

STUDIES ON THE MEDIAN NERVE NEUROSECRETORY
SYSTEM IN THE COCKROACH,
PERIPLANETA AMERICANA

A Thesis
Submitted to
the Department of Biology
Kansas State Teachers College, Emporia, Kansas

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

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May, 1972

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ACKNOWLEDGEMENT

I thank Dr. Katherine Smalley for guidance and assistance throughout this entire study. I thank Dr. Edward Rowe for technical assistance in many of the laboratory procedures.

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INTRODUCTION

Neurosecretion refers to the elaboration of hormones by cells otherwise definable as neurons. In general, neurosecretory systems consist of a group of neurons and a tract of secretion-bearing axons that terminate in close association with the vascular system. The endings thus form a neurohemal organ for storage and release of the secretory material.

Neurosecretion was first reported by Ernst Scharrer (1927) who described glandular neurons in the hypothalamus of several vertebrates. These studies were followed by reports showing that neurosecretory systems occur in nearly all groups of animals.

Until recently the only neurosecretory systems reported in the American cockroach, Periplaneta americana, were the subesophageal ganglion (Scharrer, 1940), the stomatogastric ganglion (Bounhiol, et. al., 1953), and the protocerebral neurosecretory cells which send their axons to the neurohemal corpus cardiacum (Weyer, 1953). Physiologically these systems are important in the regulation of molting, diurnal rhythmicity, egg diapause, pigmentation and water retention.

Investigators have recently discovered another neurohemal system. Raabe and Chalaye (1966) suggested that swellings on the median (spiracular) nerve system

could have a neurohemal function. This was established by Brady and Maddrell (1967) and Raabe and Ramade (1967) by fine structural analysis with the electron microscope. Smalley (1970) found three groups of neurosecretory cells in the abdominal ganglia of Periplaneta americana whose axons terminate in the neurohemal swellings. Smalley (1970) also discovered that these abdominal neurosecretory cells and the median nerve neurohemal organ (MNNO) both appear to have uptake mechanisms for dopamine.

One of the major objectives of this study is to investigate possible physiological roles of the MNNO neurosecretory material. Identification of the neurosecretory material by light microscope histochemistry or fine structural analysis with the electron microscope could give a correlation of this system to other known neurosecretory systems. If it could be shown that dopamine or another biogenic amine is normally stored in this system, then some possible functions for this neurosecretory system could be suggested. Biogenic amines have been found in whole body extracts (Oestlund, 1954), the brain (Frontali, 1967) and the ventral nerve cord (Welsh, 1960), of P. americana. They are known to produce a variety of neurological and behavioral effects in the cockroach. It is possible that biogenic amines could function in invertebrates, as in vertebrates, as neurotransmitters (Frontali, 1967). Biogenic amines also show metabolic effects in

insects. Epinephrine accelerates the rate at which fat body triglycerides are hydrolyzed (Bhakthan, 1967), and dopamine serves as a precursor of N-acetyl-dopamine, the substance responsible for tanning of the insect cuticle.

The products of the MNNO might also be related to two biogenic amines which have been found in the ventral nerve cord of insects. Casida and Maddrell (1970) recently found an amine in neurosecretory cells in the mesothoracic ganglionic mass of Rhodnius. This amine has been shown by Maddrell (1964) to be a diuretic hormone. Cook (1969) has established that a biogenic amine, Factor S, which is possibly an aromatic amine with an ester group, is responsible for hormonal neuromuscular excitation. The highest concentration of Factor S in P. americana is in the ventral nerve cord.

The present study has been designed to investigate possible functions of the median nerve neurohemal system. To accomplish this task, the ultrastructure of the neurohemal organ has been studied under various physiological conditions. Additional studies with the fluorescence microscope have been aimed at determining the relationship of catecholamines to the entire neurosecretory system. A preliminary hypothesis on the physiological function of the system has been suggested.

MATERIALS AND METHODS

ANIMALS

Periplaneta americana Linnaeus, the American cockroach, was used in all studies. In the early phases of this study the animals were irregularly fed and watered. To establish a "normal" animal, several standard conditions were used. Adult male and female animals were placed in separate isolated colonies one week before use. Rhythmic light-dark cycles were established with fifteen hours light and nine hours dark. The animals were fed Gaines dog food, oatmeal, and apples once a week. Water was available at all times.

ELECTRON MICROSCOPY

Experimental Conditions. Various techniques were employed to investigate changes in the MNNO neurosecretory material. Pharmacological agents with known effects upon either catecholamines or neurosecretory systems in general were utilized in an effort to relate fine structural changes in the MNNO to a specific hormone. Adult males were used exclusively under all experimental conditions. All injections were 0.05 ml in volume and were given intra-abdominally. Yamasaki-Narahashi (1959) saline was used in all situations requiring a saline solution. For treatment with reserpine, 2.5 mg/ml serpasil phosphate (Ciba)

was prepared in distilled water and injected twenty-four hours before dissection. Distilled water control injections were also given twenty-four hours before dissection. A 10^{-3} M solution of dopamine was prepared in saline solution and injected one hour before dissection. Control saline was injected one hour before dissection. DDT was administered by injection in the form of a slightly turbid suspension prepared by dissolving the chemical in 95% ethanol and injecting the ethanol solution below the surface of saline in a 1:50 ratio. This was followed by immediate mixing and use. The animals were dissected when completely paralyzed (about thirteen hours). DDT dosage was seven mg/kg. Ethanol, 2%, was injected as a control for the DDT study and the animals were dissected at thirteen hours. Adult males were also cooled or dehydrated to investigate fine structural alterations in relation to physiological stresses. To dehydrate them, animals were placed in an isolated colony without food or water for either three or ten days. For cold treatment the animals were placed in the refrigerator at $0-5^{\circ}\text{C}$ until all movement ceased (usually fifteen to thirty minutes). At the end of each treatment period MNNO A1 and A2 were removed and processed as described below.

Fixation and Embedding. Males or females were injected with 0.5 ml fixative (ice-cold 4% glutaraldehyde,

maintained at pH 7.4 with 0.1 M cacodylate buffer unless otherwise specified). The MNNO were exposed by dissection and the glutaraldehyde fixative pipetted onto them. The organs were removed immediately from the body and placed in fresh fixative for one-half to one hour. The swellings were then rinsed in buffer, post fixed in 0.1 M cacodylate buffered 1% osmium tetroxide, dehydrated in ethanol series, rinsed in propylene oxide, and embedded in an Epon-Araldite mixture (Epon 812 25 ml, Araldite 6005 15 ml, dibutyl phthalate 4 ml, dodecenylsuccinic anhydride 55 ml, DMP-30 tridimethylaminomethylphenol 0.3 ml/10 ml mixture). Thin sections were cut on an LKB ultramicrotome and stained with 2% aqueous uranyl acetate and Reynold's lead citrate (Reynold, 1963). The sections were examined on a Hitachi HS-8 electron microscope. Photographs were taken at the discretion of the investigator and not in a random sampling fashion.

Interpretation of E.M. Data. The purpose for the E.M. studies was to investigate changes in the neurosecretory material at the ultrastructural level. Specifically, changes in granule-vesicle content of each axon were of major interest. For determination of the relative proportions of vesicles to granules in each axon, the granules and vesicles in individual axon endings were counted. After calculation of the percentages of granules and

vesicles in each individual ending, mean percentages for all axon endings were determined. In Table 1 these mean percentages for each treatment group are related to the number of endings counted and the number of vesicles and granules counted. To give numbers of granules and vesicles per unit area in each axon, an area 16×10^8 microns² was measured and all granules and vesicles within this area counted. Granule or vesicle size was determined by measuring the diameter of fifty granules and fifty vesicles taken at random from at least five micrographs from two different animals.

FLUORESCENCE MICROSCOPY

Experimental Conditions. Solutions of 10^{-3} M d- λ -dopamine, d- λ -norepinephrine or d- λ -dopa were prepared in saline. All animals were injected with 0.05 ml intra-abdominally and dissected one hour after injection. A 3×10^{-3} M solution of the monamine oxidase inhibitor, parnate (trancypromine sulfate, Smith Kline, and French), in saline was injected two hours before dissecting the animals. Brains, removed from normal adult animals not pretreated with a drug, were used as controls for all of the previous conditions. Upon dissection, ganglia A1 and A2 with their MNNO attached were removed and treated for catecholamines fluorescence as described below.

Table 1. Comparison of data of the granule to vesicle relationship in type 2 axon endings in the MNNO under various experimental conditions.

Experimental Condition	No. of Pictures Analyzed Animals Observed	Total Count granules vesicles No. of axon endings	Average % of granules or vesicles per ending	No. of granules vesicles per 16×10^8 microns ² at 24,000 X	Total No. of granules plus vesicles per 16×10^8 microns ² at 24,000 X
Normal Cecodylate	10 3	1930 G 302 V 34 E	86.5 % G 13.5 % V	53.4 G 9.0 V	62.4
Cecodylate Erratic-Fed	10 2	22 G 216 V 6 E	7.2 % G 92.8 % V	5.2 G 55.6 V	60.8
1 hour glutaraldehyde	5 2	211 G 20 V 6 E	86.1 % G 13.9 % V	47.6 G 12.7 V	60.3
$\frac{1}{2}$ hour glutaraldehyde	4 2	201 G 19 V 6 E	85.0 % G 15.0 % V	53.2 G 7.9 V	61.1
3 day dehydration	4 1	119 G 371 V 9 E	24.9 % G 75.1 % V	18.7 G 35.6 V	54.3
10 day dehydration	6 2	443 G 103 V 26 E	82.0 % G 18.0 % V	15.8 G 3.6 V	19.4
DDT injection	5 2	362 G 105 V 25 E	81.8 % G 18.2 % V	19.1 G 4.3 V	23.4
Dopamine injection	9 3	514 G 49 V 11 E	96.7 % G 2.3 % V	79.6 G 2.6 V	81.6
Saline injection	5 2	410 G 632 V 20 E	42.2 % G 57.8 % V	35.9 G 39.3 V	75.2
Reserpine injection	8 2	271 G 290 V 10 E	49.6 % G 50.4 % V	34.9 G 36.4 V	71.3
Dist. Water injection	10 2	859 G 646 V 22 E	58.1 % G 41.9 % V	38.5 G 27.8 V	66.3
Cooled animals	5 2	814 G 72 V 15 E	79.8 % G 20.2 % V	61.3 G 5.7 V	67.0

Tissue Processing and Inspection. Adult male animals were anaesthetized by cooling in the refrigerator until all movements ceased. The first and second abdominal ganglia (A1 and A2) were quickly dissected out with the MNNO attached. The ganglia were frozen in isopentane cooled with liquid nitrogen and processed according to the technique described in detail by Falck and Owman (1965) for rendering catecholamine containing structures fluorescent. The lyophilized ganglia were treated with formaldehyde gas by heating them in a one liter bottle containing five gm paraformaldehyde (previously equilibrated with air at 70% relative humidity) at 80°C for one to three hours. After vacuum-embedding the tissues in paraffin, sections were cut at eight microns in the sagittal plane. The tissues were observed under dark field optics with a Leitz fluorescence microscope with Wratten barrier filter no. 15, BG-12 excitation filter and high pressure mercury arc lamp. Photographs were taken with Ektachrome X, ASA 64.

LIQUID SCINTILLATION

Male and female animals were injected intra-abdominally with 0.1 ml (ten microcuries) or 0.05 ml (five microcuries) of d- ℓ -dopamine-1- H^3 -HBr (New England Nuclear: Specific activity 100 mc/mM). At intervals of five, fifteen, thirty, sixty, and one hundred and

twenty minutes animals were killed by decapitation, the abdominal cavity washed with 5% glutaraldehyde and the following nerve tissues removed: brain, first abdominal ganglion (A1), second and third abdominal ganglia (A2-A3), fourth and fifth abdominal ganglia (A4-A5), sixth abdominal ganglion (A6), MNNO in front of A1, MNNO in front of A2 and, as control tissues, nerve connectives located between A5-A6 and A2-A3. Each individual tissue group from two animals was placed in a scintillation vial containing one ml NCS solubilizer. The tissue was digested in the NCS solution for twelve hours at 60°C and then ten ml of PPLO liquid scintillation fluid was added to each vial. The tritium content in each tissue was determined by counting in a Nuclear-Chicago Unilux II liquid scintillator.

RESULTS

GENERAL ANATOMY

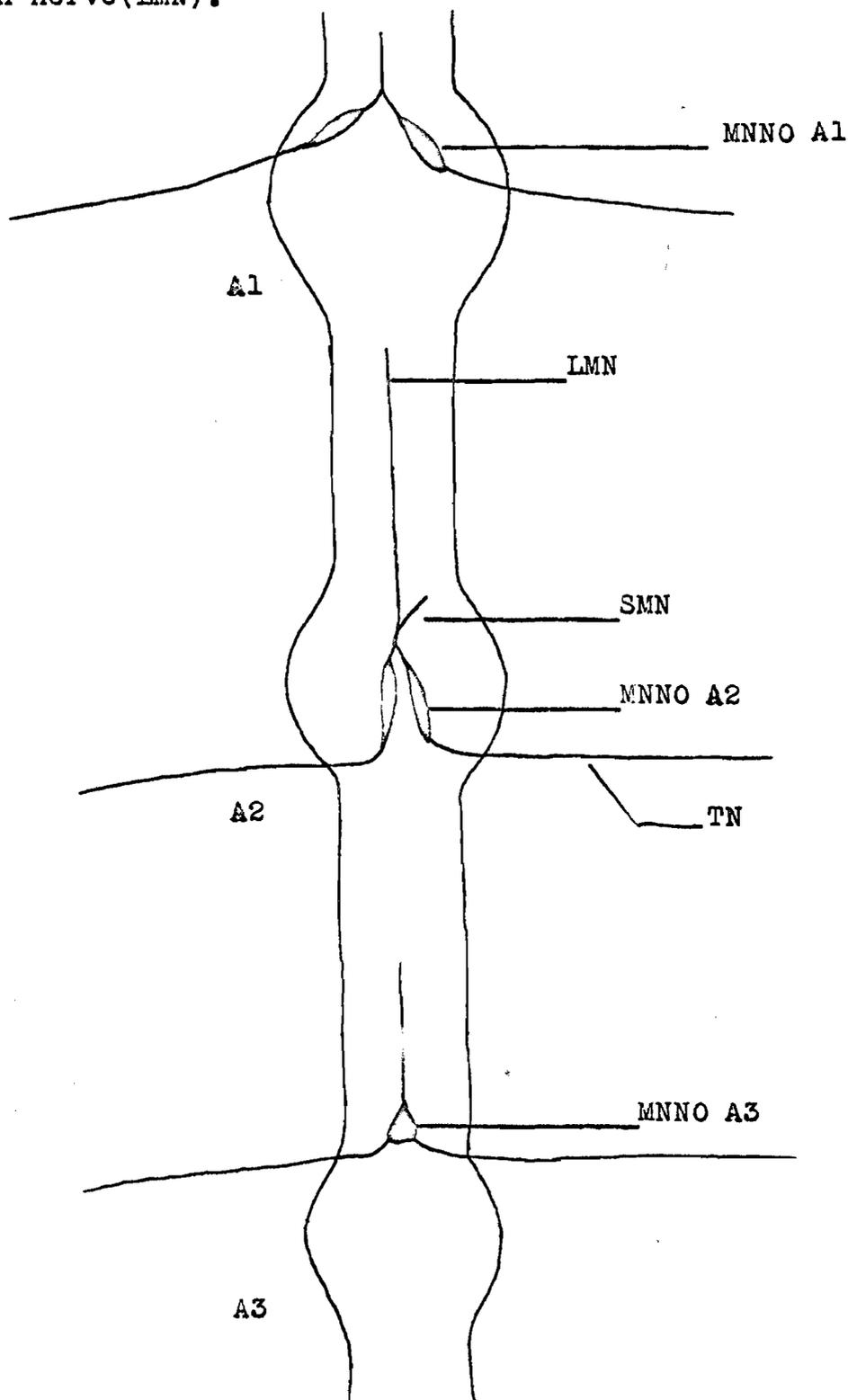
The median nerve neurohemal organs of Periplaneta are inconspicuous swellings arranged segmentally on the median nerve system (Fig. 1). The median nerve extends posteriorly from each abdominal ganglion to just in front of the following ganglion, where it connects with the neurohemal organ. From the neurohemal organ a pair of transverse nerves pass laterally to spiracles and a short median nerve extends downward into the ganglion.

ELECTRON MICROSCOPY

1. Ultrastructure of the MNNO in Normal Animals

The MNNO is encased by a continuous outer sheath of thick connective tissue which has profuse ramifications into the neurohemal organ proper. Axons, arising apparently from the short median nerve, branch extensively to form a large area for storage and release of the neurosecretory material (Figs. 2, 3). Nerve tracts, presumably the spiracular motor nerves, pass in most cases down the middle of the neurohemal organ. These nerve tracts, containing several axons, are surrounded by the axon endings containing the neurosecretory material. Most of the endings contain very large numbers of vesicles and granules in the normal animal (Fig. 2). Two types

Fig. 1. Ventral nerve cord of Periplaneta americana showing ganglia A1, A2, A3, transverse nerve (TN), median nerve neurohemal organs (MNNO), short median nerve (SMN), and long median nerve (LMN).



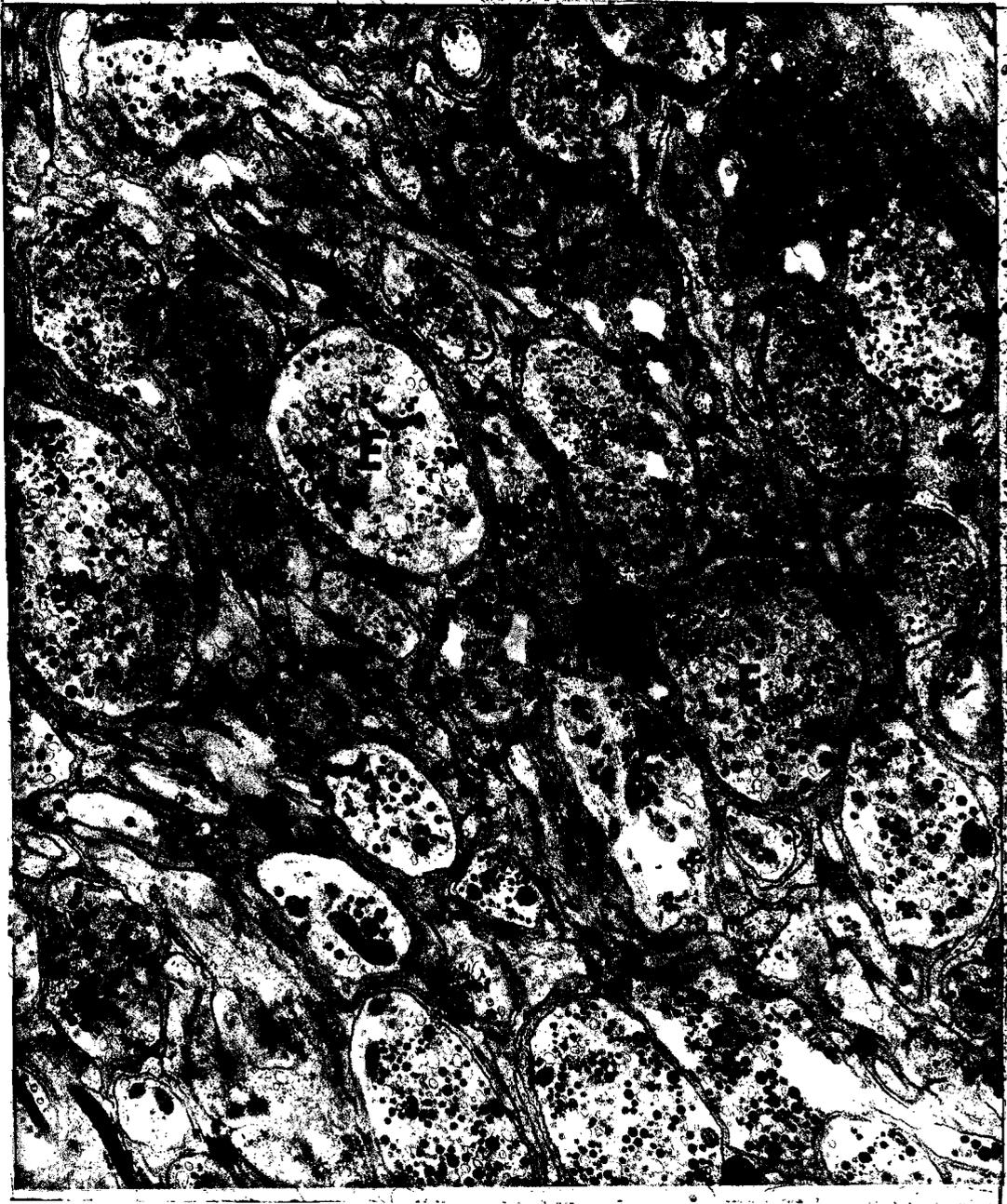


Fig. 2. Electron micrograph of the MNNO from the second abdominal ganglion of a normal male *P. americana*. Type 2 axon endings (E) containing small electron dense granules and electron transparent vesicles are clearly evident. About X 12,000



Fig. 3. MNNO from the second abdominal ganglion of a normal female. Type 2 endings are surrounded by glial membranes (GM) and contain mitochondria (M). A type 1 ending, containing large electron dense granules, can be seen surrounded by connective tissue sheath. About X 12,000

of neurosecretory endings have been found: type 1, containing large electron-dense granules 1000-2500 Å in diameter, and type 2, containing small electron dense granules 600-1600 Å in diameter and electron transparent vesicles 800-1900 Å in diameter (Fig. 3). No distinguishable size variations of the neurosecretory material in type 2 endings were found in any of the animals either normal or under experimental conditions. However, the relative proportion of granules and vesicles of type 2 endings did vary depending upon the physiological state of the animal and the buffer used with the primary fixative. The type 1 endings most frequently lie in direct contact with the outer connective tissue sheath or its inward continuations (Fig. 3). The large electron dense granules show no noticeable changes in any of the experimental conditions. The type 2 axon endings are usually invested by glial membranes. Nuclei found in the neurohemal organ are glial cell nuclei. Mitochondria are found freely distributed throughout the axons.

2. Neurosecretory Material in the MNNO

a. Male and Female; Regular Feeding and Watering.

No differences could be found in the neurosecretory material in type 2 endings between the normal male and female (Figs. 2, 3). The granule and vesicles in each individual ending are quite similar (Table 1). Also,

the granules and vesicles per unit area and the total number of granules plus vesicles per unit area does not show a significant difference. These data suggest that the neurosecretory material has a similar function in both the male and female.

b. Irregular Feeding and Watering. Irregular feeding and watering apparently causes a variation in the granule to vesicle relationship. The small electron dense granules that are the prominent feature in the type 2 endings in the normal animal become greatly reduced in number (Fig. 4). The total number of granules plus vesicles does not change; however, the percentage of granules to vesicles undergoes a dramatic shift (Table 1, Fig. 23, 24). These animals appear similar to those described by Brady and Maddrell (1967). It seems possible that their animals were slightly dehydrated or underfed, much as in this series.

c. Phosphate Buffered Fixative. Preliminary studies with irregularly fed and watered animals indicate that phosphate buffered glutaraldehyde may change the granule to vesicle relationship. Small electron dense granules are rarely found in any of the type 2 axon endings (Fig. 5). The number of vesicles found per unit area is similar to the number of granules plus vesicles in control animals. Comparison of the normal animals with irregularly fed and watered animals treated with phosphate



Fig. 4. Section of MNNO from the first abdominal ganglion of an irregularly fed and watered male *P. americana* fixed with cacodylate buffered glutaraldehyde. About X 12,000



Fig. 5. Electron micrograph of the MNNO from the second abdominal ganglion of a normal male *P. americana*. Phosphate rather than cacodylate buffered fixative was used in this preparation. About X 12,000

or cacodylate buffered fixative shows no difference in the total number of granules plus vesicles but a change from granules predominating to vesicles predominating (Figs. 23, 24). Apparently, the electron transparent vesicles are empty (containing no neurosecretory material) electron dense granules.

d. Three Day and Ten Day Dehydration. In animals dehydrated for ten days, neurosecretory material in the type 2 endings has been severely depleted (Fig. 6). Both the average number of granules and the average number of vesicles per unit area are greatly reduced compared to the normal animal (Fig. 24). However, granule-vesicle percentages in individual endings are similar to the normal animal (Fig. 23). A multi or single lamellate structure, possibly a lysosomal body appears in many of the depleted axons (Fig. 6).

The animals dehydrated for three days display only a slight depletion of the total neurosecretory material in type 2 endings (Table 1, Fig. 7). However, the proportion of granules in the dehydrated animals drops to one-third of that found in the controls (Fig. 23).

e. DDT Injection. A pharmacologically active amine is released in insects treated with DDT (Cook, 1967). DDT injection in the cockroach causes depletion of the neurosecretory material in type 2 axon endings. The endings appear strikingly similar to the ten day dehydrated

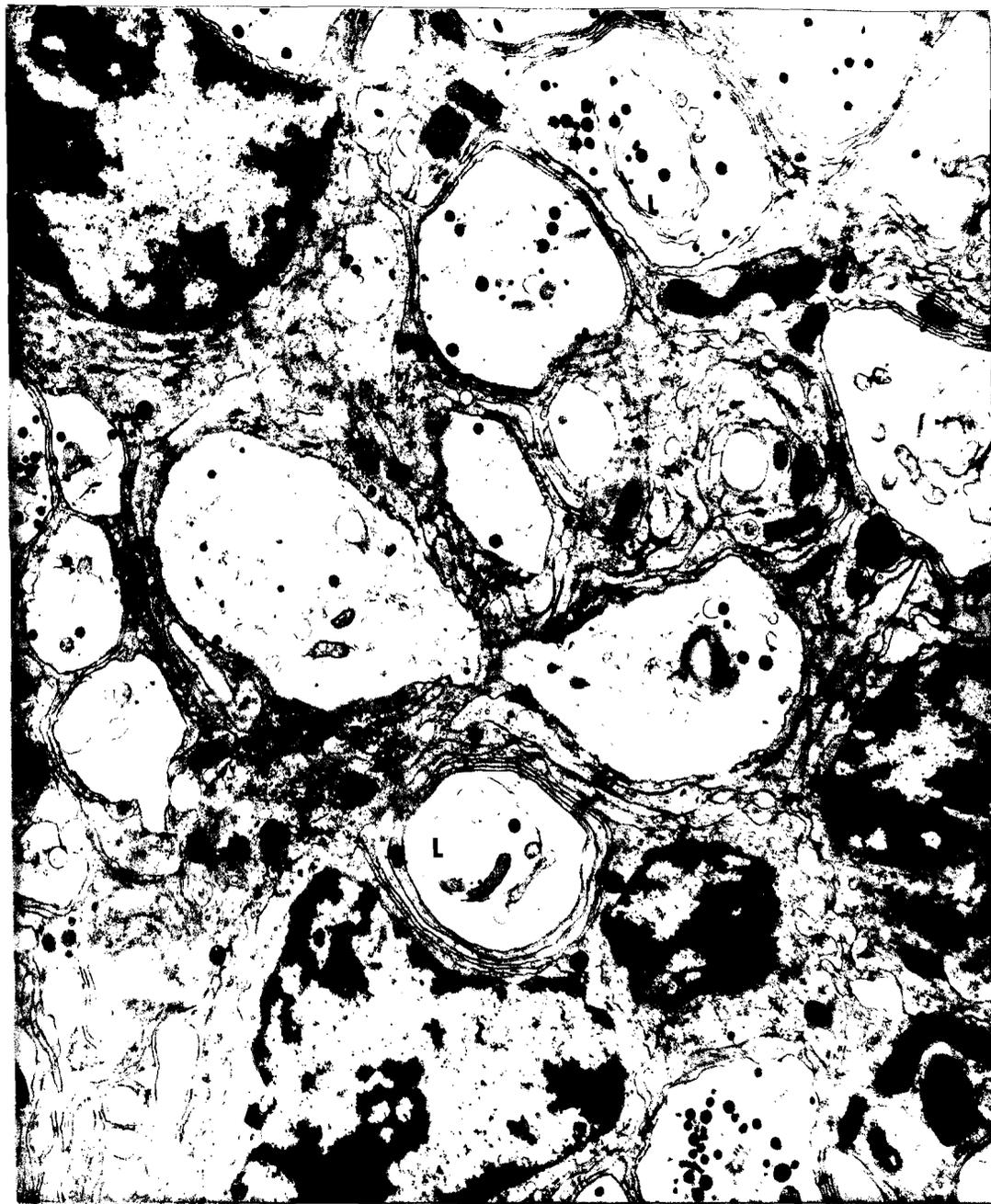


Fig. 6. Section of MNNO from the second abdominal ganglion of a 10 day dehydrated male. Notice the multiple and singular lamellate structures (L) in the type 2 axon endings. About X 12,000



Fig. 7. Section of MNN0 from the second abdominal ganglion of a 3 day dehydrated male. About X 12,000

animal (Fig. 8). Granule and vesicle percentages in individual endings are near normal (Fig. 23), but the numbers of both granules and vesicles per unit area are greatly reduced (Fig. 24). The lamellate structures are again found in many of the depleted endings (Fig. 8).

f. Dopamine Injection. Intra-abdominal injection of dopamine causes very noticeable effects in the granule-vesicle relationship compared to the saline controls. All of the endings appear completely packed with granules and show few electron transparent vesicles (Fig. 9). This suggests that dopamine or a metabolic product is being taken up and stored in the MNNO. The average number of granules per unit area is about double that of the saline control and somewhat higher than the normal animal (Figs. 23, 24, Table 1).

g. Saline Injection. Injections of saline cause immediate reduction in the percentage of granules and increase in the percentage of vesicles found in axon endings (Fig. 23). The total number of granules plus vesicles is slightly higher than the normal animal; however, it is comparable to the dopamine injection (Table 1). It seems probable that injections of saline cause release of neurosecretory material from the type 2 endings (Fig. 10).

h. Reserpine Injection. Reserpine has long been known to deplete intraneuronal stores of catecholamines (Frontali, 1969). In the present study, no distinguishable

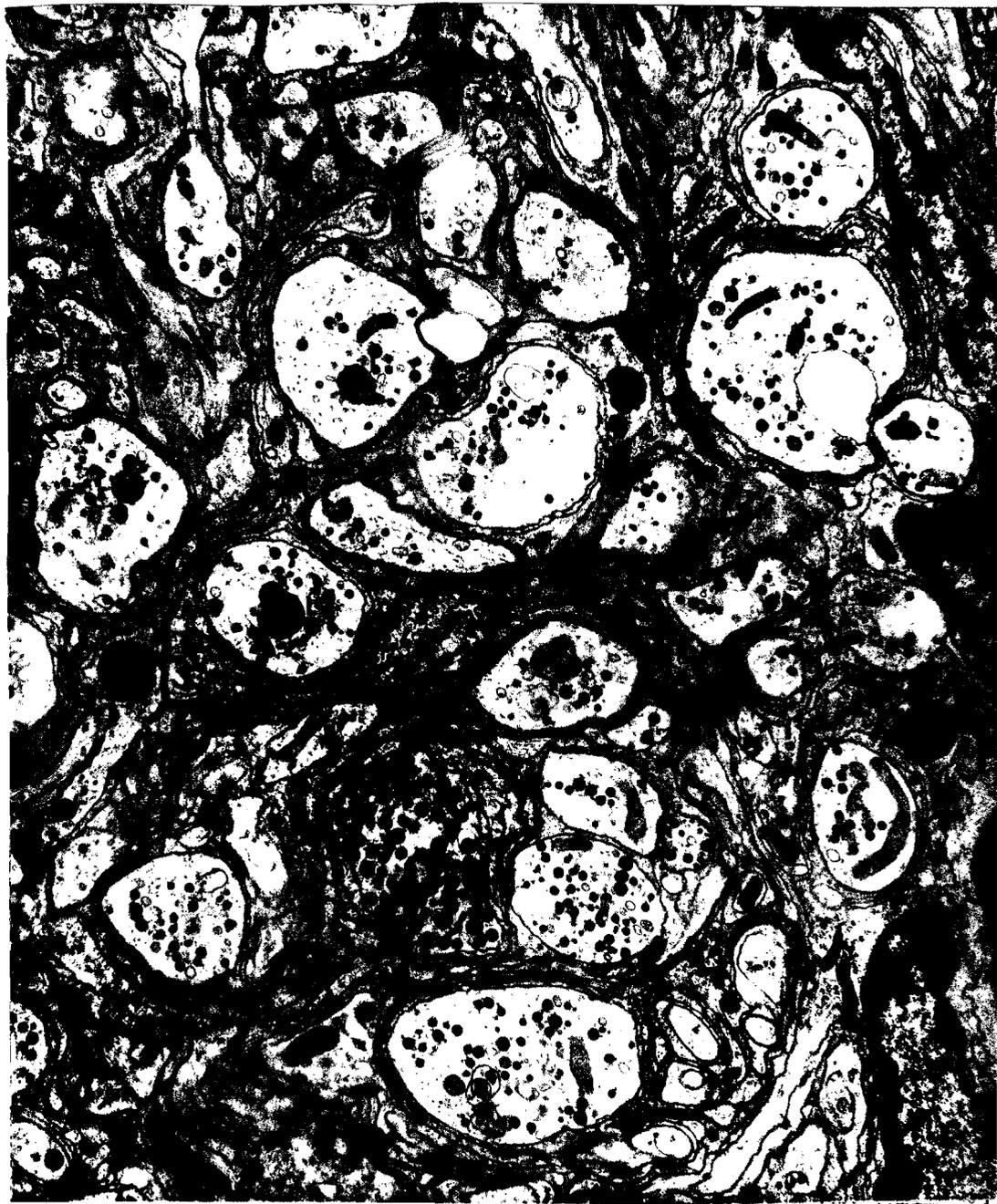


Fig. 6. Section of MNNO from the second abdominal ganglion of a male treated with DDT. Several of the type 2 endings contain lamellate structures (L) much like those found in the dehydrated animals. About X 12,000

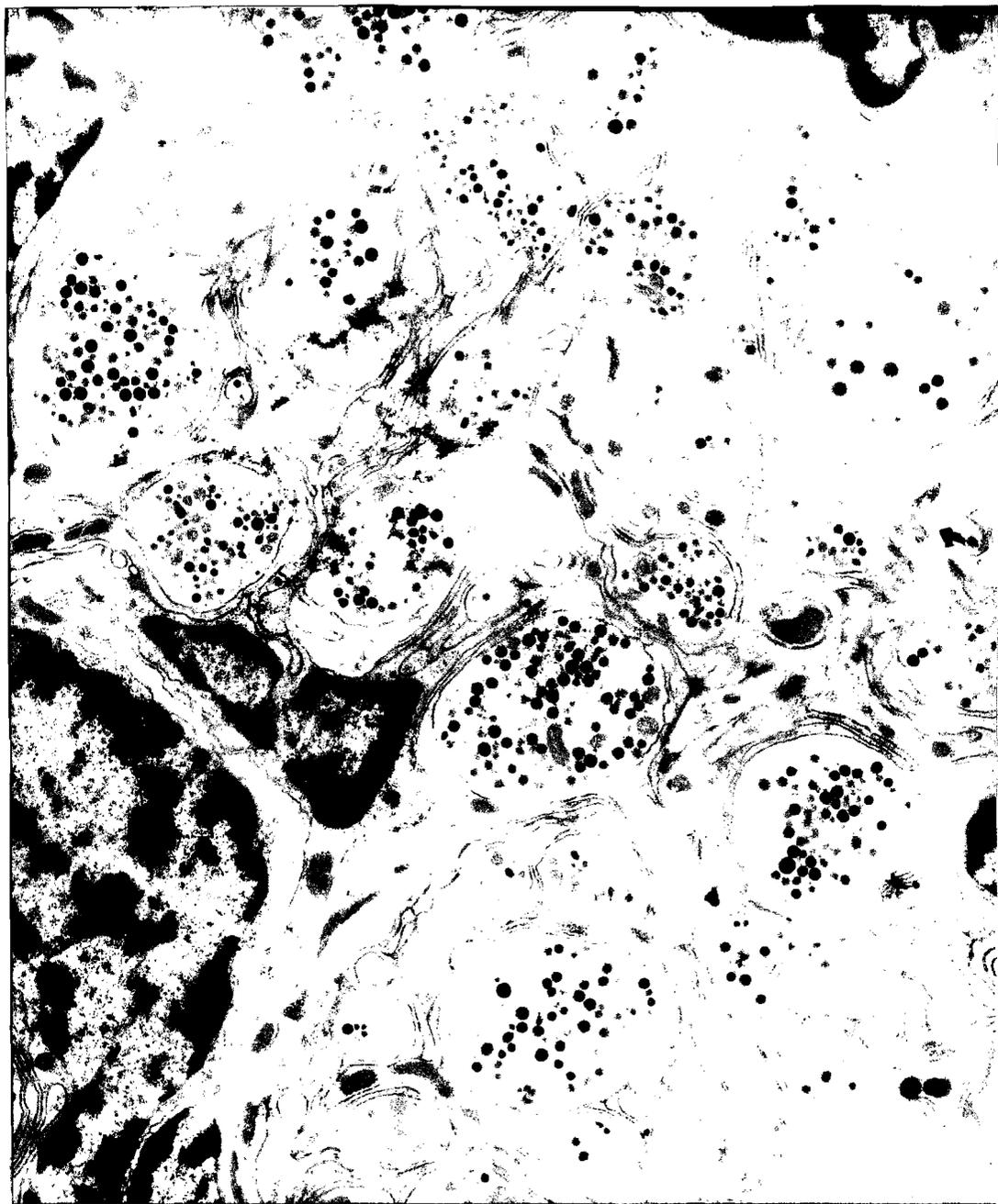


Fig. 9. Section of MNO from the first abdominal ganglion of a male injected with dopamine. About X 12,000



Fig. 10. Section of MNNO from the first abdominal ganglion of a male injected with saline. About X 12,000

change in neurosecretory material could be observed between the control animals injected with distilled water and the experimental animals injected with reserpine (Fig. 11). Both the granule-vesicle percentages (Fig. 23), and the numbers of granules and vesicles per unit area (Fig. 24) are very similar to the control values.

i. Distilled Water Injection. Injections of distilled water cause an increase in the percentage of vesicles per axon ending (Fig. 23) but the total number of granules plus vesicles (Table 1) is similar to the saline injections. Injections of either saline or distilled water seems to cause release of some of the neurosecretory material from the type 2 endings (Fig. 12).

j. One-half or One Hour Fixation. Length of time of primary fixation with glutaraldehyde makes little difference in the granule or vesicle content (Figs. 13, 14). Granule and vesicles percentages per axon ending, the numbers of granules and vesicles per unit area, and the total number of vesicles plus granules all remain similar to the normal animal (Table 1).

k. Cooling Animal Before Fixation. Preliminary results indicate no noticeable changes in the neurosecretory material (Table 1, Fig. 15). Percentage of granules to vesicles in individual axon endings, average number of granules and vesicles per unit area, and the total number

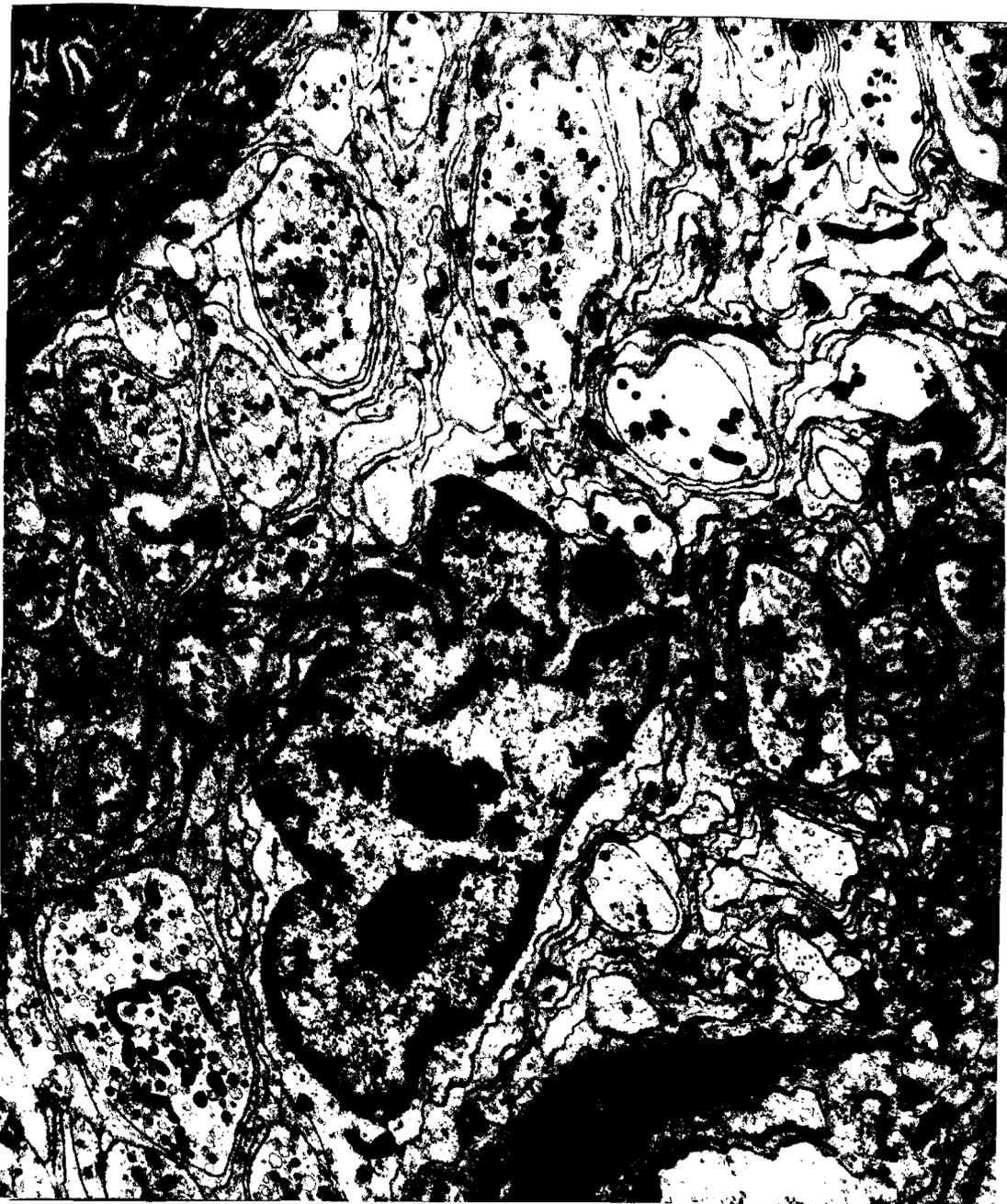


Fig. 11. Section of MNNO from the second abdominal ganglion of a male treated with reserpine. About X 12,000

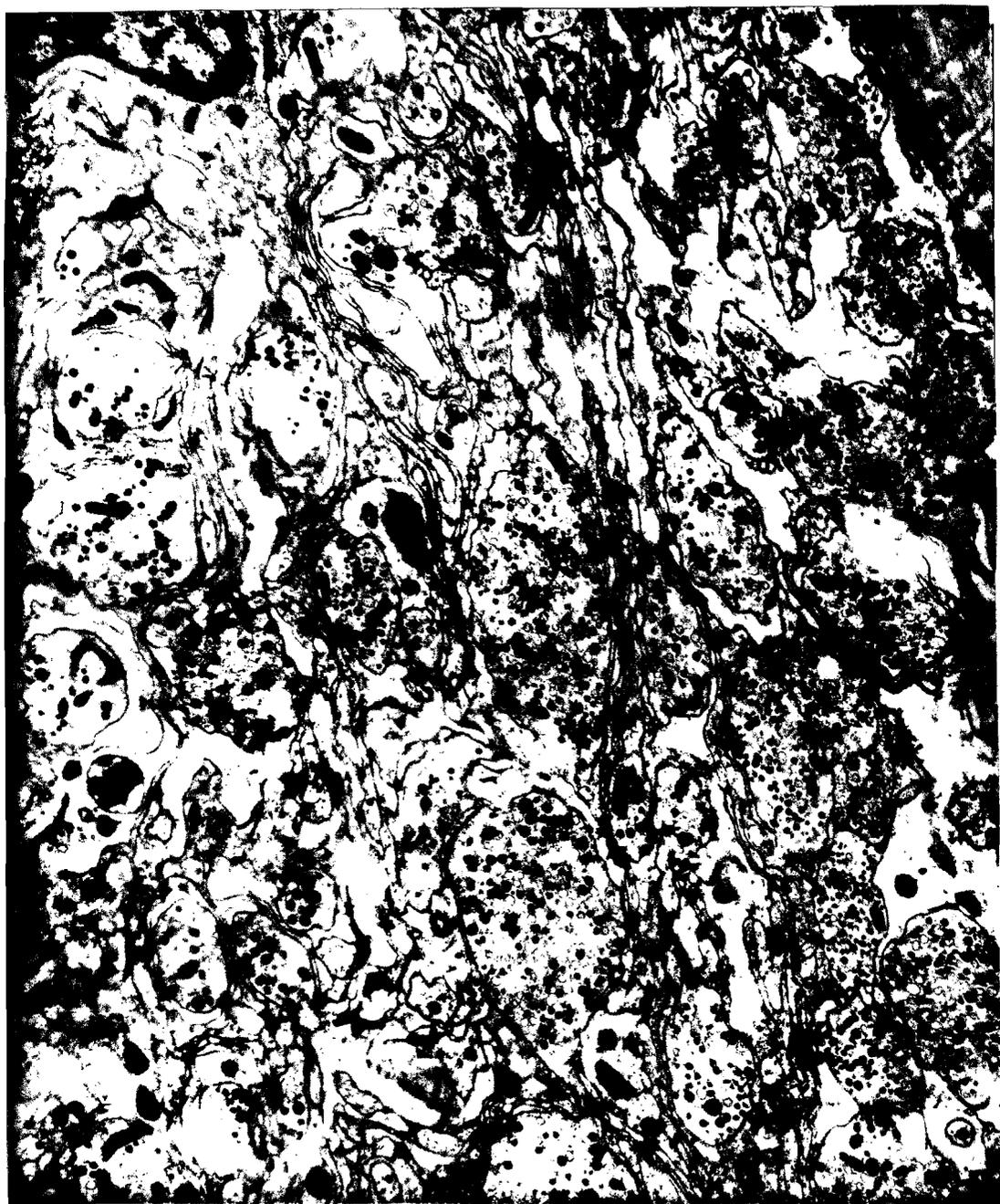


Fig. 12. Section of LMN0 from the second abdominal ganglion of a male injected with distilled water. About X 12,000



Fig. 13. Section of MNNO from the second abdominal ganglion of a normal male fixed with cacodylate buffered glutaraldehyde for $\frac{1}{2}$ hour. About X 12,000

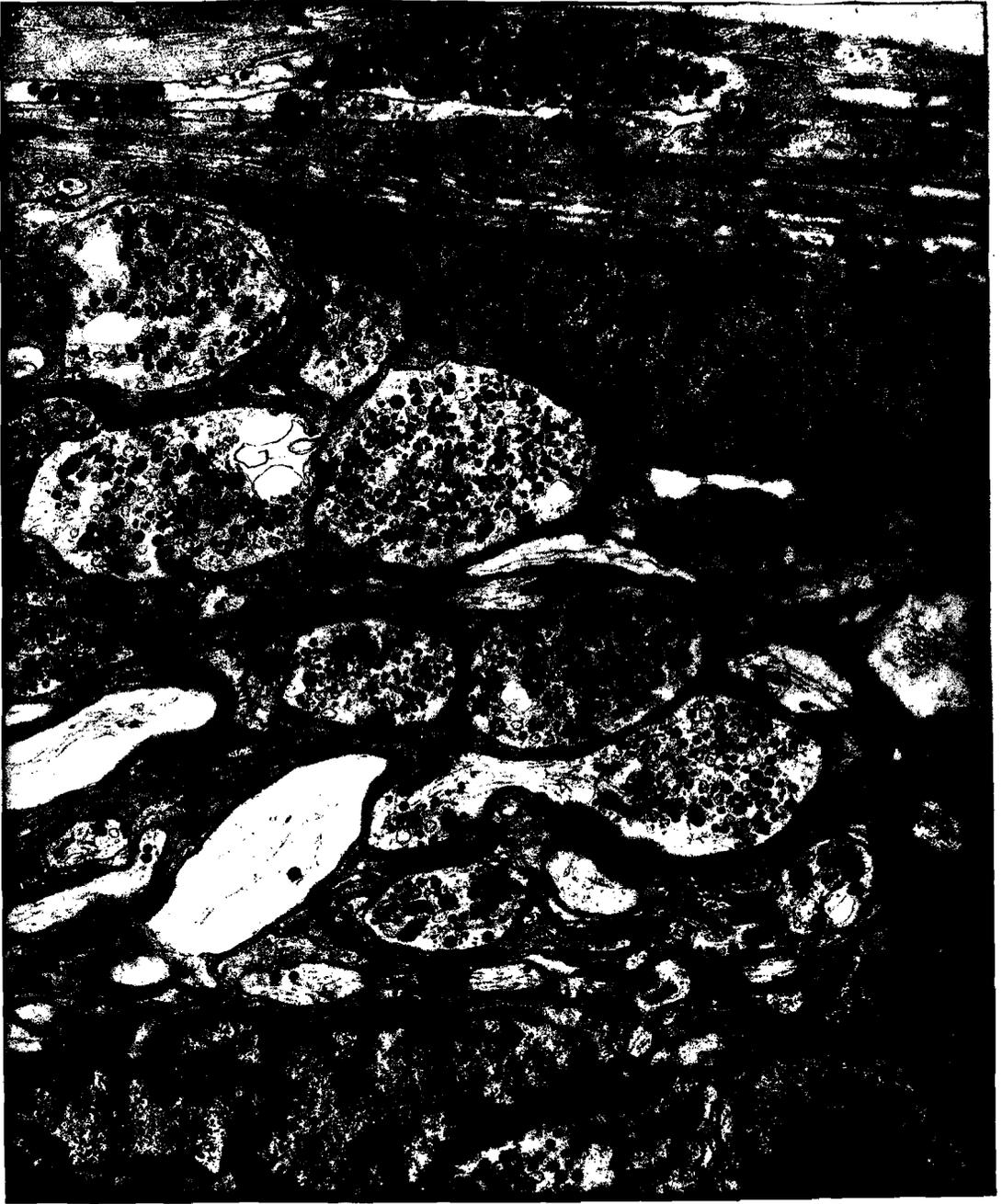


Fig. 14. Section of MNNO from the second abdominal ganglion of a normal male fixed with cacodylate buffered glutaraldehyde for 1 hour. About X 12,000

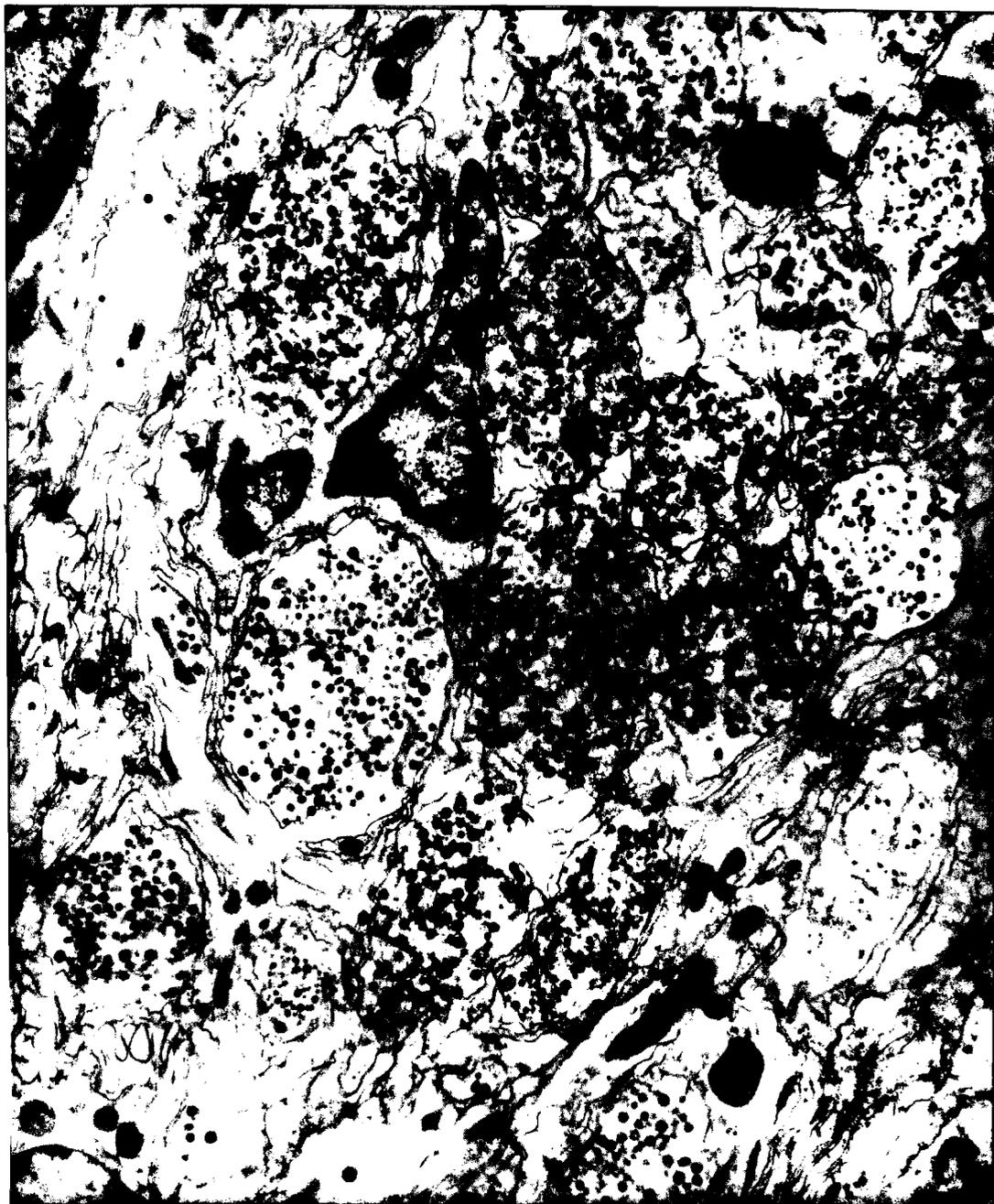


Fig. 15. Section of MNNO from the second abdominal ganglion of a male cooled in the refrigerator for 30 minutes before primary fixation. About X 12,000

of granules plus vesicles all closely resemble the normal animal (Table 1).

3. Normal Structure of the Transverse Nerve.

Electron micrographs of the transverse nerve from normal males show nerve tracts surrounded by an extensive connective tissue sheath. Woven in and out of the connective tissue sheath are several axons containing large electron dense granules 1200-1800 Å in diameter, with an average diameter of 1750 Å (Fig. 16). No other granules or vesicles are present in any of the axons. It seems probable that these large dense granules are the same type as described in the MNNO paper.

FLUORESCENCE LIGHT MICROSCOPY

The fluorescence histochemical method developed by Falck and Hillarp (Falck and Owman, 1965) has provided a means for studying the localization of amines on a cellular level. With this method nerve cells containing catecholamines or 5-hydroxy-tryptamine can be identified. This technique has been used in the following studies to aid in identification of the neurosecretory material in the midline median nerve cells, MMNC, whose axons form the type 2 endings in the MNNO (Smalley, 1970).



Fig. 16. Electron micrograph of the transverse nerve from a normal male. Large electron dense granules (G) typical of those in type I axon endings are seen surrounded by connective tissue sheath. About X 18,000

1. MNNO in Normal Male and Female

After one hour treatment with gaseous formaldehyde although no fluorescent cell bodies could be seen in the abdominal ganglia several areas of the neuropile developed yellow fluorescence. Sections of abdominal ganglia 1 and 2 not treated with formaldehyde showed only dark-yellow fluorescent dots. Prolonged treatment of three hours did not enhance fluorescence or bring about the appearance of fluorescent cells or new fluorescent areas of the neuropile. Control sections of the brain of both male and female showed fluorescence in the central body and the corpora pendunculata.

2. MNNO Under Experimental Conditions

a. Dopamine Injection. Serial sagittal sections of abdominal ganglia 1 and 2 from dopamine-injected adult males showed strong yellow-green fluorescence in cell groups 1, 2 and 3 (as defined by Smalley, 1970) (Figs. 17, 18). Only one MNNO was found in the sections and it also displayed a yellow-green fluorescence. The majority of MNNO were probably lost in tissue preparation. The brain control showed intense fluorescence in the central body and corpora pendunculata (Fig. 20).

b. Norepinephrine Injection. Norepinephrine is apparently not taken up in the MNNO under the conditions of these experiments. Serial sagittal sections of abdominal

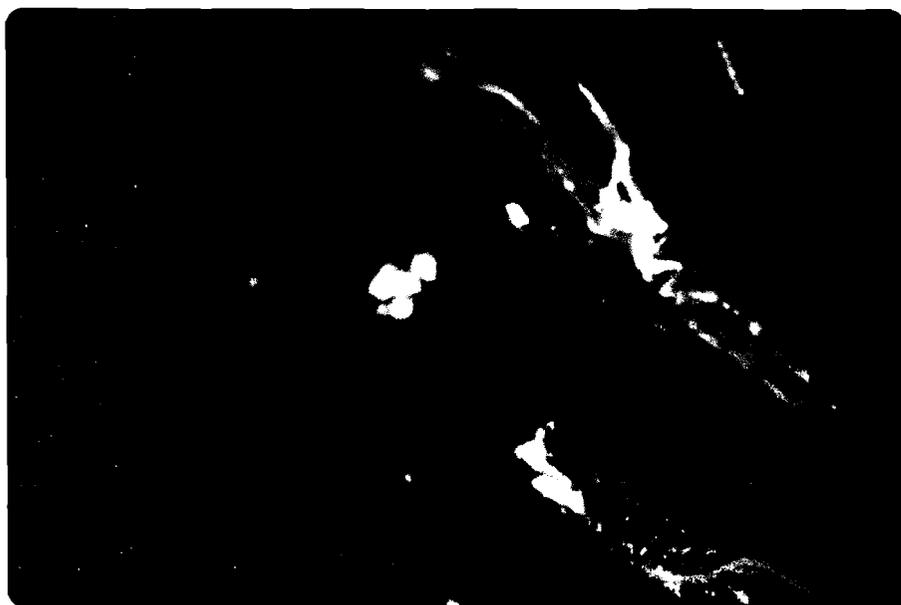


Fig. 17. Fluorescence photomicrograph (sagittal section) of second abdominal ganglion showing MMNC group 2 after pretreatment with dopamine. About X 200



Fig. 18. Fluorescence photomicrograph (parasagittal section) of a first abdominal ganglion showing MMNC group 2 after pretreatment with dopamine. About X 200



Fig. 19. Fluorescence photomicrograph of MNNO from the second abdominal ganglion of a male after injection of dopamine. About X 400

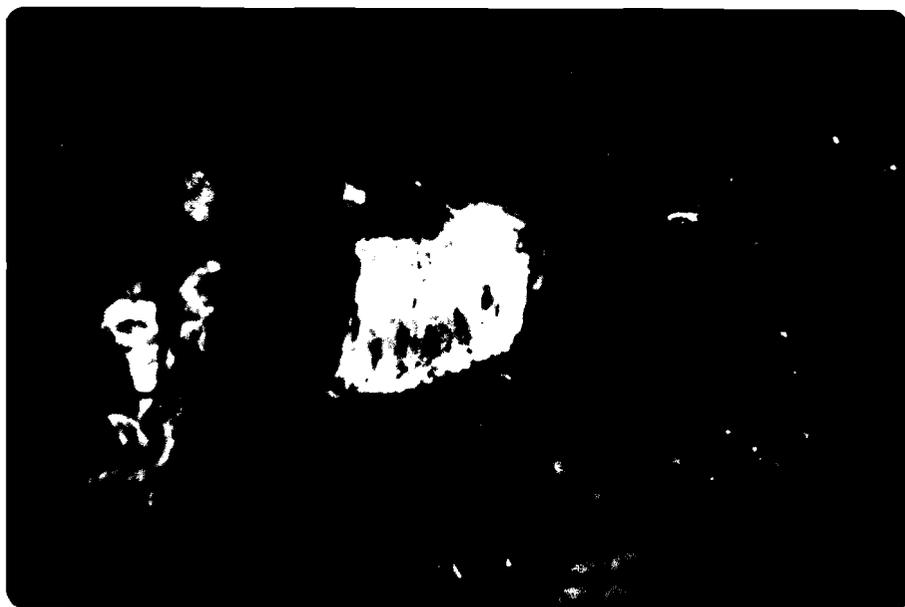


Fig. 20. Fluorescence photomicrograph (frontal section) of the central body in the brain of a normal animal. About X 200

ganglia 1 and 2 revealed no fluorescent areas resembling cell bodies even after prolonged formaldehyde treatment of three hours. However, no controls were run concurrently with the norepinephrine injected animals.

c. Dopa Injection. Examination of serial sagittal sections of abdominal ganglia 1 and 2 revealed no fluorescent areas resembling cell bodies. Prolonged formaldehyde treatment did not give any further evidence of fluorescence in any of the MNNO. The brain controls showed yellow green fluorescence in the central body and the corpora pedunculata.

d. Parnate Injection. Parnate is known to be a strong monoamine oxidase inhibitor in insects (Chaudhary, 1967). Animals injected with parnate showed extreme hyperactivity. The injection of parnate two hours before dissection did not reveal any biogenic amines in the MNNO even after prolonged formaldehyde treatment of three hours.

LIQUID SCINTILLATION

1. Uptake of Dopamine-1- H^3 in the MNNO.

Males and females show little difference in uptake of tritiated dopamine into the MNNO. Figure 21 shows the relation of MNNO to control tissues (nerve connectives from in front of A3 to just behind A2) in animals injected with dopamine-1- H^3 and dissected at five to fifteen,

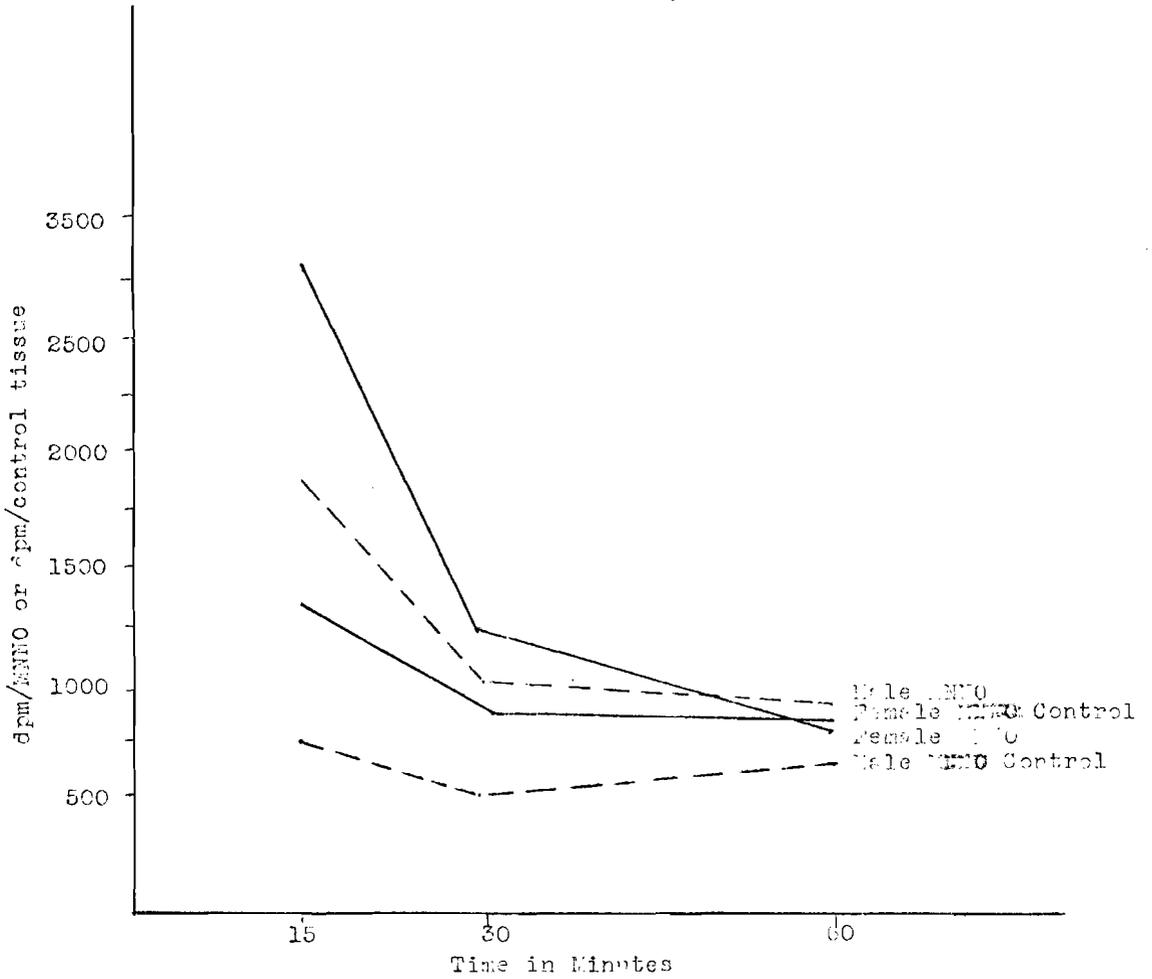


Fig. 21. Uptake and release of 10^{-3} M tritiated dopamine by the MNVO of male and female in relation to control nerve connectives AE-23.

fifteen to thirty, forty-five to sixty minutes. The MNNO appears to have a rapid uptake of tritium labeled dopamine. Highest disintegrations per minute/MNNO are found in the five to fifteen minute injection time range. The tritium label is, however, either rapidly released or diffused from the neurohemal organ. At sixty minutes, the MNNO of both the female and male show only slight variations from the control tissue. The wet weight of the MNNO could not be determined because of its very small size and consequently, the counts are expressed as dpm/MNNO or dpm/control tissue. It must be kept in mind that the MNNO is actually many times smaller in weight than the control tissue. From these data it cannot be determined whether the initial high concentration of tritium label in the MNNO is due to an actual uptake or rather an equilibration of the high concentration of dopamine in the hemolymph with the MNNO.

2. Uptake of Dopamine-1- H^3 in Various Nervous Tissues

Figure 22 relates the uptake of label in the brain, MNNO, A4-A5, A1-A3, A6, and control tissue (connective nerve tissue A5 to A6, A3 to A4) in males injected with dopamine-1- H^3 and dissected at fifteen, thirty, sixty, and one hundred and twenty minutes. The brain (Frontali, 1967) and the sixth abdominal ganglion, A6 (Farley, 1967) have been shown to have stores of biogenic amines.

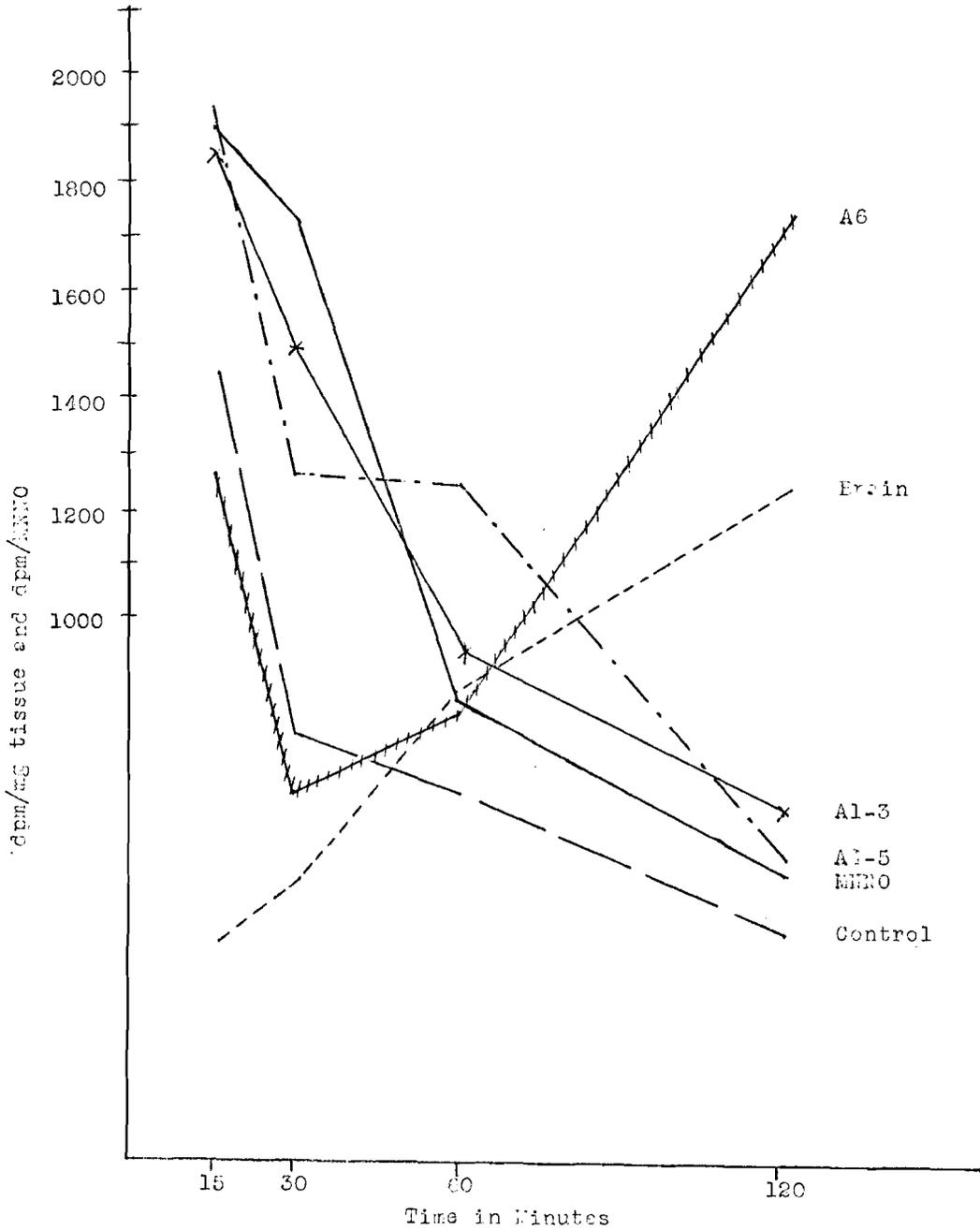


Fig. 22. Uptake and release of 10^{-3} M tritiated dopamine by the MICRO, A6, Brain, A1-A3, A4-A5, and control nerve connective A5-A6 over the time span 15-120 minutes.

Figure 20 demonstrates that the brain and A6 take up label from injected dopamine. At fifteen or thirty minutes both of these tissues have a low tritium content with a gradual but consistent rise up to the end of the experiment at one hundred and twenty minutes.

A significant point is the gradual uptake of label in the brain and A6. The MNNO, A4-A5, and A1-A3 all appear to follow a different trend. They all show high initial uptake of label followed by rapid release. The control tissue follows much the same pattern with a lower initial uptake of label. Dopamine or a metabolized product is apparently either taken up rapidly or is equilibrated between the high concentration of tritium in the hemolymph and lower concentrations in the MNNO, A1-A3, A4-A5 and then rapidly diffuses out. The brain and sixth abdominal ganglion on the other hand, take up and store the tritiated dopamine or a metabolized product.

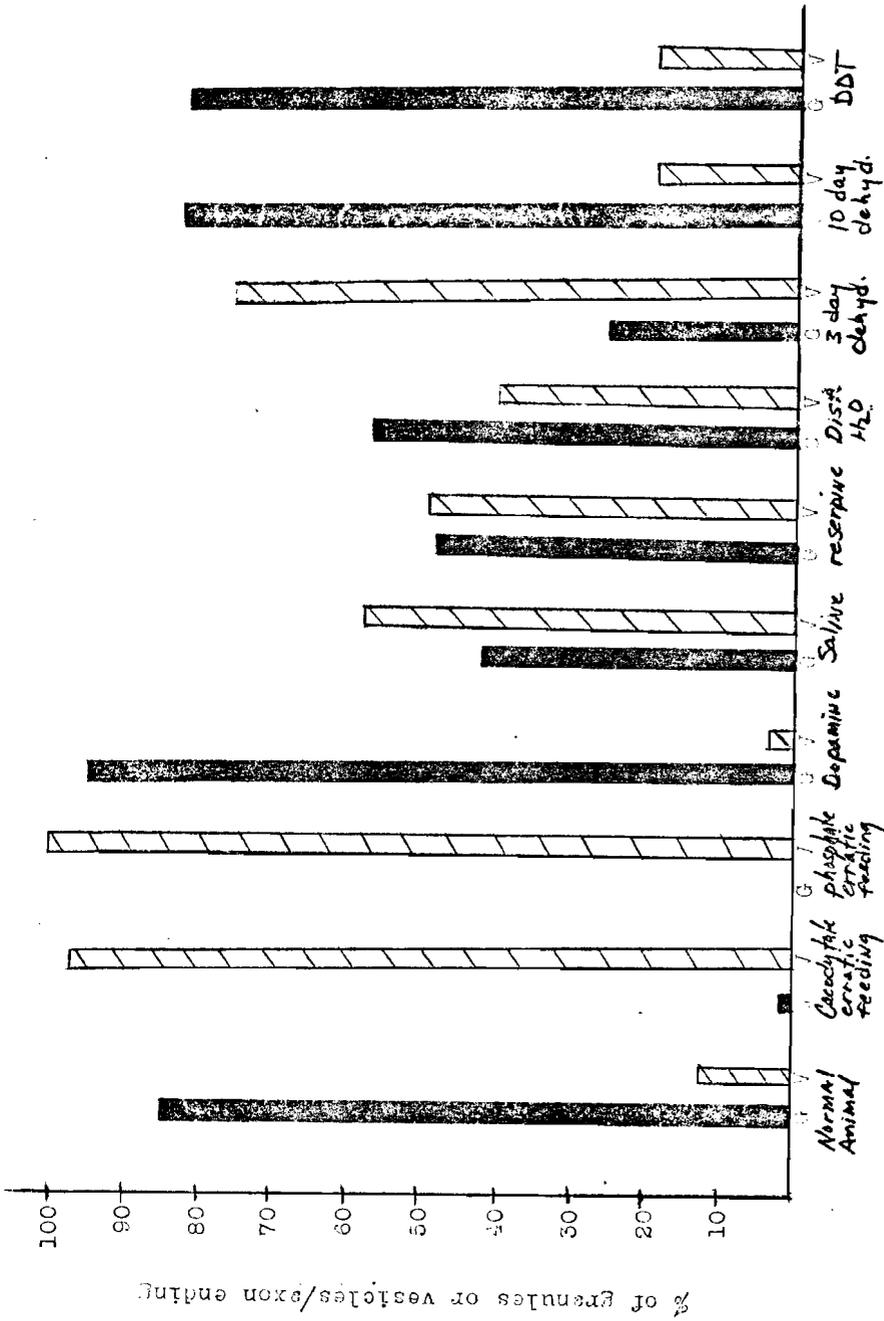


Fig. 23. The average percentage of granules and vesicles found in axon endings in the HNF0 under the various experimental conditions.

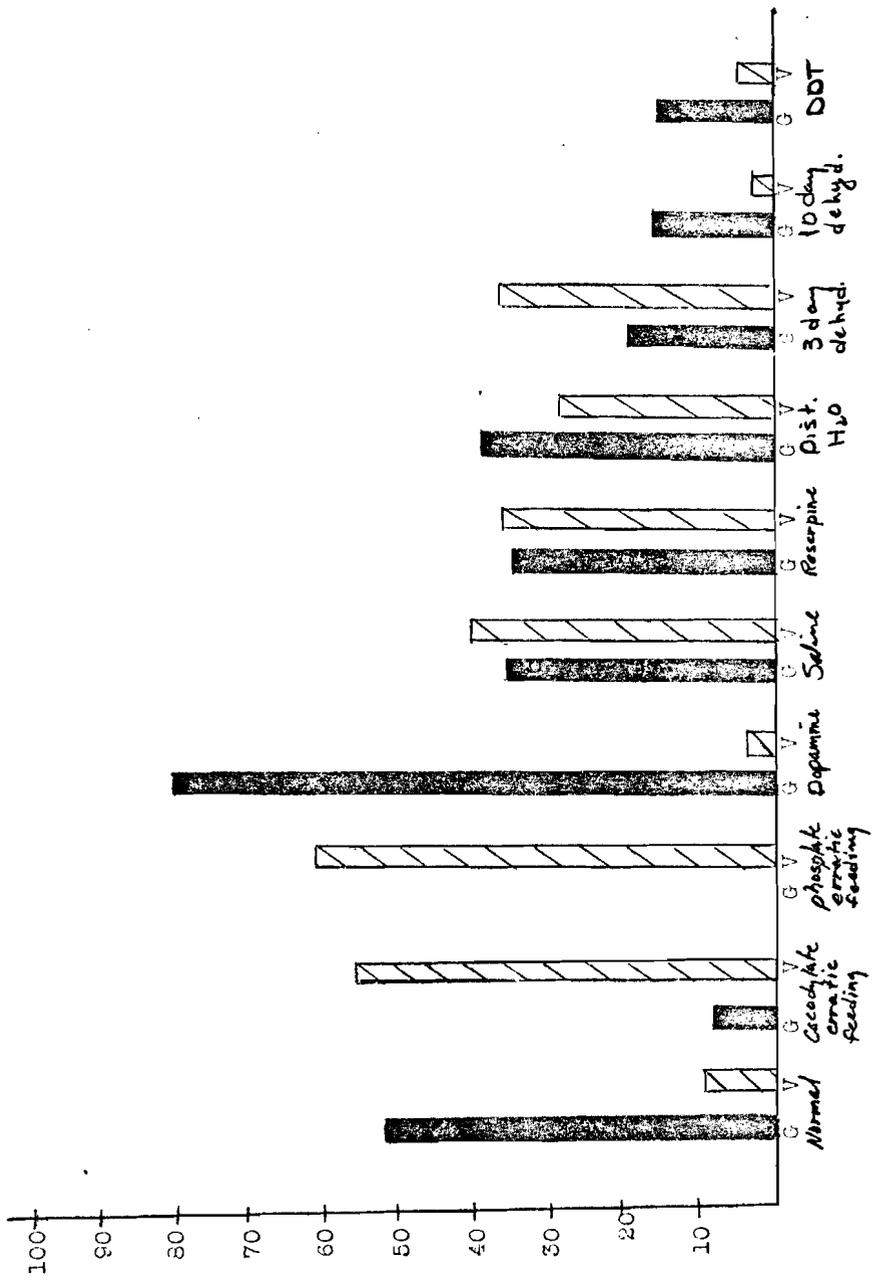


Fig. 24. The average number of vesicles and granules found in 16×10^8 microns² of exon endings for various experimental conditions.

DISCUSSION

Two types of neurosecretory material have been found in the median nerve neurohemal organ. In type 1 endings there are large electron dense granules 1000-2500 Å in diameter and in type 2 endings there are small electron dense granules 600-1600 Å in diameter as well as electron transparent vesicles 800-1900 Å in diameter. Several experimental conditions cause variations in granule and vesicle percentages in axon endings, but under most of these conditions the total number of granules plus vesicles does not change significantly. It is logical to assume that if the total number of granules plus vesicles does not change under conditions affecting the ratio of granules to vesicles, the granules and vesicles are probably interrelated. This suggests that the electron transparent vesicles are probably empty (containing no neurosecretory material) small electron dense granules. No evidence of release sites like those described by other workers (Smith, Smith, 1966; Scharrer, Kater, 1967) have been found in any of the normal or experimental animals (for either type 1 or type 2 endings). It seems probable that the neurosecretory material in type 2 endings is diffusing out of the small electron dense granule membrane and through the outer connective tissue sheath into the hemolymph.

Assuming that the change in relative proportions of granules to vesicles is indicative of release of neurosecretory material, then the conditions under which the change occurs could suggest possible functions for the hormone. Any type of intra-abdominal injection, distilled water or saline, seems to deplete some of the neurosecretory material from the small electron dense granules. Maddrell (1964) has shown a hormone to be released in Rhodnius by distention of abdominal stretch receptors sensitive to vertical abdominal distention. Such may be the case when large volumes of saline or distilled water are injected into the abdomen of the cockroach. It is also possible that the injection causes osmotic changes in the animal resulting in releases of the neurosecretory material.

Dehydration of the animal for ten days leaves the type 2 endings severely depleted of neurosecretory material. Presently, it cannot be determined whether the decrease in neurosecretory material is due to dehydration, lack of food, or a combination of these. It is interesting to note that irregular feeding and watering does not change the total number of granules plus vesicles in type 2 endings, but the neurosecretory material appears to have diffused completely from the small electron dense granules. Only under ideal conditions of feeding and watering can the type 2 endings be shown to contain small electron dense granules. These results suggest a

correlation between the availability of food and or water to the storage and retention of the neurosecretory material.

Treatment of the MNNO with DDT causes depletion of the type 2 endings with striking similarity to the ten day dehydrated animal. Maddrell (1970) has shown Rhondius paralyzed by DDT, releases a diuretic hormone. Again, it seems possible that water metabolism is important in neurosecretory material release in the cockroach.

From the above data it can be deduced that no real correlation can be made between the DDT induced depletion of neurosecretory material and a specific function for the MNNO. Also, all of the previous physiological circumstances are stress conditions. Possibly, the neurosecretory material is in some way functioning during stress conditions. Cook (1969) has shown that a biogenic amine released from the ventral nerve cord of P. americana by DDT poisoning functions as a neuromuscular excitatory substance. All of the results seem to point toward a relationship between stress conditions and the release of neurosecretory material in the type 2 endings.

Type 1 neurosecretory endings have been found in the transverse nerve some distance from the MNNO. It is possible that peripheral neurosecretory cells are contributing large electron dense granules to the neuroehmal organ via the transverse nerve. Finlayson and Osborne (1968) have described such a system in Carausius and peripheral neurons

have been found on branches of the segmental nerve of P. americana (Smalley, personal communication). In no circumstance was there any observed change of the type 1 neurosecretory material or endings under normal or experimental conditions.

Using the fluorescence technique of Falck and Owman, no catecholamines have been found in either the MMNC or the MNNO of normal animals. Nevertheless, autoradiographs have shown that tritiated dopamine is taken up by the MNNO, MMNC, and short median nerve (Smalley, 1970). The present study has also demonstrated the uptake of dopamine into the MNNO and MMNC. When animals were injected with dopamine, both the MMNC and the MNNO were rendered fluorescent. Since this fluorescence is specific for catecholamines or 5-hydroxy-tryptamine in other animals (Jonsson, 1967) it seems likely that the fluorescence observed in this case is due to the uptake of exogenous dopamine by the MMNC and MNNO. Under similar conditions neither dopa nor norepinephrine were taken up in the MMNC. This suggests that the uptake mechanism is quite specific for dopamine, quite unlike the uptake mechanism found in a mammalian catecholamine system (Hillarp et. al, 1965).

The presence of a dopamine uptake mechanism in cells which do not ordinarily contain a catecholamine is similar to the situation found in the parafollicular cells of the mouse thyroid. These cells can selectively take

up exogenous dopa and dopamine, but no trace of catecholamines can be demonstrated in the normal animal using the fluorescent technique (Larson et. al, 1966). Larson (1966) has speculated that in the parafollicular cell system some amine is operating which does not form fluorophores with formaldehyde or the amine is not retained by cellular storage mechanisms in concentrations high enough to be demonstrated histochemically. This situation seems to be very similar to the preliminary observations of the MENC and MNNO made by this investigator.

Jaim-Etchevery and Zieder (1968) have suggested that thyrocalcitonin and 5-hydroxy-tryptamine are stored together in mouse parafollicular cell granules. They have implied that the amine may act in some stage of the processes of metabolism, storage or liberation of the hormone. Dopamine or a metabolized product could possibly have a similar function in the MNNO. It is perhaps significant in this regard, that injection of dopamine causes an increase in the number and percentage of small electron dense granules in the type 2 endings.

Injections of tritiated dopamine into the cockroach show interesting relationships between known catecholamine-containing systems and the median nerve neurosecretory system. The brain (Frontali, 1967) and the sixth abdominal ganglion (Farley, 1967) have endogenous stores of catecholamines. Both these tissues gradually accumulate

label over the two hour experimental period. The MNNO, and abdominal ganglia containing the MMNC, on the other hand, both have high counts soon after injection, but rapidly lose the label thereafter. At two hours after injections, concentrations of the tritium label are very high in the brain and sixth abdominal ganglion while concentrations of the label in the MNNO and MMNC appear to be only slightly above the control nerve tissue. It is presently not clear whether the initial high count of tritium in the MNNO and MMNC is due to an actual uptake of dopamine into the system or rather an equilibration of the high concentrations of dopamine in hemolymph with the interstitial spaces of these tissues. The difference between the median nerve neurosecretory system and the known catecholamine containing tissues suggests that either dopamine is not being taken up selectively in the MNNO and MMNC or that these latter tissues do not have intraneuronal storage capacity for large quantities of dopamine.

The results of this study indicate that the median nerve neurosecretory system is probably not a catecholamine containing system. However, it is possible that a biogenic amine closely related in structure to dopamine is present in this system. Either this amine is not stored in concentrations high enough to be detected by the histochemical methods of Falck and Owman, or it is not amenable to this

fluorescence technique. Most preliminary results suggest a close correlation between physiological stress conditions, especially in relation to water metabolism, and the release of neurosecretory material from type 2 axon endings in the MNNO.

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