized for 3 minutes in a 10% ethanol solution, removed from the alcohol solution, and quickly rinsed in cold tap water. The worms that were designated as controls were then placed

in moist paper toweling and refrigerated. Those worms that constituted the experimental group were all debrained. This was accomplished by making a dorsal longitudinal incision from the second to the fifth segment which exposed the suprapharyngeal ganglion. The circumpharyngeal connectives were then cut and the brain gently lifted out. Careful observation was made to insure that the whole brain was removed. These worms were also placed in moist paper toweling and refrigerated. The experimental and the control worms remained in paper toweling up to 3 days. In the first steps of the experimental procedure, those worms which were debrained 1 and 2 days prior to studying, were kept in moist paper toweling 1 or 2 days respectively before experimentation. If the worms were to be studied at a time greater than 3 days after debraining, the worms were returned to the soil. This type of treatment was also done on the controls even though no incision was present. All the animals were randomly selected and any of the specimens that showed extensive bodily damage or unusual sluggishness, were discarded.

The two types of tissues used for this respiration study were the intestinal wall and the body wall. In removing these tissues, the worms were removed from the cold paper toweling, a dorsal longitudinal incision was made from the fifteenth segment to the posterior end, and the body wall pinned down to expose the intestine. Following the severing of the segmental partitions from the intestine, the intact intestine was slit preceding its removal from the body. After the intestine was removed, it was thoroughly rinsed in cool 0.8% saline to remove the intestinal contents. The tissue

was weighed and an average 250±25 milligrams sample was deposited in an appropriate volume of saline solution in the basin of a Warburg reaction flask. The flask was stoppered and placed in the refrigerator until all remaining flasks had been prepared.

The body wall which came from the same area as did the intestinal wall was prepared by carefully scraping away the blood vessels, the remains of the ventral nerve cord that were attached, and the intestinal contents. It was thoroughly washed in cool 0.8% saline solution. The tissue was also weighed and an average 250±25 milligrams sample deposited in a flask with the appropriate volume of saline solution. The flask was stoppered and refrigerated until testing time. Approximately 90 minutes elapsed from the beginning to the end of dissection and preparation of the tissues for any experimental determination of oxygen consumption. The flasks with their contents were then attached to the manometers and a twenty minute temperature and pressure equilibration period was allowed before the manometer stopcocks were closed. Manometric changes for the first hour after closing the stopcocks was not recorded to allow the flasks and their contents to become fully adjusted to the cooler temperature that was being maintained by the water bath. After this period of time, hourly manometric readings of pressure changes in millimeters were taken for 4 to 5 hour periods and converted to volumes of oxygen consumed by the tissues contained in the flask basin. A tape recorder was used to record the manometric changes of the 22 to 26 manometers in the shortest period of time

which was in the range of 2±0.5 minutes. From these hourly manometric changes, hourly oxygen quotients were calculated. These hourly oxygen quotients were added and a mean oxygen quotient was determined with units of microliters of oxygen consumed per milligram of dry tissue weight per hour of time.

It became apparent that the only reliable expression for the tissue weights was milligrams of dry weight rather than fresh weight. The factors of evaporation, tissue blotting inconsistency, and variability in the tissue water content could not be maintained at any constant level to allow for any degree of reliability in fresh weight determination. The values for the dry weights were determined by finding constant weights of small aluminum pans with 3 centimeters base diameters. The tissues, in numbered pans for identification, were then placed in a Cenco oven for 1 2±2.0 hours at 100 degrees centigrade. After removing the tissues from the oven, they were placed in a dessicator with dry-rite for 4±2.0 hours for the tissues to return to room temperature before weighing on a Metler H-20 balance which was used for all weight measurements.

Results obtained in the first parts of this investigation indicated that only worms debrained three days prior to experimental use should constitute the experimental animals to be used for all subsequent experimentation. Numerous observations were then made to compare the experimentals' intestinal wall and body wall oxygen consumption 3 days following debraining to that of the controls to establish with greater reliability if a difference existed due to

the loss of the suprapharyngeal ganglion.

All experiments were run between 10:00 and 16:00 E.S.T. with the exception of one set of data which was run at 14:00 to 19:00 hours E.S.T. The reason for running at the latter hour was to observe if any cyclic pattern of oxygen consumption existed that differed from that of the majority of experimental runs.

An attempt was made to eliminate the suprapharyngeal ganglion from influencing the tissues being studied while the tissues were being removed from the earthworm. This was done by quickly decapitating the standard controls and experimentals before dissecting them. The worms were removed from the refrigerator with the least possible amount of handling, laid out on a moist paper towel, and decapitated immediately down to the tenth anterior segment. Dissection of the worm then preceded as above. Tissues from these decapitated worms were studied in comparisons with tissues from both non-decapitated standard controls and decapitated and non-decapitated experimentals.

The suprapharyngeal ganglia that were removed from the earthworms were collected in ten brain lots and frozen in 1 milliliter of the 0.8% saline until needed. Crude whole brain homogenates were prepared using ground glass homogenizers. The brains and the saline solution in which the brains were frozen were poured into the homogenizer and processed for 5 minutes. Then the ground mixture was poured into a 10 milliliter graduated cylinder. Fresh 0.8% saline was then poured into the homogenizer and swirled about to insure that all the homogenate was rinsed out of the homogenizer.

This saline was then poured into the graduated cylinder with the brain homogenate, and the volume was brought up to that volume which was needed by adding additional quantities of saline until the desired volume had been reached. A 1 milliliter aliqot (which contained the appropriate number of brains) was then pipetted into the side arm of each reaction flask. When a homogenate was going to be added to a flask, the bathing saline medium was reduced by 1 milliliter. By this means, the same total fluid was still present in the flask and the flask constant (K) would remain the same. The homogenates were administered to the tissues by simply tilting the whole set-up of manometer and flask to allow the homogenate to run out of the side arm into the reaction flask basin. The bathing saline and homogenate were tilted back and forth from side arm to basin in order to insure that all the homogenate was in the reaction flask basin with the tissue. Two concentrations of brain homogenates were used to determine effect of substances present in the brains of earthworms upon oxygen consumption of the tissues studied. Due to the small size of the brains, average mean weight of one brain being 3.86×10^{-4} grams, homogenate concentrations were determined by the number of brains per one milliliter of saline. Concentrations of 1 brain per 1 milliliter saline and 2 brains per 1 milliliter saline were used. It should be noted that since the flasks had a total saline volume of 3.0 milliliters, in order to add a homogenate that would result in a concentration of 1 brain per 1 milliliter saline, 3 brains would have to be added. This same consideration

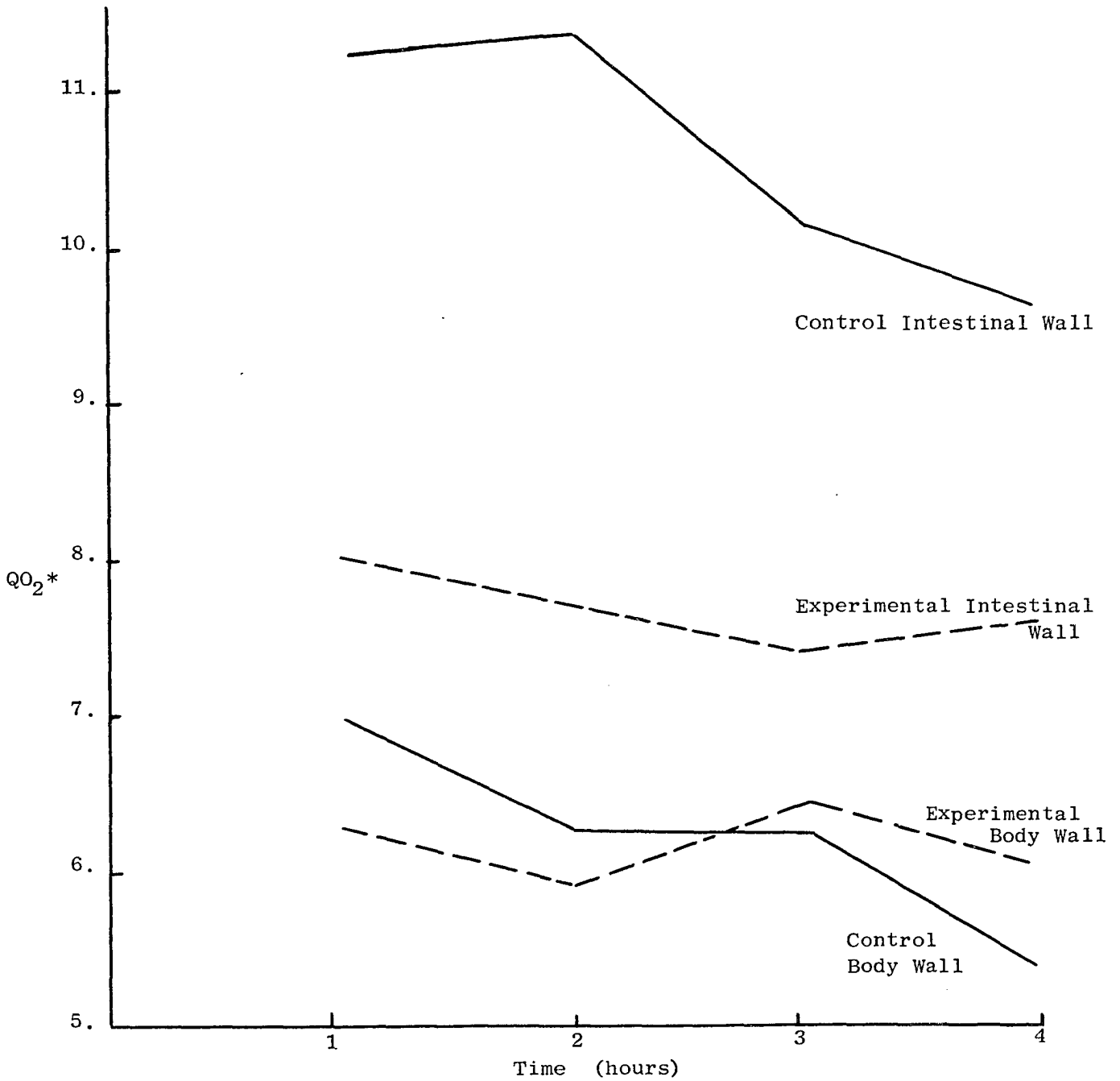
was also taken for 2 brains per 1 milliliter saline situations.

The hourly oxygen consumption rates were converted to oxygen quotients with the units of microliters of oxygen consumed per milligram of dry tissue weight per hour of time. Then these hourly oxygen quotients were totaled and a mean oxygen quotient derived from units of microliters of oxygen consumed per milligram of dry tissue weight per hour of time. These mean oxygen quotients for the different treatment groups were analysized by the one-way Anovar Dunnett's test, the Dunnett multiple comparison procedure test, and the Dunn's rank sum test which is treatment versus control. These tests were done by Mr. Joseph A. Soda of the Upjohn Company under unit 9951 of the research statistical methods division.

RESULTS AND DISCUSSION

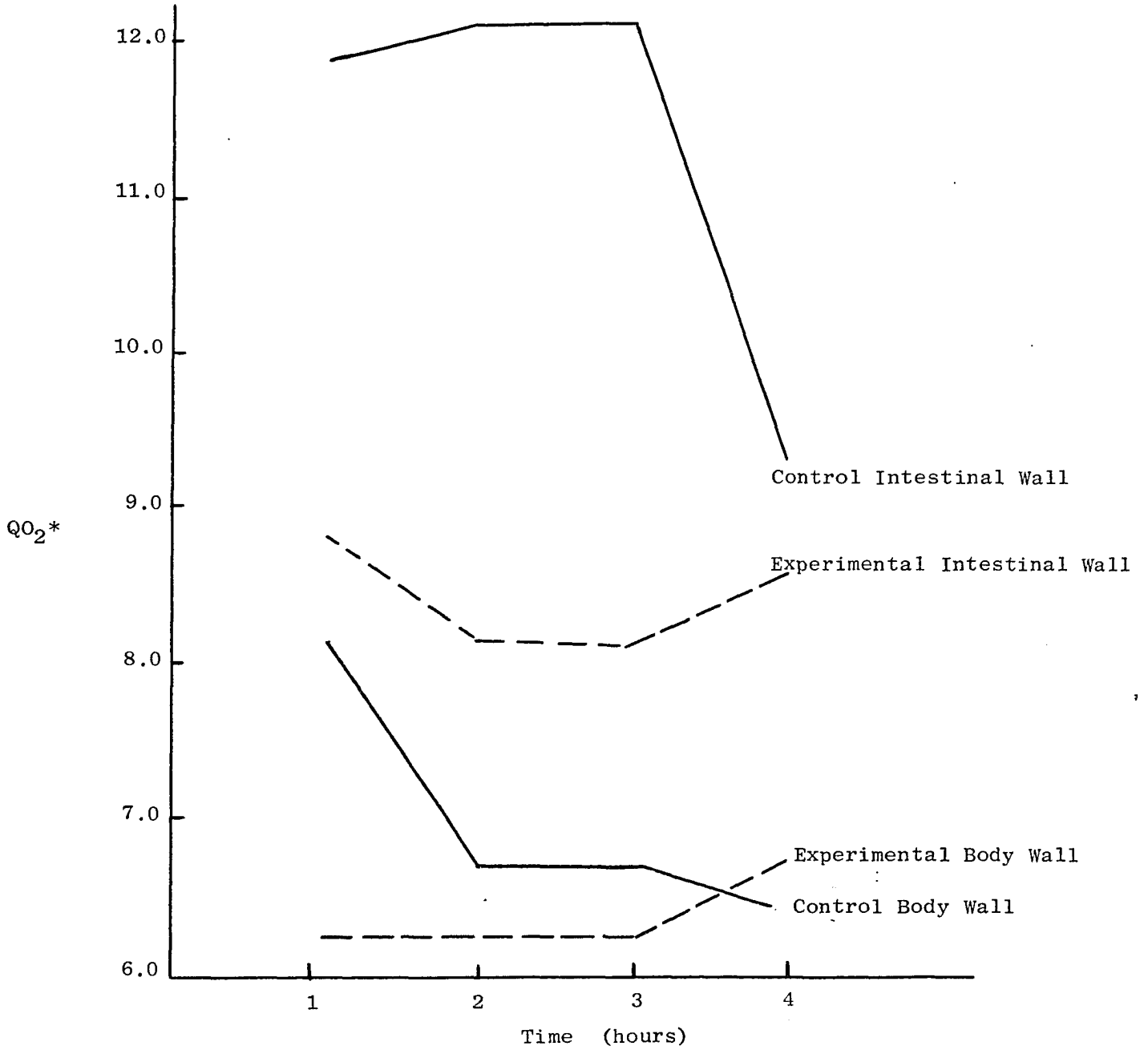
An examination of the oxygen quotients derived for each hour of each experiment demonstrated that the tissue respiration did not maintain any consistent hourly rate. It was extremely variable and oscillated from hour to hour (Figures 1a and 1b). An unsuccessful attempt was made to establish some pattern for this hourly variation. As a result it was considered necessary to determine a mean rate of oxygen consumption for the four or five hour period in the form of a mean oxygen quotient calculated from the oxygen quotients for each hour. It was on these values, which represented the mean oxygen consumption for the experimental period, that all analyses, including the statistical analyses, were based.

In the first portion of this investigation the effect of the



*QO_2 = ul of O_2/ gram dry wt./ minute of time

Figure 1a. Hourly oxygen quotients of intestinal and body wall tissues from non-decapitated controls and non-decapitated experimentals.



*QO_2 = ul of O_2/ gram dry wt./ minute of time

Figure 1b. Hourly oxygen quotients of intestinal and body wall tissues from non-decapitated controls and non-decapitated experimentals.

removal of the brain on the oxygen consumption of the intestinal and body wall tissues was determined for periods of one to twenty days following debraining. No decapitation procedures were used in preparing these tissues for these oxygen consumption determinations. The mean oxygen quotients for the intestinal wall and the body wall for those worms debrained for one to twenty days of those of the controls are listed in Table 2 and plotted in Figures 2a and 2b.

In the experiments using intestinal tissue results indicated that those worms that had been debrained for three days prior to experimentation showed the greatest consistent depression in the rate of oxygen consumption (Table 2, Figure 2a). No statistical analysis was done because of the small sample sizes used in this determination. The intestinal tissue studied from worms that had been debrained for one and two days prior to experimentation did not have as great a depression in oxygen consumption rates. Their mean oxygen quotients were not as consistently below that of the controls as were those oxygen quotients observed in three day debrained worms. After four days oxygen consumption of the experimental intestine tissues rose above that of the controls.

One day following debraining the oxygen consumption of body wall tissues was lower than that of the controls. From this point until thirteen days following debraining the body wall tissue from experimental animals demonstrated a slightly greater oxygen consumption than the controls. However, it should be noted that more data accumulated later (Table 5) indicated that the body wall tissue also consumed less oxygen three days following debraining

Table 2. The oxygen quotients of intestinal and body wall tissue from earthworms that had their supraapharyngeal ganglia removed from 1 to 20 days before experimentation. Control worms are listed with the experimental debrained worms for comparisons to be made.

Condition	Intestinal Wall QO_2*	Body Wall QO_2
Controls for 1 day worms	0.622 (2)**	0.396 (2)
1 day debrained earthworms	0.575 (4)	0.366 (4)
Controls for 2 day worms	0.572 (1)	0.252 (1)
2 days debrained earthworms	0.496 (1)	0.386 (1)
Controls for 3 day worms	0.628 (2)	0.373 (2)
3 days debrained earthworms	0.459 (4)	0.383 (4)
Controls for 4 day worms	0.523 (2)	0.381 (2)
4 days debrained earthworms	0.614 (4)	0.450 (4)
Controls for 8 day worms	0.623 (2)	0.380 (1)
8 days debrained earthworms	0.637 (2)	0.380 (1)
Controls for 13 day worms	0.623 (1)	0.380 (1)
13 days debrained earthworms	0.645 (2)	0.366 (2)
Controls for 15 day worms	0.550 (1)	0.357 (1)
15 days debrained earthworms	0.533 (2)	0.225 (2)
Controls for 20 day worms	0.623 (1)	0.380 (1)
20 days debrained earthworms	0.648 (4)	0.420 (2)

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

**The numbers in parenthesis indicate the numbers of samples for that particular experiment. No S.D. were determined due to the small samples used for this preliminary study.

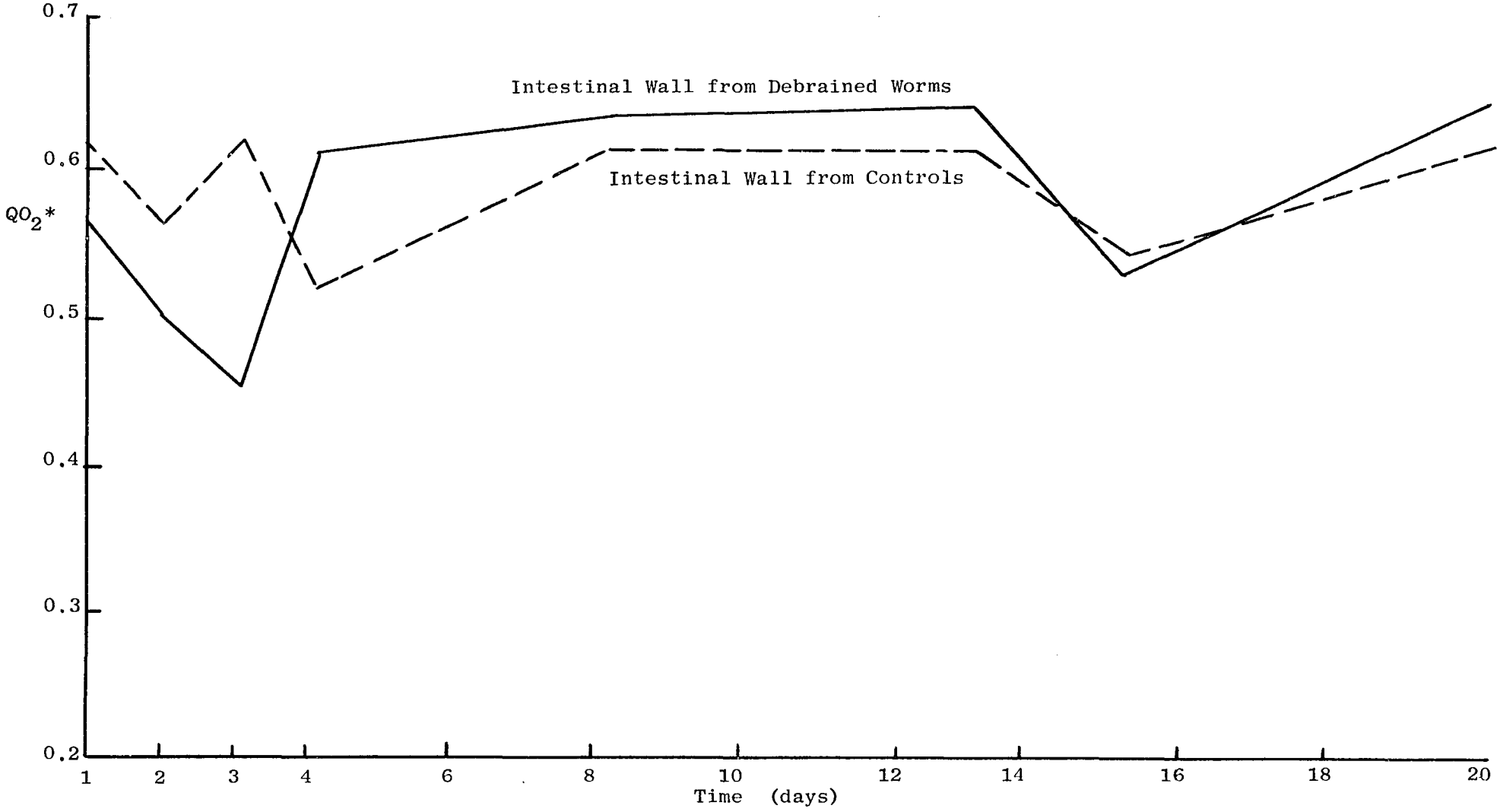


Figure 2a. Mean oxygen quotients of intestinal wall tissue from controls and from worms debrained from 1 to 20 days before experimentation.

*QO_2 = ul of O_2/ mg. dry wt./ hour of time

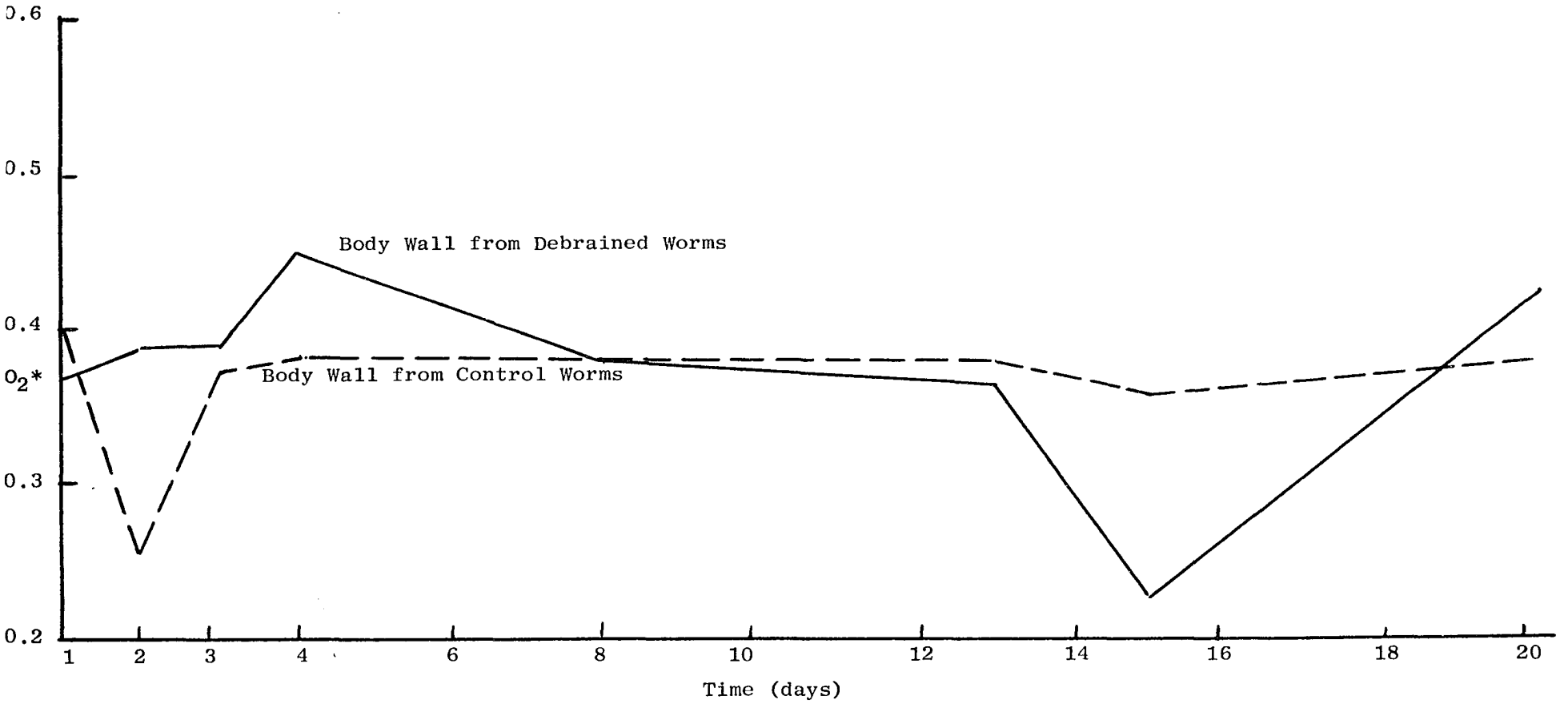


Figure 2b. Mean oxygen quotients of body wall tissues from controls and from worms debrained from 1 to 20 days before experimentation.

*QO_2 = ul of O_2/ mg. dry wt./ hour of time

than did control tissues although the difference was not statistically significant.

Elzinga (1963) observed in his studies on whole body respiration of L. terrestris that within the first 36 hours after debraining the lowest rate of oxygen consumption was displayed by the worms. This was followed by a slight recovery but not to the level of oxygen consumption rates of the controls. In the present study, the intestinal tissue from those worms that had been debrained for longer than 3 days did not show this continued depression in oxygen consumption. Thus, the recovery exhibited by these tissues seemed much more complete than Elzinga (1963) found for the whole body. In fact, those worms in this study debrained from 4 to 20 days before testing had intestinal and body wall tissue respiration levels higher than that of these same tissues from controls worms (Table 2, Figure 2a and 2b).

Since neurosecretory cells are present in the ventral nerve cord ganglia and the subpharyngeal ganglia of earthworms (Gabe, 1966) and amine secreting cells are also present in the same areas (Bianchi, 1967), it is possible that after four days following the debraining, these structures could begin to release substance(s) previously suppressed which had a similar influence on oxygen consumption as the suprapharyngeal ganglion had before its removal and thus would continue to influence the various tissues involved. In addition to this explanation, the observable oxygen consumption pattern exhibited by intestinal and body wall tissues of the debrained worms of first hypoactivity followed by hyperactivity,

might be also explained by the loss of the suprapharyngeal ganglion. If the brain of the earthworm is to be classified as a true regulator of oxidative metabolism it must be able to suppress as well as to stimulate the oxidative metabolic processes. At this point an assumption can be made that the brain of the earthworm may be a true regulator of oxidative metabolism. This assumption will be substantiated later on in the discussion. Upon the basis of this assumption, then, perhaps this hypoactivity as reflected by low oxygen quotient exhibited by the intestinal and body walls from worms that had been debrained for 1 to 3 days prior to experimentation was due to the inability to respond to the surgical stimulus of tissue dissection. This inability could be due to the trauma brought about by the removal of the brain which caused the hypoactivity observed in 1, 2, and 3 days debrained worms. The observable hyperactivity of high level oxygen quotient of intestinal and body wall tissues from worms that had been debrained 4 to 20 days prior to experimentation, might possibly be due to the lack of the brain with its suppressive action. The hyperactivity as reflected by an increased rate of oxygen consumption observed in intestinal tissues from 4 to 20 days debrained worms (Table 2, Figure 2a), might be due to either substance(s) released from the brain or the absence of some factor(s) upon brain removal. The assumption made of the brain being a true regulator of oxidative metabolism will be specifically dealt with further on in the discussion.

A sham operation was done on a number of earthworms three days prior to experimentation. This was done to determine if the

consistent depression in oxygen consumption observed at 3 days was actually due to the loss of the brain or merely the trauma resulting from slitting the dorsal segments. It was found that these worms showed an oxygen consumption like that of the controls (Table 3). The results from the slitting of the first few dorsal segments indicate that the initial operation of removing the brain is not the cause for the observable depression of the oxygen quotient seen in three days debrained worms. However, this operation does not eliminate the possibility that the initial depression of the oxygen quotient is due to surgical trauma because merely slitting the dorsal segments might not have the same effect as the whole operation of debraining.

Table 3. The mean oxygen quotients of intestinal and body wall tissue from sham operated worms and control worms.

Condition	Intestinal Wall QO_2*	Body Wall QO_2
Three days sham operated	0.494±0.012 (4)**	0.300±0.073 (4)
Control	0.466±0.020 (2)	0.315±0.013 (2)
Probability	n.s.	n.s.

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

**Values expressed as averages ± S.D. with numbers of replicate tests in parenthesis.

Craig (1966) found in L. terrestris that the glucose levels of the blood decreased to zero within the first 24 hours after debraining and although some later recovery was shown, blood levels

remained below normal. In this study, three concentrations of glucose were added to the bathing saline media. The total concentrations present in the various flasks were 0.0166 M., 0.0333 M., and 0.100 M. This procedure was done to determine if the oxidative metabolic rate was dependent on substrate availability. The results (Table 4) showed that the glucose substrates did not increase the oxidative metabolism of either the intestinal wall or the body wall of the experimental group. Keeley and Friedman (1967) also found no increase in oxygen consumption of fat bodies of cardiatectomized cockroaches when glucose was added. The results from this study indicated that the depression of the oxygen quotient of intestinal and body wall tissues from three days debrained worms was not due completely to lack of available glucose in body fluids.

Table 4. The oxygen quotients of intestinal and body wall tissues from worms debrained three days prior to experimentation in the presence of a non-nutritive bathing media and those oxygen quotients from these same tissues when a nutritive bathing media was used.

Condition	Intestinal Wall QO_2*	Body Wall QO_2
Experimentals	0.426±0.028 (2)**	0.286±0.001 (2)
Experimentals + 0.0166 M. glucose	0.399±0.029 (2)	0.262±0.080 (2)
Experimentals + 0.0333 M. glucose	0.426±0.003 (2)	0.297±0.004 (2)
Experimentals + 0.1000 M. glucose	0.388±0.002 (2)	0.192±0.026 (2)

*QO_2 = the mean oxygen quotient in units of microliters of oxygen consumed per milligram of dry tissue weight per hour of time.
**Values expressed as averages ± S.D. with numbers of replicate tests in parenthesis.

After it was determined that those worms debrained three days prior to experimentation were to constitute the experimental group, numerous determinations were made of the oxygen quotients of the intestinal wall and the body wall tissues from both experimental and control animals. Statistical analysis demonstrated that the mean oxygen quotients determined from the experimental and control intestinal tissue were statistically significantly different ($P<0.01$) (Table 5). There was no statistically significant difference ($P>0.05$) between the mean oxygen quotients of the body wall from the experimental and from the control earthworms (Table 5). Explanations are uncertain for this lack of a real difference between the oxygen quotients for body wall tissues from experimental and control worms. However, it was observed in the initial experiments (Table 2, Figure 2b) that the body wall from one day debrained worms had a lower oxygen consumption than body wall from those of controls. Complete recovery of this depressed oxygen quotient was present earlier than in the intestinal tissues. (Figure 2a and 2b). Also, perhaps the body wall tissue is less sensitive than the intestinal tissue and more stable. This possibility was indicated by the lesser degree of hourly fluctuation of oxygen consumption by the body wall tissue. (Figure 1a and 1b)

A further examination of the data presented in tables 2,3,4, and 5 showed that the oxygen consumption of the intestinal tissue was higher at all times than that of the body wall tissue. This is further illustrated in Figures 1a, 1b, 2a, and 2b. Statistical analysis indicated that this difference was statistically significant.

Table 5. The comparison of the oxygen quotients from intestinal and body wall tissues from control earthworms and three days debrained experimental earthworms.

Condition	Intestinal Wall QO_2*	Body Wall QO_2
Controls	0.530±0.093 (55)**	0.309±0.082 (52)
Experimentals	0.443±0.120 (37)	0.294±0.074 (37)
Probability	0.01	n.s.

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

**Values expressed as averages ± S.D. with numbers of replicate tests in parenthesis.

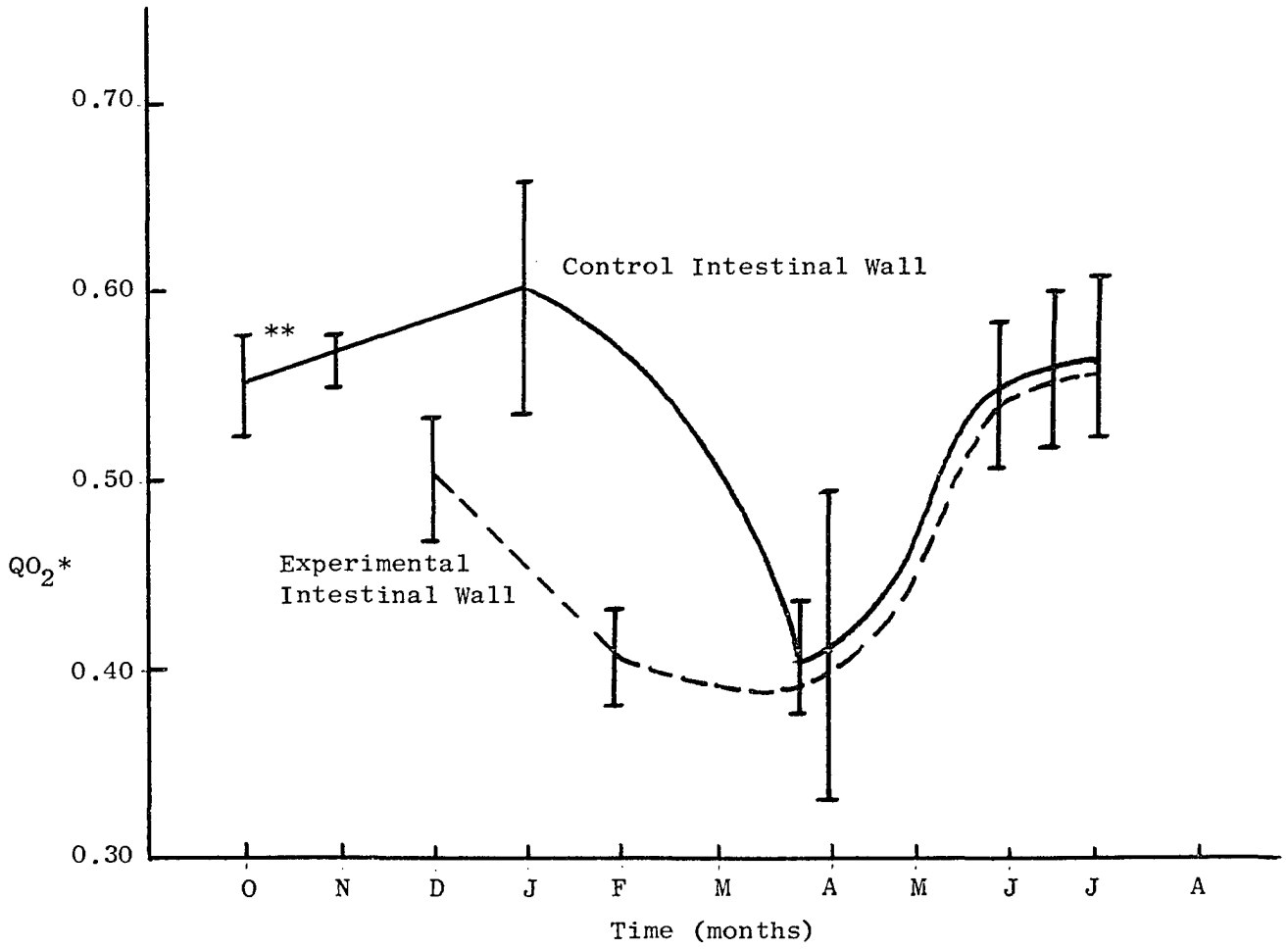
The body wall of the earthworm has been reported to have only one type of muscle fiber present with very low oxidative enzyme activity (Ogata and Morl, 1964). This lends support to this observation that the intestinal wall did have a higher rate of oxidative metabolism than did the body in both controls and experimentals. Urich (1964) determined the oxygen consumption rates of various types of tissues and organs of L. terrestris and found that the oxygen consumption of intestinal tissue was higher than for other tissues. From his study, he suggested that due to the relatively high oxygen consumption of the intestinal tissue of earthworms that this tissue perhaps has other metabolic functions besides digestion, absorption, and accumulation or storage.

A hypothesis has been formulated by Keeley and Friedman (1969) from their observations on Blaberus discoidalis that a depression of the fat bodies oxygen quotients following cardiatectomy could

indicate a malfunctioning of the fat body mitochondria. There may be a similar explanation for the results observed in the earthworms following debraining.

It has also been shown that extracts of the corpus cardiacum have been effective in increasing phosphorylase activity in insects (Mordue and Goldsworthy, 1968). The depression in oxygen consumption in earthworms could be due to a decrease in active phosphorylase following debraining. If the phosphorylase system was inactivated, the conversion of glycogen to glucose-1-phosphate would be retarded. Without the products of glycogenolysis being present, oxidative metabolism would decrease and this would be indicated by a drop in the rate of oxygen consumption. Since the addition of the glucose substrates did not restore the depressed rate of oxygen consumption, the possibility exists that the oxidative metabolism suspression was not due to an inactivated phosphorylase system.

The major portion of this study was conducted between October 5, 1968, and July 23, 1969. From an examination of the mean oxygen quotients obtained throughout this period it was found that the intestinal wall from the controls but not the body wall from the same controls had a 30 percent lower rate of respiration in April and May than during any other time of the study. (Figure 3a) Following this time period the oxygen consumption rates returned to the same level as seen prior to April and May for the intestinal wall. The intestinal wall from the experimental worms followed the same pattern as the intestinal wall from the control, but the amount of oxygen consumption depression was not as great as before.



*QO_2 = ul of O_2/ mg. dry wt./ hour of time

**Indicate the range above and below the mean oxygen quotient

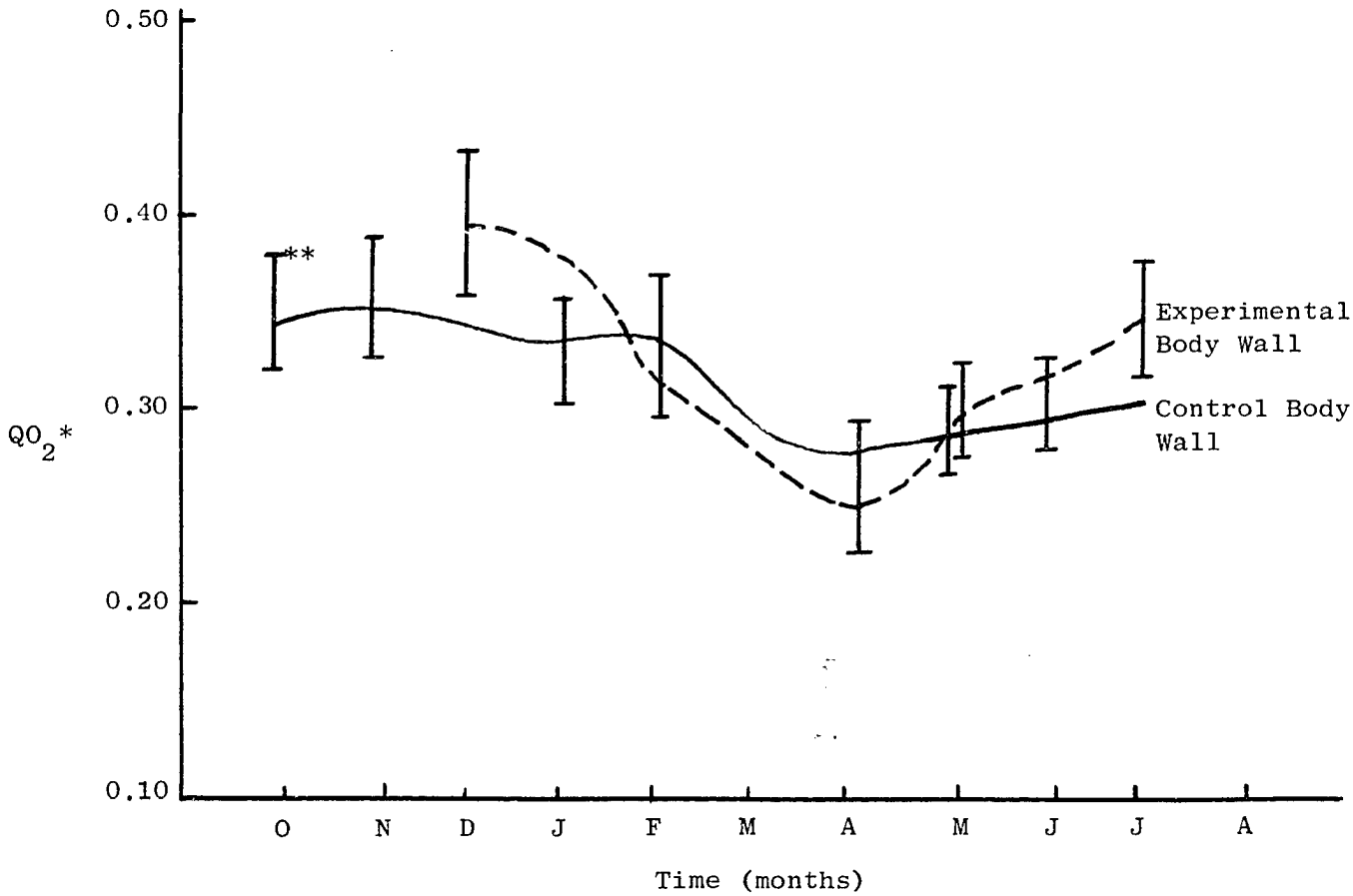
Figure 3a. The mean oxygen quotients of intestinal walls from controls and experimental worms between October, 1968, and July, 1969.

This was demonstrated by the fact that the difference between the oxygen consumption of intestinal wall of the experimental and of the controls was no longer of statistical significance ($P>0.05$).

There was a similar drop in the oxygen consumption of body wall tissues. However, proportionately it was much less, amounting to only about a ten percent drop. (Figure 3b) This may be further evidence for the greater stability of the body wall tissue.

Possible explanations for this seasonal drop in oxidative metabolism in these two tissues require additional data. However, this time coincides with reproductive prepardness of the earthworm and possibly the majority of activity has shifted from the digestive system to the reproductive system.

The major portion of the determination of the oxygen quotients was done between 10:00 and 16:00 E.S.T. This was done to minimize any variations brought about by the presence of a circadian rhythm that would make it difficult to compare results obtained at different time periods. To check the presence of such a circadian rhythm a run was conducted between the times of 15:00 and 19:00 E.S.T. (Table 6) Since only two runs were made, no statistical analysis was done. Since such a small sample size was used, it was difficult to draw any conclusions. However, there was some indication that the mean oxygen quotients of both the intestinal wall and the body wall of the controls was depressed in the experiment run at this later time period. These same tissues from the experimental worms appeared to have increased their rate of oxygen consumption in this later time period. Reasons for this apparent shift in



*QO_2 = ul of O_2/ mg. dry wt./ hour of time
**Indicate the range above and below the mean oxygen quotient

Figure 3b. The mean oxygen quotients of body wall tissue from controls and experimental worms between October, 1968, and July, 1969.

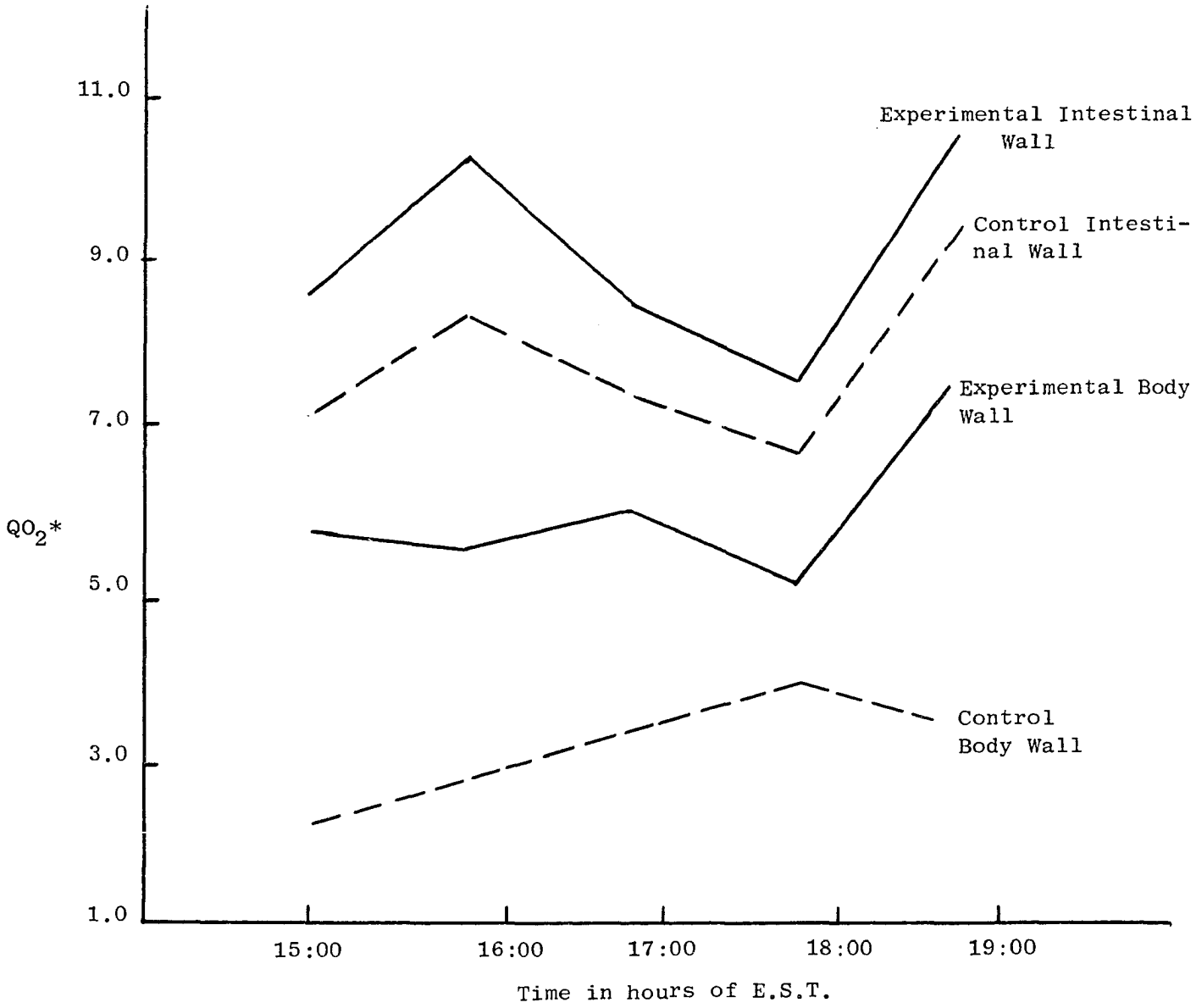
pattern are obscure. Bennett and Willis (1966) have found a faster rate of locomotion and withdrawal by L. terrestris at 19:00 E.S.T. as compared with worms run at 12:00 E.S.T. It is interesting to note that worms from which brains had been removed performed at the same rate at the two time periods. Ralph (1957) indicated a greater oxygen consumption from 19:00 to 24:00 of intact earthworms than at 12:00 E.S.T. However, although the mean oxygen quotient of controls showed a depression upon examining the individual hourly oxygen quotients (Figure 4) the controls do have a higher rate at 19:00 hours than they showed at 15:00 E.S.T. which agrees with the results found by Ralph (1957). This same higher level of oxygen consumption at 19:00 hours as compared to that at 15:00 hours was also seen in the experimental groups by both types of tissues. This indicated that some type of factor(s) were affecting both groups of tissues

Table 6. The oxygen quotients from control intestinal wall and body wall and from experimental intestinal wall and body wall observed in a late afternoon-early evening study from 15:00 to 19:00 E.S.T.

Condition	Intestinal Wall QO_2*	Body Wall QO_2
Controls	0.456±0.053 (2)**	0.162±0.063 (2)
Decapitated controls	0.426±0.058 (3)	0.198±0.082 (3)
Experimentals	0.551±0.051 (3)	0.329±0.010 (3)

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

**Values expressed as averages ± S.D. with numbers of replicate tests in parenthesis.



*QO_2 = ul of O_2/ gram dry wt./ minute of time

Figure 4. Hourly oxygen quotients of intestinal and body wall tissues from non-decapitate controls and non-decapitated experimentals obtained from experimentation from 15:00 to 19:00 hours E.S.T.

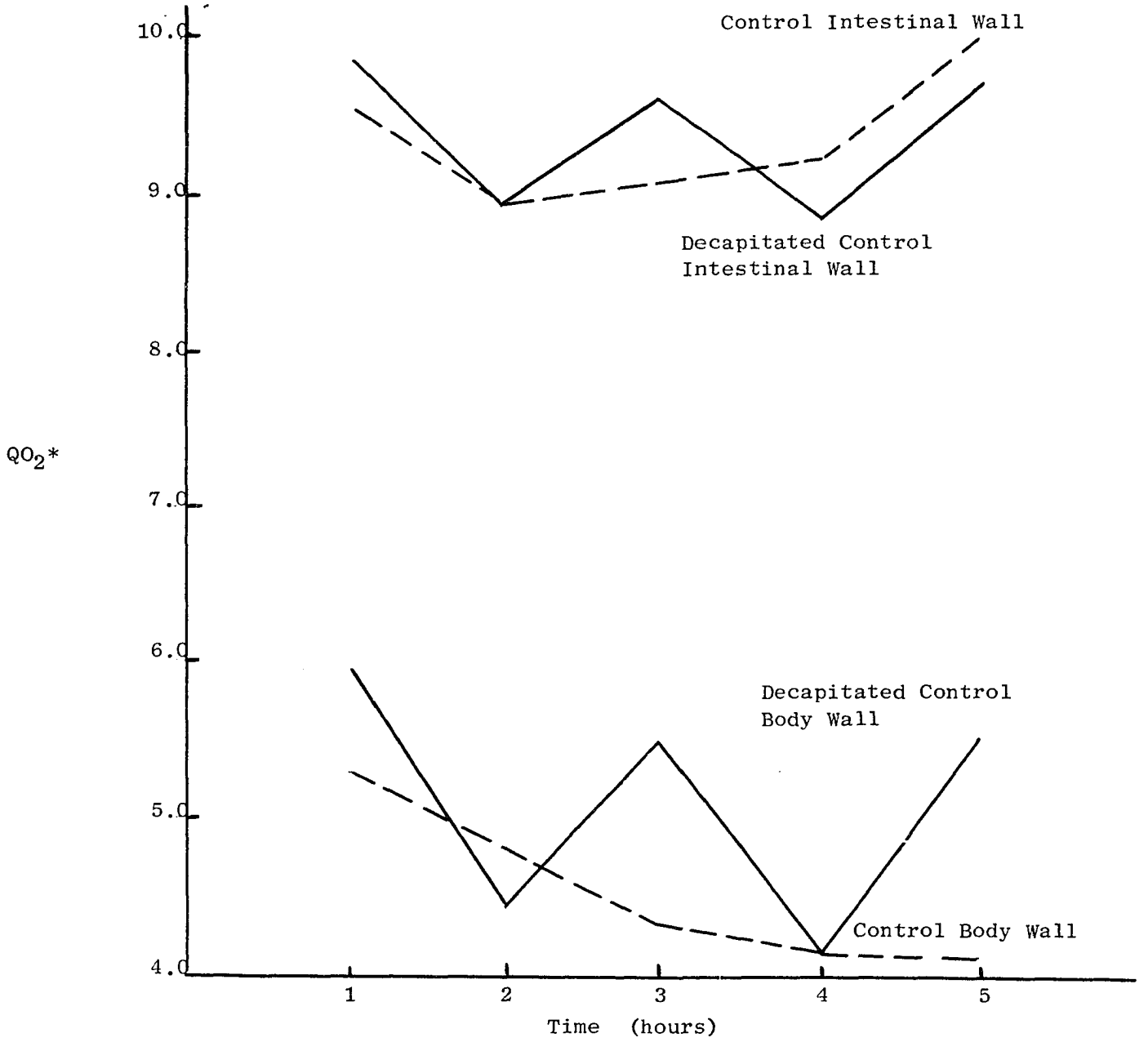
similarly and that, perhaps, oxygen consumption of these isolated tissues reflect the increased nocturnal activity shown by the intact worm.

Up to this point the work reported here and that of Elzinga (1963) have demonstrated (Table 5) that the brains of earthworms have a stimulatory effect on the oxidative metabolism of the intestinal tissues as well as the intact organism because removal resulted in depression of oxygen consumption. However, in order to assume that the brain regulates oxidative metabolism, it must also be able to slow down the process of oxidative metabolism depending on the needs of the whole organism. In order to demonstrate this regulatory function of the earthworm brain, it was necessary to establish a basal level of oxidative metabolism for this study. It was decided that upon decapitation of the anterior end of the earthworm, all effects of the brain and its associate structures would be eliminated during the stimulus of surgical dissection of the tissues. This then would represent a condition of a resting level of oxidative metabolism. It cannot be overemphasized that speed in performing the decapitation operation was of the utmost importance in order to prevent the brain from releasing any substance(s) which could affect oxidative metabolism of the tissues. Intestinal and body wall tissues' oxygen quotients from decapitated control earthworms were compared to the oxygen quotients of these same tissues from non-decapitated controls (Table 6, Table 7). The decapitation procedure appeared to have opposite effects on the two types of tissues. The intestinal tissue from decapitated controls

had a lower rate of oxygen consumption than the intestinal tissue from the non-decapitated controls (Table 7). However, the body wall from the decapitated control worms in this series of experiments had a higher rate of oxygen consumption than that of body wall tissue from non-decapitated control worms. No statistically significant difference ($P>0.05$) was demonstrated between the two types of tissues from decapitated controls and non-decapitated controls (Table 7).

It would seem reasonable that tissues from decapitated controls would have a lower oxygen quotient than those same tissues from non-decapitated controls because of the time required to prepare and remove the tissues from the living worm, which was about 90 seconds. The suprapharyngeal ganglion would be stimulated by this surgical trauma which could result in the brain's release of its substance(s) at an augmented rate. This could produce an increased rate of oxygen consumption. Upon decapitation, the release of secretions from these structures would thus be eliminated, resulting in a lower rate of oxygen consumption. However, the body wall from decapitated controls did not show this pattern of a depressed oxygen quotient as did the intestinal tissues. However, in later experiments (Table 8) body wall tissues from decapitated controls exhibited lower oxygen quotients than this series (Tables 7 and 8).

In addition, when the oxygen quotients of the intestinal and the body wall tissues from decapitated controls were compared to these same tissues from decapitated experimental worms, it was observed that the intestinal wall and the body wall from these



*QO_2 = ul of O_2/ gram dry wt./ minute of time

Figure 5. Hourly oxygen quotients of intestinal wall and body wall tissues from non-decapitated controls and decapitated controls.

Table 7. Oxygen quotients of intestinal and body wall tissues from decapitated controls and non-decapitated control earthworms.

Condition	Intestinal Wall QO_2*	Body Wall QO_2
Non-decapitated controls	0.570±0.150 (10)**	0.272±0.050 (10)
Decapitated controls	0.558±0.174 (10)	0.305±0.067 (10)
Probability	n.s.	n.s.

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

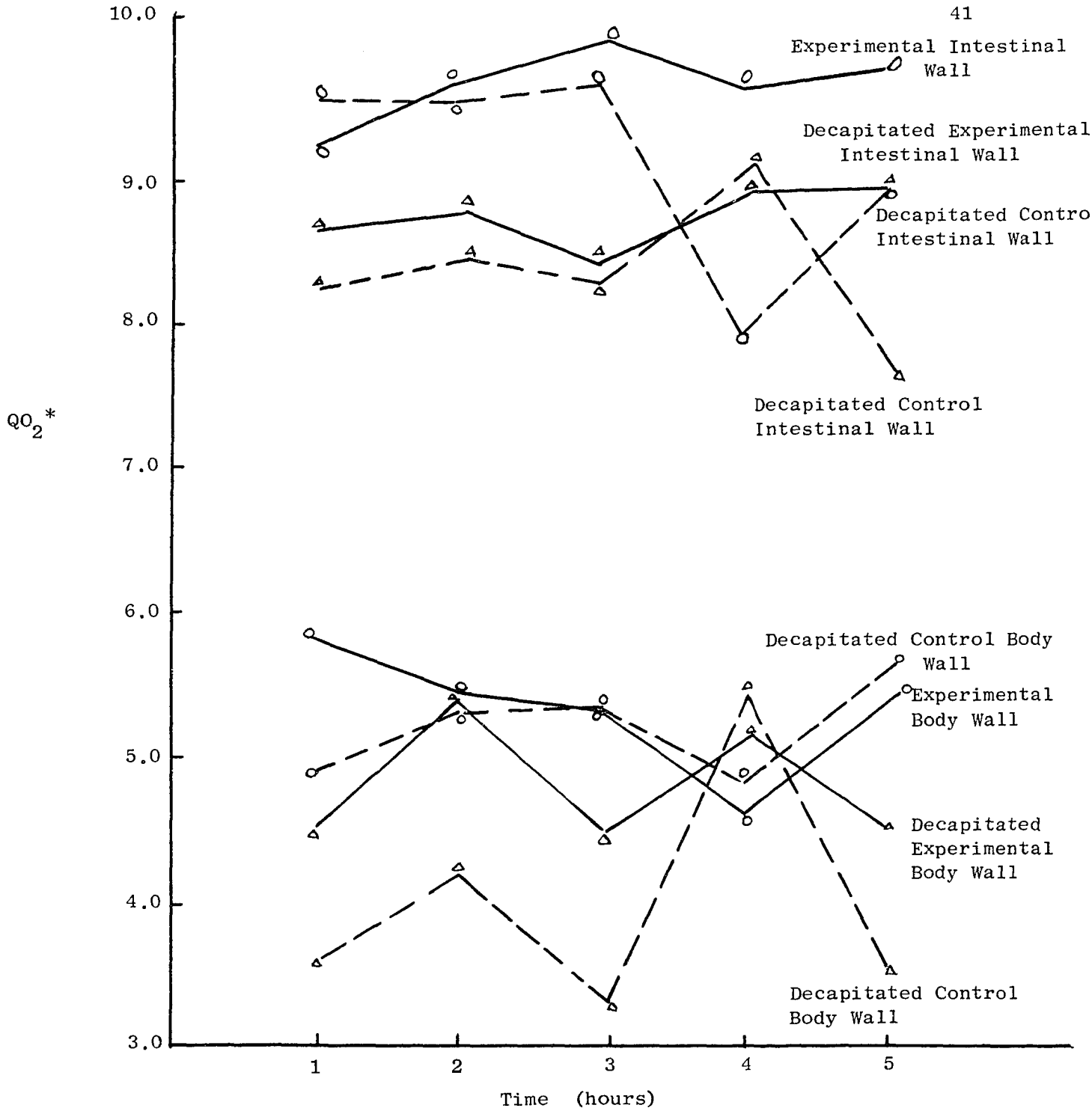
**Values expressed as average ± S.D. with numbers of replicate tests in parenthesis.

decapitated control worms had a lower oxygen quotient than did these same tissues from the decapitated experimental group (Table 8 and 10b, Figure 6). These results were opposite to those results observed from tissues when decapitation procedures were not used, (Tables 5, 8, and 10b, Figures 1a, 1b, and 6). In these experiments the experimental intestinal wall tissues consumed less oxygen than did the controls in which there were no significant differences.

Table 8. Oxygen quotients of intestinal and body wall tissues from decapitated controls and from decapitated experimental worms.

Condition	Intestinal Wall QO_2*	Body Wall QO_2
Decapitated controls	0.467±0.114 (14)**	0.229±0.041 (14)
Decapitated experimentals	0.522±0.051 (16)	0.300±0.056 (16)
Probability	n.s.	0.01

*QO_2 = the mean oxygen quotient in units of microliters of oxygen consumed per milligram of dry tissue weight per hour of time.
**Values expressed as average ± S.D. with numbers of replicate tests in parenthesis.



*QO_2 = ul of O_2/ gram dry wt./ minute of time

Figure 6. Hourly oxygen quotients of intestinal and body wall tissues from decapitated controls ( ) and decapitated experimentals ( ). Also hourly oxygen quotients of intestinal and body wall tissues from decapitated controls ( ) and non-decapitated experimentals ( ).

Since the oxygen consumption rate from the intestinal wall and the body wall tissues from decapitated control worms were considered as the basal oxidative metabolic rate for this study, these same tissues from decapitated and non-decapitated experimental worms were found to respond to the stimulus of tissue dissection in a stimulatory manner. The intestinal wall's oxygen quotient from decapitated controls was not statistically significantly different from intestinal tissue from both decapitated and non-decapitated experimental worms ($P > 0.05$) (Tables 7 and 10b). The body wall's oxygen quotient from decapitated controls was statistically significantly different from body wall oxygen quotient from decapitated experimental worms ($P < 0.01$) (Table 7, Figure 6). However, these same body wall tissues from decapitated controls were not statistically significantly different from body wall tissues from non-decapitated experimentals in respect to their oxygen consumption rates ($P > 0.05$) (Table 10b). This would indicate that with the absence of the brain, the body wall and to some extent the intestinal wall do not have the ability to slow down their rate of oxidative metabolism upon stimulation (Figure 6).

These results may also help to explain why the tissue respiration from worms debrained 4 to 20 days prior to experimentation showed a higher rate of oxidative metabolism than those same tissues from intact earthworms (Table 2, Figure 2a and 2b). The control worms responded positively to the stimulus of tissue dissection, but not to the extent these same tissues from greater than 4 days debrained worms responded. The possible explanation might be that the control

worms, although responding in a hyperactive manner, were restricted by the substance(s) in the brain or merely to the presence of the brain. The debrained worms could also respond positively to tissue dissection, but these tissues could not restrict their response which was indicative of a higher oxidative metabolic rate than experienced by the control worm's tissues (Table 2).

In an earthworm without its suprapharyngeal ganglion, stimulation elicits an excitory response by augmented tissue respiration with no suppressive check to slow down the oxidative metabolic rate. The intestinal and body wall tissues from control worms with their brains intact during dissection showed an excitory response to dissection, too (Table 2), but not to the extent these same tissues from debrained worms showed. Further, the oxygen quotient of the body wall from decapitated controls was statistically significantly different from the oxygen quotient of the body wall from the decapitated experimental animals ($P<0.01$) (Table 8). This difference tends to indicate that the body wall as well as the intestinal wall was affected by the brain. Earlier in the study, the oxygen consumption of the body wall seemed not to be affected by the brain (Table 5, Figures 1a and 1b) to any statistical significant difference ($P>0.05$). However, it seemed that upon decapitation of control worms, the body wall showed a statistically significant difference ($P<0.01$) in oxygen quotient than decapitated experimentals (Table 7) and to a less degree ($P>0.05$) from non-decapitated experimental worms (Table 10b). In other words, the tissues from worms whose brains were instantly removed had an oxygen consumption

rate lower than did experimental tissues.

Perhaps the effect of the brain was not previously observed in the body wall because this tissue had a lower rate of oxidative metabolism as contrasted to that of the intestinal tissue. Therefore, it may be less sensitive to the effects of the brain than that of the intestinal tissue and special precautions must be undertaken in body wall tissue preparations in order to observe effects elicited by the brain of earthworms. The body wall also showed little effect by the time of year studied (Figure 2b) which indicated that this tissue was more stable than the intestinal tissue (Figure 2a). Therefore, the brain in the intact earthworm apparently can speed up and slow down the oxidative metabolism of its tissues, and perhaps can be classified as a true regulator of oxidative metabolism of L. terrestris.

Up to this point all experimental work to demonstrate the effects of the brain on oxygen consumption of intestinal and body wall tissues was concerned with the effect of brain removal. In order to show that these observed effects were due to substance(s) present in the brain and not to neurogenic mechanisms, a series of experiments were performed in which the effect on oxygen consumption of the addition of brain homogenates to the tissues was studied. When sufficient brain homogenate to effect a final concentration of one brain per one milliliter of the bathing saline in the reaction flask was added to both types of tissues from controls and experimentals, the oxygen quotients from all the tissues increased (Table 9). The data showed that the addition of brain

homogenates of this concentration to these in vitro systems increased the oxygen quotient of the intestinal tissue from the experimental worms by 11% over the same tissue not exposed to the homogenate. This rise of 11% of the experimental's intestinal wall oxygen quotient almost restored the depression in oxygen quotient which resulted from debraining (Table 9). The homogenate had a lesser effect on the control intestinal wall as it produced only a 5.8% increase in oxygen consumption, which was about one-half the response displayed by the experimentals' intestinal tissue.

The addition of the brain homogenate of this concentration also increased the oxygen consumption rate of the body wall of both the controls and the experimentals. This increase was 12.9% above those same tissues of both controls and experimentals not exposed to the homogenate. This lends support to the hypothesis developed above that perhaps the body wall is affected by the brain and its contents.

However, this crude whole brain homogenate could contain many substance(s) that elicit this excitatory type of response. This excitatory type response observed as a result of homogenate addition could be due to a direct contact with these tissues of substance(s) present in the brain that would normally not be in contact with them. It is known that monamines are present in the ventral nerve cords of L. terrestris (Rude, 1969), and perhaps the brain has these amines present, too. Bianchi (1967) demonstrated the presence of amine secreting neurons in the central nervous system of earthworms. Thus both catecholamines and indolamines may be present in the crude homogenate made from the brains of these earthworms, and it is these

substance(s) that are affecting the oxidative metabolism. However, none of these results were found to be of statistical significant difference ($P > 0.05$), but were just indications that brain homogenates had an effect on both body wall and intestinal wall tissues of earthworms as reflected by changes in the observable oxygen quotients.

Table 9. The results from the addition of brain homogenates of final concentrations of one brain per milliliter of bathing saline on oxygen quotients of intestinal and body wall tissues from both control and experimental earthworms.

Condition	Intestinal Wall QO_2*		Body Wall QO_2	
Controls	0.396±0.070	(6)**	0.278±0.026	(6)
Controls + brain homogenate	0.419±0.088	(3)	0.314±0.036	(3)
Probability	n.s.		n.s.	
Experimentals + brain homogenate	0.385±0.099	(12)	0.317±0.087	(12)
Experimentals	0.346±0.029	(12)	0.282±0.083	(12)
Probability	n.s.		n.s.	

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

**Values expressed as average ± S.D. with numbers of replicate tests in parenthesis.

When sufficient brain homogenate to effect a final concentration of two brains per one milliliter of bathing saline was administered to the tissues, two opposite effects were observed (Tables 10a and 10b). In the first experiment that was run on May 9, 1969, the brain homogenate of this concentration stimulated oxygen consumption of both

control's body wall and the experimental's body wall. Only the intestinal wall of the experimentals was stimulated by this brain homogenate, while the intestinal tissue from the control seemed to be inhibited (Table 10a). On June 25, 1969, and June 27, 1969, the opposite effect was observed. In this test, the intestinal wall and the body wall of the experimental worms had a lower oxygen quotient when the brain homogenate was added then did those same tissues from experimental worms that were not exposed to the brain homogenate. In the June testing, the control worms were decapitated while the experimental worms were not. It was observed in this test that the intestinal tissue from decapitated controls was the only tissue which responded positively to the brain homogenate administration (Table 10b). However, the body wall as reflected by no change in its oxygen quotient, from the decapitated control worms was not affected by the brain homogenate of this concentration. It would have seemed logical that a positive response would have been observed from brain homogenate addition (ie. intestinal tissues response to brain homogenate seen in Table 10b) but the lack of response by the body wall from the decapitated control worms was unexplainable.

However, the brain homogenate of the greater concentration did not elicit as great a response as the weaker brain homogenate concentration did. The explanation for this type of response is uncertain. It is, also, uncertain why a dose response is not seen upon homogenate addition of two different strengths. It was unlikely that a Crabtree effect was present because of the results obtained

Table 10a. The results from the addition of brain homogenates of final concentrations of two brains per milliliter of bathing saline on oxygen quotients of intestinal and body wall tissues from both non-decapitated controls and non-decapitated experimentals. The data was collected during May 9, 1969.

Condition	Intestinal Wall QO_2*	Body Wall QO_2
Controls	0.490±0.000 (2)**	0.389±0.024 (2)
Controls + brain homogenate	0.404±0.000 (1)	0.404±0.000 (1)
Probability	n.s.	n.s.
Experimentals	0.358±0.167 (4)	0.290±0.058 (4)
Experimentals + brain homogenate	0.401±0.047 (4)	0.312±0.018 (3)
Probability	n.s.	n.s.

Table 10b. The results from the addition of brain homogenates of final concentrations of two brains per milliliter of bathing saline on oxygen quotients of intestinal and body wall tissues from decapitated controls and non-decapitated experimentals. The data collected was on June 25 and 27, 1969.

Condition	Intestinal Wall QO_2*	Body Wall QO_2
Decapitated controls	0.542±0.032 (4)**	0.306±0.024 (4)
Decapitated controls + homogenate	0.568±0.052 (4)	0.305±0.046 (4)
Probability	n.s.	n.s.
Experimentals	0.571±0.065 (6)	0.313±0.057 (6)
Experimentals + homogenate	0.508±0.032 (6)	0.271±0.070 (6)
Probability	n.s.	n.s.

*QO_2 = the mean oxygen quotient in units of ul O_2 consumed / mg. dry wt./ hour

**Values expressed as averages ± S.D. with numbers of replicate test in parenthesis.

when different glucose substrates were added to the intestinal and body wall tissues (Table 4).

These results, where homogenates of different concentrations had opposite affects on tissue respiration at different times are not completely improbable. Muller and Engelman (1968) found in Leucophaea maderae that if basal respiration were high, corpus cardiacum homogenates elicited an inhibitory response. And if the basal rates were low, the corpus cardiacum homogenates stimulated the respiration rates. This type of situation may be present in this study with the greater concentration of brain homogenates. In the June testing, the oxygen consumption rates of the experimental intestinal wall was relatively high (Table 10b). It was then observed that brain homogenate addition resulted in a depression of the oxygen consumption of the experimental worm's intestinal wall. When these same brain homogenates were added to intestinal tissue which displayed relatively low basal respiratory rates as found in May, the results were stimulatory as reflected by an increase in the observable oxygen quotient of the experimental worm's intestinal tissue (Table 10a).

The above results suggested that perhaps the effect of whole brain homogenates on tissue respiration in this study can not be exclusively attributed to one single circumstance(s). Several things appeared to contribute to the observable results. For instance, the condition of the target tissue may be important as seen by the different responses depending on the level of metabolic activity. The amount and condition of the substance(s) present in the brain could have an effect on responses observed on tissue oxidative metabolism.

It cannot be precisely determined what effect the brain homogenates have on tissue oxidative metabolism because of the nature of the homogenates. They are whole brain homogenates and not pure identified fractions. This could mean that the homogenates used might have a whole series of active substance(s) that elicit specific responses in the intact organism. Therefore, the brain could have specific products for regulation of osmotic and ionic balance, control of cardiac rates, reproductive processes, and many others. However, in this study when the whole brain was made into a single homogenate and administered to a designated tissue, the response observed can only be grossly viewed and reported.

Keeley and Friedman (1969) concluded that oxygen consumption depression from cardiactectomized-allatectomized cockroaches could be due to a malfunctioning of fat body mitochondria. Since the mitochondria are the site of cellular respiration, it is reasonable to assume that any factor(s) that would influence oxidative metabolism, would act directly on the mitochondria. By using mitochondrial extracts which are more uniform than whole tissues, the influence of the suprapharyngeal ganglia and its contents could be analyzed in greater detail.

Tata et. al. (1962) found thyroid hormones affected both basal metabolism and mitochondrial activity. With the administration of thyroid hormones, tissue respiration was enhanced and both the electron transport system and phosphorylation increased (Tata et. al, 1963). Klitgaard (1966) observed upon thyroidectomy that both the cytochrome-c and oxygen uptake of rat muscle decreased. This

makes for interesting analogy between the vertebrate and invertebrate world. It is conceivable that the suprapharyngeal ganglia of earthworms regulates basal metabolism and influences mitochondrial activity as does the thyroid in the mammals.

SUMMARY

1. The present study was done in order to determine if the oxidative metabolism of intestinal and body wall tissues of earthworms, Lumbricus terrestris, was affected by substance(s) present in the suprapharyngeal ganglion.

2. The removal of the suprapharyngeal ganglia of earthworms resulted in a depression of the oxygen consumption of intestinal tissue within the first three days following debraining.

3. The depression in the oxygen consumption of the intestinal tissue from worms debrained for longer than three days disappeared.

4. The depression in the oxygen consumption of intestinal tissues of earthworms within the first three days prior to experimentation seemed to be due to the loss of the brain.

5. The greatest and most consistent depression in oxygen consumption of intestinal wall was observed three days after debraining and this depression of oxygen consumption of intestinal tissues following removal of the brain was statistically significant ($P<0.01$).

6. The body wall tissue had its greatest depression at one day following debraining, followed by hyperactivity as indicated by a oxygen consumption rate greater than that of the controls from the second day after debraining.

7. The respiratory rates of the intestinal tissues from both experimental and control worms was always statistically significantly higher from body wall tissue respiratory rates ($P<0.01$).

8. Oxygen consumption rates of intestinal tissues were found to be lower during April and May, 1969.

9. This apparent time of year did not seem to influence the body wall oxygen consumption to as great an extent.

10. The isolated tissues of earthworms tended to have a higher rate of oxygen consumption at 19:00 E.S.T. than at 15:00 E.S.T. although the mean QO_2 of these tissues was lower during the later time period.

11. There was some indication that decapitation of earthworms before dissection of tissues to be tested for oxygen consumption resulted in a lowering of oxygen consumption. It was considered that this was the best representative of a resting metabolic rate of the tissues involved.

12. Intestinal and body wall tissues from debrained earthworms had a higher oxygen quotient than those same tissues from decapitated control worms. This indicated that the tissues from the experimental worms did not have the ability to slow down their rate of oxidative metabolism following stimulation.

13. The addition of brain homogenates of 1 brain per 1 milliliter bathing saline solution resulted in a observable increased oxygen consumption rate of all intestinal and body wall tissues from both controls and experimentals. The body walls of both controls and experimentals were affected the most by a rise of 12.9% in the oxygen consumption in both cases.

14. The brain homogenate of 1 brain per 1 milliliter of bathing saline appeared to restore the oxygen consumption rate of the

experimentals' intestinal tissue to that of the standard controls' intestinal tissue. The brain homogenate also increased the oxygen consumption of control intestinal tissue, but only by one-half the amount the experimental intestinal tissue exhibited.

15. Administration of brain homogenates with twice the concentration as above had two affects:

a. When administered to intestinal tissue which had low metabolic rates, the brain homogenates seemed to stimulate respiratory metabolism.

b. When administered to intestinal tissue which had high metabolic rates, the brain homogenates seemed to inhibit respiratory metabolism.

16. The greater concentration of brain homogenate either stimulated, depressed, or had no affect on the body wall oxygen consumption of both control and experimental worms.

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