7-1964

An Investigation of the Influence of the Suprapharyngeal Ganglia upon Water Balance and on Osmoregulation in Lumbricus Terrestris

Richard M. Dennany

AN INVESTIGATION OF THE INFLUENCE OF THE SUPRAPHARYNGEAL GANGLIA UPON WATER BALANCE AND ON OSMOREGULATION IN LUMBRICUS TERRESTRIS

by

Richard M. Dennany

A thesis presented to the
Faculty of the School of Graduate
Studies in partial fulfillment
of the
Degree of Master of Arts

Western Michigan University
Kalamazoo, Michigan
July, 1964

ACKNOWLEDGEMENTS

The investigator wishes to express his sincere appreciation to the many persons for their assistance in obtaining materials and providing advice for the production of this paper.

Special mention is due to the members of my graduate committee Dr. Jean McVay Lawrence, Dr. Imy Holt, Dr. Jack Wood, and Dr. Leo VanderBeek. The author also wishes to thank Dr. Richard Brewer for the use of his laboratory.

Richard M. Dennany

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

INTRODUCTION

In the central nervous system of most animals which have been examined some neurons show cytological characteristics of gland cells. These cells are now recognized as a specialized cell type and designated neurosecretory cells.

These neurosecretory cells are capable of elaborating secretory material which is accumulated as granules in the body of the nerve cell and in the axon-endings. They release complex organic substances, neurohormones, which act as hormones and are often liberated into the blood stream (Welsh, 1958). These specialized neurons are capable of receiving impulses from other neurons although their axons do not end in contact with other neurons or effectors (Scharrer, 1959). Examples are the hypothalmus-pituitary axis of vertebrates and the sinus-x gland complex of crustaceans (Laverack, 1963).

In many of the invertebrates, especially Annelida, these neurosecretory cells make up a large proportion of the central nervous system (Hagadorn, 1959; Scharrer, 1959; Herlant-Meewis, 1961). According to Scharrer (1959) they may well be the only source of hormones in these organisms.

Because neurosecretory cells of various types have been demonstrated in all the ganglia of the central nervous system of lumbricid ogliochaetes, (Herlant-Meewis, 1961) this investigation was undertaken to establish a function for the neurohormones produced by the neurosecretory cells of the suprapharyngeal ganglia of Lumbricus terrestris.

Neurosecretory cells of L. terrestris were first described in the suprapharyngeal ganglia by Scharrer and Scharrer (1939). Schmid (1947) confirmed their presence and also obtained evidence of the occurrence of a cyclic variation in their activity. Hubl (1956a) described a cell type in the subpharyngeal ganglion which he designated as a u-cell. This cell was oval shaped with a nucleus located in its center which was frequently surrounded by secretory granules. Brandenburg (1956) described the neurosecretory cells found in the suprapharyngeal ganglia, the u-cell originally described by Hubl (1956a) in the subpharyngeal ganglia, and neurosecretory cells in all the ganglia of the nerve cord. He concluded that in the suprapharyngeal ganglia there was only one cell type which had undergone changes depending on the functional state of the organism. On the other hand, Marapao (1957) found that mechanical stimulation such as needle pricking of earthworms revealed two types of secretory cells in the suprapharyngeal ganglia. One type resembled ordinary nerve cells and was, apparently, a true neurosecretory cell. It was found in three apparently successive stages: (1) an

acidophilic or A-stage, (2) a basophilic or B-I stage, and (3) a final, vacuolated or C-stage. The second type of secretory cell was a small, strongly basophilic cell distinct from the A, B-I, C cell series. Scharrer and Brown (1961) studied the neurosecretory cells of the suprapharyngeal ganglia using an electron microscope and described the submicroscopic structure in great detail. They concluded that, in general, their activity was similar to that of other cells.

The functions of the secretions of these cells have not been fully established. However, it is assumed that they are hormonal in nature (Scharrer and Brown, 1962; Marapao, 1959). Most of the investigations which have been done to determine possible functions of these secretions in the oligochaetes have involved the removal of the supraparyngeal ganglia. Prosser (1939) described the effect of the removal of the suprapharyngeal ganglia upon the total behavior of L. terrestris as follows:

> The anterior segments are lifted upward; the worm crawls normally, appears restless and active, it can right itself, can copulate, eat, and it burrows in a half hour as compared with the normal time of one to two minutes.

Bennett and Suttle (1960) removed the suprapharyngeal ganglia from L. terrestris and found that the experimental worms lost more weight than the controls. They also noted that the presence of the suprapharyngeal ganglia seemed to have an inhibitory effect on the seminal vesicles

and receptacles of the earthworm. Kovaleva (1961) found that the removal of the suprapharyngeal ganglia led to itensification of the motor activity of the animal. In addition, he found that the oxygen consumption of the earthworm at rest decreased and the body weight gradually diminished. Elzinga (1963) confirmed that the removal of the suprapharyngeal ganglia caused a decrease in the oxygen consumption of earthworms at rest. He concluded that these ganglia contained factors which influenced gaseous exchange in the resting state. Clark and Clark (1959) discussed the findings of Hubl (1959b) on the effects of the removal of the suprapharyngeal ganglia on regeneration of posterior segments in lumbricids. Their extirpation, at the same time that a number of posterior segments were removed, inhibited regeneration of the posterior segments. However, removal of the ganglia twenty-four to forty-eight hours after the posterior segments were amputated did not inhibit posterior regeneration. Therefore, it appeared that the suprapharyngeal ganglia had a hormonal effect and that this anterior portion of the central nervous system was essential to the process of regeneration.

Definite evidence has accumulated from experimental work with polychaete annelids that the secretions of the neurosecretory cells of the suprapharyngeal ganglia governed the regenerative phenomena in several species of this class. Clark and Bonney (1960) found that the suprapharyngeal

ganglia in Nereis diversicolor played an essential role during initial regeneration of posterior segments but once regeneration was initiated their influence declined. Thus, if the suprapharyngeal ganglia of this species were removed before or during the first three days after posterior segments had been amputated no new segments were grown. If the ganglia were removed more than three days after the loss of segments then regeneration was not prevented. Clark and Evans (1961) confirmed these findings and also found that, at least in some cases, the implantation of an extirpated suprapharyngeal ganglia into decrebrated, tail-less worms resulted in regeneration. Clark, et.al. (1961) found that for implanted ganglia to be effective in regeneration of posterior segments in Nephyts the ganglia had to be stimulated before they were transplanted. Thus, if the suprapharyngeal ganglia of a worm in which the posterior segments were removed at least three days before, were then removed and transplanted into a decrebrated tail-less worm, regeneration took place. They concluded from these experiments that regenerative hormones were not present in the suprapharyngeal ganglia in effective quantities until the second or third day after the loss of posterior segments. Herlant-Meewis (1961) investigated regeneration of nervous tissue in Eisenia foetida and found that when the suprapharyngeal ganglia were regenerated three to four weeks after their removal, the restored "brain" contained more than two categories of neurosecretory cells

occuring in the normal worm. The regeneration of the subpharyngeal ganglia was similar to that of the suprapharyngeal ganglia with the neurosecretory cells becoming very active in those ganglia posterior to the cut during regeneration.

The water relationships of L. terrestris have been the subject of several research projects. Maloeuf (1940) found that worms transferred from moist soil to tap water had a thirty-one percent average weight gain attributed to osmosis. He also noted that chloride ions were taken up from fresh water at a rate sufficient to maintain a concentration of ten millequivalents of chloride ions in the coelomic fluid. Ramsay (1949) in his studies on osmotic relationships of the earthworm found that, as the concentration of sodium chloride in the medium increased, the osmotic pressure of the body fluids also increased and remained greater than the medium. Although the chloride content of the coelomic fluid increased as the concentration of sodium chloride in this medium increased from .025 percent to 1.27 percent, the chloride content of the coelomic fluid became less than the chloride concentration of the medium as it exceeded 0.35 percent. The urine remained hypotonic to the body fluids except when the earthworms were placed in a medium in which the concentration of sodium chloride was greater than one percent. In a medium of a 1.27 percent sodium chloride, the urine of the earthworm was isotonic to the body fluids. Kamemota, et.al. (1961) found that when

earthworms were placed in a solution which had a concentration of 0.6 percent sodium chloride, the worms increased the concentration of sodium ions in both blood and coelomic fluid. Furthermore, when the worms were placed in this 0.6 percent sodium chloride medium, the organisms maintained a greater concentration of sodium ions in the body fluids than that of the medium.

From the above evidence, it would appear that L. terrestris is an osmoregulating organism. Since neurosecretions may well be the only source of hormones in these organisms and because in other animals water balance is controlled hormonally, it appeared that an investigation of the control of water balance in these forms might prove fruitful.

In this investigation an attempt was made to determine the effects of the removal of the suprapharyngeal ganglia on osmoregulation of L. terrestris in various dilutions of sodium chloride solutions and on water balance under normal environmental conditions.

CHAPTER II

METHODS

All earthworms used in this study were purchased either locally or from a supply house in Pittsburgh, Pennsylvania. They had a mature clitellum and were identified as Lumbricus terrestris by the investigator (Eddy and Hodson 1961) using the following characteristics: (1) the position of the sperm duct on segments thirteen or fifteen; (2) the complete division of the peristomium; and (3) the position of the clitellum in the thirty-first segment or in the thirty-second to the thirty-seventh segments.

The worms were maintained in plastic containers filled with a mixture of fresh sphagnum and soil and covered with perforated aluminum foil. A measured amount of water was added every third day. The earthworms were refrigerated at 2°C since room temperatures are lethal over extended periods of time. (Wolf, 1938).

The procedure used for extirpation of the supra-pharyngeal ganglia was as follows:

1. The worms were anesthetized by first placing them in a three percent ethyl alcohol solution in distilled water and then in a freezer for twenty minutes.
2. An incision was made along the dorsal surface of the second through the fifth segment.

3. A curved tip finder was used to expose the suprapharyngeal ganglia. The ganglia were lifted up and quickly excised with a small sharp scissors.
4. The worms were placed in foil covered containers containing wet absorbent paper for one week to allow for the incision to heal. They were then transferred to fresh sphagnum and refrigerated for twenty-three additional days. The thirty day period between extirpation of the ganglia and determination of water content was arbitrarily chosen by the investigator.
5. Steps 1,2, and 4 were performed on all controls.

After a period of thirty days the total water content of both experimental and control worms was determined as follows.

1. The experimental and control worms were divided into groups of five.
2. Each worm was rinsed in tap water to remove surface dirt and placed on absorbent paper for thirty seconds to remove excess water.
3. To obtain a wet weight each worm was placed in a pre-weighed covered Petri dish and weighed to the 0.0001 of a gram.

4. The weighed worms were placed in an oven at 110°C for six hours, removed, placed in a desiccator, and allowed to cool to room temperature. It had been previously determined by the investigator that six hours at 110°C was sufficient time for complete removal of water.
5. After cooling, a dry weight was obtained by weighing to the 0.0001 of gram.

Using the wet and dry weights of the worms, the percentage of total water content was determined for each worm. From these data a mean water content for each group of five worms was calculated. Following the determination of the mean for each group, the standard deviation and standard error of the means were determined for both experimental and control groups. To evaluate the significance of the difference between the grand mean of the experimental groups and the grand mean of the control groups, a standard error of the difference between the grand means was calculated. This standard error was used in applying the students "t" test to the data. The five percent level of confidence was used in the test for significance (Downie and Heath, 1959).

For an investigation of the role of the suprapharyngeal ganglia in osmoregulation, the ganglia were removed as described previously. As before, experimental and control worms were placed in wet absorbent paper and refrigerated.

Seven days later they were transferred to fresh sphagnum and refrigerated again. Since it appears that the earthworm is an osmoregulating organism when placed in 0.025 percent to 1.27 percent sodium chloride (Ramsay, 1949) and that the removal of the suprapharyngeal ganglia may affect this regulation in either hypotonic, isotonic or hypertonic solutions, the increase or decrease in weight of experimental and control worms was determined in either 0.2 percent sodium chloride, a hypotonic solution, 0.8 percent sodium chloride; a relatively isotonic solution, or in 1.2 percent sodium chloride; a hypertonic solution.

Two weeks after extirpation this increase or decrease in weight was determined as follows:

1. The experimental and control worms were divided into groups of five.
2. Each worm was rinsed with tap water to remove excess dirt. The worms were then placed on absorbent paper for thirty seconds to remove excess water.
3. A wet weight was obtained for each group of worms by placing them in a pre-weighed flask and weighing to the nearest 0.0001 of a gram.
4. Each group of weighted worms was placed in either 0.2 percent, 0.8 percent or 1.2 percent sodium chloride at room temperature for five hours. The five hour immersion time was arbitrarily chosen by the investigator.
5. After five hours the excess water was removed

as in step 2 and a wet weight was obtained as in step 3.

Using the initial weight and the five hour weight the percentage increase or decrease in weight was determined. The mean increase or decrease in weight was calculated from this, as well as the standard deviation and standard error of the mean for both the experimental and the control groups. A standard error of the difference between the means was determined and used in applying the students "t" test to these data, in order to determine if the difference that existed between the experimental and the control groups was significant at the .05 level of confidence.

CHAPTER III

RESULTS

The effect of the removal of the suprapharyngeal ganglia of L. terrestris on water balance under normal environmental conditions was to decrease the total water content of the experimental worms as compared to normal worms under the same conditions.

The results of the experiment concerning the effect of the suprapharyngeal ganglia on the water content are summarized in Table I. The mean water content of the normal worms was 83.40 percent of the wet weight as compared to 81.35 percent forthe experimental worms. Statistical analysis shown in Table I indicates a significant difference at the .05 level of confidence. The t-value was 3.94 with 22 degrees of freedom which exceeded the value of 1.72 necessary for significance at the .05 level of confidence. Therefore, these values tend to support the hypothesis that a real difference exists.

Tables II, III, and IV summarize the results of the experiment concerning the effect of the suprapharyngeal ganglia on osmoregulation in 0.2 percent, 0.8 percent, and 1.2 percent sodium chloride.

The effect of the removal of the suprapharyngeal ganglia on osmoregulation in the earthworm in various

dilutions of sodium chloride was to cause a greater uptake of water in the experimental worms when placed in 0.2 percent sodium chloride for five hours at room temperature. However, when the worms were placed either in 0.8 percent or 1.2 percent sodium chloride for five hours at room temperature, the experimental worms lost less weight than the normal worms.

The mean weight gain for the experimental worms in 0.2 percent sodium chloride for five hours at room temperature (Table II) was 10.53 percent as compared to a 10.16 percent weight gain for the normal worms. Statistical analysis shown in Table II does not indicate a significant difference at the .05 level of confidence. The t-value was 0.37 with 24 degrees of freedom which was less than the value of 1.71 necessary for significance at the .05 level of confidence. Therefore these values do not support the hypothesis that a real difference exists.

When the worms were placed in 0.8 percent sodium chloride for five hours at room temperature, the average weight loss of the experimental groups was 11.67 percent compared to 13.27 percent for the control groups (Table III). Statistical analysis shown in Table III does not indicate a significant difference at the .05 level of confidence. The t-value was 1.58 with 27 degrees of freedom which was less than the value of 1.70 necessary for significance at the .05 level of confidence. Therefore, these

values do not support the hypothesis that a real difference exists.

The average weight loss after five hours at room temperature for the experimental worms placed in 1.2 percent sodium chloride was 18.03 percent as compared to an average decrease in weight of 20.24 percent for the normal worms (Table IV). Statistical analysis shown in Table IV indicates a significant difference at the .05 level of confidence. The t-value was 2.27 with 22 degrees of freedom which exceeded the value of 1.71 necessary for significance at the .05 level of confidence. Therefore, these values tend to support the hypothesis that a real difference exists.

CHAPTER IV

DISCUSSION OF RESULTS

The neurosecretory cells in the Hirudinea, Polychaeta, and Oligochaeta have been investigated in some detail. These cells appear to be the only source of hormones in these organisms.

In the Hirudinea, Hagadorn (1959) has done considerable work on neurosecretory phenomena in the leech, Theromyzon rude. He found two secretory cell types, both of which show axonal transport of their secretory products. The direction of the secretory material is from the ventral nerve cord to the brain as shown by an accumulation of secretory material at the posterior side of the cut after transection of the cord. The secretory products leave the brain either through the nerves or the vascular system.

Extensive work has been done on the effect of the suprapharyngeal ganglia on regeneration in polychaetes (Clark and Bonney, 1960; Clark and Clark, 1959; Clark et.al., 1961; Clark and Evans, 1961).

Clark et.al. (1961) demonstrated that there was a rapid response of the neurosecretory cells in the suprapharyngeal ganglia of both Nephyts and Nereis to a loss of posterior segments. In Nephyts one group of cells within the suprapharyngeal ganglia began a secretory cycle within six hours after the loss of posterior segments. Furthermore,

after eighteen to twenty-four hours a second group of neurosecretory cells, located in a different portion of the same ganglia, also began producing neurosecretory material. Clark and Bonney (1960) reported comparable occurrences in the suprapharyngeal ganglia of Nereis.

In Oliogochaeta, it appears that the only reported work on regeneration in lumbricids is Hubl's (1956b). He has shown that neurosecretory cells located in the suprapharyngeal ganglia become active during the regeneration of posterior segments.

Marapao (1959) investigated the possible hormonal nature of the neurosecretory material of the suprapharyngeal ganglia in L. terrestris. Injections of extracts made from the suprapharyngeal ganglia affected the neurosecretory cycle of the cells within the ganglia. The injection caused a decrease in the number of the A, B-1, and C stages of the first type of secretory cell, and either increased the number of B-2 (second type) secretory cell or had no effect on it.

In addition to the effects of the removal of the suprapharyngeal ganglia on regeneration, it has been found that, at least in L. terrestris, its removal caused a loss of body weight, an increase in size of seminal vesicles and receptacles, intensified the worm's motor activity and decreased its oxygen consumption at rest. (Kovaleva, 1961; Elzinga, 1963; Bennett and Suttle, 1960).

The investigations of Ramsay (1949) on water balance in L. terrestris have provided the greatest amount of information on this subject. From his work it appears that the

earthworm is an osmoregulator.

By analogy it would appear possible that the control of osmoregulation is most likely hormonal. Therefore, it would seem logical that the removal of the suprapharyngeal ganglia, which is one of the sources of hormones in the earthworm, would affect either water balance under normal environmental conditions or osmoregulation under stress conditions, or both.

The results of this investigation show that the effect of the removal of the suprapharyngeal ganglia in L. terrestris was to cause a decrease in the total water content of the experimental worms as compared to that of the control worms. It therefore appears that the suprapharyngeal ganglia may contain factors which control water balance in the earthworm under normal environmental conditions, since the experimental worms showed a greater decrease in weight than the control worms due to a greater water loss. It has also been shown that the removal of the ganglia affected osmoregulation in a hypertonic solution (1.2 percent sodium chloride), since the experimental worms lost less weight than the control worms when they were placed in 1.2 percent sodium chloride for five hours. The removal of the ganglia had no effect on osmoregulation in either an isotonic solution (0.8 percent sodium chloride) or a hypotonic solution (0.2 percent sodium chloride), as there was no significant difference in the increase or decrease

in weight of the experimental worms as compared to the control worms when placed in either 0.8 percent or 0.2 percent sodium chloride. It therefore appears that the ganglia may also contain factors which influence osmoregulation in hypertonic solutions as shown by the decreased water loss of the experimental worms as compared to that of the control worms, when they were placed in a hypertonic solution for five hours.

Since it appears that the suprapharyngeal ganglia may contain factors which control water balance in the earthworm and osmoregulation in hypertonic solutions and that ionic balance is usually associated with both these phenomenon, it may prove fruitful to investigate the effect of the removal of the suprapharyngeal ganglia on ionic balance in both the blood and coelomic fluid.

CHAPTER V

SUMMARY

The purpose of the present study was to investigate the influence of extirpation of the suprapharyngeal ganglia on water balance under normal environmental conditions and on osmoregulation in a hypertonic, an isotonic and hypotonic solution of sodium chloride of Lumbricus terrestris.

In order to determine the influence of the suprapharyngeal ganglia on water balance, extirpation of the ganglia was performed on 115 experimental worms. The control and experimental worms were maintained at 2° C. for thirty days. Following the thirty day period the total water content of both the experimental and control worms was determined.

To obtain data on the influence of the suprapharyngeal ganglia on osmoregulation in the earthworm, the ganglia were removed from 390 worms. After two weeks the increase or decrease in weight of both experimental and control worms was measured in either 1.2 percent, 0.8 percent or 0.2 percent sodium chloride.

The results of this study show that the total water content of the experimental worms was significantly less than that of the control worms and that the decrease in weight of the experimental worms was significantly less than

that of the control worms in 1.2 percent sodium chloride, a hypertonic solution.

From the above results it was concluded that the suprapharyngeal ganglia contains factors which influences water balance under normal environmental conditions and osmoregulation in hypertonic solutions.

REFERENCES

Bennett, M.R. and G.E. Suttle, 1960. Effects of the removal of the suprapharyngeal ganglia in earthworms. Anat. Rec., 137 (3):339.

Brandenburg, J., 1956. Neurosekretorische zellen des regerwurms. Naturwissenschaftern, 43;453.

Clark, R.B. and D.G. Bonney, 1960. Influence of supr-oesophageal ganglion on posterior regeneration in Nereis diversicolor. Jour. Embryol. and Expl. Morphol., 8:112-118.

Clark, R.B. and M.E. Clark, 1959. Role of the suprapharyngeal ganglion during the early stages of caudal regeneration in some errant polychaetes. Nature, 183 (4678):1834-35.

Clark, R.B., M.E. Clark and J.G. Ruston, 1961. The endo-crinology of regeneration in some errant polychaetes. Memoirs of the Society of Endocrinology. New York & London: Academic Press. 12:275-286.

Clark, and S.M. Evans, 1961. The effect of delayed brain extirpation and replacement on caudal regeneration in Nereis diverisicolor. Jour. Embryol. and Expl. Morphol., 9:97-105.

Eddy, S. and A.C. Hodson, 1961. Taxonomic Keys. 3rd ed. Minneapolis: Burgess Co. pp v 162.

Downie, N.M. and R. W. Heath, 1959. Basic Statistical Methods. New York: Harper & Brothers. 123-136.

Elzinga, W.E., 1963. Investigation of influence of extirpation of suprapharyngeal ganglia upon oxygen uptake of the earthworm. Master's Thesis. Western Michigan University, Kalamazoo, 1-20.

Hagadorn, I. R. 1959. Neurosecretion and brain of rynchob-dellid leech, Thermyzon rude. Jour. of Morph., 102: 55-90.

Herlant-Meewis, H., 1961. Neurosecretory phenomena during regeneration of nervous centers in Eisenia foetida. Neurosecretion No. 12, Proc. of the Third Internat. Group Symp. on Neurosecretion, Ed. H. Heller and R. B. Clark, London & New York: Academic Press. 267-274.

Hubl, H., 1956a. Ein neurer typ neurosekretorischer zellen im unterschlundganglion der regenwurmen. Naturwissen-schaften, 43:284.

_____,__., 1956b. Uber die beziehungen der neurosekretion zum regenerationsgeschehen bei lumbriciden nebst beschreibung eines neuartigen neurosekretorischen zell types im unterschlundganglion. Arch. Entw-mech. Crg., 149:73-87.

Kamemota, F.I., A.E. Spalding, and S. Keister, 1961. Ionic balance in blood and coelomic fluid of earthworms. Biol. Bull., 122:229-31.

Kovaleva, N.E., 1961. The effects of destruction of parts of the central nervous system on gas exchange and other physiological functions in the earthworm. Fiziol. Zhur. USSR (trans.), 47 (1):114-20.

Laverack, M.S., 1963. The Physiology of Earthworms. International series of Monographs on Pure and Applied Biology, New York: Macmillan Co. 15:128-37.

Maloeuf, N.S.R., 1940b. Osmo- and volume regulation in the earthworm with special reference to the ailmentary tract. Jour. Cell. Comp. Physiol., 16:175-78.

Marapoa, B.P., 1959. The effects of nervous tissue extracts on neurosecretion in the earthworm. Lumbricus terrestris. Catholic U. Amer., Biol., 55:1-34.

Prosser, C.L., 1939. Physiology of the nervous system of earthworms. Quart. Rev. Biol. 9:181-200.

Ramsay, J.A., 1949a. The osmotic relations of the earthworm. Jour. Exp. Biol., 26:46-56.

_____,_____, 1949b. The site of formation of hypotonic urine in the nephridium of Lumbricus. Jour. Exp. Biol., 26: 65-75.

Scharrer, E., and S. Brown, 1961. Neurosecretory granules in the earthworm, Lumbricus terrestris. Zeit. Zellf. 54:531-40.

_____,_.and_,_____, 1962. Neurosecretion in Lumbricus terrestris. General Comparative Endocrinology, New York: Academic Press 2:1-3.

Scharrer, B., 1959. The role of neurosecretion in neuroendocrine integration. Comparative Endocrinology, Ed. A. Gorbmann, New York: Wiley 134-145.

Scharrer, B. and E. Scharrer, 1937. Uber drusennervenzellen und neurosekretorische organe bei wirbellosen und wirbeltieren. Biol. Rev. 12: 185-216.

Schmid, L.A., 1947. Induced neurosecretion in Lumbricus terrestris. Jour. Exp. Zool. 104:365-377.

Welsh, J.H., 1958. Neuroendocrine substances. Comparative Endocrinology, Ed. A. Gorbmann, New York: Wiley 121-131.

Wolf, A.V., 1938. Notes on the effect of heat in Lumbricus terrestris L. Ecology 19:246-48.

TABLE I

A summary of the mean water content of experimental and control groups with five worms per group at 2° C for 30 days

Group	Percent Water Content Experimental	Control
1	83.61	82.75
2	81.28	82.48
3	82.75	84.03
4	81.30	83.28
5	81.61	82.63
6	81.89	81.21
7	84.18	82.04
8	82.83	82.96
9	82.65	81.93
10	78.52	81.93
11	75.11	85.42
12	78.17	84.11
13	78.54	85.20
14	78.75	85.02
15	72.49	85.98
16	81.27	-
17	81.08	-
18	80.88	-
19	81.63	-
20	83.59	-
21	83.39	-
22	83.98	-
23	84.61	-
$\bar{X}$	81.35	83.40
ΣX	1871.00	1251.00
ΣX^2	152,341.00	104,359.00
$s_{\bar{X}}$	2.32	1.35
sX	0.48	0.35

$t = 3.94$
$df = 22$

TABLE II

A summary of the increase in weight of experimental and control groups with five worms per group in 0.2% NaCl for five hours at room temp.

Group	Percent Increase in Weight	
	Experimental	Control
1	6.07	9.39
2	8.90	4.67
3	8.46	6.51
4	8.90	6.86
5	9.93	7.14
6	7.75	7.21
7	9.08	9.85
8	6.78	7.56
9	6.45	6.69
10	7.64	8.18
11	6.55	16.78
12	7.71	13.27
13	6.26	11.94
14	3.55	11.89
15	6.02	10.17
16	3.89	10.52
17	0.49	10.94
18	4.34	10.14
19	2.85	17.35
20	3.81	15.97
21	2.36	-
22	2.32	-
23	0.27	-
24	2.39	-
25	0.42	-
$\bar{X}$	10.53	10.16
ΣX	263.00	203.00
ΣX^2	2998.00	2305.00
s	1.98	1.13
$s_{\bar{X}}$	0.123	0.175

$t = .37$

$df = 24$

TABLE III

A summary of the decrease in weight of experimental and control groups with five worms per group in 0.8% NaCl for five hours at room temperature

Group	Percent decrease in weight	
	Experimental	Control
1	5.62	5.10
2	6.36	6.06
3	0.69	6.12
4	2.43	8.96
5	8.30	14.17
6	3.87	12.04
7	7.26	10.70
8	11.51	11.83
9	10.25	13.87
10	10.90	14.26
11	12.55	17.75
12	13.74	16.80
13	11.75	16.27
14	12.80	16.56
15	11.18	10.83
16	12.25	11.33
17	11.71	17.28
18	10.55	14.69
19	15.12	14.60
20	13.01	17.26
21	12.70	18.72
22	13.79	11.94
23	15.63	16.82
24	20.89	14.04
25	14.76	14.42
26	17.53	14.95
27	11.97	-
28	14.25	-
$\bar{X}$	11.67	13.27
ΣX_2	354.00	347.00
ΣX^2	4488.00	4988.00
s	3.22	3.72
$s_{\bar{X}}$	0.107	0.143

t = 1.58
df = 27

TABLE IV

A summary of the decrease in weight of experimental and control groups with five worms per group in 1.2 % NaCl for five hours at room temperature

Group	Percent decrease in weight	
	Experimental	Control
1	18.83	24.58
2	20.23	29.38
3	19.98	24.25
4	20.02	24.86
5	20.04	23.70
6	20.46	21.24
7	17.78	25.67
8	16.97	19.00
9	19.36	18.76
10	17.97	15.94
11	16.81	16.29
12	16.53	16.53
13	15.41	14.32
14	14.38	14.96
15	16.29	16.32
16	16.96	20.99
17	20.37	21.02
18	17.17	17.90
19	19.12	21.35
20	17.42	19.02
21	15.10	19.39
22	23.94	17.67
23	20.05	20.38
$\bar{X}$	18.03	20.24
ΣX_2	421.00	466.00
ΣX^2	7821.00	9739.00
s	2.29	3.75
$s_{\bar{X}}$	0.099	0.163

$t = 2.27$

$df = 22$