SOME ASPECTS OF THE HOST/ PARASITE RELATIONS OF NEWFOUNDLAND BLACKFLIES AND THEIR MERMITHID PARASITES

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SOME ASPECTS OF THE HOST/PARASITE RELATIONS OF NEWFOUNDLAND BLACKFLIES AND THEIR MERMITHID PARASITES

A Thesis

Presented to

the Department of Biology

Memorial University of Newfoundland

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

William John Condon

©

October, 1975

ABSTRACT

Two species complexes of larval blackflies, Prosimulium mixtum/fuscum Syme and Davies and Simulium venustum Complex, susceptible to mermithid parasitism, were collected from two Newfoundland streams. The anatomical relations of the neuroendocrine centers of these simuliid larvae were histologically examined. The neurosecretory system showed certain morphological similarities to other Nematocera (viz. three pairs of neurosecretory cell clusters in the brain, one pair of nervi corporis cardiaci entering the corpora cardiaca). The retrocerebral endocrine complex is comprised of cellular corpora cardiaca glands, a single corpus allatum and surrounding peritracheal gland. The morphology of the blackfly neuroendocrine system is discussed in relation to other Nematocera and higher Diptera. Two experimental groups were chosen consisting of uninfected larvae with dark histoblasts (controls) and nematode infected larvae of similar body dimensions. Histochemical studies showed no significant differences between the endocrine glands of mermithid infected and control P. mixtum/fuscum larvae. However, the mermithid parasite did significantly increase the nuclear DNA/RNA activity of the corpus allatum gland and the corpus cardiacum gland volume and stored neurosecretory material in S. venustum Complex larvae. Such endocrine effects of mermithid parasitism are discussed in relation to their significance within the host/parasite relationship. A marked decline in the amount of fat body tissue and their glycogen concentrations in both the infected simuliid species was recorded. However, fat body nucleic acid activity

was unaffected by mermithid parasitism. Mermithid-parasitized P. mixtum/fuscum larvae had a lower overall dry weight than uninfected controls, but the biomass of S. venustum Complex did not appear to be affected by mermithid parasitism.

TABLE OF CONTENTS

	Page
INTRODUCTION	1
HISTORICAL INTRODUCTION	4
MATERIALS AND METHODS	12
Histological observations on the neuroendocrine systems of the larval blackflies Prosimulium mixtum/fuscum and Simulium venustum Complex	16
Effects of N. flumenalis on the neuroendocrine system of the larval blackflies Prosimulium mixtum/fuscum and Simulium venustum Complex	20
Some effects of N. flumenalis parasitism on the levels of storage metabolites of Prosimulium mixtum/fuscum and Simulium venustum Complex	21
RESULTS	23
Histological observations on the neuroendocrine system of the larval blackflies Prosimulium mixtum/fuscum and Simulium venustum Complex	23
Effects of N. flumenalis on the neuroendocrine system of the larval blackflies Prosimulium mixtum/fuscum and Simulium venustum Complex	34
Some effects of N. flumenalis parasitism on the levels of storage metabolites of Prosimulium mixtum/fuscum and Simulium venustum Complex	47
DISCUSSION	54
SUMMARY	66
REFERENCES CITED	68
APPENDICES	75

LIST OF TABLES

Table		Page
1.	Comparison of size and weight measurements between control and mermithid infected larvae of two Newfoundland blackfly species	24
2.	Effects of *N. flumenalis* parasitism on the corpus allatum gland of the larval blackfly hosts *P. mixtum/fuscum* and *S. venustum* Complexes	38
3.	Effect of *N. flumenalis* parasitism on the corpora cardiaca gland cells of the larval blackfly hosts *P. mixtum/fuscum* and *S. venustum* Complexes	39
4.	Effects of *N. flumenalis* parasitism on the fat body tissues of the larval blackfly hosts *P. mixtum/fuscum* and *S. venustum* Complexes	51

LIST OF FIGURES

Figure		Page
1a.	A mature terminal instar *P. mixtum/fuscum* larva.	14
1b.	A mermithid-infected *P. mixtum/fuscum* larva.	14
2a.	A mature terminal instar *S. venustum* Complex larva	15
2b.	A mermithid-infected *S. venustum* Complex larva	15
3a.	Head region of adult female *N. flumenalis*.	17
3b.	Vulva area of adult female *N. flumenalis*	17
4.	Tail region of adult male *N. flumenalis*.	18
5.	Reconstruction of the neuroendocrine system of the larval stages of *P. mixtum/fuscum* and *S. venustum* Complex.	25
6.	Three paired neurosecretory cell clusters in the pars intercerebralis of *P. mixtum/fuscum* larvae	26
7.	Anterior neurosecretory cell clusters.	28
8.	Intermediate and posterior neurosecretory cell clusters. . .	28
9.	Nervi corporis cardiaci of *P. mixtum/fuscum* larvae	30
10.	Nervi corporis cardiaci of *S. venustum* Complex larvae. . . .	30
11.	Thin sectioned corpus allatum of *S. venustum* Complex larvae	31
12.	Corpus allatum of *P. mixtum/fuscum* larvae.	32
13.	Corpus allatum of *S. venustum* Complex larvae	32
14.	Cellular corpora cardiaca of *P. mixtum/fuscum* larvae	33
15.	Cellular corpora cardiaca of *S. venustum* Complex larvae	33
16.	Peritracheal gland of *P. mixtum/fuscum* larvae.	35
17.	Peritracheal gland of *S. venustum* Complex larvae	35

Figure		Page
18.	Pericardial cells of P. mixtum/fuscum larvae	36
19.	Pericardial cells of S. venustum Complex larvae	36
20.	Comparison of the corpus allatum gland volume between control and infected larvae of P. mixtum/fuscum and S. venustum Complex	40
21.	Comparison of the corpus allatum nucleic acid concentrations between control and infected larvae of P. mixtum/fuscum and S. venustum Complex	41
22.	Comparison of the corpus allatum nuclear/cytoplasmic ratios between control and infected larvae of P. mixtum/fuscum and S. venustum Complex	42
23.	Comparison of the corpora cardiaca gland volume between control and infected larvae of P. mixtum/fuscum and S. venustum Complex	43
24.	Comparison of the corpora cardiaca neurosecretory material concentrations between control and infected larvae of P. mixtum/fuscum and S. venustum Complex	44
25.	Comparison of the corpora cardiaca nuclear/cytoplasmic ratios between control and infected larvae of P. mixtum/fuscum and S. venustum Complex	45
26.	Comparison of the corpora cardiaca nucleic acid concentrations between control and infected larvae of P. mixtum/fuscum and S. venustum Complex	46
27.	Densely packed fat body tissues of P. mixtum/fuscum control larvae	48
28.	Mermithid nematode within the hemocoel of P. mixtum/fuscum infected larvae	48
29.	Densely packed fat body tissue of S. venustum Complex control larvae	49
30.	Mermithid nematode within the hemocoel of S. venustum Complex infected larvae	49
31.	Comparison of the fat body glycogen concentrations between control and infected larvae of P. mixtum/fuscum and S. venustum Complex	52
32.	Comparison of the fat body nucleic acid concentrations between control and infected larvae of P. mixtum/fuscum and S. venustum Complex	53

ACKNOWLEDGEMENTS

I would like to express my appreciation to the following individuals for their contribution in making this study possible:

To Dr. Roger Gordon, my supervisor, for suggesting this topic, for his continued encouragement and interest throughout this study and for his patience in aiding the author to correct and edit the manuscript.

To Dr. C. H. Bailey for suggesting most interesting avenues of research and various techniques of study; also for his help in correcting the manuscript and for discussing some of his recent unpublished research programs which are relevant to the discussion of the data presented.

To Dr. Gordon F. Bennett for his helpful suggestions regarding the direction of the program and his most useful editing of the manuscript.

To Mr. Roy Ficken, Department of Biology Photographer, for his assistance with the photographs.

To Mr. Eugene Fitzgerald, Department of Biology Supervisor of Technicians, for his assistance in acquiring various chemicals and apparatus required by the author.

To Christine Jackman and Ramona Raske for typing the thesis.

To the Research Unit on Vector Pathology, Memorial University of Newfoundland, for the kind use of the Zeiss Microvideomat.

To the Provincial Government of Newfoundland and Labrador, and to Memorial University of Newfoundland for their direct financial assistance.

INTRODUCTION

In recent years, increasing emphasis has been given to the possibility of using biological control agents to selectively limit populations of their target insect hosts (Laird, 1971). Deployment of biocontrol agents contributes a promising alternative to existing chemical control procedures (e.g. use of DDT) which pollute the environment, cause non-discriminative destruction to beneficial organisms in the environment and which may become useless in controlling the target organisms if the latter develop resistance toward it (Petersen, 1973). Entomophilic nematodes share a range of associations with their insect host(s) (Gordon and Webster, 1974) and nematodes of the family Mermithidae offer considerable biocontrol potential because they invariably kill and/or sterilize their hosts (Welch, 1958, 1962b; Nickle, 1972; Petersen, 1973; Gordon et al., 1973). Such nematodes appear to possess a high degree of host specificity and are obligate parasites of many kinds of invertebrates, especially insects.

Hematophagous blackflies (Diptera:Simuliidae) may transmit debilitating parasitic diseases to man and domestic livestock and certainly represent a considerable nuisance problem via their ravenous blood feeding habits (Jamnback, 1973). These insects are susceptible to parasitism by mermithid nematodes (Welch, 1965; Gordon et al., 1973). Welch and Rubtsov (1965) showed that under natural conditions, mermithids effectively eradicated a population of larval simuliids from a stream in the USSR. Thus, mermithid nematodes would seem to have potential for controlling blackflies under applied conditions.

Various authors have reviewed the usefulness of using mermithid nematodes in future programs to control blackflies (Welch, 1965; Gordon et al., 1973; Jamnback, 1973). In the absence of any satisfactory methods for rearing larval blackflies in the laboratory, mass colonization of blackfly mermithids by in vivo techniques would appear non-feasible. Therefore, the successful outcome of future control programs will probably depend on the establishment of in vitro techniques for mass cultivating the mermithids' infective stages (Gordon et al., 1973). However, there is a paucity of information concerning the development, nutrition, and host/parasite interactions (esp. hormonal and physiological) between mermithids and their simuliid hosts. Moreover, the physiology of blackflies (esp. their larval stages) has been neglected except for recent studies of free amino acid concentrations of larval S. venustum Say hemolymph by Gordon and Bailey (1974). Virtually no information is available regarding such important physiological processes as nutrient metabolism and storage, excretion, somatic and reproductive development along with hormonal control mechanisms of simuliid larvae. Baseline physiological data could prove crucial for devising artificial culture media for mermithids simulating the host (i.e. simuliid) microenvironment.

From studies done on other mermithid/insect host associations, the host fat body appears to be a source of proteinacious and carbohydrate nutriment for the developing nematode (Gordon and Webster, 1971; Gordon et al., 1971; Gordon et al., 1973; Bailey and Gordon, 1973; Rutherford and Webster, 1974). From gross morphological evidence, mermithids cause considerable depletion of fat body tissue in larval simuliids (Strickland, 1911; Phelps and DeFoliart, 1964; Ebsary, 1973) but no studies have

been done to ascertain the extent to which fat body storage metabolites are exhausted by mermithid parasitism. It seemed important to study the effect of mermithid parasitism upon the fat body metabolism of the simuliid host because the insect fat body is known to be an important tissue for nutrient storage and metabolism (Wigglesworth, 1972). Accordingly, one objective of my investigation was to quantitatively determine the effects of mermithid parasitism on carbohydrate storage in the larval fat bodies of two species complexes of hematophagous simuliids indigenous to Newfoundland. Carbohydrates are the major energy fuel in parasitic nematodes (Lee, 1965; Von Brand, 1966).

A diversity of homeostatic and developmental processes are hormonally controlled in insects (Highnam and Hill, 1969; Wigglesworth, 1972; Willis, 1974). Moreover, certain documented pathogenic effects (e.g. suppression of pupal histoblast development, inhibition of pupation) of mermithid parasitism of larval blackflies are normally controlled by the insects' neuroendocrine system. Thus, the possibility exists that parasitic mermithids share an endocrinological association with their larval simuliid hosts. Therefore, a further objective of my present investigation was to ascertain and compare the effects of mermithid parasitism on the neuroendocrine system of the two species complexes of larval simuliids. As a prerequisite to this component of my study, it was necessary to fully elucidate the morphology of the neuroendocrine system of the two blackfly species complexes, which had not yet been investigated.

HISTORICAL INTRODUCTION

The taxonomy of mermithids from blackflies has been somewhat of an enigma as many authors (invariably entomologists) refer to them loosely as a species of Mermis, Paramermis, Limnomermis and Hydromermis (Gordon et al., 1973). The Mermithidae are a notoriously difficult family of nematodes to classify. The most widely accepted taxonomic designation for mermithids from North American blackflies is based on adult morphology as described by Welch (1962b) and Anderson and DeFoliart (1962). At present the three species of mermithids Isomermis wisconsinensis Welch, Gastromermis viridis Welch and Neomesomermis flumenalis Nickle are the only members of this family reported from North American blackflies. The problem of obtaining an acceptable taxonomic scheme for blackfly parasitic mermithids has been tried by Rubstov (1965) who based their identification on larval characteristics. However, such a key is at variance with more conventional methods of nematode taxonomy based on adult morphological structures. The most prevalent mermithid species infecting simuliid species complexes of P. mixtum/fuscum and S. venustum on the Avalon Peninsula of Newfoundland has been recorded as Neomesomermis flumenalis with Isomermis wisconsinensis and Gastromermis viridis less common (Ebsary, 1973; Ezenwa, 1974).

The life history of blackfly parasitic mermithids has been recorded by Phelps and DeFoliart (1964) for G. viridis and I. wisconsinensis infecting Simulium vittatum Zetterstedt larvae of Wisconsin and by Ebsary (1973) and Ezenwa (1974) on N. flumenalis parasitizing P. mixtum/fuscum and S. venustum Complex larvae of Newfoundland. The cycle involves gravid

female nematodes ovipositing their eggs in the stream whereupon these eggs continue to embryonate. The eggs hatch (dependent upon natural temperatures) to release motile preparasites which infect susceptible species of larval simuliids. In Newfoundland these preparasites have a somewhat short-lived survival time requiring successful infection within 1-2 days after hatching (Ebsary, 1973). Larval blackflies were alleged to be infected by ingesting the mermithids' preparasitic larvae, which subsequently penetrate the gut wall of the host to gain access to the hemolymph (Phelps and DeFoliart, 1964; Rubstov, 1971). However, recent studies have shown that N. flumenalis infects its simuliid host by actively penetrating the soft intersegmental cuticle of early simuliid instars using its' long whip-like tail for anchorage to the host while it burrows into the cuticle (Bailey, personal communication).[1] After entering the hemocoel, the parasitic larva(e) begins to obtain host nutrients to be utilized for its development and nutrient storage for use by later free-living adult stages. The mermithid N. flumenalis of Newfoundland simuliids is located in the abdominal region of the host, which appears somewhat transparent due to fat body depletion by the nematode. Further, these late infected blackfly larvae apparently lack any development of the pupal and/or imaginal histoblasts and are unable to pupate as observed by Strickland (1911), Phelps and DeFoliart (1964) and Ebsary (1973). Upon completion of parasitic development, the mermithid emerges from the host by piercing the weak intersegmental areas of the host's abdomen or via natural host openings such as the anus or

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[1] Dr. C. H. Bailey, Research Unit on Vector Pathology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada.

mouth. Such emergence results in death of the simuliid host due to hemolymph loss. These postparasitic mermithids position themselves on a substrate within the stream (either aquatic vegetation or rocks) and remain tightly coiled around it. Postparasitic juveniles molt to adult nematodes dependent upon the temperature; however, the type of molt (single or double molt) has not yet been agreed upon (Ebsary, 1973; Bailey, personal communication).[2] The adult mermithids mate within a few hours of molting, producing gravid females with developing eggs which will later be oviposited into the stream.

While this mermithid life cycle pattern is adhered to by N. flumenalis infecting species complexes of P. mixtum/fuscum and S. venustum larvae in Newfoundland, there is an apparent difference in the seasonal synchronization of life cycles between the two hosts. According to Ebsary (1973) the univoltine N. flumenalis infects the univoltine P. mixtum/fuscum larvae in mid-to late October coinciding with the P. mixtum/fuscum first instar hatch. These mermithids overwinter within the P. mixtum/fuscum host having a parasitic phase of about seven months which is completed around late April or early May of the following year. The newly emerged mermithids require a developmental time of at least 129 days to produce another infective generation; therefore any assumption that they infect the S. venustum generation about 30-40 days later would seem unfounded. Instead it is suggested that the mermithid which emerges in the spring from the Prosimulium host develops around October to infect the following years' generation of P. mixtum/fuscum. The N. flumenalis larvae which infect the first generation of the multivoltine S. venustum

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[2] See footnote #1.

larvae in mid-May to early June complete parasitic development in 4-8 weeks emerging around mid-June to early July. Emergence at this time should allow for development of preparasites around mid-December. However, the low stream temperature throughout December to March may delay hatching until the spring (Ebsary, 1973) to allow the presence in the stream water of preparasites (which originate from mermithids infecting the previous year's S. venustum Complex generation) to coincide with the hatching of S. venustum larvae in late May to early June. However, it is not definitely known whether or not mermithid eggs hatch continually throughout the winter months. It appears, therefore, that the Newfoundland bionomics of P. mixtum/fuscum and S. venustum Complex larvae dictates the change of synchronization of life cycles with respect to their N. flumenalis infections.

Several detailed accounts of the taxonomy and bionomics of Canadian blackflies have been documented (Wood et al., 1963; Lewis and Bennett, 1973, 1974, 1975), but the physiology of blackflies has been neglected due to difficulties of rearing simuliids under laboratory conditions. Hence, studies must at present be made on field collected specimens. A few isolated studies have been done on the ultrastructure of adult blackfly oocytes and activities of midgut digestive enzymes (Pacaud, 1945, 1950; Yang and Davies, 1968a, b, c; Liu and Davies, 1971). The physiology of larval blackflies has been almost entirely ignored except for recent studies on the free-amino acid composition of larval S. venustum hemolymph by Gordon and Bailey (1974).

Although the neuroendocrine system of P. mixtum/fuscum and S. venustum Complex larvae has not yet been examined, Cazal and Maurand (1966) outlined the morphology of the glandular portion of the neuro-

endocrine system of the larval blackfly, Simulium ornatum Meig. The latter authors did not, however, examine the cerebral portion (including distribution of neurosecretory cells within the pars intercerebralis) of the neuroendocrine system of this European blackfly species.

The possibility that entomophilic parasites (in particular, nematodes) may interact with the hormonal milieu of their insect hosts was reviewed by Davey and Hominick (1973), who designated hormonal interactions between entomophilic nematodes and insects into three categories: a) certain nematodes require either direct or indirect hormonal stimulation from the host for their development (Gordon, 1968, 1970; Nadakal and Nayar, 1968); b) nematodes may be unaffected by the hosts' hormonal state (Yoeli et al., 1962; Hansen and Bueeher, 1971; Hominick and Davey, 1972; Gwadz and Spielman, 1974); c) nematodes may affect the hosts' endocrinology either to enhance its own development and/or as a stressful phenomenon (Palm, 1948; Stoffalano, 1967). Evidence for the first category of nematodes has been afforded from studies done on thelastomatid nematodes resident in the hindgut of various species of cockroaches. From results of ablation experiments on the host neuroendocrine system, Gordon (1968, 1970) concluded that the oxyurid nematode Hammerschmidtiella diesingi Hammerschmidt parasitic in the hindgut of the cockroach Blatta orientalis L. is dependent for its development on the hormone(s) of the median neurosecretory cells/corpora cardiaca complex of its host. However, Hominick and Davey (1972) interpreted the effects of surgical manipulation of the host hormonal milieu upon resident pinworm populations to be indirect, since microsurgery invariably reduced host food intake. Inconclusive evidence was advanced by Nadakal and Nayar (1968) that the corpus allatum hormone(s) of Periplaneta

americana (L.) affects the fecundity of oxyurid nematodes in the hindgut. The second category of an entomophilic nematode may include the rhabditoid nematodes Neoaplectana dutkyi Jackson and N. glaseri Steiner in which developmental processes such as egg laying and cuticle exsheathment are unaffected by juvenile hormone compounds in vitro (Hansen and Buecher, 1971). Gwadz and Speilman (1971) showed that the development of the larval filarioid nematode Brugia pahangi (Buckley and Edeson) within the hemocoel of the adult female mosquito Aedes aegypti (L.) appears independent of both synthetic treatments of either ecdysone or juvenile hormone, and also develop normally in brain or allata excised hosts. Similarly, the development of the filarioid nematode Dirofilaria immitis (Leidy) appears independent of the endocrinology of its host Anopheles quadrimaculatus Say (Yoeli et al., 1962). The third category of nematodes includes the tylenchid nematode Sphaerularia bombi Dufour which parasitizes female bumblebees causing the host's ovaries and corpora allata to degenerate. Palm (1948) suggested that this nematode inhibited the corpora allata of its host by secreting a toxin(s). It has been similarly suggested that the tylenchid nematode Heterotylenchus autumnalis Nickle infecting the face fly Musca autumnalis de Geer causes destruction of the hosts' ovaries by actively secreting toxic substances to impair corpus allatum activity (Stoffolano, 1967).

It has not been established whether or not mermithid nematodes share an association with the neuroendocrine system of the host. While there is some evidence which would tend to preclude such an interaction, other studies yielded circumstantial evidence to indicate that the neuroendocrine system of the host is affected by mermithid parasitism. Craig and Webster (1974) showed no change in the ecdysone levels of the locust

Schistocerca gregaria Forskål when parasitized by the mermithid Mermis nigrescens Dujardin but rather a decrease in fat body protein synthesis. These authors concluded that the nematode suppressed molting by the host due to the nutritional demands made by the parasite for proteins and/or amino acids normally required for insect molting and differentiation. Jutsum and Goldsworthy (1974) studied the effect of M. nigrescens parasitism on the flight performance of the locust Locusta migratoria R. and F. The nematode caused a reduction of lipid content in flight muscles of infected locusts, but injection of corpora cardiaca extracts into infected hosts did not restore lipid mobilization; so Jutsum and Goldsworthy (1974) concluded that the effect of parasitism upon flight performance was not mediated by the host hormone(s).

According to the host/parasite system, mermithids have been reported to cause a variety of pathogenic symptoms in their hosts -- e.g. parasitic castration, intersexes and gynandromorphs, malformed wings, suppression of molting and/or pupation, inhibition of imaginal disc development and fat body depletion (Gordon et al., 1973; Gordon and Webster, 1974). In parasitized blackflies, several of the above effects of mermithid parasitism (viz. fat body depletion, imaginal bud suppression and inhibition of pupation) normally controlled by the insect's neuroendocrine system, have been documented (Strickland, 1911; Phelps and DeFoliart, 1964; Gordon et al., 1973). Additional evidence which would suggest that mermithid parasites interfere with the hormonal balance of their hosts may be deduced from studies done on the physiology of the host/parasite relationship between M. nigrescens and S. gregaria. This mermithid profoundly alters the homeostatic balance of hemolymph and fat body metabolites as well as interfering with excretion, a process normally

regulated by the insect's endocrine system (Gordon and Webster, 1971; Gordon et al., 1971; Gordon and Webster, 1972; Gordon et al., 1973; Craig and Webster, 1974; Rutherford and Webster, 1974).

MATERIAL AND METHODS

All specimens of larval blackflies were field collected from two streams near St. John's, Newfoundland, selected on the basis of a preliminary survey of host populations and mermithid parasites conducted in April/July, 1973. One of these streams (Broad Cove River) was especially suitable for collection of large numbers of mermithid parasitized P. mixtum/fuscum larvae, while the other stream (Long Pond Tributary) was used for collection of mermithid parasitized S. venustum Complex larvae. All larvae for experimental replicates of a single host species were obtained from the same stream and were collected by the technique of Bailey et al. (1974).

All simuliid larvae were examined alive in the laboratory using a stereomicroscope and the species were identified according to the classification scheme of Wood et al. (1968) for Ontario simuliids. For the purposes of this study, Prosimulium mixtum Syme and Davies and Prosimulium fuscum Syme and Davies were considered as one host species complex in view of the difficulties inherent in taxonomically separating these species both of which are parasitized by N. flumenalis in Newfoundland. For the same reason, Simulium venustum Say, Simulium tuberosum Lundstrom and Simulium verecundum Stone and Jamnback were considered as a single species complex and are referred to hereafter in this study as the Simulium venustum Complex. Only P. mixtum/fuscum and S. venustum Complex larvae were used in this study since these are the most prevalent Newfoundland blackfly hosts of mermithids.

At present, different numbers of larval instars have been reported

in the literature for different simuliid species by various authors and probably the number varies according to species and prevailing environmental conditions. No reliable data for assessing larval aging in blackflies was available, therefore I employed a larval aging technique based on external features by Sommerman et al. (1955). In each study a control group and a mermithid infected group of each host species were compared. The controls (Figs. 1a, 2a) were larvae with dark histoblasts (mature terminal larval instars having black pupal respiratory filaments nearly fully differentiated for oncoming pupation). The infected group (Figs. 1b, 2b) were larvae of similar size with a large prominent mermithid coiled within a swollen host abdomen -- usually averaging one nematode per larval host. The stage of infected larvae was difficult to ascertain because the parasite caused suppression of the imaginal/pupal disc development. Consequently, for the initial study on the histology of the endocrine system of the larval blackfly hosts only the mature dark histoblast stage was examined since this is the only stage of blackfly development which can be determined with any degree of precision. For the subsequent experiments on the effect of the parasite upon the hosts' physiology and endocrinology, both groups (infected and dark histoblast controls) previously mentioned, were compared for each of the blackfly species under study. In view of the difficulties inherent in determining precise stages of development within field populations of simuliids, head capsule widths and body lengths were measured within each of the two experimental groups (ten insects chosen at random from within each group). Based on the assumption that head capsule width and body length measurements are reliable indicators of stage of development, such measurements were made to insure that specimens within each of the groups

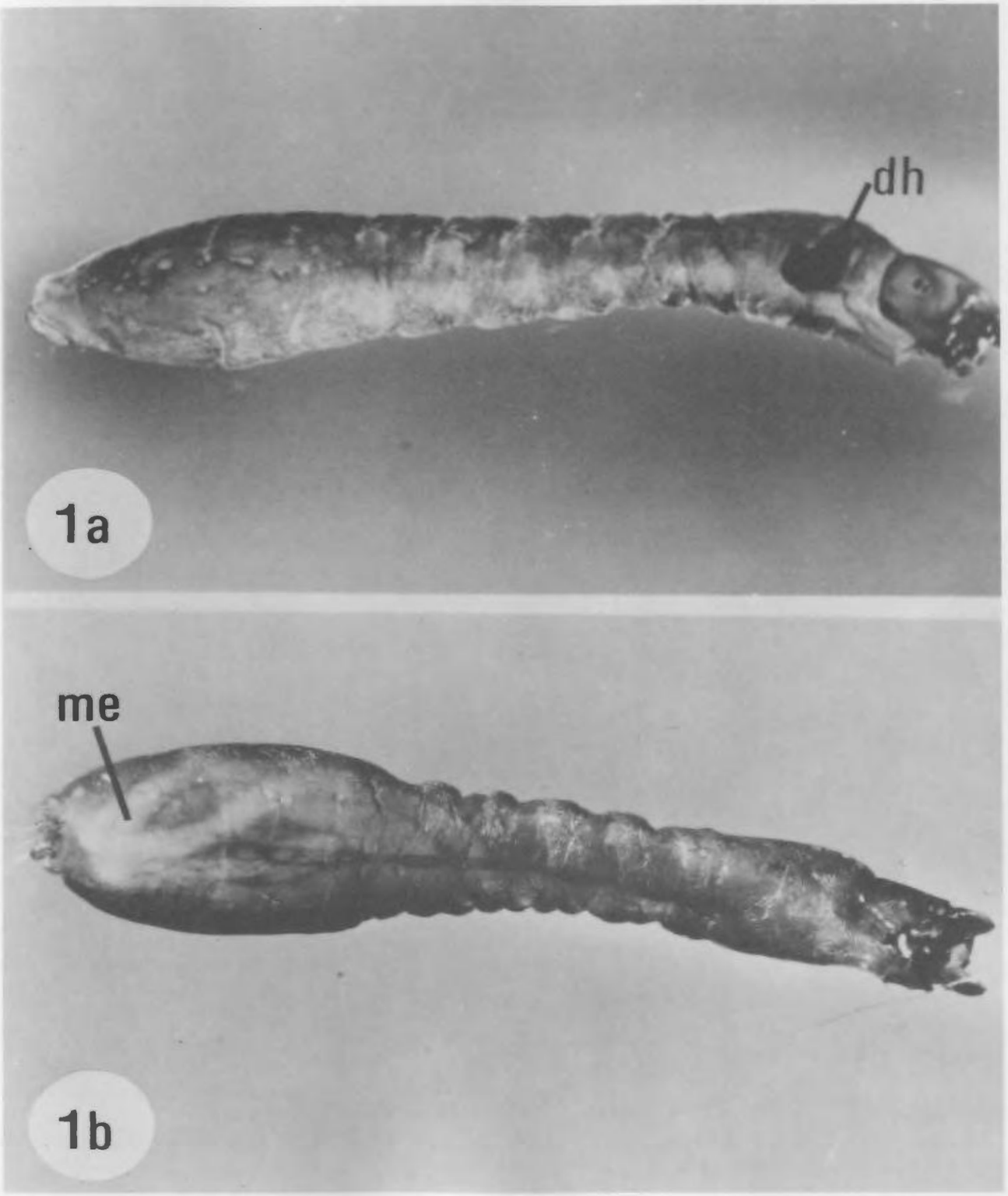

dh
1a
me
1b

Figure 1a



A mature terminal instar P. mixtum/fuscum larva having black pupal histoblasts on the dorso-lateral sides of its prothorax.

Abbreviations: dh, dark histoblast.

Magnification: 20X

Figure 1b

A mermithid-infected P. mixtum/fuscum larva recognized by the prominent mermithid coiled within the swollen abdomen of the blackfly larva. Also the infected larva lacks prominent histoblasts.

Abbreviations: me, mermithid.

Magnification: 20X

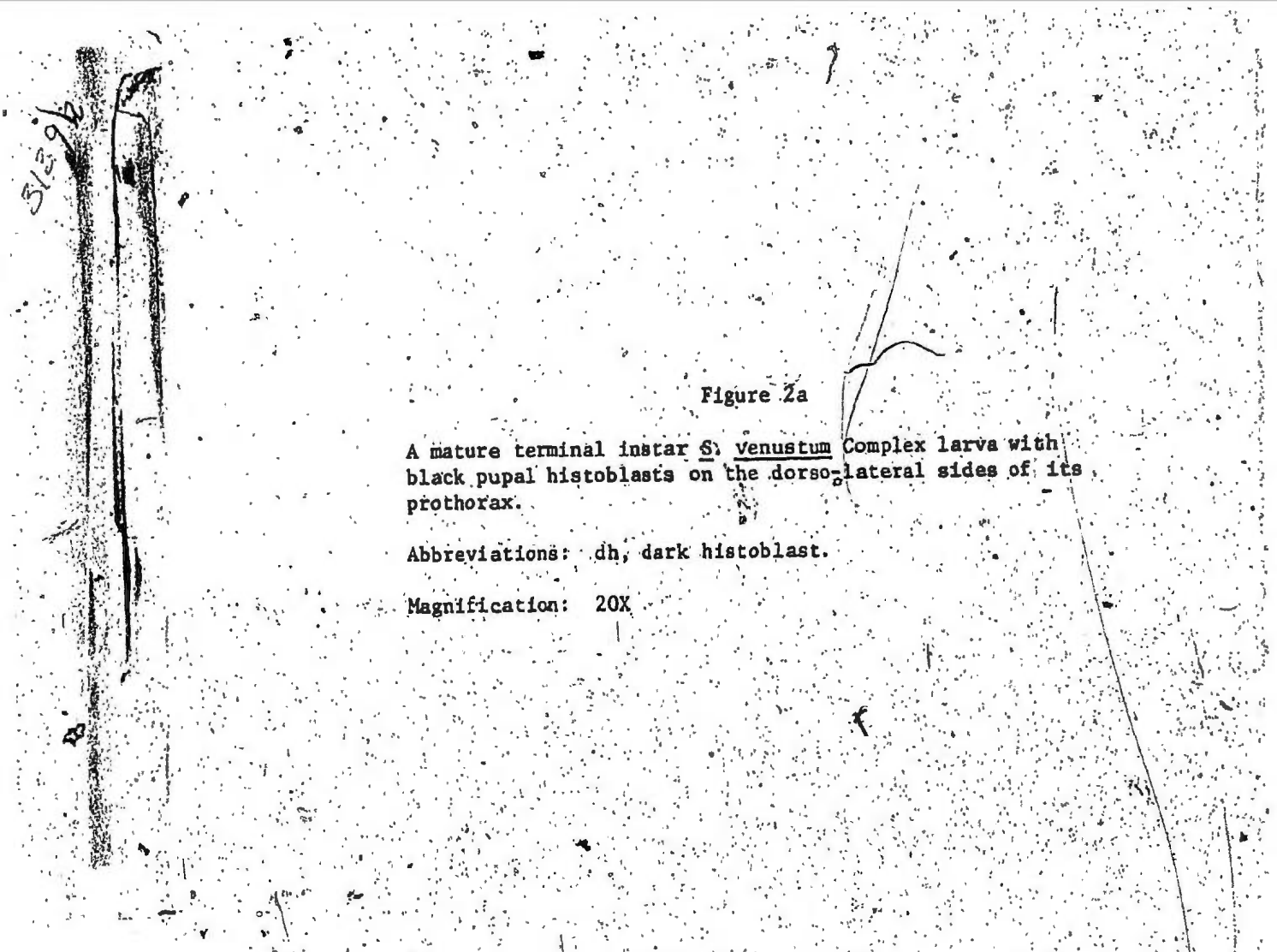


Figure 2a

A mature terminal instar S. venustum Complex larva with black pupal histoblasts on the dorso-lateral sides of its prothorax.

Abbreviations: dh, dark histoblast.

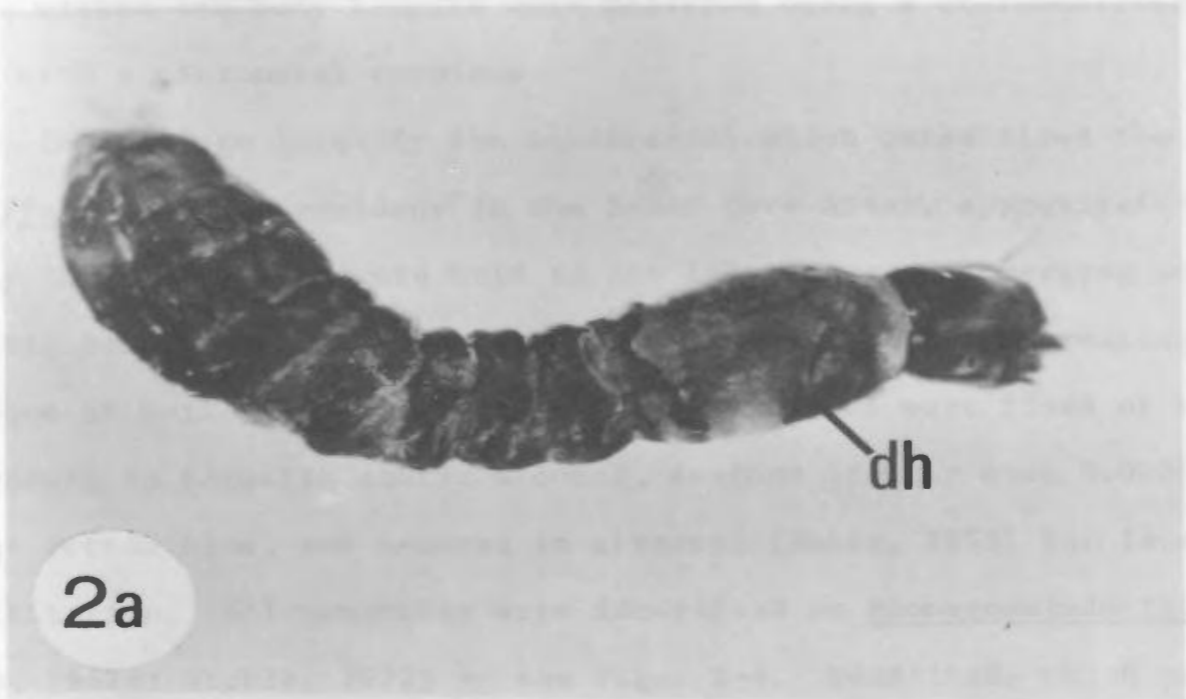
Magnification: 20X

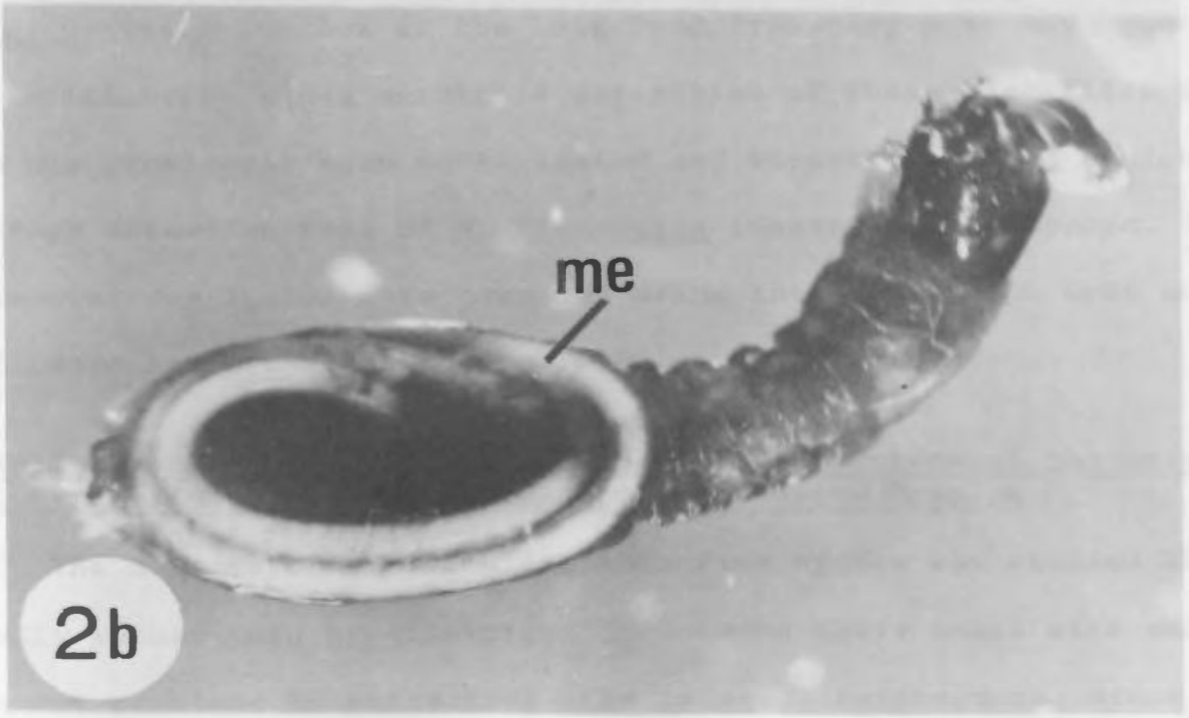
Figure 2b

A mermithid-infected S. venustum Complex larva recognized by the prominent mermithid coiled within the swollen abdomen of the blackfly larva. Also the infected larva lacks prominent histoblasts.

Abbreviations: me, mermithid.

Magnification: 20X


dh
2a


me
2b

were at approximately the same stage of development and that the stage of development of infected larvae (irrespective of effects of parasitism) corresponded to that of the dark histoblast controls. Both the head capsule widths and body lengths were measured using a stereomicroscope fitted with a micrometer eyepiece.

In order to identify the nematode(s) which parasitized the P. mixtum/fuscum larvae resident in the Broad Cove River, approximately seventy infected larvae were held in the laboratory and emerging post-parasitic larval nematodes were reared to adults, using the rearing technique of Bailey et al. (1974). Adult nematodes were fixed at room temperature in formalin acetic alcohol, stained lightly with 0.0025 percent cotton blue, and mounted in glycerol (Baker, 1953) for later identification. All nematodes were identified as Neomesomermis flumenalis (Welch, 1962a; Nickle, 1972) — see Figs. 3-4. Mermithids which parasitized S. venustum Complex in the Long Pond Tributary were not mounted for identification since mermithid parasitism of these blackflies in the stream has previously been investigated and reported to have a high percentage infection rate of N. flumenalis (Ebsary, 1973; Ezenwa, 1974). Experimental replicates were compared using the Student's t test and the significance level was taken as $p < 0.05$.

Histological observations on the neuroendocrine systems of the uninfected larval blackflies P. mixtum/fuscum and S. venustum Complex

The anatomy of the simuliid endocrine system was studied histologically rather than by dissection because of their small size and the consequent problems in extracting them intact. Furthermore, since simuliid larvae have soft cuticles, the fixative solutions infiltrate quite rapidly. All specimens for paraffin sectioning were fixed in

31392

Figure 3a

N. flumenalis adult female showing head region with cephalic papillae.

Abbreviations: cp, cephalic papilla; h, hypodermis.

Stain: lactophenol/cotton blue.

Magnification: 3800X

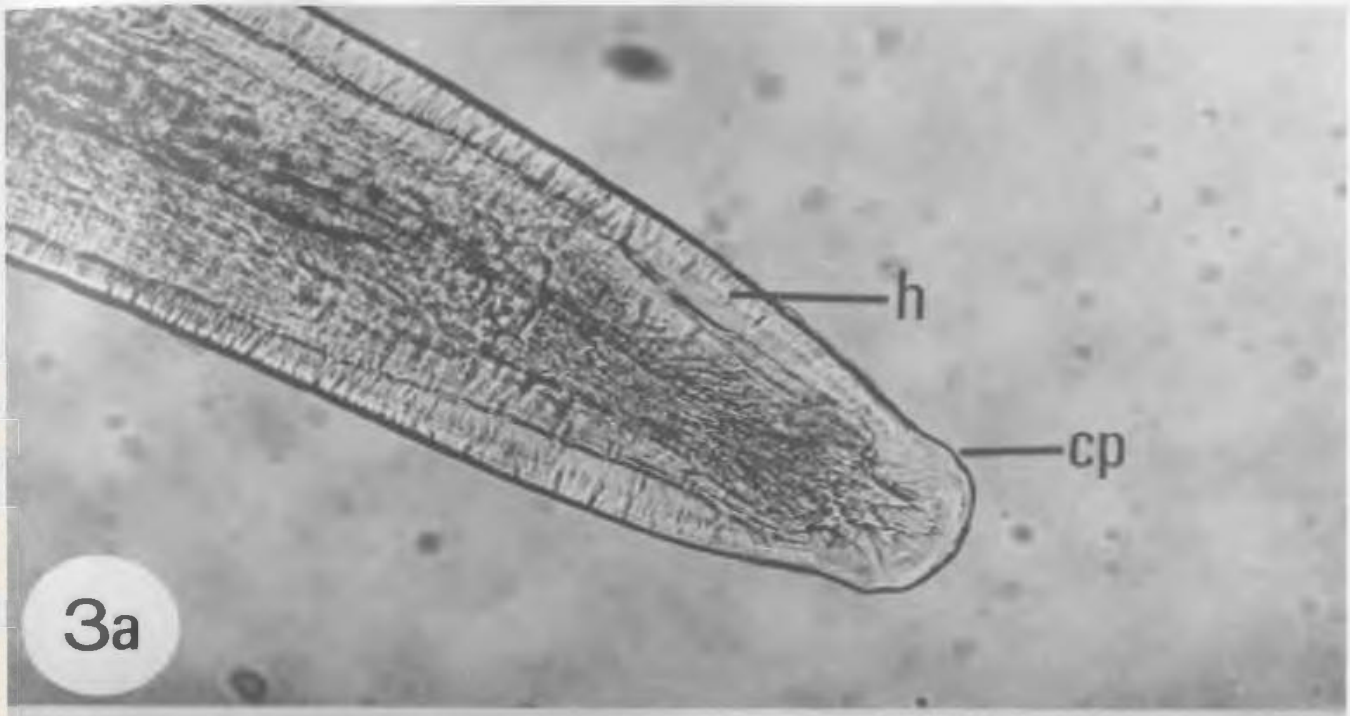
Figure 3b

N. flumenalis adult female vulval area with short barrel-shaped vagina. The vulva is a transverse slit with prominent anterior lips. The densely packed trophosome almost completely fills the pseudocoelom.

Abbreviations: l, lips; v, vagina; vu, vulva; t, trophosome.

Stain: lactophenol/cotton blue.

Magnification: 3800X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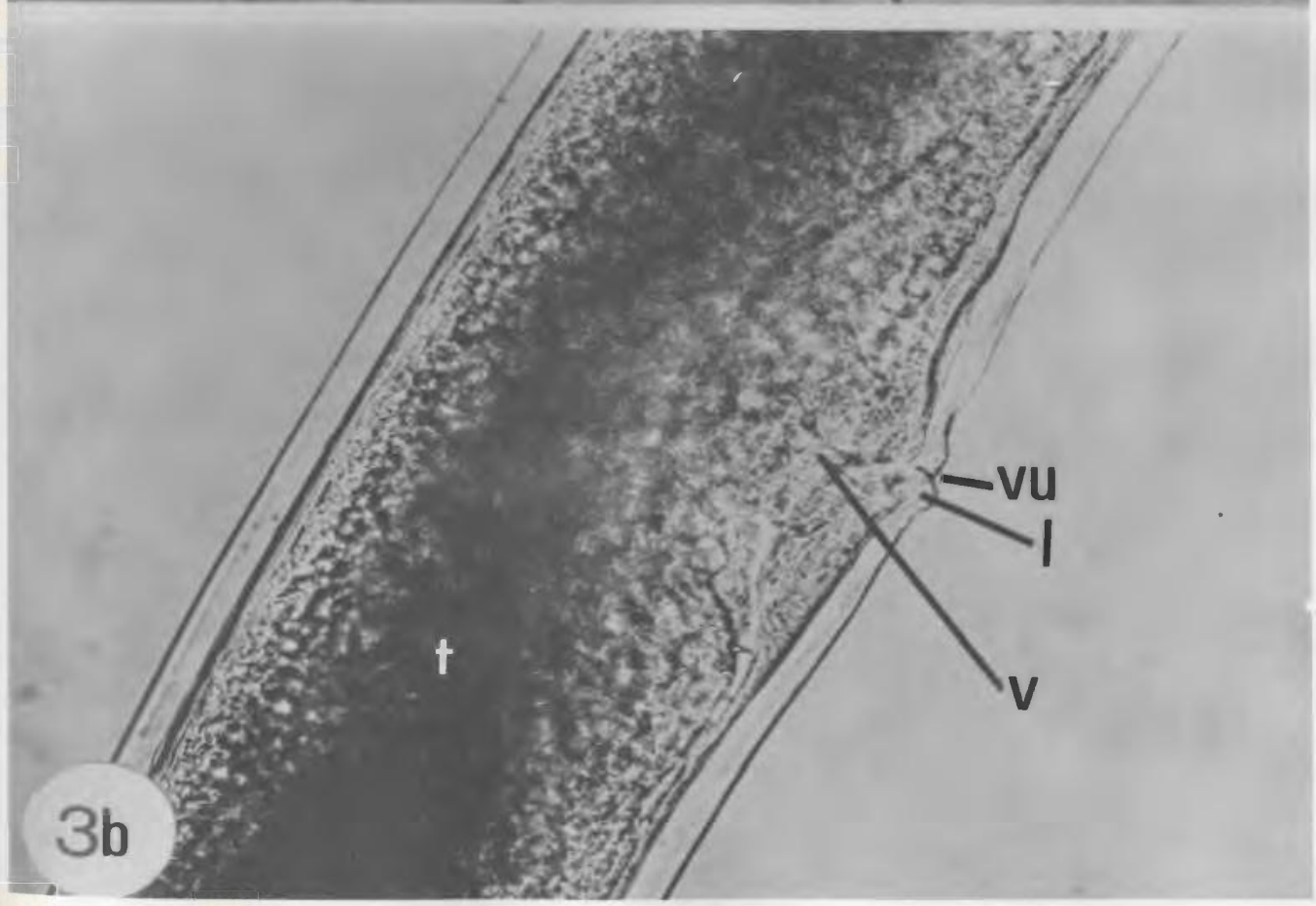
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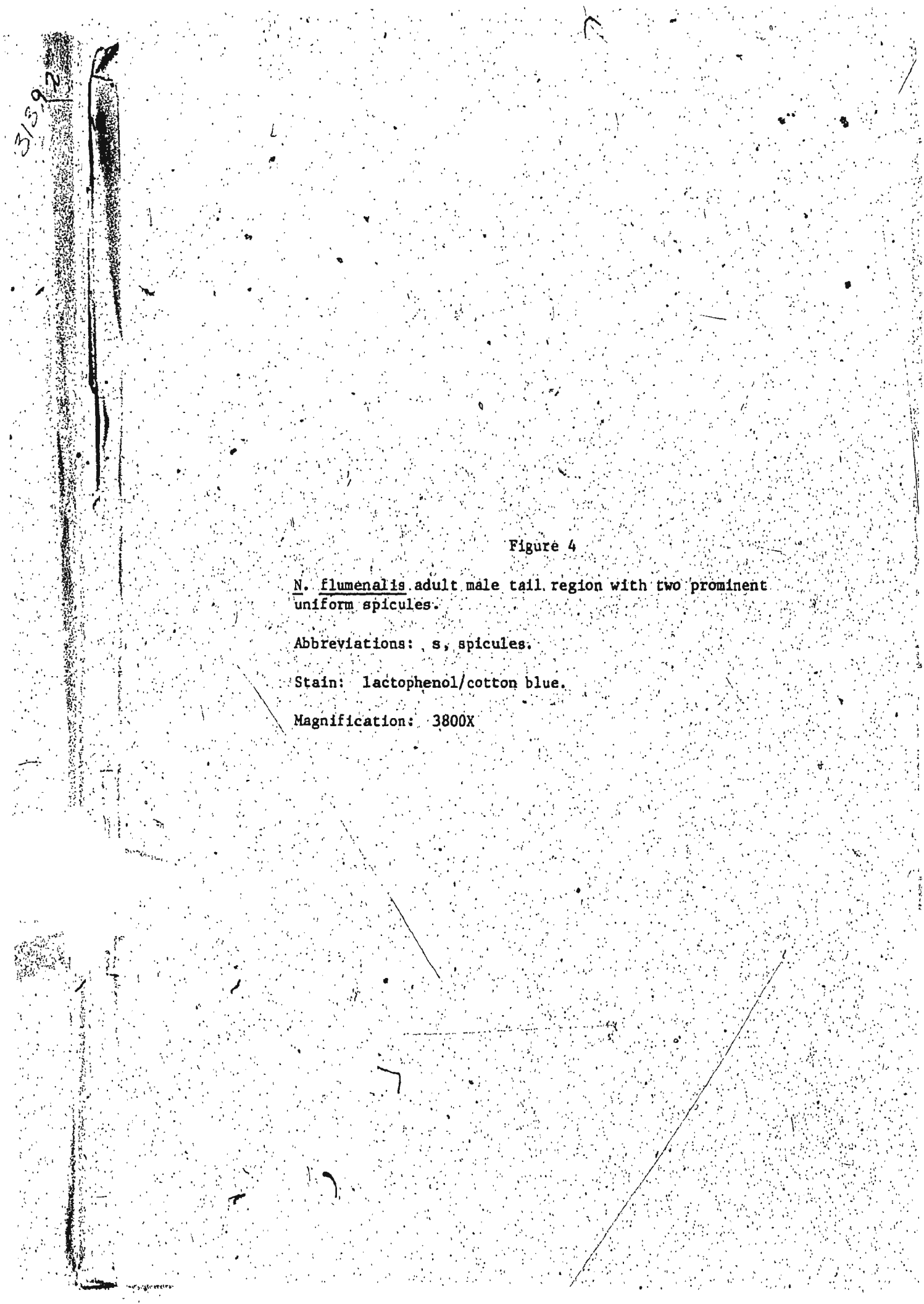


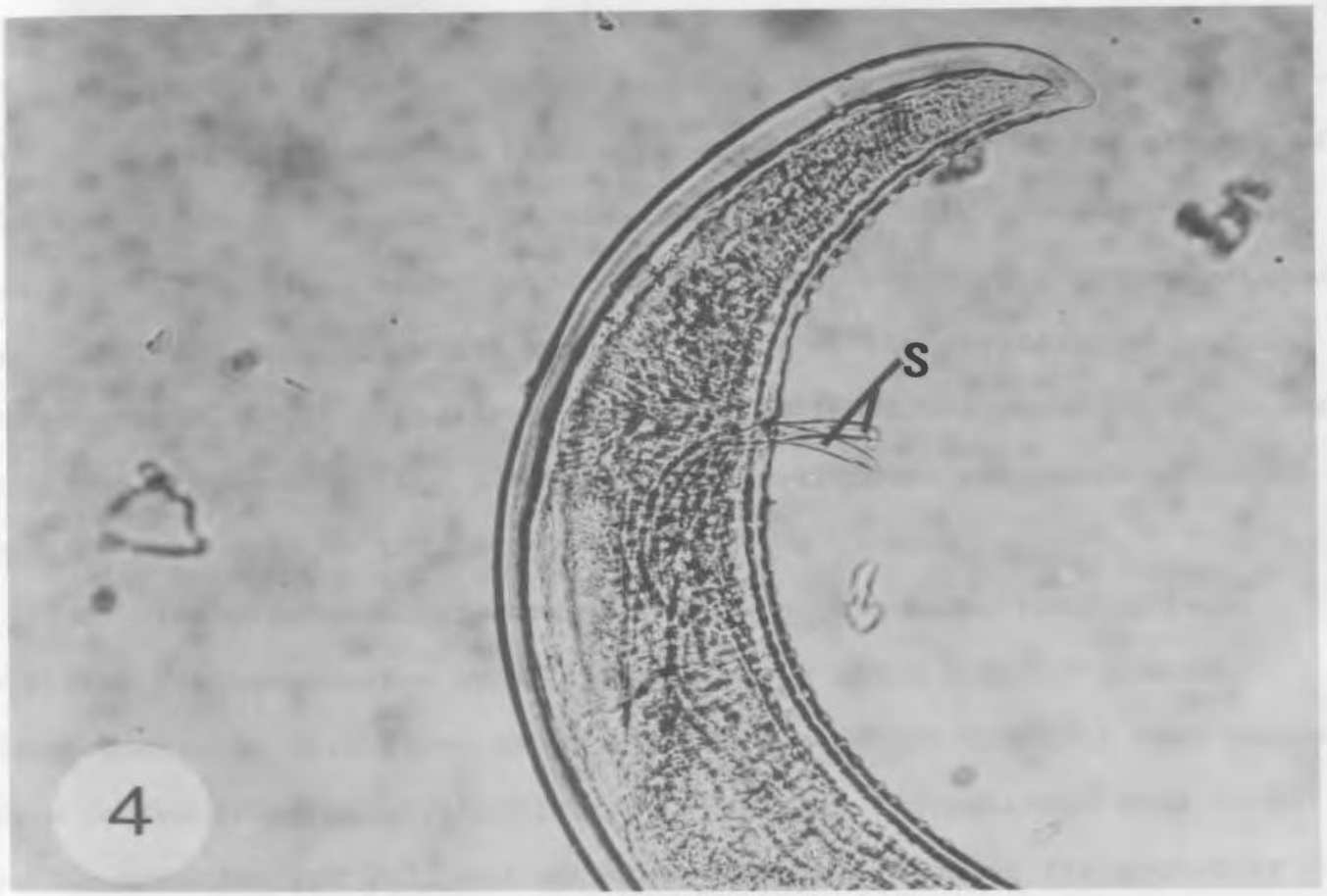
Figure 4

N. flumenalis adult male tail region with two prominent uniform spicules.

Abbreviations: s, spicules.

Stain: lactophenol/cotton blue.

Magnification: 3800X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cold aqueous Bouin's fluid (24 hr; 4°C), stored in 70% ethanol at 4°C (minimum of 24 hr.), dehydrated with a gradual ethanol series, cleared progressively in methyl benzoate/benzene and embedded in paraplast (mp 56-57°C) (Davenport, 1960). Specimens were initially sectioned at 8 μm but later at 4 μm using a Sorvall microtome (Model JB-4).[3] All sections were mounted on albuminized slides with a thin film of water, dried overnight at 40°C, then stained for neurosecretory material using the aldehyde-fuchsin technique (Ewen, 1962) or the paraldehyde-fuchsin technique (Meola, 1970). Since the latter method produced better clarity of the neurosecretory material it was preferred for most of the investigations. All sections were stained within a two-day period after sectioning to minimize oxidation; which reduces uptake of the paraldehyde-fuchsin stain (Meola, 1970). Sections were mounted in Fisher permount resin and dried overnight at 40°C. A total of ten individual specimens of each species were used in this study.

Five uninfected specimens of S. venustum larva Complex were prepared for examination by light microscopy using thin sectioning. These specimens were fixed in 3% glutaraldehyde (24 hr; 4°C) then washed in a Sodium 5:5 - diethylbarbiturate (0.04M) - hydrochloric acid (0.2N) buffer solution (pH 7.2) and embedded in Epon following the method by Luft (1961). All specimens were sectioned at 0.5 μm on a Sorvall ultrathin microtome (Model MT-1)[4] with glass knives. Sections were stained with 1% toluidene blue, rinsed in distilled water, mounted in Histoclad

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[3] Sorval Inc., Norwalk, Connecticut, U.S.A.

[4] See footnote #3.

resin, then photographed immediately before fading could occur.

Effects of N. flumenalis on the neuroendocrine system of the larval blackflies P. mixtum/fuscum and S. venustum Complex

Effects of the mermithid on the host endocrine system were monitored by comparing the two groups (infected and control) of insects with respect to: a) gland volumes (corporus cardiacum and corpus allatum); b) nuclear/cytoplasmic ratios within the endocrine glands; c) concentration of neurosecretory material and/or nucleic acids within the endocrine glands. For determinations of gland volumes, field collected specimens (a total of five individuals per each species group) were fixed in aqueous Bouin's fluid (4°C; 24 hr.), embedded in paraplast (Davenport, 1960), serially sectioned at 4 μm then mounted in permount. These specimens were stained with paraldehyde-fuchsin (Meola, 1970) since the technique was found superior to others tested in affording contrast between the endocrine glands and unassociated host tissues. Gland volumes were determined by using a Zeiss Micro-Videomat (Model 1)[5] to measure the area of the respective endocrine glands as they appeared on serial sections. This method was tested initially for consistency and found to be reliable. The total surface area of each endocrine gland section was multiplied by the section thickness (4 μm) and the summation of these individual cells produced the total gland volume (Wigglesworth, 1964; Highnam, 1964). For determinations of nuclear/cytoplasmic ratios and concentration of neurosecretory material and/or nucleic acids within the endocrine glands of parasitized and non-parasitized hosts, insects were

[5]Carl Zeiss, Oberkochen, W. Germany.

fixed in Zenker's fluid (24 hr.; 4°C) for nuclear/cytoplasmic ratios and nucleic acid determinations and in aqueous Bouin's fluid (24 hr.; 4°C), for neurosecretory material measurements. All specimens were stored in 70% ethanol at 4°C and embedded in paraplast (Davenport, 1960). Sections for nuclear/cytoplasmic ratios (a total of four or five individual specimens of each species group) and nucleic acid (four or five individual specimens of each species group) determinations were stained with Feulgen stain (Feulgen and Rossenbeck, 1924) using 0.05% fast green FCF counterstain, while the sections showing neurosecretory material (seven individual specimens of each species group) were stained with paraldehyde-fuchsin stain (Meola, 1970). Nuclear/cytoplasmic ratios within each endocrine gland were ascertained and compared using the Zeiss Microvideomat. The concentration of neurosecretory material and/or nucleic acids within the endocrine glands was compared between infected and non-infected hosts by determination of stain intensity using a Zeiss microspectrophotometer (Model 01).[6]

Some effects of N. flumenalis parasitism on the levels of storage metabolites of P. mixtum/fuscum and S. venustum Complex

The control and infected groups (a total of five or seven individual specimens of each species group) were fixed in aqueous Bouin's fluid (4°C; 24 hr.), embedded in paraffin, serially sectioned at 4 μm, then stained with Periodic Acid Schiff's Reagent for fat body glycogen (Davenport, 1960). Similar numbers of infected and control larvae (both species complexes) were fixed in Zenker's fluid (4°C; 24 hr.), embedded in paraffin, sectioned at 4 μm then stained with Feulgen stain (Feulgen

[6] See footnote #5.

and Rossenbeck, 1924) to indicate fat body DNA/RNA activity and consequent rate of protein synthesis. Relative estimates of both host fat body glycogen and nuclear DNA/RNA levels were made using the Zeiss micro-spectrophotometer.

In order to obtain an overall assessment of the effects of mermithid parasitism upon the host, dry weight measurements were made of ten insects chosen at random within each group (infected and controls) for both host species complexes. The dry weights were determined by air drying the specimens, including the total biomass of host and parasite in the infected host (approximately four weeks at room temperature) then weighing the specimens using a Cahn Gram Electrobalance (Model-G).[7]

[7] Cahn Division, Vention Corp., Paramount, California, U.S.A.

RESULTS

The head capsule widths and body lengths of each of the two groups (infected and non-infected) of P. mixtum/fuscum and S. venustum Complex showed low variability within each group of the replicates (Table 1). This consistency within each group indicates a high degree of uniformity amongst the experimental groups chosen. This data shows the head capsule width and body length measurements (commonly used indicators of developmental stage) of the late infected larvae of both host species to correlate closely with dark histoblast controls suggesting that late infected larvae are of the same instar as controls.

Histological observations on the neuroendocrine systems of the larval blackflies P. mixtum/fuscum and S. venustum Complex

The neuroendocrine system (comprised of neurosecretory and glandular portions) was examined histologically in both species of blackfly larvae. However, information regarding the neurosecretory system per se was based primarily on studies of the P. mixtum/fuscum larvae because neurosecretory cells did not stain intensely in S. venustum Complex larvae. The glandular portion of the neuroendocrine system stained very conspicuously in both species.

From the combined histological data a reconstruction of the neuroendocrine system of the larval complexes of P. mixtum/fuscum and S. venustum has been formulated (Fig. 5). The neurosecretory system is composed of three paired clusters of neurosecretory cells located within the pars intercerebralis of the brain (Figs. 5, 6). These cells range in neurosecretory material content from being prominently stained with

TABLE 1

Comparison of Size and Weight Measurements Between Control and Mermithid-Infected Larvae of Two Newfoundland Blackfly Species +

Host Species	Head Capsule Width (mm)		Body Length (mm)		Dry Weight (mg)	
	Control	Infected	Control	Infected	Control	Infected
P. mixtum/fuscum	.068 ± .0015(10)	.065 ± .0012(10)	.596 ± .0046(10)	.562 ± .0082(10)	.649 ± .030(10)	.491 ± .0078(10) ****
S. venustum Complex	.059 ± .0008(10)	.055 ± .0009(10)	.521 ± .0058(10)	.482 ± .0076(10)	.508 ± .017(10)	.496 ± .015(10)

+ Values given are mean values ± standard errors. Shown in parenthesis are the number of replicates involved in each determination. For detailed data see Appendix 1.

**** Significantly different from control values at $p < 0.001$.

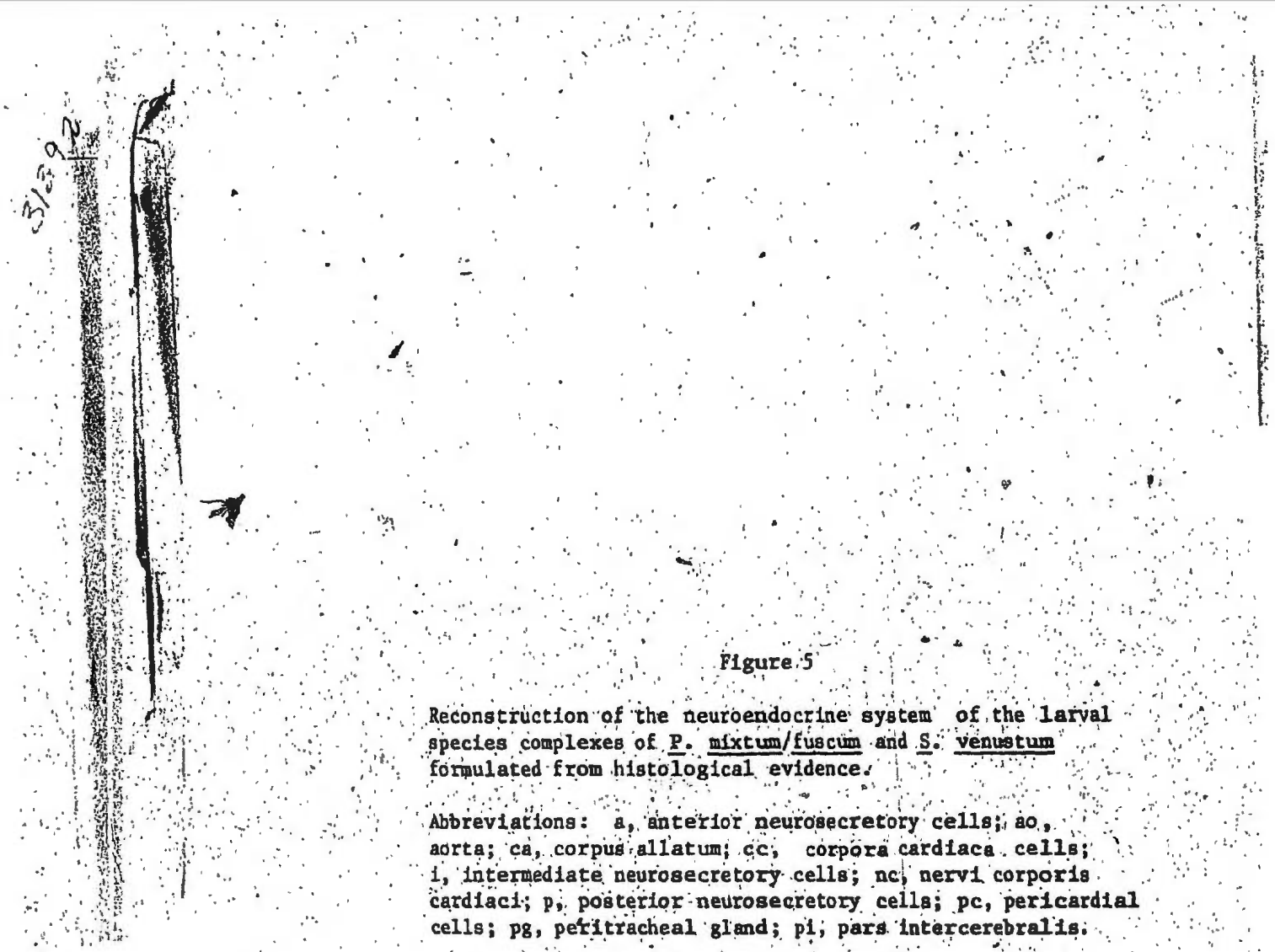
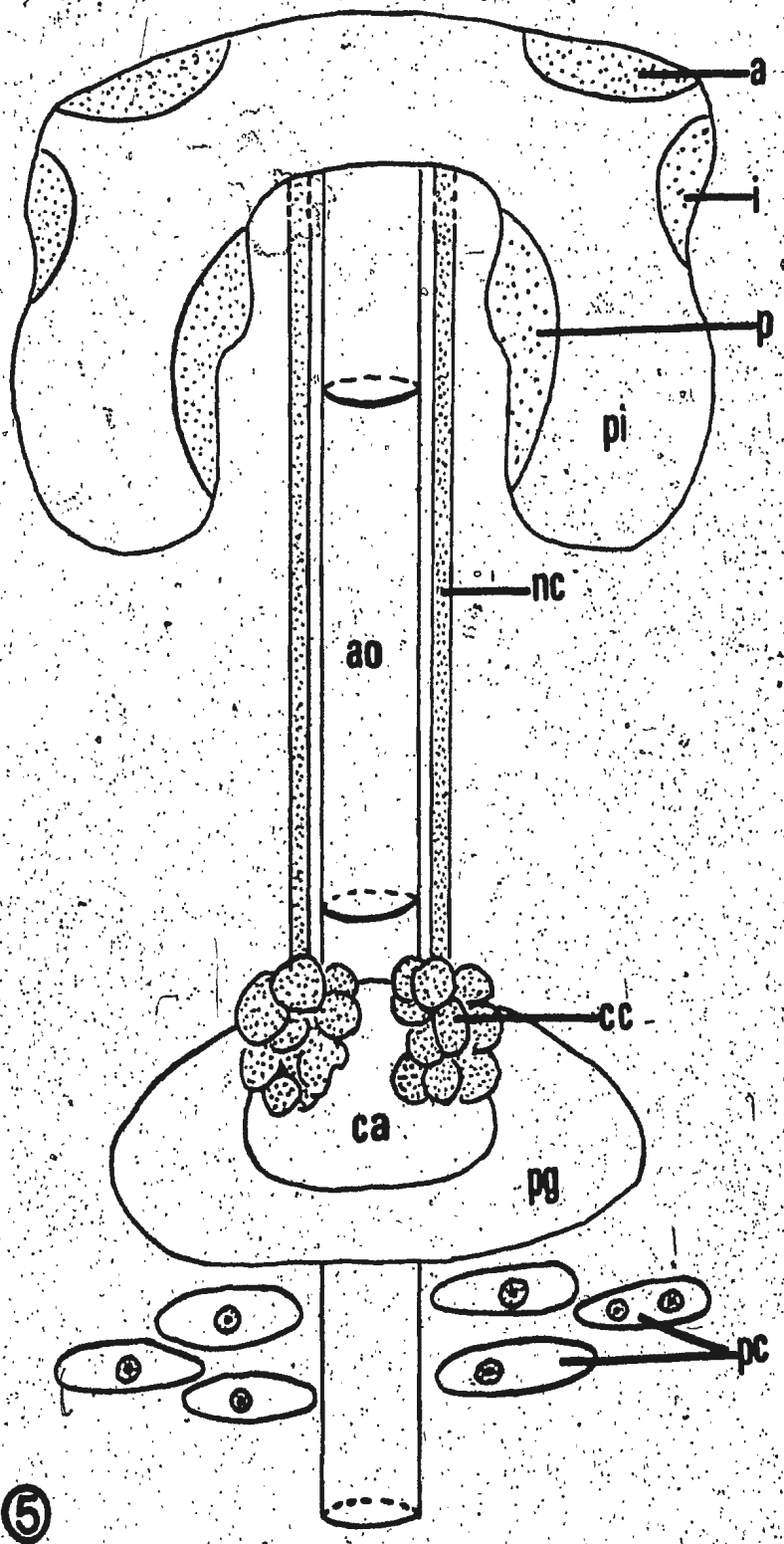


Figure 5

Reconstruction of the neuroendocrine system of the larval species complexes of P. mixtum/fuscum and S. venustum formulated from histological evidence.

Abbreviations: a, anterior neurosecretory cells; ao, aorta; ca, corpus allatum; cc, corpora cardiaca cells; i, intermediate neurosecretory cells; nc, nervi corporis cardiaci; p, posterior neurosecretory cells; pc, pericardial cells; pg, peritracheal gland; pi, pars intercerebralis.



⑤

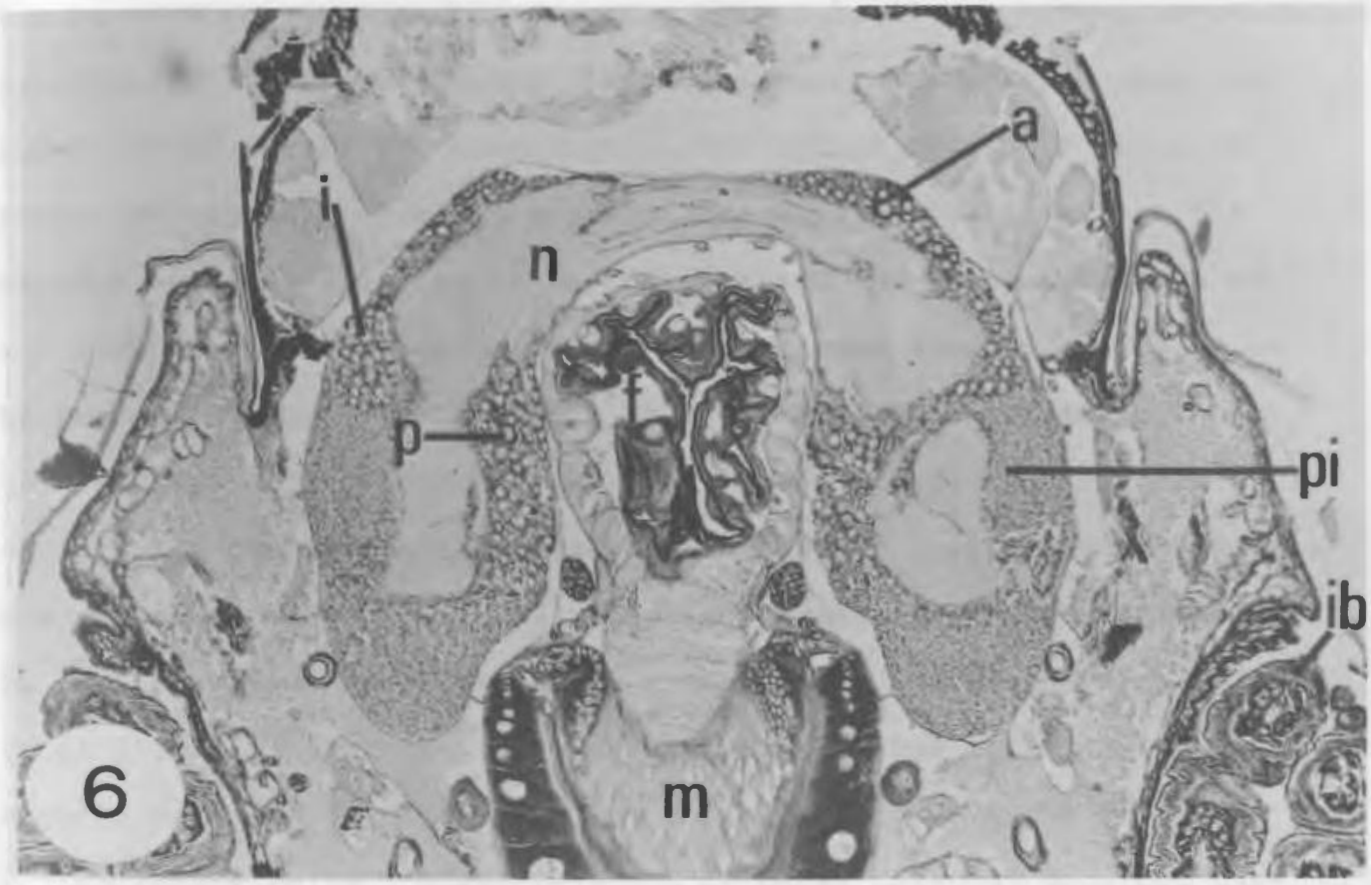
Figure 6

Longitudinal section of the anterior region of a P. mixtum/fuscum terminal larva showing the three pairs of neurosecretory cell clusters in the pars intercerebralis of the protocerebrum.

Abbreviations: a, anterior neurosecretory cells; f, foregut; i, intermediate neurosecretory cells; ib, imaginal buds; m, midgut diverticulum; n, neuropile mass; p, posterior neurosecretory cells; pi, pars intercerebralis.

Stain: paraldehyde fuchsin/Halmi's counterstain.

Magnification: 950X


a
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paraldehyde-fuchsin to less prominently stained. The cytoplasm of these cells stained positively with paraldehyde-fuchsin (showing the neurosecretory material as intense bluish-purple granules), while the cytoplasm of the surrounding cells and the neuropile mass stained with the green counterstain. The group of neurosecretory cells positioned most anteriorly with respect to both lobes of the brain, bounded on each lateral side by more non-neurosecretory cells (Figs. 5, 7) are termed the anterior neurosecretory cells following the terminology adopted by Burgess and Rempel (1966) for larval mosquitoes. The second and third pairs of neurosecretory cell clusters are situated more posteriorly than the anterior neurosecretory cells on the external and internal region of each lobe of the brain respectively and are separated on each side by the neuropile mass. The external lateral clusters being in an intermediate location are referred to as the intermediate neurosecretory cells, while the internal lateral neurosecretory cell clusters having a more posterior position on the pars intercerebralis are designated as the posterior neurosecretory cells as designated by Burgess and Rempel (1966) (Figs. 5, 8). The neurosecretory cell axons entering the nervi corporis cardiaci (including chiasmic axonal supply to the "medial neurosecretory cells," characteristic of other insects) were not discerned. Therefore, analogies of the three groups of neurosecretory cells reported herein for simuliids with those of other insects are somewhat speculative.

The axonal pathway transferring neurosecretory material from the brain to the corpora cardiaca for storage is composed of a single pair of nervi corporis cardiaci (a common trait for most Nematocera). These nervi corporis cardiaci originate from fused neurosecretory



Figure 7

P. mixtum/fuscum longitudinal section of one of the anterior neurosecretory cell clusters.

Abbreviations: a, anterior neurosecretory cells; f, foregut; n, neuropile mass.

Stain: paraldehyde fuchsin/Halmi's counterstain.

Magnification: 4378X



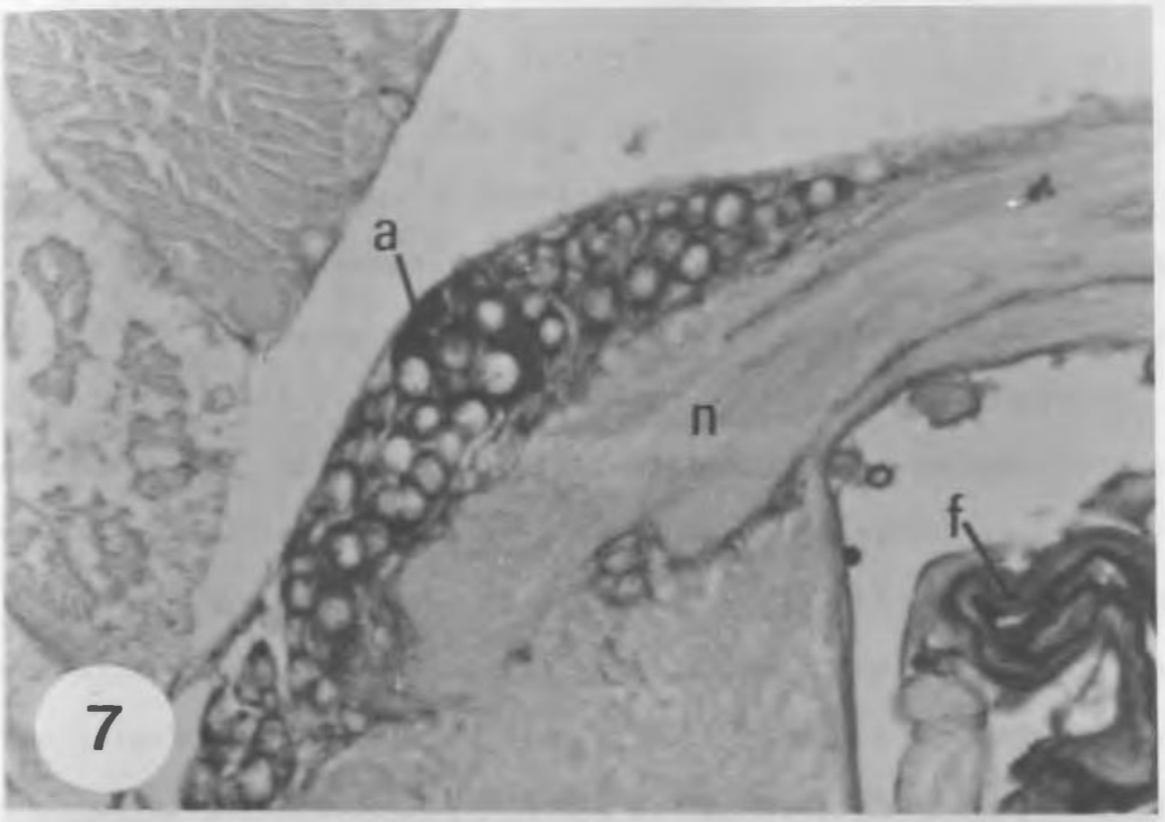
Figure 8

P. mixtum/fuscum longitudinal section showing both the intermediate and posterior neurosecretory cell clusters on one side of the brain.

Abbreviations: f, foregut; i, intermediate neurosecretory cells; n, neuropile mass; p, posterior neurosecretory cells.

Stain: paraldehyde fuchsin/Halmi's counterstain.

Magnification: 4378X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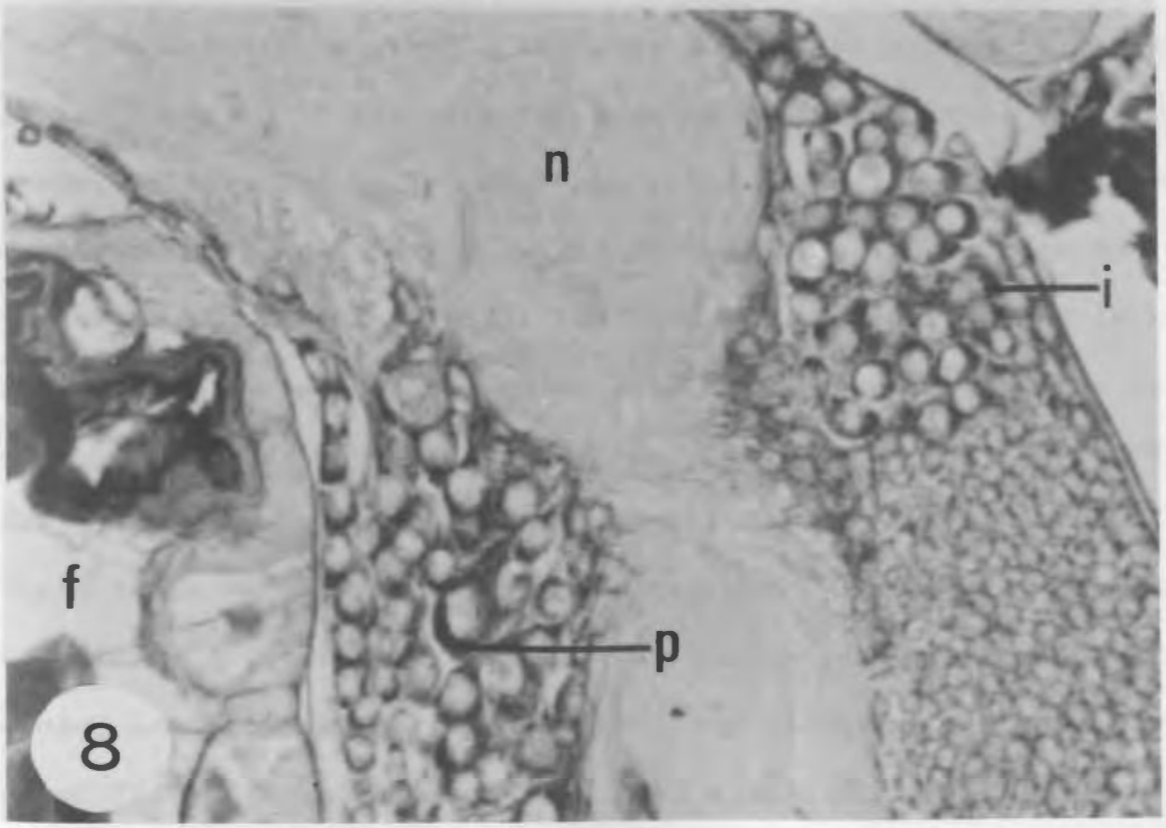
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cell axons and extend posteriorly, bordering either side of the dorsal aorta to terminate into the glandular region located within the prothorax of the larval insect (Figs. 5, 9, 10).

The retrocerebral glandular system of both P. mixtum/fuscum and S. venustum Complex (Fig. 5) consists of a corpus allatum, paired corpora cardiaca and a peritracheal gland analogous to the prothoracic gland of other insects (Thomsen, 1951). In both blackfly species the corpus allatum is the most prominent gland, but unlike other Nematocera (Possompes, 1946, 1948; Cazal, 1948; Thomsen, 1951; Burgess and Rempel, 1966) this gland is a single structure with no dividing membrane -- as discerned by thin sectioning of this gland (Fig. 11). The cells of the corpus allatum are basophilic and therefore stain with a light green dye of the Halmi's counterstain (a standard background stain for cytoplasm because paraldehyde-fuchsin will not stain basic cytoplasm). These cells possess characteristic granulated areas around the periphery of their nuclei as observed in corpus allatum cells of other larval Diptera (Thomsen, 1951). The nuclei of these cells stain lightly with dark nucleoli as their acidophilic nature is stained by the orange dye within the counterstain (Figs. 12, 13). Each corpus cardiacum gland is composed of a number of characteristic cells located in proximity to the corpus allatum. These cells had a very densely grandulated paraldehyde-fuchsin positive cytoplasm showing a bluish-purple color. The nucleus of each of these cells was distinctly light (Figs. 14, 15). Unlike the tightly packed cells of the corpus allatum, the corpus cardiacum cells were more discrete, less compact cells. The peritracheal gland (syn. peritracheal gland of Tipulidae -- Thomsen, 1951) was a more diffuse structure than

Figure 9

Cross section of P. mixtum/fuscum prothoracic region showing the nervi corporis cardiaci bordering each side of the aorta.

Abbreviations: ao, aorta; g, gut; nc, nervi corporis cardiaci.

Stain: paraldehyde fuchsin/Halmi's counterstain.

Magnifications: 3800X

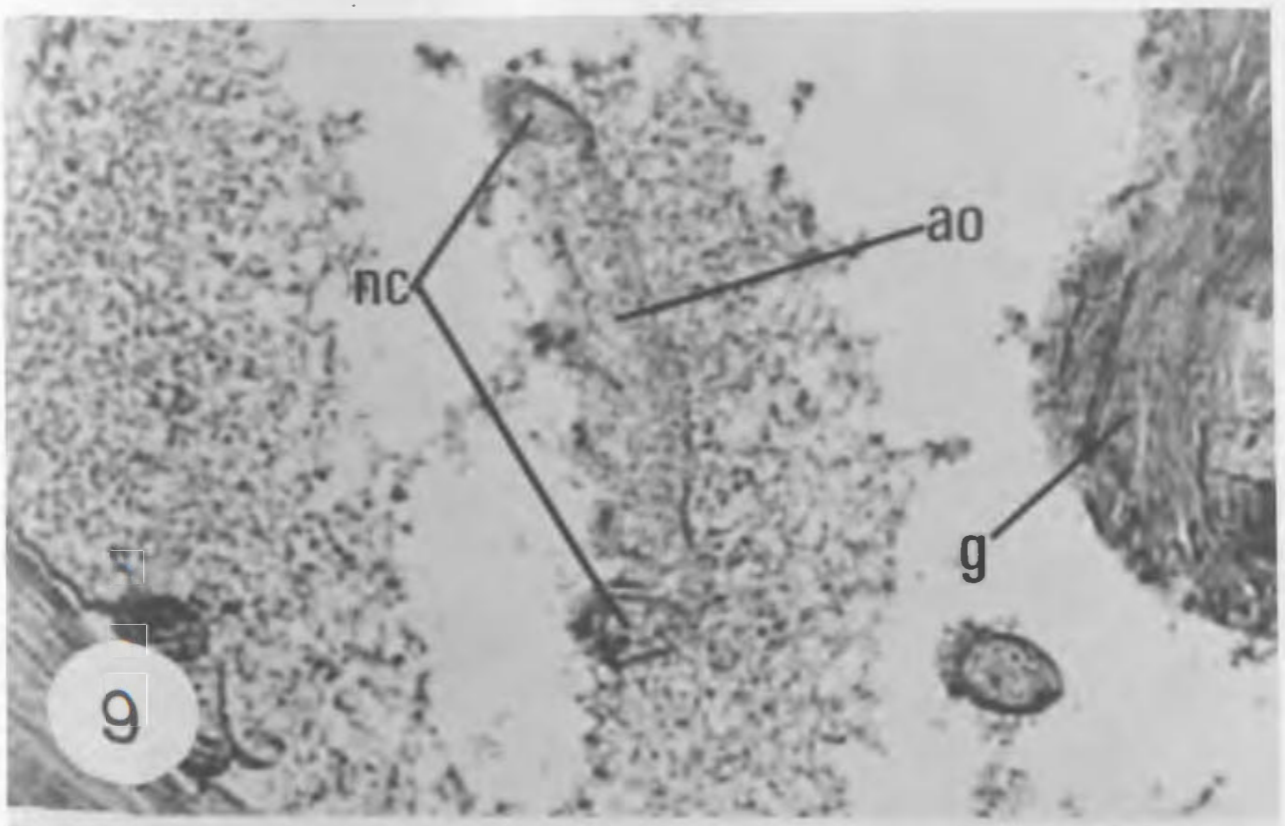
Figure 10

Cross section of S. venustum Complex prothoracic region showing the nervi corporis cardiaci bordering each side of the aorta.

Abbreviations: ao, aorta; ca, corpus allatum; m, midgut; nc, nervi corporis cardiaci.

Stain: paraldehyde fuchsin/Halmi's counterstain.

Magnification: 3800X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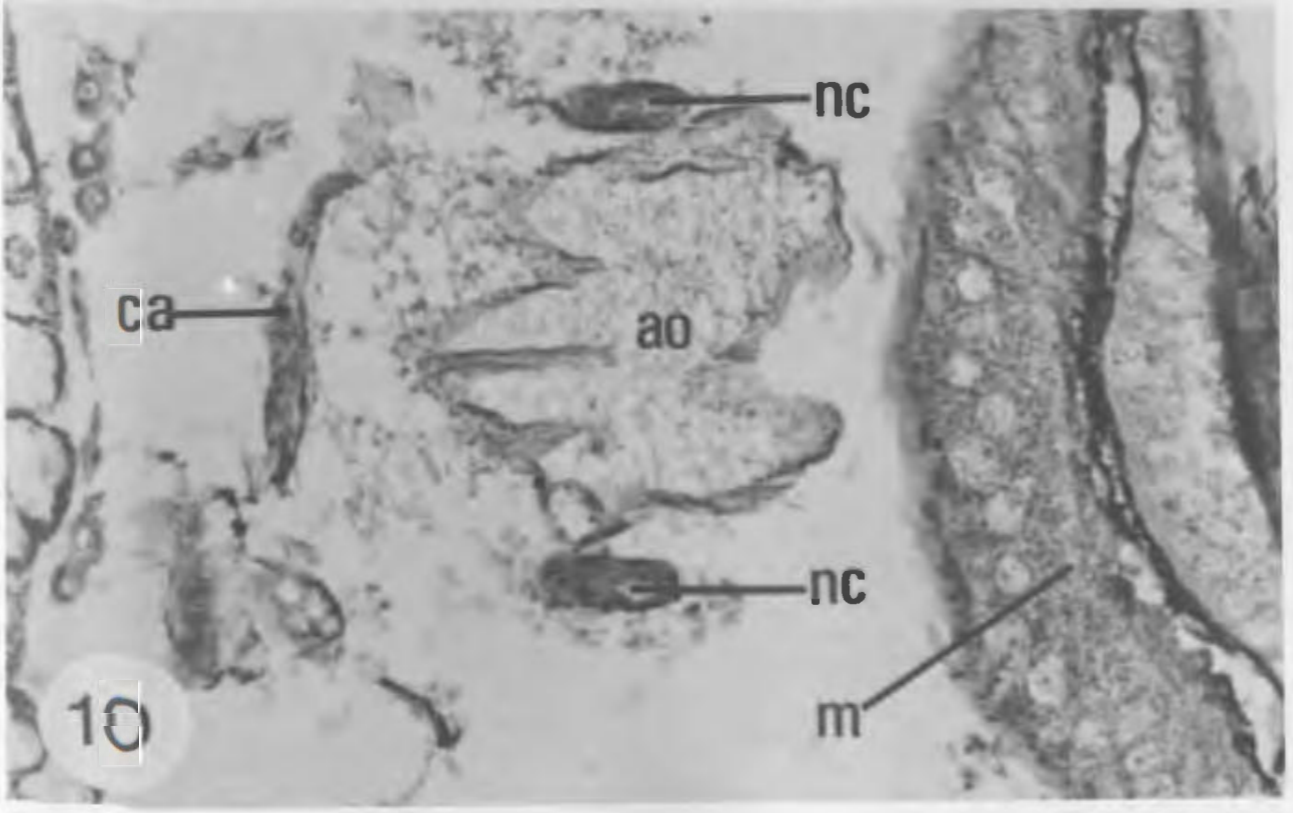
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Figure 11

Cross section of S. venustum Complex prothoracic region showing the corpus allatum as a single structure.

Abbreviations: ao, aorta; ca, corpus allatum.

Stain: toluidine blue (Epon embedded).

Magnification: 3800X

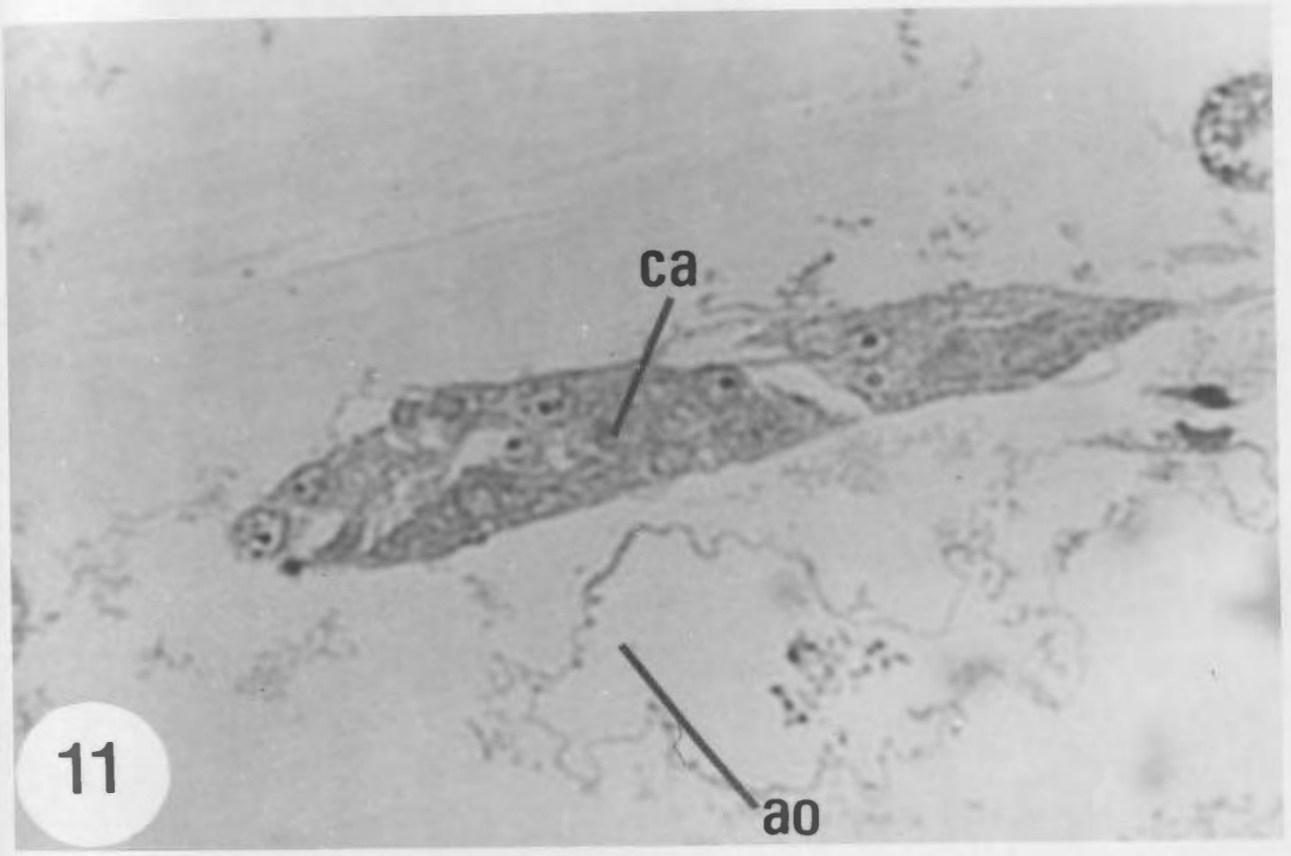
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Figure 12

Cross section of the corpus allatum of P. mixtum/fuscum bordered by corpora cardiaca cells on either side; in a dorsal position to the aorta. The prothoracic glands are shown on either side of the aorta.

Abbreviations: ao, aorta; ca, corpus allatum; cc, corpora cardiaca cells; fb, fat body cells; m, epithelium of midgut diverticulum; pg, peritracheal gland.

Stain: paraldehyde fuchsin/Halmi's counterstain.

Magnification: 3800X

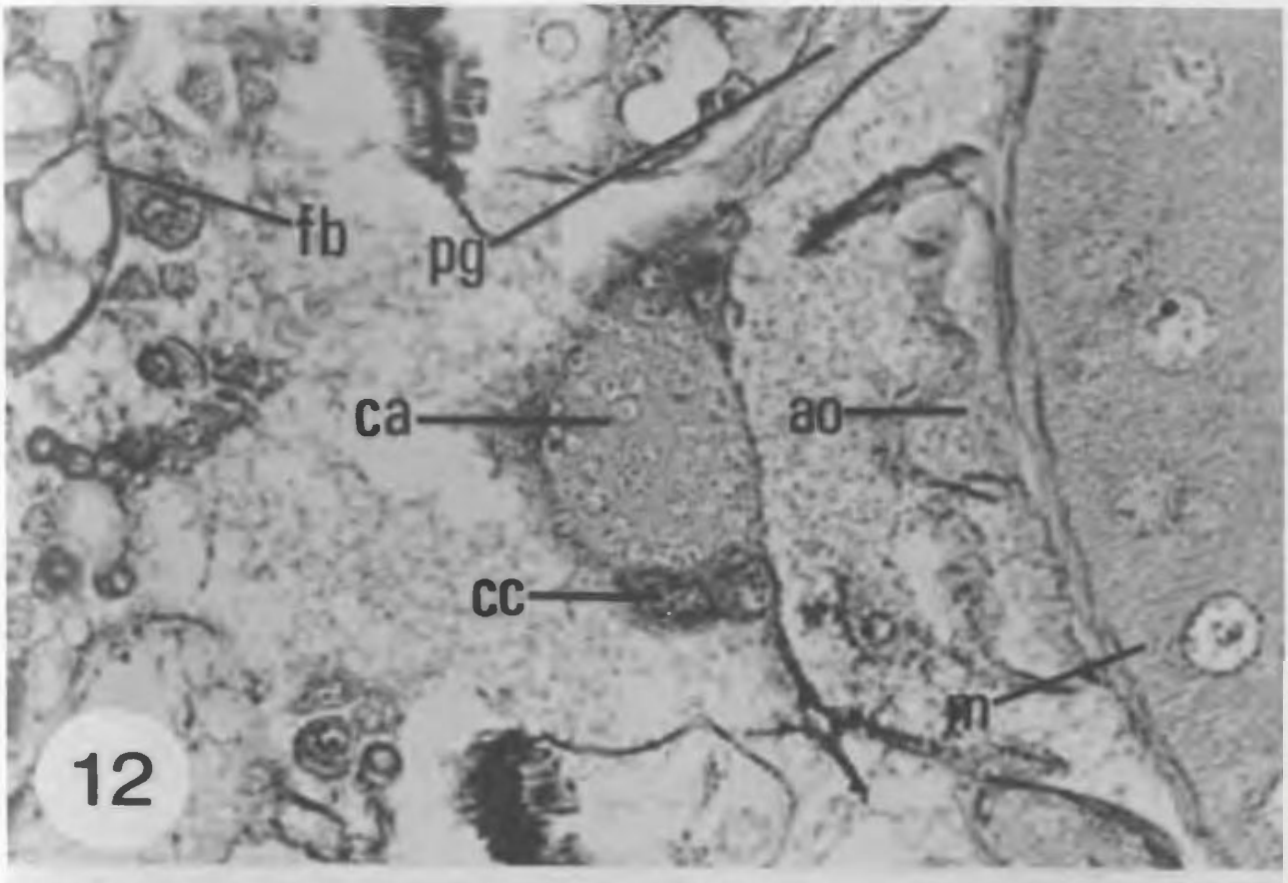
Figure 13

Cross section of the corpus allatum of S. venustum Complex bordered on either side by the corpora cardiaca cells; in a dorsal position to the aorta. The peritracheal glands are beginning to appear on either side of the aorta.

Abbreviations: ao, aorta; ca, corpus allatum; fb, fat body cells; m, midgut; nc, nervi corporis cardiaci.

Stain: paraldehyde fuchsin/Halmi's counterstain.

Magnification: 3800X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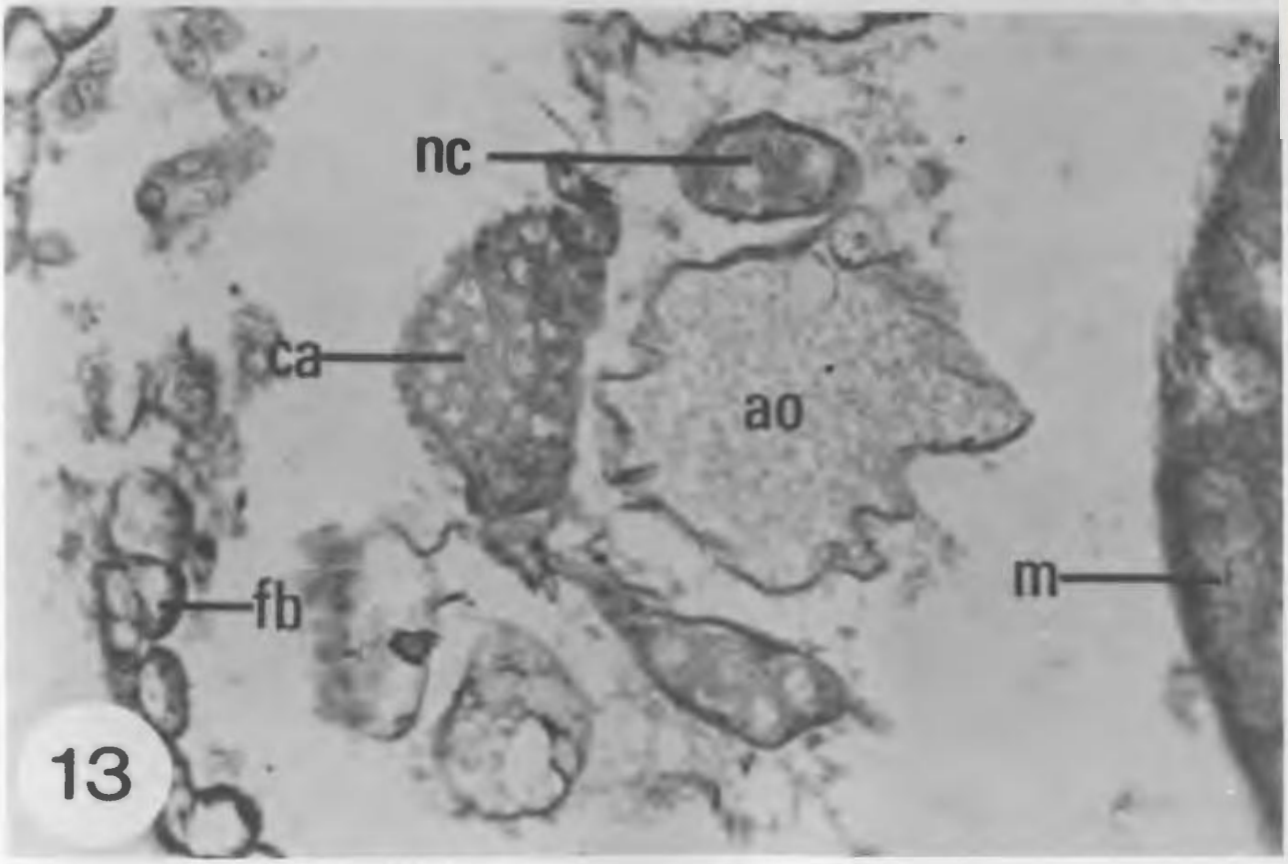
nc
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Figure 14

Cross section of P. mixtum/fuscum prothoracic region showing the cellular structure of the corpora cardiaca and of the corpus allatum.

Abbreviations: ca, corpus allatum; cc. corpora cardiaca cells.

Stain: paraldehyde fuchsin/Halmi's counterstain.

Magnification: 5472X

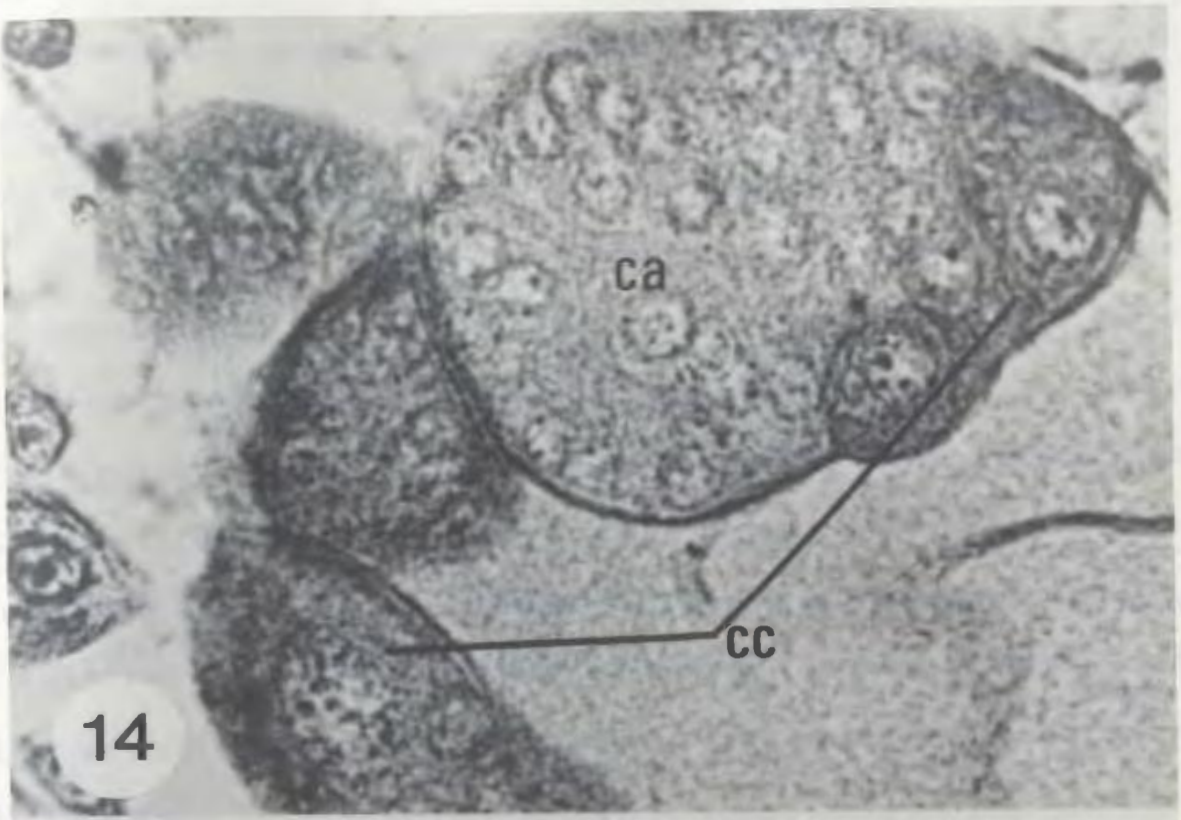
Figure 15

Cross section of S. venustum Complex prothoracic region showing the corpora cardiaca cells on either side of the corpus allatum.

Abbreviations: ca, corpus allatum; cc. corpora cardiaca cells; m, midgut.

Stain: paraldehyde fuchsin/Halmi's counterstain.

Magnification: 4320X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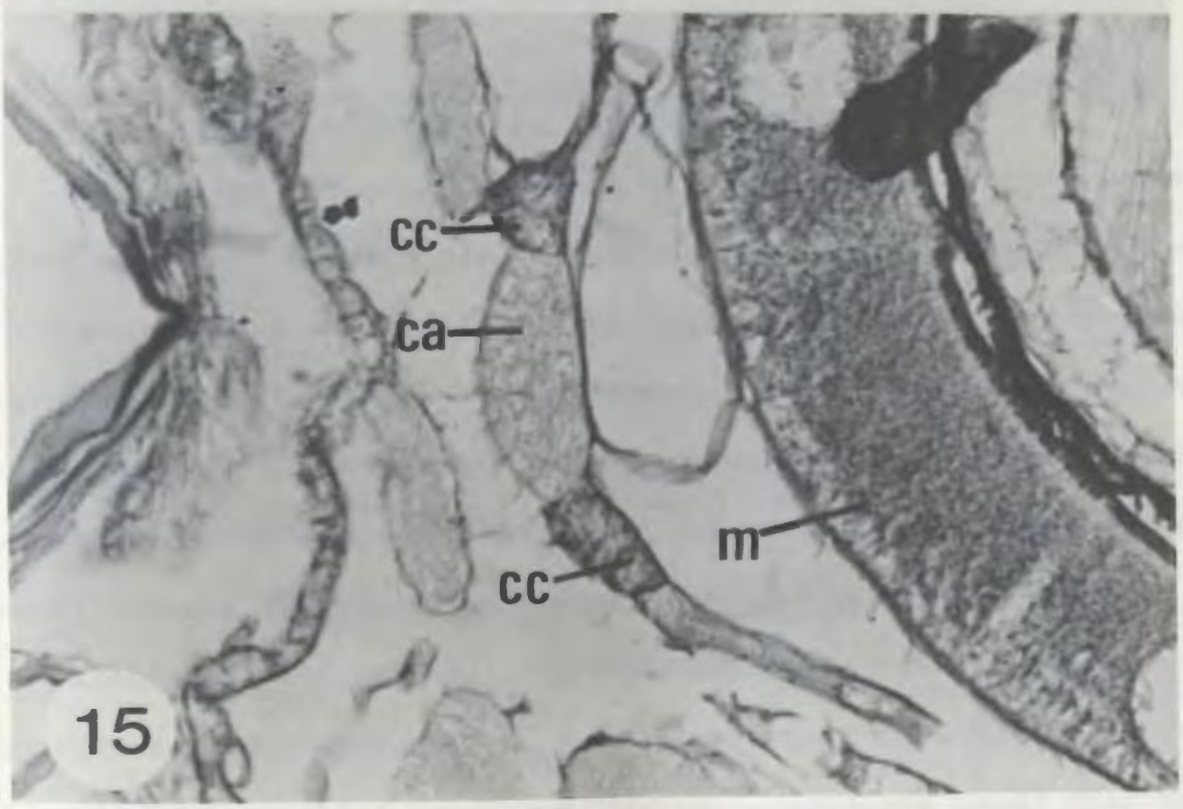
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either the corpus allatum or corpus cardiacum appearing intermittently throughout successive sections. This gland appears to encircle the corpus allatum and corpus cardiacum complex. The cells of the peritracheal gland lacked any paraldehyde-fuchsin positive granules but stained with light green of the Halmi's counterstain (Figs. 16, 17).

The pericardial cells are situated on either side of the midgut region and posterior to the endocrine gland complex. Although these cells are not considered by most authorities to be part of the neuroendocrine system, they are important in regulating heart rate. When stimulated via a trophic hormone from the corpora cardiaca, these pericardial cells (Figs. 18, 19) accelerate heart rate by secreting a substance similar in chemical activity to serotonin (Davey, 1961a, 1961b) and thus permit increased circulation of prohormones and hormones to the target tissues.

Effects of N. flumenalis on the neuroendocrine system of the larval blackflies P. mixtum/fuscum and S. venustum Complex

None of the infected larvae of either blackfly species showed any gross histological evidence of morphogenic disturbances in cells of the corpus cardiacum or corpus allatum that would suggest any obvious endocrinological effects due to parasitism. Therefore, more sensitive indicators of endocrine activity were studied (nuclear DNA/RNA intensities; nuclear/cytoplasmic ratios; gland volumes; stored neurosecretory material) in both P. mixtum/fuscum and S. venustum Complex larvae. There was no significant effect at all on the endocrines of infected P. mixtum/fuscum larvae when compared with their counterpart dark histoblast controls, thereby suggesting (at least for the parameters used) that the

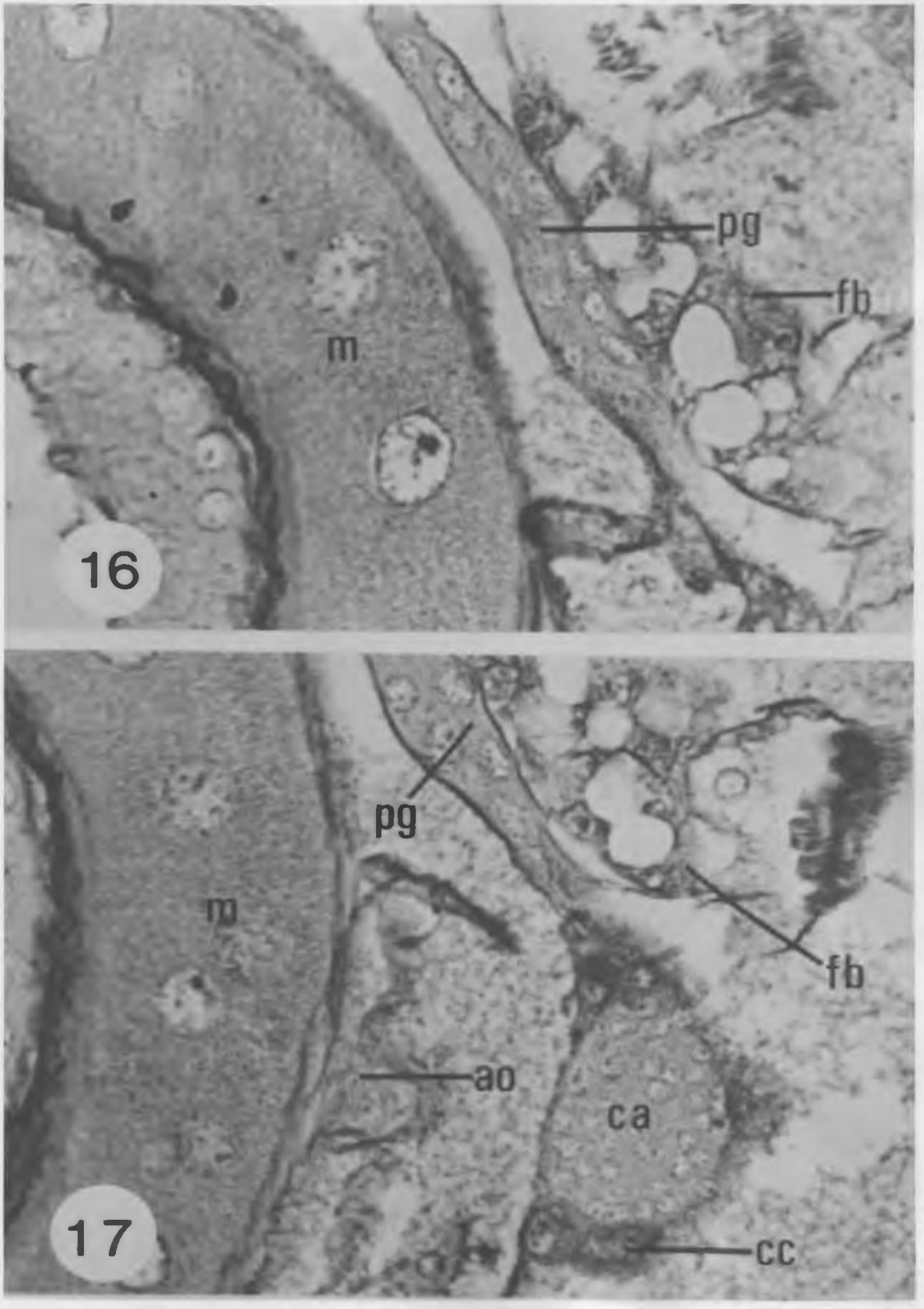
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Figure 16

Cross section of P. mixtum/fuscum prothoracic region showing the peritracheal gland in relation to the gut epithelium.

Abbreviations: fb, fat body cells; m, midgut epithelium; pg, peritracheal gland.

Stain: paraldehyde fuchsin/Halmi's counterstain.

Magnification: 5472X

Figure 17

Cross section of S. venustum Complex prothoracic region showing the peritracheal gland.

Abbreviations: ao, aorta; ca, corpus allatum; cc, corpora cardiaca cells; m, midgut; pg, peritracheal gland.

Stain: paraldehyde fuchsin/Halmi's counterstain.

Magnification: 3420X

Figure 18

Cross section of P. mixtum/fuscum showing nucleated pericardial cells bordering the gut.

Abbreviations: fb, fat body cells; m, midgut epithilium; pc, pericardial cells.

Stain: paraldehyde fuchsin/Halmi's counterstain.

Magnification: 4864X

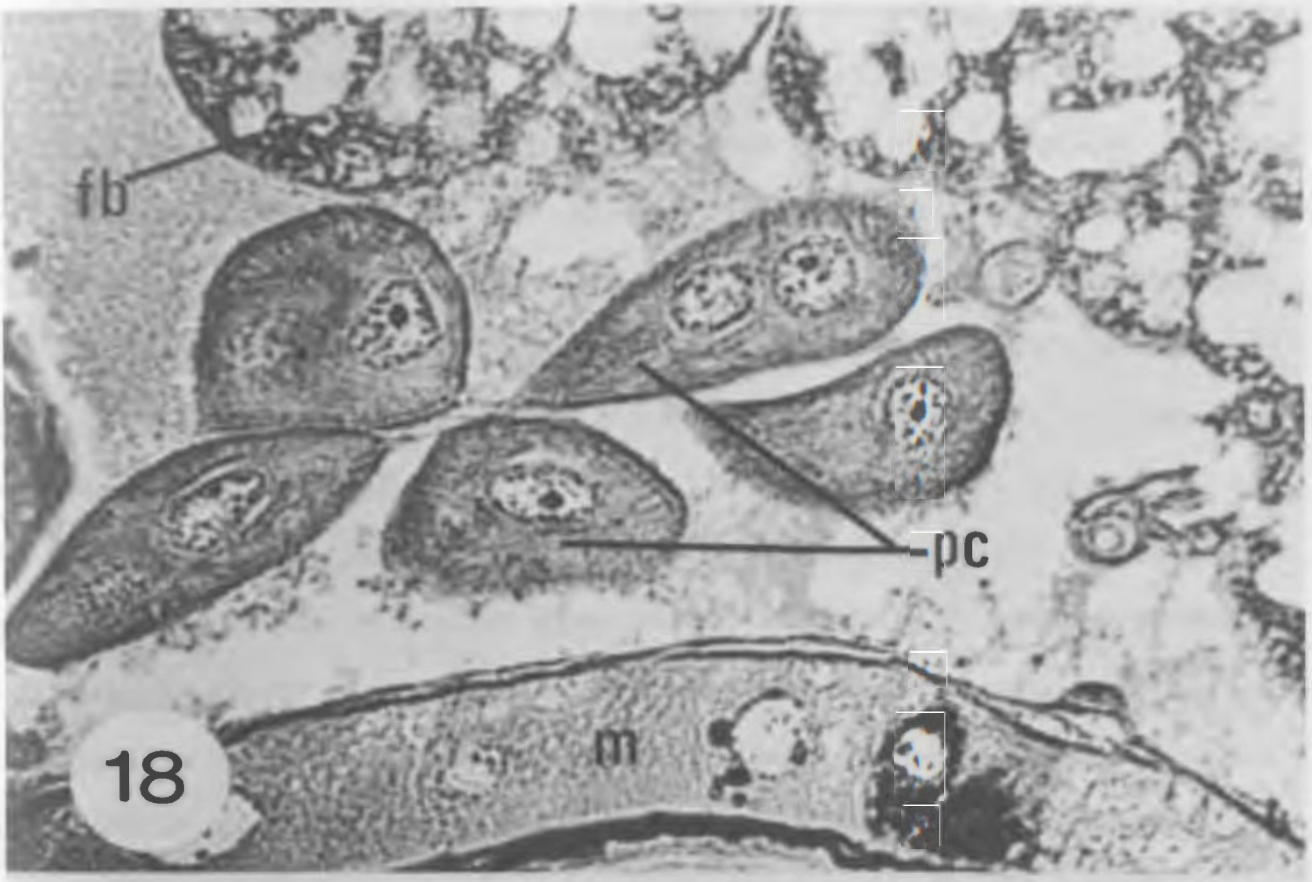
Figure 19

Cross section of S. venustum Complex showing nucleated pericardial cells bordering the gut.

Abbreviations: fb, fat body cells; m, midgut; pc, pericardial cells.

Stain: paraldehyde fuchsin/Halmi's counterstain.

Magnification: 3800X


fb
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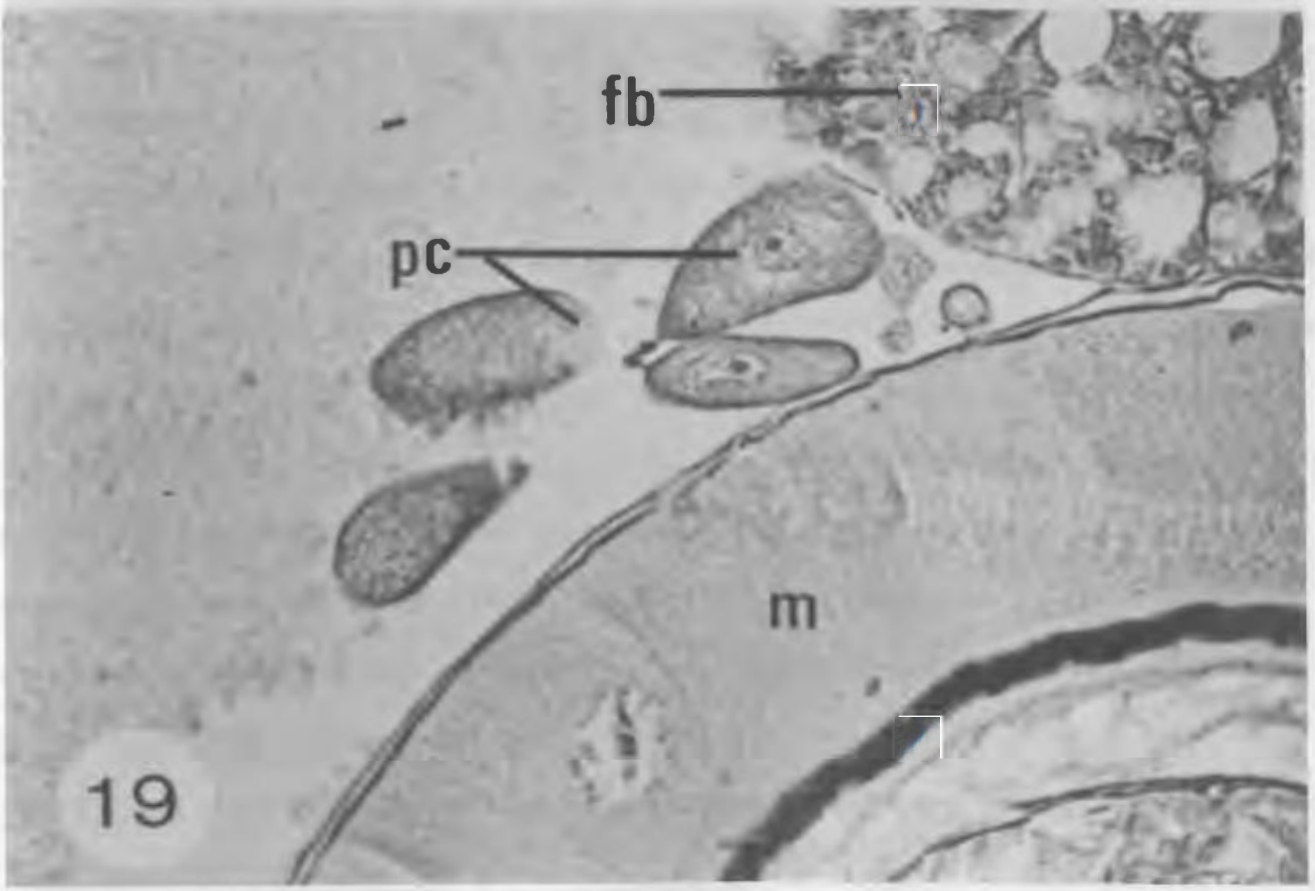
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19

mermithid parasite does not manipulate nor consequently cause a change in this simuliid host endocrine system (Tables 2, 3; Figs. 20-26). However, the mermithid parasite of infected S. venustum Complex larvae did significantly increase the nuclear DNA/RNA activity in the corpus allatum gland (Table 2; Fig. 21), indicating that mermithid parasitism enhanced protein synthesis within the corpus allatum of this simuliid host. Although no other statistically significant changes of the corpus allatum (nuclear/cytoplasmic ratios; gland volumes) were discerned (Table 2; Figs. 20, 22), a possible reduction of the gland volumes in the parasitized hosts of both species was suggested by my measurements, but large standard errors in the data made this difference statistically insignificant. However, it may be that gland volumes in simuliids are poor indicators of endocrine activity, especially in field populations.

Similar investigations of gland volumes, nuclear/cytoplasmic ratios and nuclear DNA/RNA activities of the corpora cardiaca cells were done with an additional study on neurosecretory material stored within these glands. The results showed a significant increase in the overall corpora cardiaca gland volume of the infected S. venustum Complex larvae as compared to the dark histoblast controls (Table 3; Fig. 23). Further, a similar significant increase was obtained in the concentration (and thus total amount within the gland) of neurosecretory material stored in the corpus cardiacum cells of parasitized S. venustum Complex larvae as compared to the controls (Table 3; Fig. 24). Such data indicates a retention of neurosecretory material by the corpus cardiacum cells of the S. venustum Complex larvae when parasitized by N. flumenalis. No significant difference between the infected hosts and controls were found

TABLE 2

Effects of *N. flumenalis* Parasitism on the Corpus Allatum Gland of the Larval Blackfly Hosts of *P. mixtum/fuscum* and *S. venustum* Complexes +

Host Species	Corpus Allatum DNA/RNA Concentrations (Absorbance Values)		Corpus Allatum Gland Volumes ($\mu^3 \times 10^4$)		Corpus Allatum ‡ Nuclear/Cytoplasmic Ratios	
	Control	Infected	Control	Infected	Control	Infected
P. mixtum/fuscum	.118 ± .011(7)	.098 ± .004(4)	2.470 ± .523(5)	1.594 ± .149(5)	.463 ± .036(4)	.461 ± .017(4)
S. venustum Complex	.122 ± .003(6)	.158 ± .005(5) ***	2.290 ± .219(5)	1.709 ± .194(5)	.335 ± .033(5)	.332 ± .030(4)

+ All data are expressed as mean values ± standard errors. More detailed presentation of this data is given in Appendices 2-4.

‡ Nuclear/cytoplasmic ratios = $\frac{\text{Area occupied by nuclei}}{\text{Area occupied by cytoplasm}}$

*** Significantly different from control values at $p < 0.01$.

TABLE 3

Effect of N. flumenalis Parasitism on the Corpora Cardiaca Gland Cells of the Larval Blackfly Hosts, P. mixtum/fuscum and S. venustum Complexes +

Host Species	Corpora Cardiaca Gland Volumes ($\mu^3 \times 10^4$)		Corpora Cardiaca Neurosecretory material Concentrations (Absorbance Values)		Corpora Cardiaca Nuclear/cytoplasmic ratios		Corpora Cardiaca DNA/RNA Concentrations (Absorbance Values)	
	Control	Infected	Control	Infected	Control	Infected	Control	Infected
P.mixtum/fuscum	3.727±.679(5)	2.846±.436(5)	.108±.004(7)	.108±.002(7)	.209±.017(5)	.169±.007(4)	.126±.020(6)	.122±.006(4)
S.venustum Complex	1.937±.265(5)	5.200±1.098(5)*	.106±.008(7)	.133±.007(7)*	.140±.030(4)	.123±.017(4)	.131±.020(5)	.125±.005(6)

+ All data are expressed as mean values ± standard errors. For more detail see appendices 5-8.

* Significantly different from control values at $p < 0.05$.

Figure 20

Comparison of the corpus allatum gland volume between the control and infected larvae of P. mixtum/fuscum and S. venustum Complex.

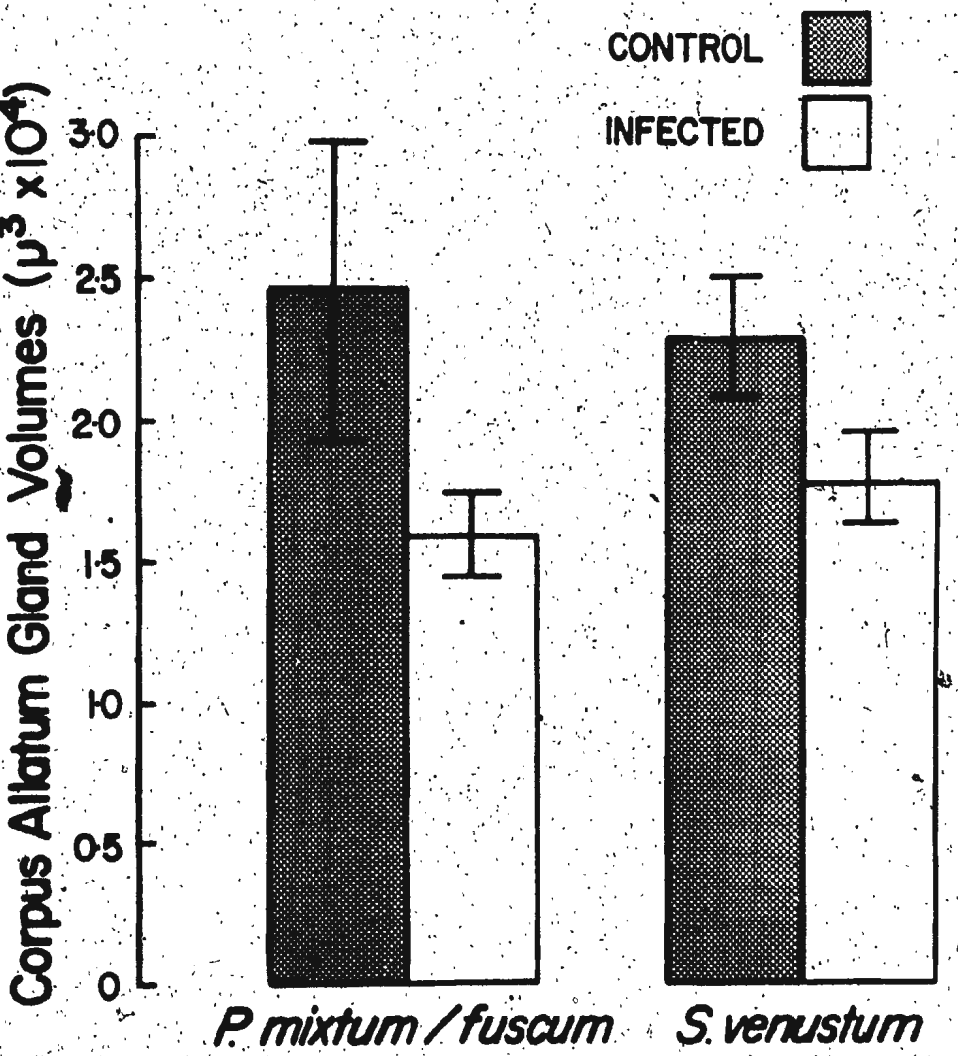

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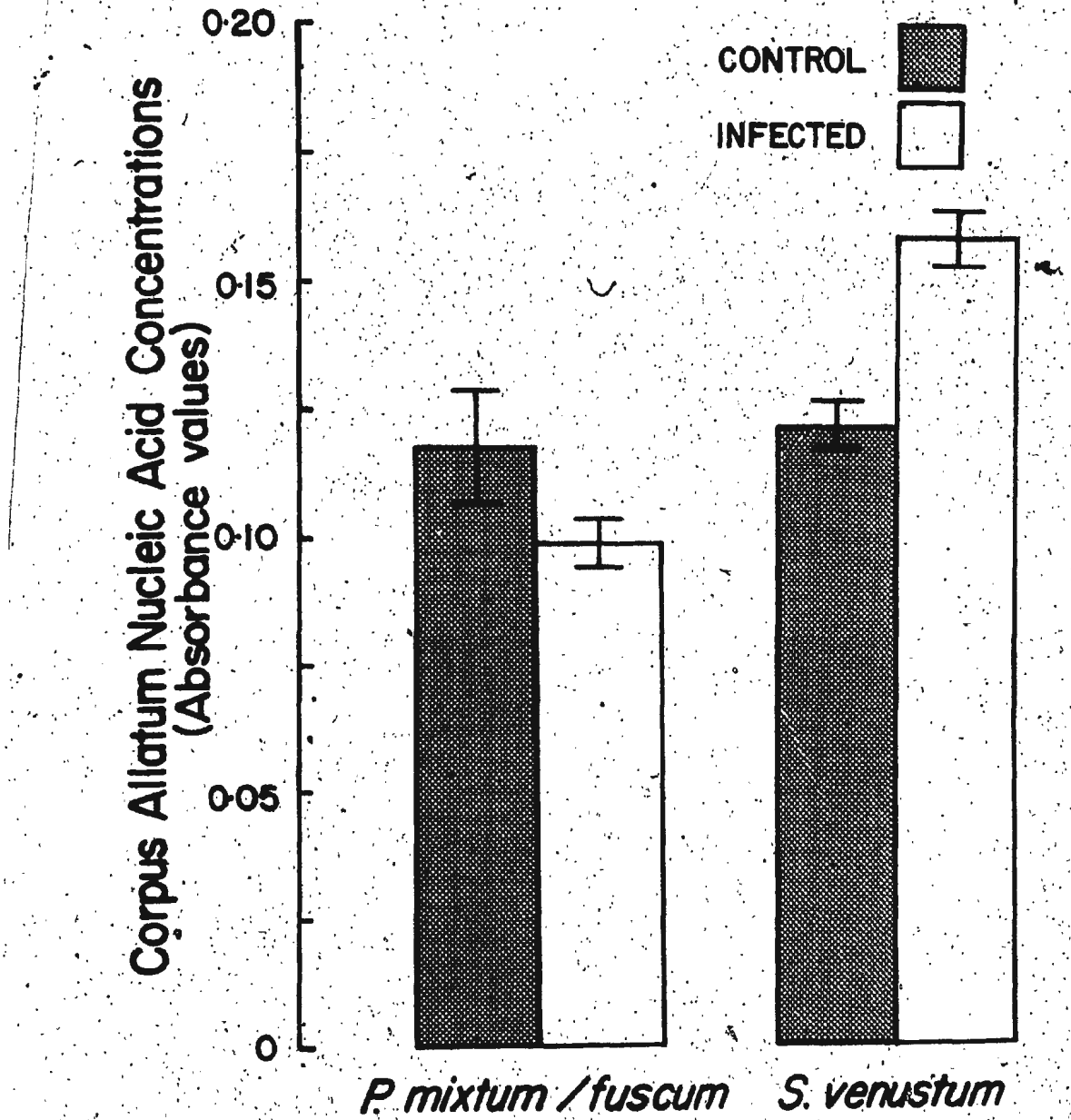
Figure 21

Comparison of the corpus allatum nucleic acid concentrations between control and infected larvae of P. mixtum/fuscum and S. venustum Complex.



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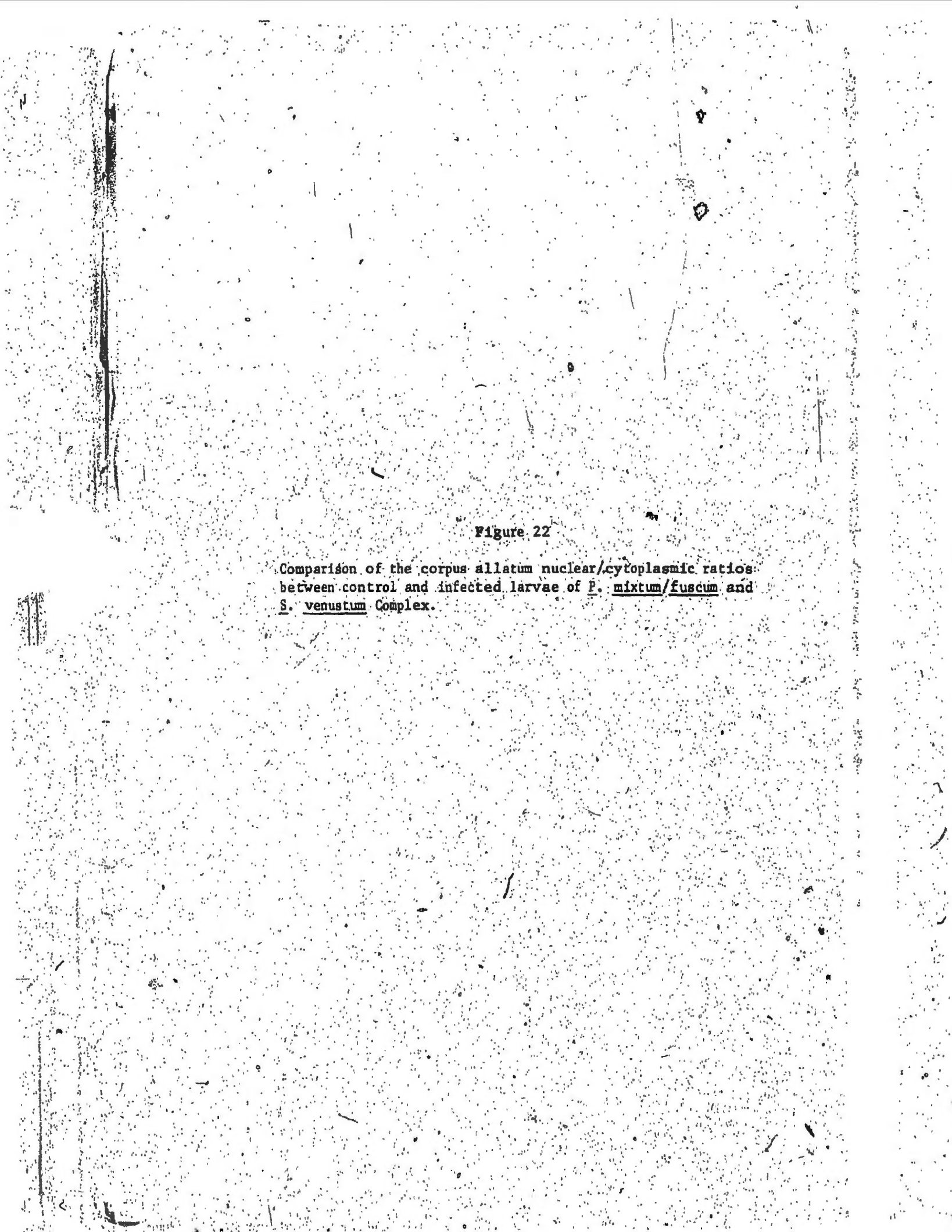
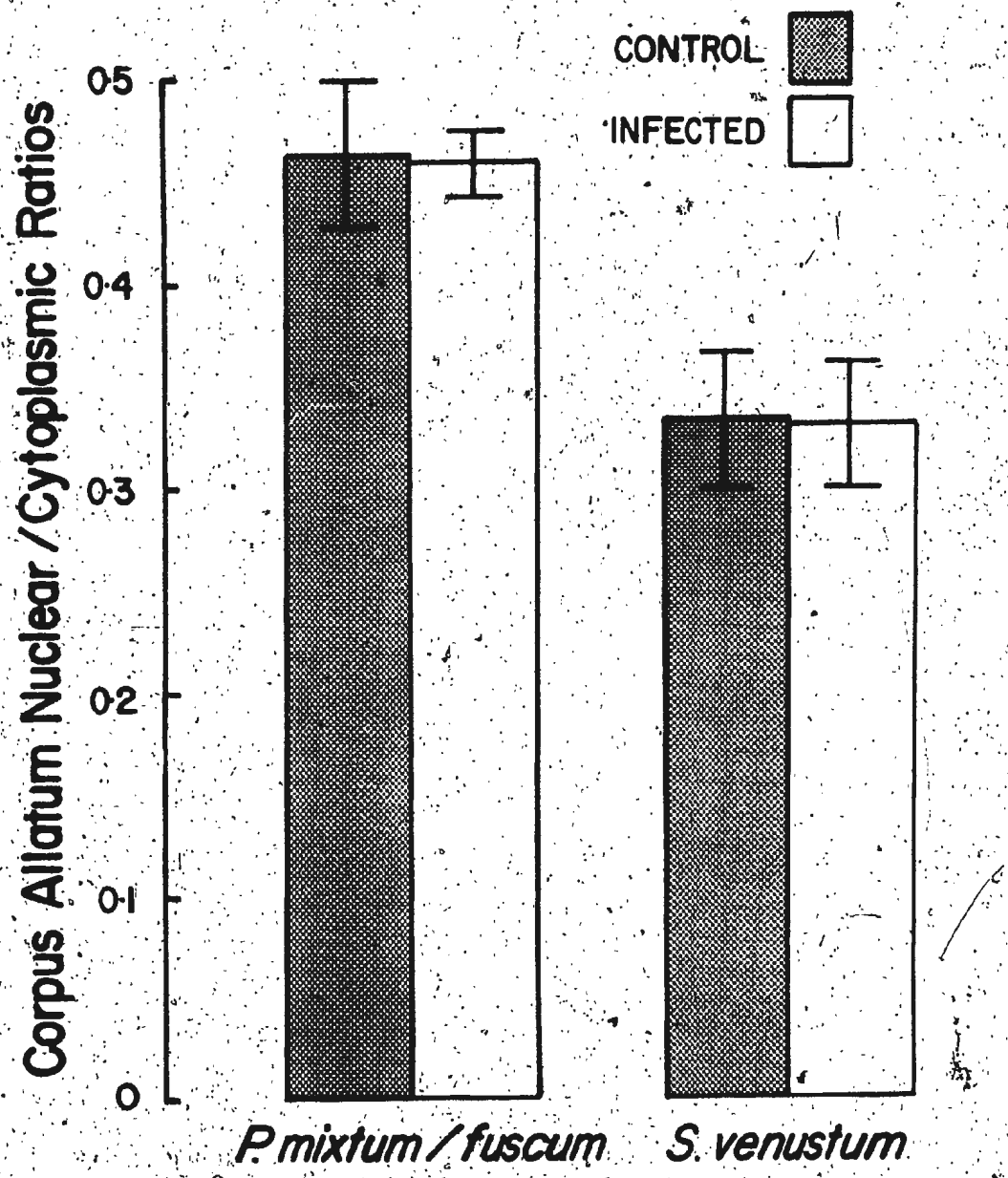


Figure 22

Comparison of the corpus allatum nuclear/cytoplasmic ratios between control and infected larvae of P. mixtum/fuscum and S. venustum Complex.


CONTROL
INFECTED
Corpus Allatum Nuclear / Cytoplasmic Ratios
0·5
0·4
0·3
0·2
0·1
0
P. mixtum / fuscum
S. venustum

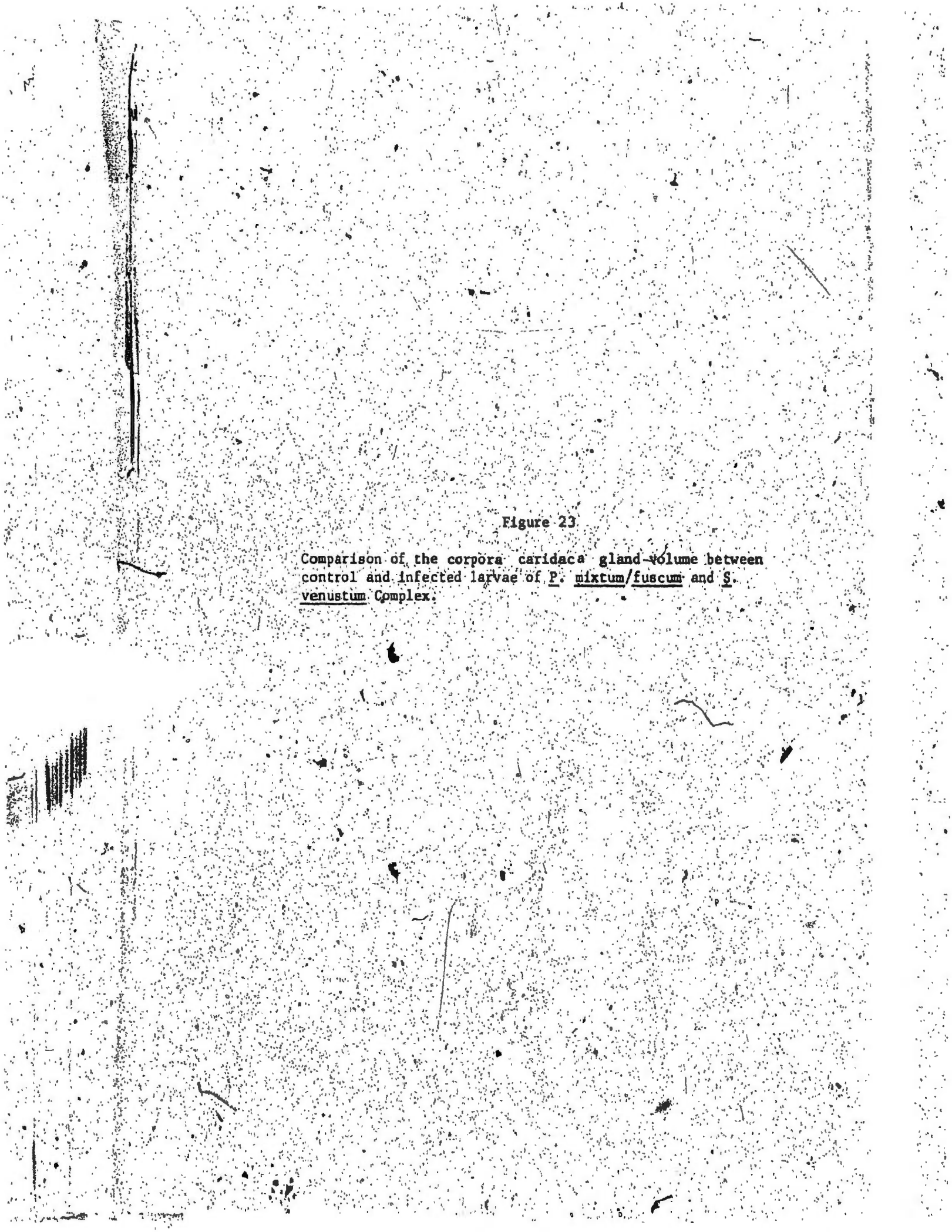


Figure 23

Comparison of the corpora caridaca gland volume between control and infected larvae of P. mixtum/fuscum and S. venustum Complex.

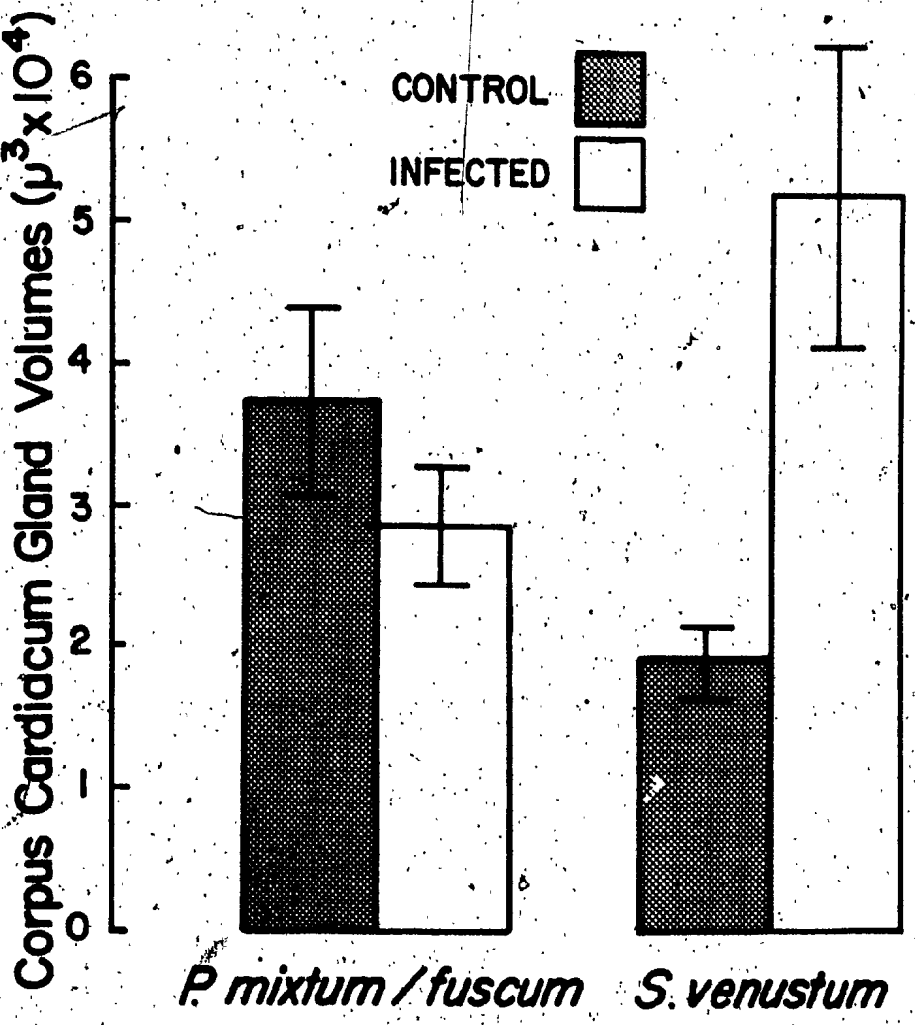

Corpus Cardiacum Gland Volumes ($\mu^3 \times 10^4$)
0
1
2
3
4
5
6
CONTROL
INFECTED
P. mixtum / fuscum
S. venustum

Figure 24

Comparison of the corpora cardiaca neurosecretory material concentrations between control and infected larvae of P. mixtum/fuscum and S. venustum Complex.

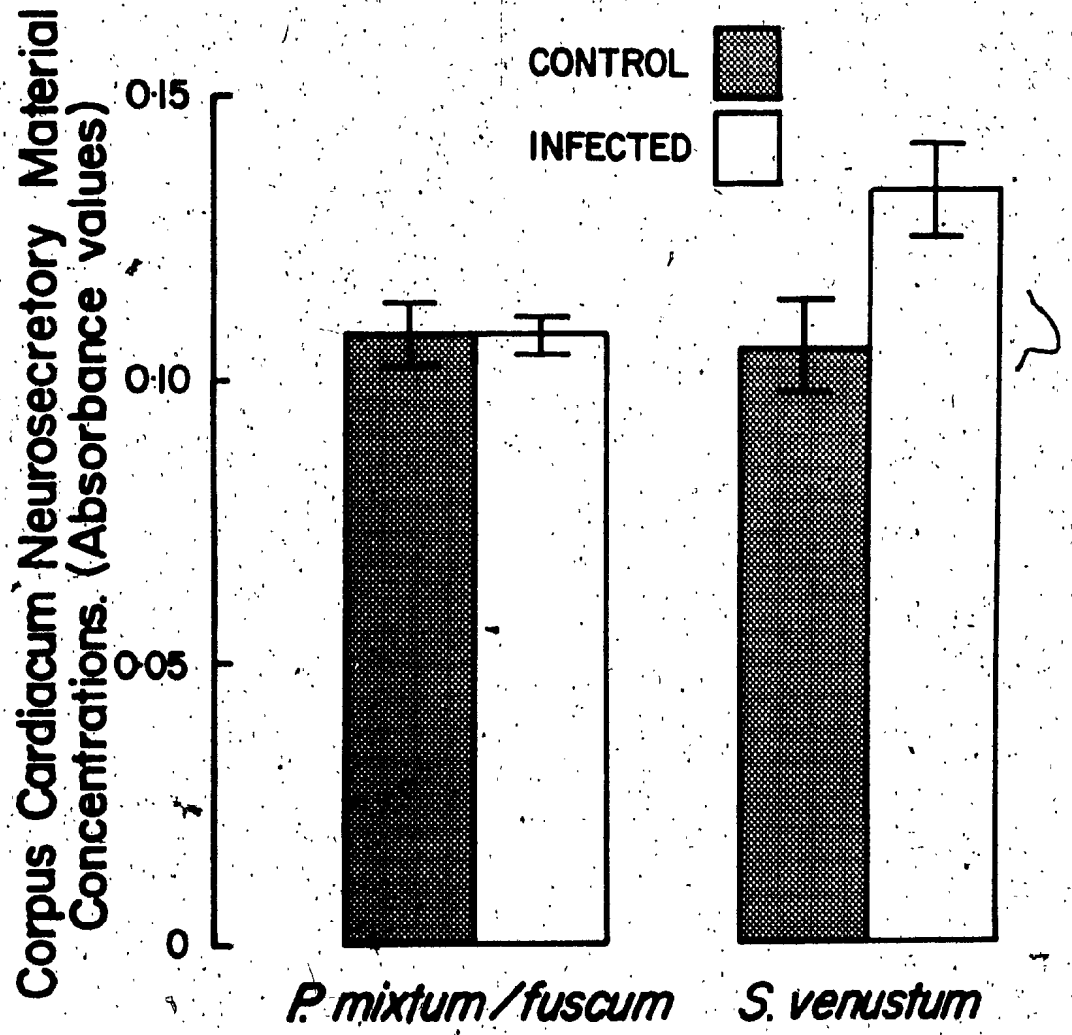

Corpus Cardiacum Neurosecretory Material
Concentrations. (Absorbance values)
0·15
0·10
0·05
0
CONTROL
INFECTED
P. mixtum/fuscum
S. venustum

Figure 25

Comparison of the corpora cardiaca nuclear/cytoplasmic ratios between control and infected larvae of P. mixtum/fuscum and S. venustum Complex.

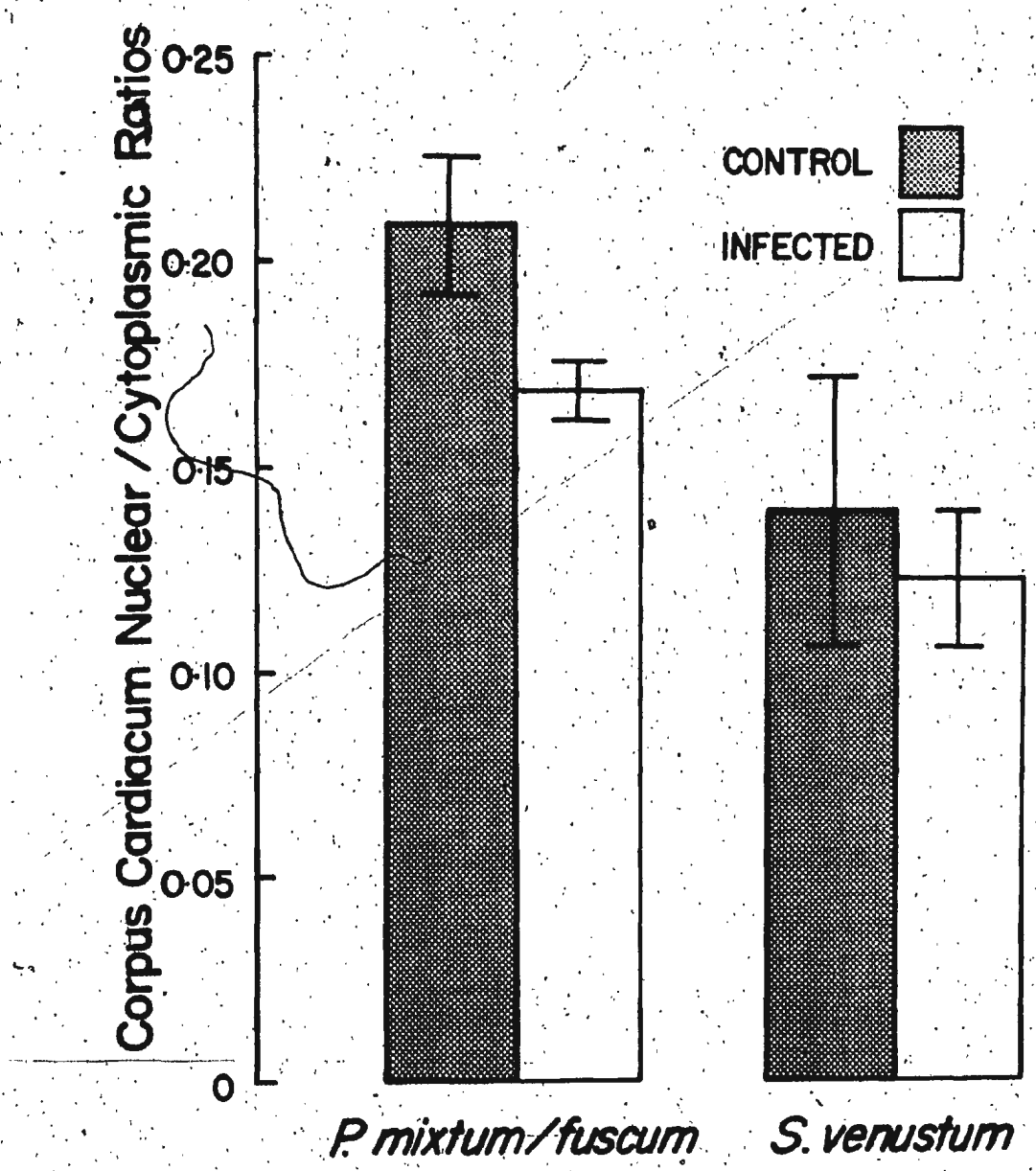
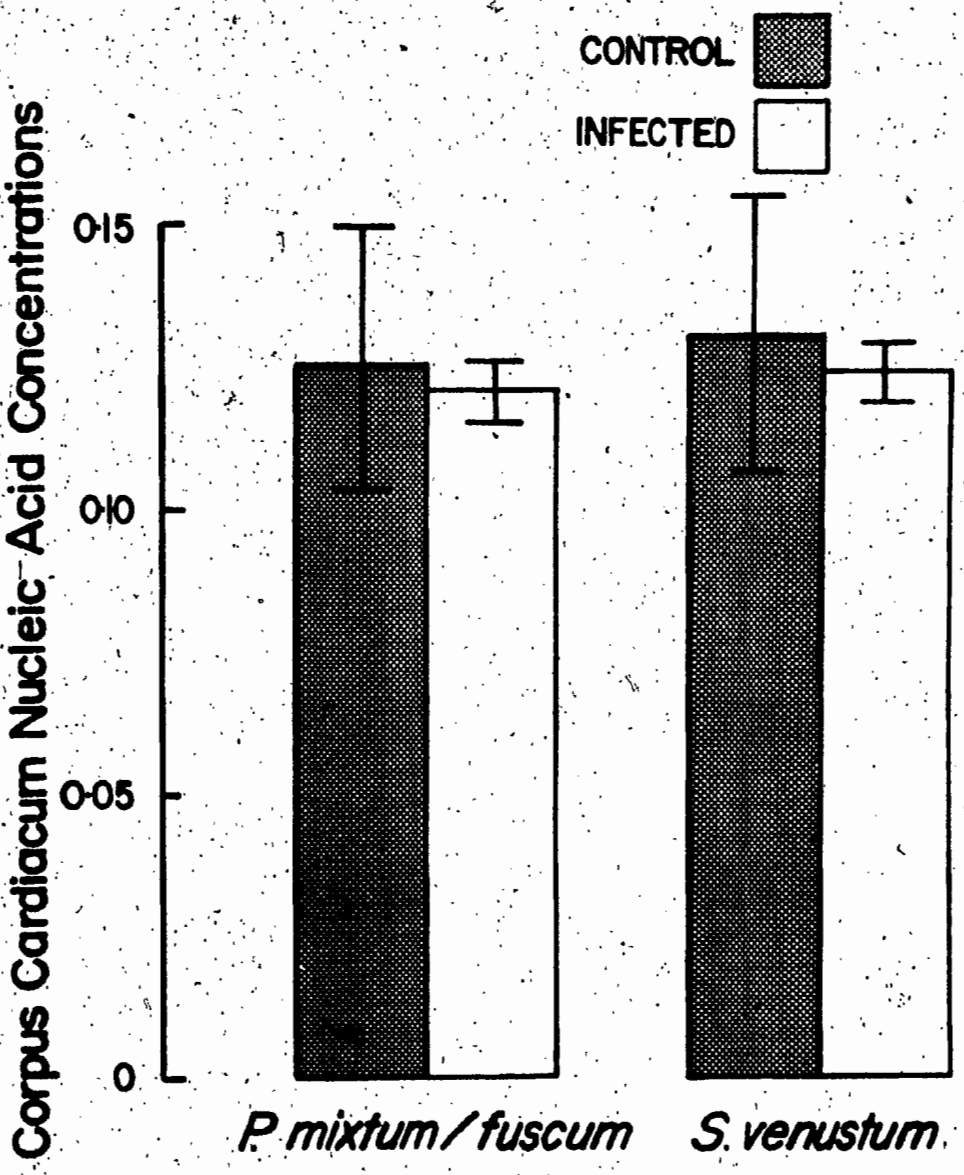

Corpus Cardiacum Nuclear /Cytoplasmic Ratios
0·25
0·20
0·15
0·10
0·05
0
CONTROL
INFECTED
P. mixtum/fuscum
S. venustum

Figure 26

Comparison of the corpora cardiaca nucleic acid concentrations between control and infected larvae of *P. mixtum/fuscum* and *S. venustum* Complex.


CONTROL
INFECTED
Corpus Cardiacum Nucleic Acid Concentrations
0·15
0·10
0·05
0
P. mixtum / fuscum
S. venustum

in the corpus cardiacum cells nuclear DNA/RNA concentrations and nuclear/cytoplasmic ratios (although there was a reduction in the nuclear/cytoplasmic ratios in both infected host species, these were not shown to be statistically significant ⊢ Table 3; Figs. 25/26). Therefore it appears that the corpora cardiaca of these infected simuliids retains the ability to synthesize its intrinsic hormones.

Such data suggests an acceleration of protein synthesis by the corpus allatum and an increased retention of neurosecretory material and large gland volumes in the corpora cardiaca of mermithid parasitized S. venustum Complex larvae. However, a trend of non-endocrine involvement by the mermithid seems to be apparent with respect to the P. mixtum/fuscum generation.

Some effects of N. flumenalis parasitism on the levels of storage metabolites of P. mixtum/fuscum and S. venustum Complex

The effect of this mermithid parasitism on the fat bodies of both species complexes of P. mixtum/fuscum and S. venustum was very evident and may be observed histologically. The depletion of fat body tissue in parasitized individuals was readily observed in both blackfly species because these larvae harbored parasites which were nearing completion of their parasitic way of life (Figs. 27-30). Densely packed fat body tissue was concentrated in the abdominal hemocoel of dark histoblast control larvae of P. mixtum/fuscum (Fig. 27) and S. venustum Complex (Fig. 29). By contrast, in both infected P. mixtum/fuscum (Fig. 28) and S. venustum Complex (Fig. 30) the hemocoel was mainly filled with the mermithid nematode(s) and only isolated remnants of fat body tissue remained. Evidently the mermithid(s) exhausted fat body nutrient reserves of the host in a non-selective manner, rather than having

Figure 27

Longitudinal section of an uninfected P. mixtum/fuscum larva (abdominal region) showing densely packed fat body tissues throughout the body cavity.

Abbreviations: fb, fat body cells; g, gut; s, salivary gland.

Stain: Periodic acid-Schiff.

Magnification: 3420X

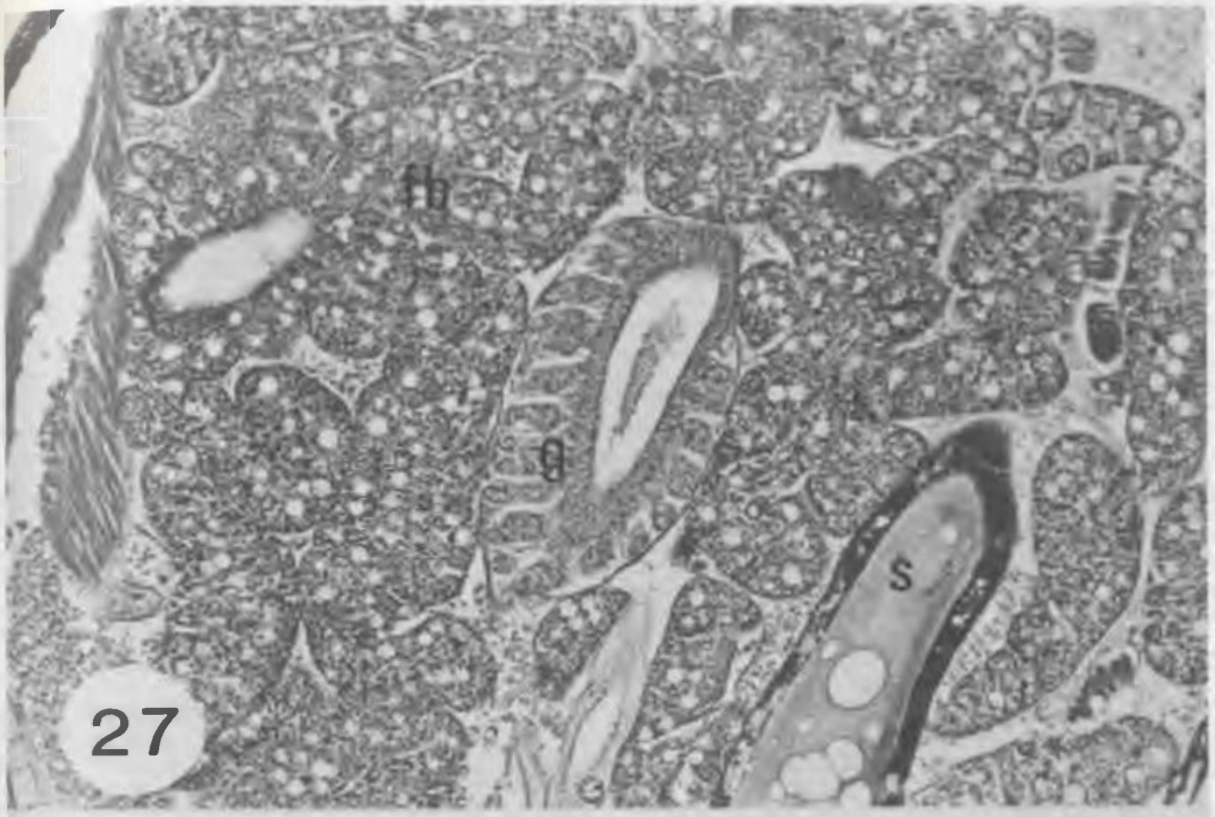
Figure 28

Longitudinal section of an infected P. mixtum/fuscum larva (abdominal region) showing the replacement of fat body tissues by a developing mermithid nematode throughout the hemocoel. Note the displacement of the gut toward one side of the insect.

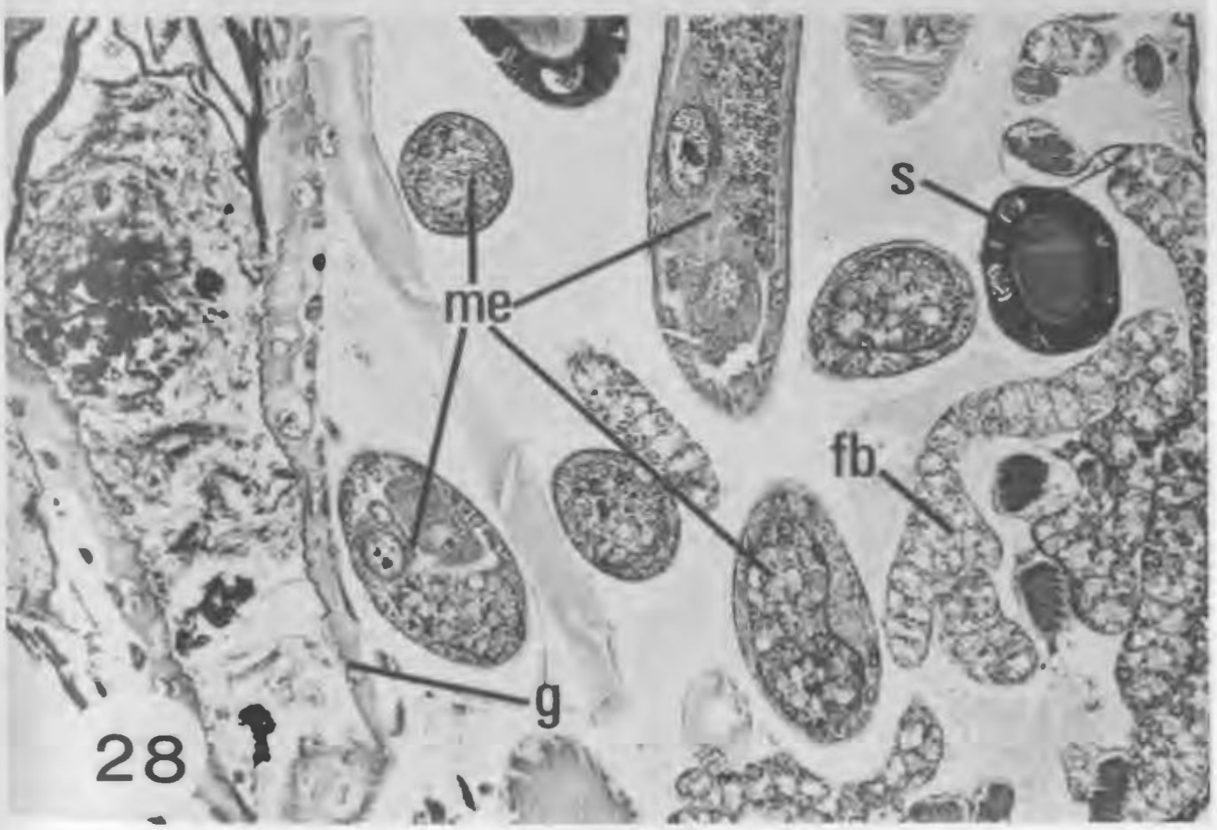
Abbreviations: fb, fat body cells; g, gut epithelium; me, mermithid; s, salivary gland.

Stain: Periodic acid-Schiff.

Magnification: 3420X



27



28

Figure 29

Longitudinal section of S. venustum Complex control larva (abdominal region) showing densely packed fat body tissues throughout the body cavity.

Abbreviations: fb, fat body cells; g, gut epithelium; s, salivary gland.

Stain: Periodic acid-Schiff.

Magnification: 3420X

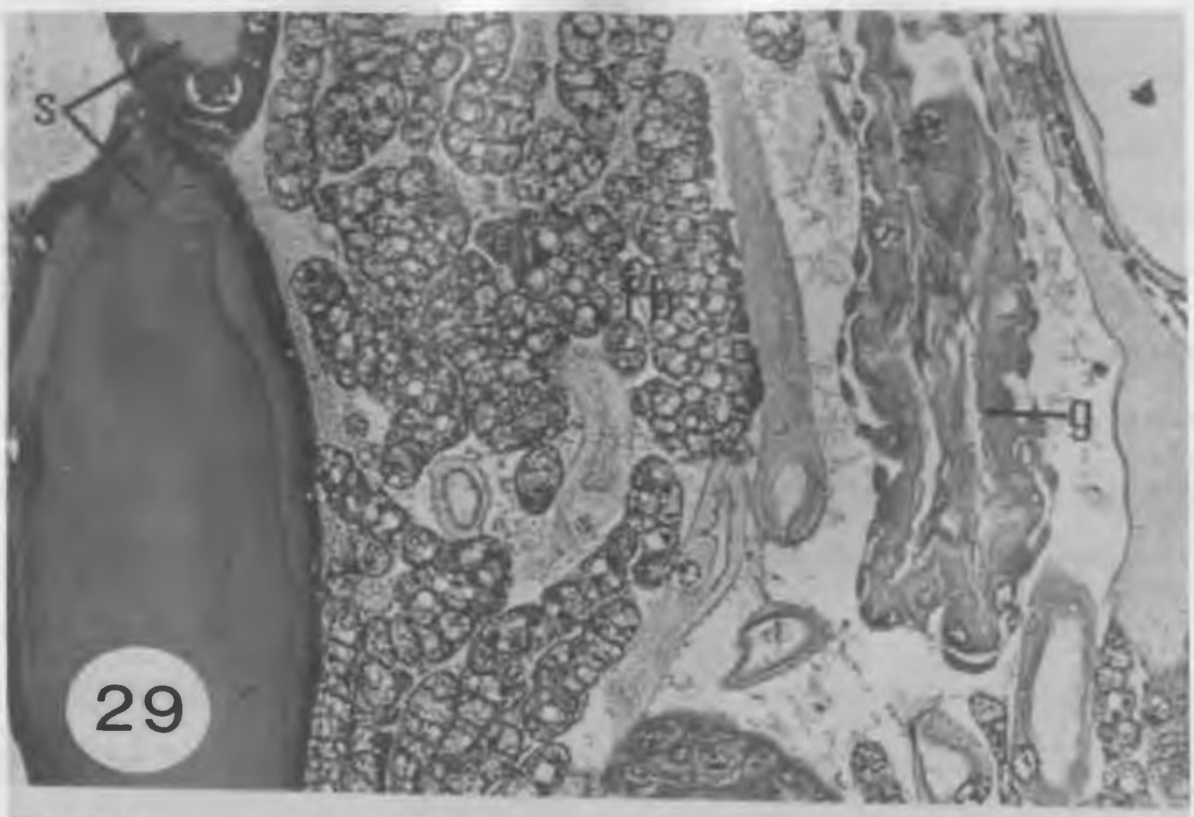
Figure 30

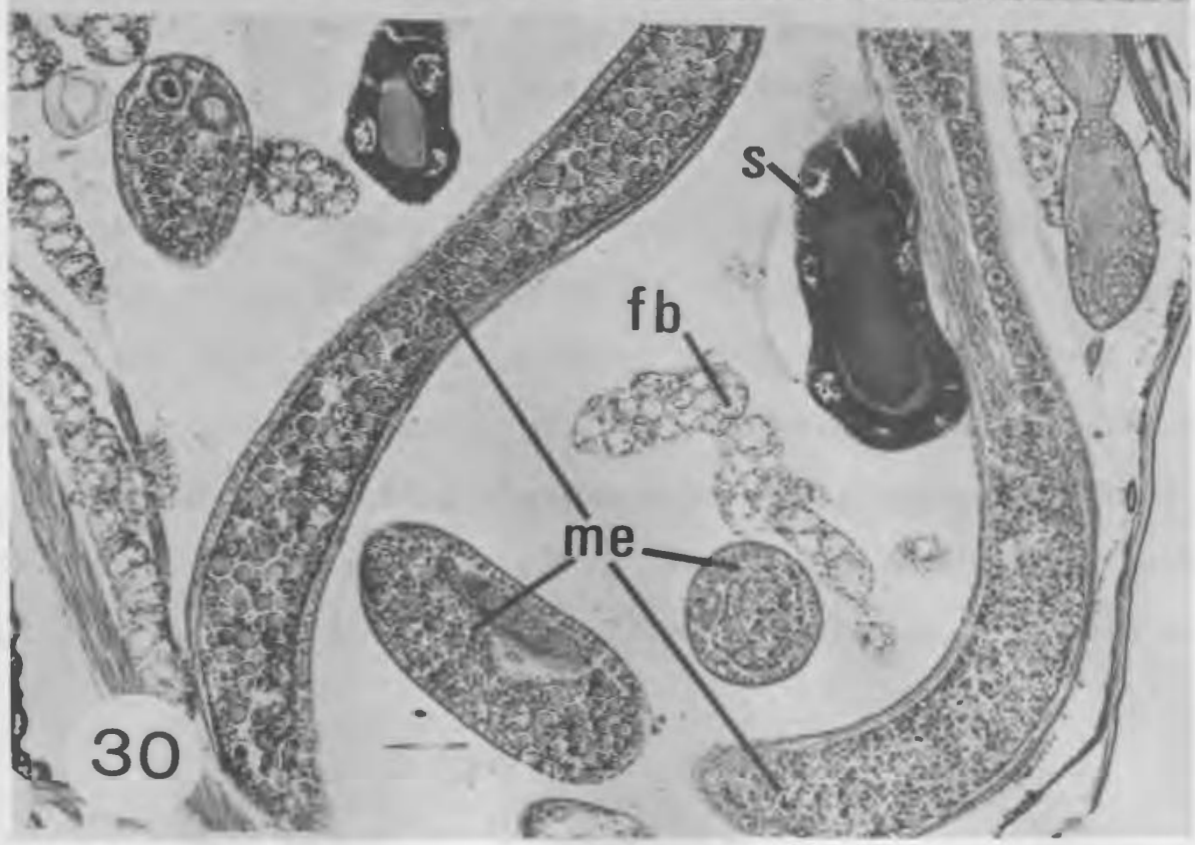
Longitudinal section of an infected S. venustum Complex larva (abdominal region) showing the replacement of fat body tissues by a developing mermithid nematode throughout the hemocoel.

Abbreviations: fb, fat body cells; me, mermithid; s, salivary gland.

Stain: Periodic acid-Schiff.

Magnification: 3420X


s
g
29


s
fb
me
30

elicited specific alterations in fat body nutrient turnover.

In mermithid parasitized P. mixtum/fuscum the concentration of fat body glycogen was significantly reduced to about two-thirds the level prevailing in the dark histoblast control larvae (Table 4; Fig. 31). Similarly the infected S. venustum Complex larvae showed a significant one-quarter reduction in the level of fat body glycogen as compared to the control larvae (Table 4; Fig. 31). Interestingly the concentration of fat body glycogen was significantly greater ($p < 0.01$) in non-parasitized P. mixtum/fuscum than in control S. venustum Complex larvae; although the fat bodies of infected hosts contained the same low concentration of glycogen of both host species.

Such depletion of host carbohydrate reserves by mermithid parasites of the blackfly species studied does not appear to be related to accelerated fat body glycogenolysis, because the nuclear DNA/RNA activities in the fat bodies of both host species were unaffected by mermithid parasitism (Table 4; Fig. 32).

The mean dry weight of non-parasitized S. venustum larva Complex was the same as that of their infected counterparts (Table 1). Interestingly, the total biomass of infected P. mixtum/fuscum larvae was significantly less than that of the dark histoblast controls (Table 1). Evidently the parasite had profoundly altered the feeding behavior and/or nutrient utilization by its host when its association with the latter was of a more protracted duration with an overwintering simuliid species complex.

TABLE 4

Effects of *N. flumenalis* Parasitism on the Fat Body Tissues of the Larval Blackfly Host, *P. mixtum/fuscum* and *S. venustum* Complexes +

Host Species	Fat Body Glycogen Concentrations (Absorbance Values)		Fat Body Nuclear DNA/RNA Concentrations (Absorbance Values)	
	Control	Infected	Control	Infected
P. mixtum/fuscum	.119 ± .003(7)	.076 ± .003(7) ****	.058 ± .002(6)	.059 ± .006(4)
S. venustum Complex	.095 ± .005(5)	.075 ± .002(5) **	.053 ± .002(6)	.054 ± .001(6)

+ All data expressed as mean values ± standard errors. More detail in Appendices 9-10.

** Significantly different from control values at $p < 0.02$.

**** Significantly different from control values at $p < 0.001$.

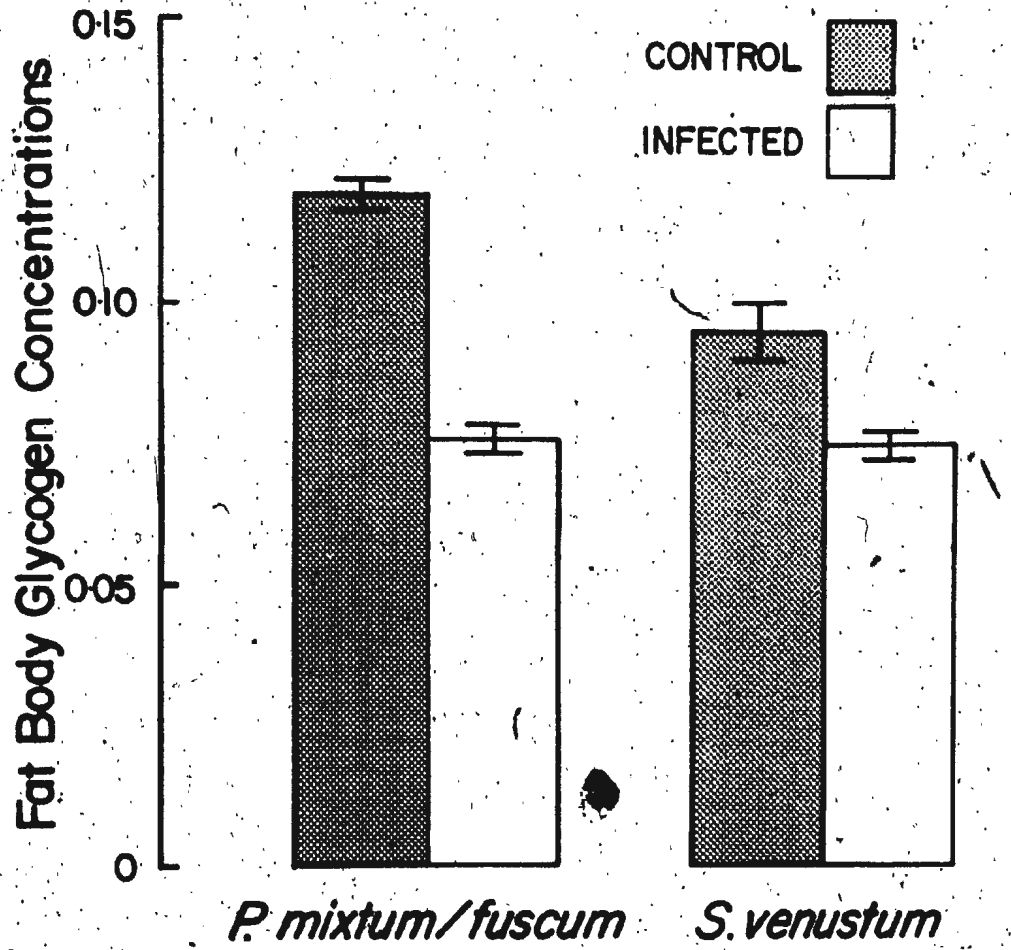

0·15
0·10
0·05
0
Fat Body Glycogen Concentrations
CONTROL
INFECTED
P. mixtum/fuscum
S. venustum

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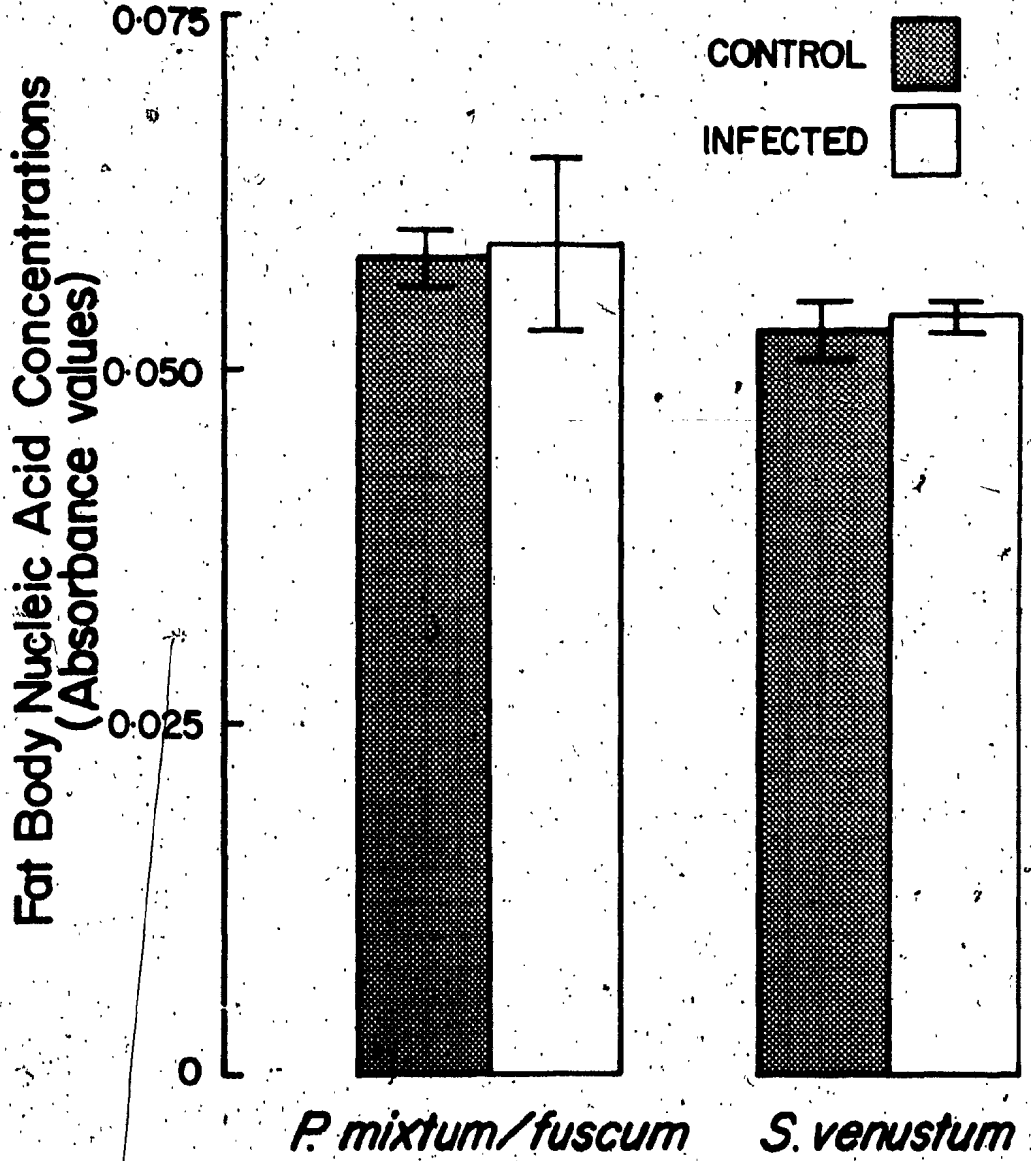
Figure 31

Comparison of the fat body glycogen concentrations between the control and infected larvae of P. mixtum/fuscum and S. venustum Complex.

Figure 32

Comparison of the fat body nucleic acid concentrations between the control and infected larvae of P. mixtum/fuscum and S. venustum Complex.



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DISCUSSION

The neuroendocrine system of P. mixtum/fuscum and S. venustum Complex larvae have been shown to consist of neurosecretory cells in the brain composed of three pairs of neurosecretory cell clusters having a similar distribution to that of the mosquito Aedes aegypti (Burgess and Rempel, 1966). Since the brain neurosecretory cells of other members (i.e. other than mosquitoes and blackflies) of the Nematocera have not yet been histologically examined, then this format of three clustered pairs of neurosecretory cells may be a common feature for this group. The axonal link between the neurosecretory cells of the pars intercerebralis and the corpora cardiaca, known in most insects as the nervi corporis cardiaca I and II, are fused within P. mixtum/fuscum and S. venustum Complex consisting of a pair bordering each side of the aorta. This fusion of the nervi corporis cardiaci was also observed in other families of the Nematocera -- including Culicidae (Burgess and Rempel, 1966), Chironomidae (Possompes, 1948) and Tipulidae (Thomsen, 1951). The retrocerebral glandular system of both P. mixtum/fuscum and S. venustum Complex consists of separate corpora cardiaca cells comprised of characteristically stained cells connected to the nervi corporis cardiaci. Closely positioned with the corpora cardiaca is the corpus allatum consisting of closely packed cells forming a single gland. This gland was studied in closer detail using thin sectioned specimens and was shown to be a single structure of cellular composition with no extending membrane within the gland. This single corpus allatum gland of simuliids is unique to the Nematocera which normally possess bilateral

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corpora allata glands (Possompes, 1948; Cazal, 1948; Thomsen, 1951). The peritracheal gland (termed by Thomsen (1951) for Tipulidae as analogous to the prothoracic glands of other insects) of these blackfly larvae appears to loosely encircle the corpora cardiaca and corpus allatum unlike the bilateral longitudinal and peritracheal glands of other Nematocera (Arvy and Gabe, 1962; Thomsen, 1951).

The characteristic structure of a single corpus allatum and closely situated cellular corpora cardiaca and peritracheal gland of simuliids appears to represent an intermediate type of glandular system between the Nematocera endocrine system and the Brachycera arrangement. The Brachycera endocrine system as exemplified by the family Tabanidae (Thomsen, 1951) also consists of a single corpus allatum gland but includes a peritracheal gland and corpora cardiaca cells fused into a ring structure encircling the aorta. This Brachycera arrangement appears to be morphologically intermediate to the Weismann's ring structure of the Cyclorrhapha, which according to evidence obtained from the family Calliphoridae (Thomsen, 1951) possess the corpus cardiacum, corpus allatum and peritracheal gland cells in the form of a single ring structure encircling the aorta. Such an evolutionary pathway from the endocrine pattern of the Nematocera to the specialized ring structure of the Cyclorrhapha has been suggested by Thomsen (1951).

In order to ascertain any effect mermithid parasitism might have on the endocrine gland activity of the simuliid hosts, three recognized histochemical parameters for monitoring endocrine activity were used (gland volumes, nuclear/cytoplasmic ratios and DNA/RNA concentrations) because of the paucity of knowledge of simuliid endocrine systems. Gland size has been used as a reliable indicator of endocrine activity for

various insects (Scharrer, 1952; Engelmann, 1957; Highnam, 1964; Wigglesworth, 1964); however, in some insects (e.g. Oncopeltus fasciatus (Dallas), Johansson, 1958; Tenebrio molitor L., Mordue, 1965; and Calliphora erythrocephala Meig., Lea and Thomsen, 1969) corpus allatum volume is not always associated with gland activity. Therefore, another indicator of endocrine activity, that of nuclear/cytoplasmic ratios, was also employed. This parameter had been used for estimating corpora allata activity in the cockroach Leucophaea maderae Fabr. (Engelmann, 1957, 1960) and for determining the relative activity of the ventral glands (somewhat similar to the molting glands of pterygotes) in the firebrat Thermobia domestica Packard (Watson, 1964). The third method of comparing gland activities involved microspectrophometric estimation of nuclear DNA/RNA concentrations. This histochemical technique has to the author's knowledge not been employed previously; however, nucleic acid synthesis has been used to estimate corpora allata gland activity via radiotracer incorporation methods (Gillott and Dogra, 1972). The present study has shown no evidence of any effect on the endocrinology of mermithid infected P. mixtum/fuscum larvae using the parameters previously stated. However, an apparent increase in nuclear DNA/RNA activity was observed in the corpus allatum of infected S. venustum Complex hosts, suggesting an acceleration in protein synthesis by this host. Furthermore, the nematode N. flumenalis significantly affects the corpora cardiaca of S. venustum Complex larvae showing increased gland volume with a correspondingly increase in stored neurosecretory material.

Such effects of parasitism upon S. venustum Complex hosts, but not on the P. mixtum/fuscum generation may be due to differences in the synchronization of life cycles between the two hosts and corresponding

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differences in the manifestation of stressful symptoms of the "disease." Ebsary (1973) recorded that infection of the univoltine P. mixtum/fuscum by N. flumenalis occurs about mid-October and the parasite overwinters within the hosts hemocoel to complete its parasitic development and commence the free living phase of its life cycle in the following spring. By contrast, infection of S. venustum Complex larvae is considered to take place during the spring and parasitic development is of shorter duration in this host species so that free-living development starts during early or mid-summer. Thus it would seem reasonable to postulate that the effect of mermithid parasitism on the endocrinology of S. venustum Complex was of greater intensity than in parasitized P. mixtum/fuscum because the nematode developed less rapidly and consequently exerted minimized nutritional demands in the overwintering host. However, in S. venustum Complex the parasite's development was more rapid and nutritional demands per unit time were of greater intensity. Such a suggestion of stressful phenomena (e.g. enforced activity; injury; etc.) altering endocrine activity in insects has been well documented (Schneiderman and Jankowitz, 1957; Highnam, 1962; Davey, 1963). It would seem plausible that mermithid parasitism constitutes a stressful situation for the blackfly host, which is of greater intensity in S. venustum Complex larvae than in P. mixtum/fuscum larvae due to the more accelerated nutritional demands of the parasite in the S. venustum Complex host.

Based on recent information, it is possible (though less likely) that the differences in the effects of mermithid parasitism upon the two blackfly species is due to the fact that the mermithid which infects S. venustum Complex may have been incorrectly identified by previous

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researchers as N. flumenalis (Bailey, personal communication).[8] Nickle (1972) commented on the difficulties associated with the taxonomic identification of mermithid nematodes. If future studies establish these suspicions, the recorded differences in effects of mermithid parasitism upon the simuliid endocrinology may be due to different mermithid parasites infecting the two host species.

The corpora allata of the female bumblebee host Bombus sp. Latr. were arrested in growth by the nematode Sphaerularia bombi, although no changes were detected in host neurosecretory cell activity (Palm, 1948; Pouvreau, 1962). Similarly, the parasitoid Stylops sp. caused reduction of corpus allatum volume and ovarian growth in the female sandbee Andrena vaga Cockerell, but exerted no detectable affect on the hosts' brain neurosecretory cells (Brandenburg, 1956). Whether or not such effects of mermithid parasitism are due to the production of a toxin(s) by the parasite (Palm, 1948; Pouvreau, 1962) or are the indirect consequence of disturbances in host physiology in the parasitized host (Brandenburg, 1956) is a debatable point.

In the present investigation the variabilities in corpus allatum gland volumes within each group of specimens were high, an unavoidable difficulty no doubt associated with performing endocrinological investigations of field populations. Thus it would be interesting to ascertain whether or not the apparent (though not statistically significant) reductions in corpus allatum volumes recorded from infected blackflies in this study would assume a greater significance if a satisfactory system for rearing blackflies was devised and an in vivo study of the

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[8] See footnote #1.

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host/parasite relationship was undertaken under controlled conditions. In S. venustum Complex larvae, the mermithid caused an increase in both the corpora cardiaca gland volume and the amount of stored neurosecretory material. A similar accumulation of neurosecretory material was observed in the brain cells on the alfalfa plant bug, Adelphocoris lineolatus Goeze infected by the fungal parasite Entomophthora sp. (Ewen, 1966). This parasite caused the neurosecretory A cells of the brain to become inactive with an accumulation of neurosecretory material occurring in the infected female hosts due to parasitic castration.

It is intriguing to consider the possibility that the recorded disturbances in S. venustum Complex endocrinology (elevated corpus allatum nucleic acid activity; increased retention of neurosecretory material by the corpus cardiacum cells) by mermithid parasitism may be related to certain developmental disturbances (e.g. prevention of pupation and inhibition of histoblast development) which invariably accompany mermithid parasitism in blackflies. Welch (1965) suggested that mermithids inhibited pupation of the insect host by directly or indirectly inactivating secretion of the hosts' prothoracic hormone. Increased retention of neurosecretory material in the corpora cardiaca of the parasitized S. venustum Complex could suggest a reduction in the level of circulating thoracotrophic hormone, which would inhibit $\propto$ ecdysone secretion by the peritracheal glands and consequently prevent pupation. Moreover, enhanced corpus allatum activity (as evidenced by increased DNA/RNA activity) might account for the retention of larval histoblasts in the parasitized S. venustum Complex. However, while such endocrinological disturbances might in part explain effects upon host development due to mermithid parasitism, it seems highly unlikely that

TIGHT BINDIN

hormonal factors constitute the only mechanism involved. In P. mixtum/fuscum mermithid parasitism did not induce any detectable changes in the host endocrinology, yet this host was also prevented by mermithid parasitism from pupating and developing normal pupal histoblasts. Thus it would seem more likely that disturbances in host development caused by the mermithid are the result of depletion of host nutrient reserves by the developing nematode.

Mermithid parasitism drastically reduced the net amount of fat body tissue in both host species and caused a significant reduction in the concentration of fat body glycogen reserves. Based on previous research conducted on M. nigrescens parasitism in locusts, the mermithid is probably depleting the hemolymph and fat body of the blackfly host of necessary precursors required for synthesis of cuticular proteins during molting as well as causing a more general reduction of host metabolites. Gordon et al. (1971) referred to this general depletion of host metabolites by mermithid nematodes as "a state of physiological starvation." In relation to the size of the insect host, mermithids are one of the largest nematodes known. By absorbing low molecular weight nutrients through their body cuticle (Gordon and Webster, 1972; Rutherford and Webster, 1974), these nematodes possess extremely high growth rates and consequently exert excessive nutritional demands upon the host. Available evidence from other mermithid/insect associations indicates that the hemolymph and fat body is severely depleted in protein nitrogen and carbohydrate nutriment by mermithid parasitism (Gordon et al., 1971; Gordon and Webster, 1972; Gordon et al., 1973; Bailey and Gordon, 1973; Craig and Webster, 1974; Rutherford and Webster, 1974). Moreover, mermithid parasitized Culex pipiens larvae will develop to pupation if fed on a diet rich in vitamins (Muspratt, 1965). Recently, Craig and

Webster (1974) showed that depending upon the time of host infection, M. nigrescens inhibited molting in Schistocerca gregaria, but ecdysone levels in infected locusts were the same as in controls while fat body protein synthesis was impaired by the mermithids. Thus these authors concluded that mermithid parasitism prevents molting in locusts by interfering with the capacity of the fat body tissue to synthesize proteins required for cuticle synthesis. Based on the available evidence, there is every reason to suppose that this hypothesis holds true for mermithid parasitism in blackflies.

The interpretation of the recorded effects of mermithid parasitism on the endocrinology of larval S. venustum Complex, as such effects may relate to the host/parasite relationship, is of necessity somewhat speculative at this time. In view of the fact that the mermithid did not induce any noticeable changes in the endocrinology of P. mixtum/fuscum, it seems somewhat unlikely that the endocrinological disturbances caused by mermithid parasitism in S. venustum Complex would have any bearing upon the development of the nematode. Accordingly, it would seem reasonable to hypothesize that the nematode is not actively manipulating the hormonal balance of its host to modify its own microenvironment and thereby enhance its parasitic development. Mermithid parasites of blackflies probably develop independently of the hosts' hormonal condition and in this respect resemble certain filarioid nematodes (Yoeli et al., 1962; Gwadz and Spielman, 1974). On the available evidence, it seems unnecessary to postulate a hormonal basis for the host/parasite relationship. It would appear more likely, as suggested earlier, that the recorded disturbances in S. venustum Complex endocrinology are one of possibly several stressful symptoms of the parasitemia, not necessarily

TI BINDIN

induced for the benefit of the nematode. Whether or not the suggested stress effect upon the host endocrines is of a direct nature (i.e. secretion of toxic materials by the parasite directly affecting the host endocrine system -- Palm, 1948; Pouvreau, 1962; Stoffalano, 1967) or due indirectly to the disturbance of host homeostasis (e.g. nutrient levels) should be determined. The type of nutritional disturbances which mermithids cause could result in endocrine changes as starvation is known to affect endocrine activity in insects (Wigglesworth, 1936; Kaiser, 1949; Engelmann, 1957; Johansson, 1958). However, P. mixtum/fuscum endocrinology appeared unaltered by mermithid parasitism, despite the fact that fat body reserves of this insect were affected in this instance in the same way as S. venustum Complex. Therefore, while the evidence is at present inconclusive, I am tentatively proposing that the mermithid directly induces the changes in host endocrinology.

It has easily been discerned that N. flumenalis parasitism causes severe depletion of fat bodies in both S. venustum Complex and P. mixtum/fuscum. More specifically, the fat body glycogen concentration was significantly decreased in both simuliid species infected by the mermithid nematode. Further, the concentration of fat body glycogen of non-infected P. mixtum/fuscum larvae was significantly greater than control S. venustum Complex hosts. P. mixtum/fuscum may store greater quantities of fat body glycogen than S. venustum Complex to prepare for the relatively longer non-feeding pupal phase. Alternatively, increased larval nutrient reserves may enable the adult P. mixtum/fuscum to conduct its first gonotrophic cycle autogenously, while adult S. venustum Complex, not possessing such reserves, must seek a blood meal for its first gonotrophic cycle (see Lewis and Bennett, 1973). However, even though there was an

GHT BINDI

increase in fat body glycogen in P. mixtum/fuscum larvae, the infected larvae had the same low glycogen concentrations as that of parasitized S. venustum Complex, indicating a further degradation process by these nematodes as they appear to deplete nutrient glycogen reserves at an ever increasing rate. A similar decrease in fat body glycogen was recorded by Bailey and Gordon (1973) in A. aegypti larvae infected by the mermithid Reesimermis nielseni Tsai and Grundmann. Such depletion of host nutrient reserves appears to be a common feature of mermithid parasitism and results from the intense nutritional requirements of these nematodes. Carbohydrates in particular are a major nutrient requirement of nematodes since these substances constitute the major energy fuel (Lee, 1965). A significant reduction of fat body glycogen also occurs in S. gregaria infected by the mermithid, M. nigrescens; but fat body glycogen levels remain constant (due to reduced fat body glycogen phosphorylase activity) at a lower than normal level after the initial reduction in infected locusts (Gordon et al., 1971). It is possible that locusts may be able to compensate for the nutrient demands of M. nigrescens by actively feeding, while the feeding pattern of the smaller immature parasitized mosquitoes and simuliids may be insufficient to prevent progressive degradation of fat body reserves (Bailey and Gordon, 1973). Further, the individual growth curves of M. nigrescens (Gordon and Webster, 1972) and R. nielseni (Bailey and Gordon, 1973) show the former nematode having its peak growth and development period during the third week of a four-week infection period with less rapid growth and development during the latter part of the infection. By contrast, the growth rate of R. nielseni appears to increase about the mid-infection period and remains high throughout the remainder of the infection. Although

TIGHT BINDING

the growth curve of N. flumenalis had not yet been recorded, continuous uptake of nutrients could explain progressive depletion of host fat body tissue in mermithid-infected blackflies. Decreased levels of fat body glycogen in mermithid-infected S. gregaria (and presumably in other insects parasitized by mermithids) result from the utilization of blood carbohydrate precursors by the nematode causing reduced glycogenesis in the host fat bodies (Gordon et al., 1971). M. nigrescens has been shown to incorporate glucose from the blood of the locust through its thin larval cuticle via facilitated diffusion. It seems that small molecules of glucose can be assimilated by the mermithid larval cuticle but not larger trehalose and glycogen molecules (Rutherford and Webster, 1974). Although the chemical identity of carbohydrate nutriment for mermithid parasites of blackflies has not been ascertained from this study, there is no reason to suppose that it is different from M. nigrescens, especially in view of the fact that many larval Diptera possess glucose and/or fructose (instead of trehalose or glycogen) as the predominant blood carbohydrate (Florkin and Jeuniaux, 1974). Although it seems probable that parasitized simuliids are in a state of 'physiological starvation' and therefore have an impaired capacity for glycogen synthesis, it is not known whether or not this is accompanied by increased glycogenolysis. However, fat body DNA/RNA activities of both simuliid host species did not differ from their counterpart controls, thereby indicating the overall enzymatic activity to be unaltered. Since there is no evidence that large scale alterations in fat body metabolism occur then it would appear unlikely that the parasite accelerates fat body glycogenolysis. Moreover, nuclear DNA/RNA activities and nuclear/cytoplasmic ratios of the corpus cardiacum cells were not significantly affected by the mermithid

parasite in either host species, suggesting that the corpus cardiacum cells could secrete intrinsic hormones (e.g. hyperglycaemic hormone, which controls levels of blood and fat body carbohydrates) normally.

This study has provided baseline data of the general morphology of the simuliid neuroendocrine system and how this is related to other members of the Nematocera. The information regarding the effects of mermithid parasitism on the endocrinology of the simuliid larvae is important as a preliminary investigation to ascertain whether or not the development of the nematode is dependent upon the availability of host hormones. Although results obtained in this study would tend to refute any endocrine requirement by the nematode, more detailed studies should be undertaken because such information is vital for devising suitable in vitro culture systems for blackfly parasitic mermithids. The studies on effects of mermithids on host nutrient reserves should be extended, because a complete knowledge of the nutritional interactions between host and parasite may also be critical for developing in vitro culture systems.

TIGHT BINDING

SUMMARY

1) Two species of larval blackflies, P. mixtum/fuscum and S. venustum Complex, collected from two local Newfoundland streams, were histologically examined to ascertain the morphology of their neuroendocrine systems.

2) Histological observations (Paraffin and Epon sections) showed the neuroendocrine system of the larval blackflies to consist of three paired cerebral neurosecretory cell clusters, fused nervi corporis cardiaci, cellular corpora cardiaca, single corpus allatum and peritracheal gland. These findings were discussed in relation to the morphology of the neuroendocrine systems of other Nematocera and higher Diptera.

3) Effects of mermithid parasitism on the host were examined by comparing two groups of insects -- a control group (dark histoblast) and a late infected group. Measurements of head capsule widths and body lengths suggested that in both the simuliid species the infected and control groups were of the same larval instar. Dry weight measurements showed that the parasite reduced the overall biomass of P. mixtum/fuscum larvae.

4) Histochemical investigations showed no effect on parasitism on the endocrines of the mermithid parasitized P. mixtum/fuscum generation. However, a significant increase in nuclear DNA/RNA activity in the corpus allatum and an increase in the corpora cardiaca gland volume with higher levels of stored neurosecretory material were recorded in infected S. venustum Complex larvae. These results were discussed in relation to their significance within the host/parasite relations of both

TIGHT BINDING

blackfly species and their mermithid parasites.

5) It was shown both histologically and histochemically that mermithid parasitism caused a significant reduction of fat body tissue and glycogen levels in both simuliid species. However, the DNA fat body activity was unaffected in both infected simuliid species. Such effects on host storage products were discussed in relation to information available on other mermithid/insect associations.

TIGHT BINDING

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APPENDIX 1

Data of Size and Weight Measurements Between Control and Mermithid Infected Larvae of P. mixtum/fuscum and S. venustum Complexes +

Blackfly Species	Experimental Group	Head Capsule Width (mm)	Body Length (mm)	Dry Weight (mg)
P.mixtum/fuscum	Control	.070	.580	.634
"	"	.065	.605	.584
"	"	.075	.600	.676
"	"	.075	.620	.688
"	"	.070	.610	.776
"	"	.070	.590	.772
"	"	.065	.600	.604
"	"	.070	.600	.610
"	"	.065	.580	.452
"	Infected	.060	.575	.690
"	"	.060	.575	.488
"	"	.070	.585	.492
"	"	.065	.540	.458
"	"	.060	.540	.480
"	"	.070	.590	.530
"	"	.060	.525	.464
"	"	.065	.550	.534
"	"	.065	.575	.484
"	"	.065	.540	.495
"	"	.070	.600	.480
S.venustum Complex	Control	.060	.500	.595
"	"	.055	.505	.536
"	"	.060	.555	.500
"	"	.055	.515	.448
"	"	.060	.525	.550
"	"	.060	.510	.440
"	"	.060	.500	.568
"	"	.055	.525	.520
"	"	.060	.535	.450
"	"	.060	.540	.470
"	Infected	.060	.515	.550
"	"	.055	.500	.540
"	"	.055	.460	.492
"	"	.055	.475	.454
"	"	.055	.500	.580
"	"	.055	.470	.464
"	"	.055	.515	.468
"	"	.050	.460	.504

... continued

TIGHT BINDING

Blackfly Species	Experimental Group	Head Capsule Width (mm)	Body Length (mm)	Dry Weight (mg)
S.venustum Complex	Infected	.055	.450	.442
"	"	.050	.470	.468

Each set of data represents one individual blackfly.

COMPARISON OF MEANS

A) Head Capsule Widths

P. mixtum/fuscum	Infected vs Controls	not significant
S. venustum Complex	Infected vs Controls	not significant

B) Body Lengths

P. mixtum/fuscum	Infected vs Controls	not significant
S. venustum Complex	Infected vs Controls	not significant

C) Dry Weights

P. mixtum/fuscum	Infected vs Controls	$t = 5.097$, $p < 0.001$
S. venustum Complex	Infected vs Controls	not significant

TIGHT BINDING

APPENDIX 2

Data of Total Corpus Allatum Gland Volume of Control and Mermithid Infected Larvae of P. mixtum/fuscum and S. venustum Complexes +

Blackfly Species	Experimental Group	Micro Videomat Reading $(mm)^2$	$\mu^2 \times 10^4$	$\mu^3 \times 10^4$
P.mixtum/fuscum	Control	394	0.7880	3.1520
"	"	207	0.4140	1.6560
"	"	194	0.3880	1.5520
"	"	526	1.0520	4.2080
"	"	223	0.4460	1.7840
"	Infected	235	0.4709	1.8800
"	"	238	0.4760	1.9040
"	"	189	0.3780	1.5120
"	"	196	0.3920	1.5840
"	"	136	0.2720	1.0880
S.venustum Complex	Control	361	0.7220	2.8880
"	"	234	0.4680	1.8720
"	"	289	0.5780	2.3120
"	"	329	0.6580	2.6320
"	"	217	0.4340	1.7360
"	Infected	307	0.6140	2.4560
"	"	216	0.4320	1.6550
"	"	184	0.3680	1.4720
"	"	173	0.3460	1.3840
"	"	245	0.4900	1.9600

+ Each set of data represents the cumulative surface area measurement of each gland reading, being converted to volume in the final column (multiplying by a factor of 4μ).

COMPARISON OF MEANS

Total Corpus Allatum Gland Volume

P. mixtum/fuscum	Infected vs Controls	not significant
S. venustum Complex	Infected vs Controls	not significant

TIGHT BINDING

APPENDIX 3

Data of Corpus Allatum Nucleic Acid Concentrations of Control and Mermithid Infected Larvae of P. mixtum/fuscum and S. venustum Complexes +

Blackfly Species	Experimental Group	Spectrophotometer Reading (% Transmittance)	Absorbance Values
P. mixtum/fuscum	Control	82.5	.08364
"	"	69.2	.16009
"	"	73.1	.13816
"	"	77.7	.10946
"	"	76.2	.11811
"	"	80.0	.09697
"	Infected	78.8	.10385
"	"	80.0	.09697
"	"	82.3	.08448
"	"	79.3	.10119
S. venustum Complex	Control	75.5	.12247
"	"	74.4	.12879
"	"	73.5	.13227
"	"	75.9	.12016
"	"	77.3	.11217
"	"	77.1	.11298
"	Infected	70.9	.15022
"	"	70.1	.15460
"	"	71.7	.14589
"	"	70.4	.15283
"	"	69.1	.16071
"	"	66.0	.18129

+ Each set of data represents the average nucleic acid concentrations of each individual blackfly (expressed in % transmittance and converted to absorbance values).

COMPARISON OF MEANS

Corpus Allatum Nucleic Acid Concentrations

P. mixtum/fuscum	Infected vs Control	not significant
S. venustum Complex	Infected vs Control	$t = 6.207$, $p < 0.01$

TIGHT BINDING

APPENDIX 4

Data of Corpus Allatum Nuclear/Cytoplasmic Ratios of Control and Mermithid Infected Larvae of P. mixtum/fuscum and S. venustum Complexes +

Blackfly Species	Experimental Group	Number of Nuclei (N) of Cytoplasm (C)	Microvideomat Reading μ^2	Conversion to N/C Ratios μ	Average N/C Ratios
P.mixtum/fuscum	Control	15(N)	7	.350	
		15(C)	20		.509
		15(N)	20	.667	
		15(C)	30		
"	"	10(N)	10	.333	
		10(C)	30		.342
		2(N)	9	.300	
		2(N)	30		
		6(N)	13	.394	
		6(C)	33		
"	"	22(N)	8	.364	
		22(C)	22		.557
		22(N)	15	.750	
		22(C)	20		
"	"	20(N)	7	.438	.438
		20(C)	16		
"	"	11(N)	12	.333	
		11(C)	36		.467
		20(N)	15	.600	
		20(C)	25		
"	Infected	7(N)	14	.467	.467
		7(C)	30		
"	"	10(N)	8	.500	.500
		10(C)	16		
"	"	12(N)	.6	.500	
		12(C)	12		.459
		12(N)	5	.417	
		12(C)	12		
"	"	10(N)	9	.450	
		10(C)	20		.418
		14(N)	10	.385	
		14(C)	26		
S.venustum Complex	Control	6(N)	11	.333	.280
		6(C)	33		
		7(N)	7	.226	
		7(C)	31		

....continued

TIGHT BINDING

Blackfly Species	Experimental Group	Number of Nuclei (N) of Cytoplasm (C)	Microvideomat Reading μ^2	Conversion to N/C Ratios μ	Average N/C Ratios
S.venustum Complex	Control	6(N)	9	.273	
		6(C)	33		.288
		7(N)	13	.302	
		7(C)	43		
"	"	10(N)	12	.375	.375
		10(C)	32		
"	"	18(N)	9	.500	
		18(C)	18		.446
		14(N)	9	.391	
		14(C)	23		
"	"	8(N)	6	.240	
		8(C)	25		.287
		7(N)	6	.333	
		7(C)	18		
"	Infected	14(N)	9	.300	
		14(C)	30		.300
		16(N)	9	.300	
		16(C)	30		
"	"	12(N)	6	.230	
		12(C)	26		.267
		21(N)	7	.304	
		21(C)	23		
"	"	15(N)	9	.429	
		15(C)	21		.405
		16(N)	8	.381	
		16(C)	21		
"	"	11(N)	13	.325	
		11(C)	40		.355
		6(N)	13	.385	
		6(C)	34		

+ Each set of data represents the average Nuclear/cytoplasmic ratios of each individual blackfly.

COMPARISON OF MEANS

Corpus allatum nuclear/cytoplasmic Ratios

P. mixtum/fuscum	Infected vs Control	not significant
S. venustum Complex	Infected vs Control	not significant

TIGHT BINDING

APPENDIX 5

Data of Corpora Cardiaca Gland Volume of Control and Mermithid Infected Larvae of *P. mixtum/fuscum* and *S. venustum* Complexes+

Blackfly Species	Experimental Group	Microvideomat Reading $(mm)^2$	$\mu^2 \times 10^4$	$\mu^3 \times 10^4$
P. mixtum/fuscum	Control	648	1.2960	5.1840
"	"	571	1.1420	4.5680
"	"	320	0.6400	2.5600
"	"	649	1.1630	4.6420
"	"	210	0.4200	1.6800
"	Infected	480	0.9600	3.8400
"	"	330	0.6600	2.4400
"	"	310	0.6200	2.4800
"	"	202	0.4000	1.6200
"	"	521	0.9620	3.8480
S. venustum Complex	Control	232	0.4650	1.8600
"	"	174	0.3480	1.3260
"	"	327	0.6540	2.4000
"	"	177	0.3540	1.4160
"	"	364	0.7280	2.6720
"	Infected	957	1.9140	7.6560
"	"	493	0.9860	3.9440
"	"	242	0.4840	1.9360
"	"	609	1.2180	4.8720
"	"	949	1.8980	7.5920

+ Each set of data represents the cumulative surface area measurement of each gland reading, being converted to volume in the final column (multiplying by a factor of 4μ).

COMPARISON OF MEANS

Total Corpora Cardiaca Gland Volume

P. mixtum/fuscum Infected vs Control not significant
S. venustum Complex Infected vs Control $t = 2.889$, $p < 0.05$

TIGHT BINDING

APPENDIX 6

Data of Corpora Cardiaca Neurosecretory Material Concentrations of Control and Mermithid Infected Larvae of P. mixtum/fuscum and S. venustum Complexes +

Blackfly Species	Experimental Group	Spectrophotometer Reading (% Transmittance)	Absorbance Values
P. mixtum/fuscum	Control	78.8	.10336
"	"	76.9	.11466
"	"	75.6	.12013
"	"	78.1	.10741
"	"	81.4	.09043
"	"	76.8	.11479
"	"	79.0	.10286
"	Infected	77.0	.11373
"	"	77.6	.11037
"	"	79.1	.10192
"	"	76.8	.11526
"	"	78.5	.10554
"	"	78.3	.10621
"	"	78.4	.10563
S. venustum Complex	Control	74.2	.12978
"	"	75.7	.12089
"	"	78.6	.10478
"	"	75.4	.12263
"	"	83.9	.07646
"	"	81.3	.09588
"	"	81.7	.08781
"	Infected	72.1	.14207
"	"	69.3	.15978
"	"	75.0	.12506
"	"	71.2	.14793
"	"	76.5	.11673
"	"	74.8	.12652
"	"	77.5	.11087

+ Each set of data represents the average neurosecretory material concentrations of each individual blackfly.

COMPARISON OF MEANS

Corpora Cardiaca Neurosecretory Material

P. mixtum/fuscum Infected vs Control not significant
S. venustum Complex Infected vs Control $t = 2.540$, $p < 0.05$

TIGHT BINDING

APPENDIX 7

Data of Corpora Cardiaca Nuclear/Cytoplasmic Ratios Mermithid Infected Larvae of P. mixtum/fuscum and S. venustum Complexes +

Blackfly Species	Experimental Group	Number of Nuclei (N) of Cytoplasm (C)	Microvideomat Reading μ^2	Conversion to N/C Ratios μ	Average N/C Ratios
P.mixtum/fuscum	Control	2(N)	25	.227	
		2(C)	110		
		3(N)	20	.286	
		3(C)	70		
		1(N)	24	.160	.208
		1(C)	150		
		2(N)	24	.160	
		2(C)	150		
	"	2(N)	13	.186	.143
		2(C)	70		
		1(N)	20	.100	
		1(C)	200		
	"	2(N)	19	.271	
		2(C)	70		
		1(N)	44	.220	
		1(C)	200		
		1(N)	44	.200	
		1(C)	220		.230
	"	2(N)	16	.160	
		2(C)	100		
		2(N)	30	.300	
		2(C)	100		.230
	"	2(N)	17	.243	
		2(C)	70		
		2(N)	24	.218	.234
		2(C)	110		
		1(N)	24	.240	
		1(C)	100		
	Infected	3(N)	15	.188	
		3(C)	80		
		2(N)	20	.182	.185
		2(C)	110		
	"	2(N)	20	.133	
		2(C)	150		
		1(N)	32	.146	
		1(C)	220		.154
		2(N)	22	.138	
		2(C)	160		

....continued

TIGHT BINDING

Blackfly Species	Experimental Group	Number of Nuclei (N) of Cyto-plasm (C)	Microvideomat Reading μ^2	Conversion to N/C Ratios μ	Average N/C Ratios
		2(N)	22	.200	
		2(C)	110		
"	"	2(N)	35	.184	
		2(C)	190		.163
		2(N)	24	.142	
		2(C)	170		
"	"	2(N)	35	.175	.175
		2(C)	200		
S.venustum Complex	Control	2(N)	23	.230	.230
		2(C)	100		
"	"	7(N)	24	.126	
		7(C)	190		.119
		1(N)	20	.111	
		1(C)	180		
"	"	1(N)	18	.090	
		1(C)	200		.104
		2(N)	13	.118	
		2(C)	110		
"	"	1(N)	20	.125	
		1(C)	160		.106
		1(N)	12	.086	
		1(C)	140		
"	Infected	1(N)	26	.152	
		1(C)	170		.165
		2(N)	25	.178	
		2(C)	140		
"	"	2(N)	10	.059	
		2(C)	170		
		2(N)	18	.100	.082
		2(C)	180		
		1(N)	22	.073	
		1(C)	300		
		2(N)	15	.094	
		2(C)	160		
"	"	2(N)	20	.167	.130
		2(C)	120		
		2(N)	14	.093	
		2(C)	150		
"	"	1(N)	22	.085	
		1(C)	260		
		1(N)	26	.118	.114
		1(C)	220		
		2(N)	26	.140	
		2(C)	100		

....continued

TIGHT BINDING

+ Each set of data represents the average Nuclear/Cytoplasmic ratios of each individual blackfly.

COMPARISON OF MEANS

Corpora Cardiaca Nuclear/Cytoplasmic Ratios

P. mixtum/fuscum	Infected vs Control	not significant
S. venustum Complex	Infected vs Control	not significant

TIGHT BINDING

APPENDIX 8

Data of Corpora Cardiaca Nucleic Acid Concentrations of Control and Mermithid Infected Larvae of P. mixtum/fuscum and S. venustum Complexes +

Blackfly Species	Experimental Group	Spectrophotometer Reading (% Transmittance)	Absorbance Values
P. mixtum/fuscum	Control	86.7	.06202
"	"	68.9	.16243
"	"	70.3	.15858
"	"	77.0	.11320
"	"	70.8	.15100
"	"	77.9	.10877
"	Infected	75.5	.12229
"	"	78.3	.10611
"	"	75.0	.12516
"	"	73.7	.13308
S. venustum Complex	Control	79.0	.10240
"	"	70.7	.15062
"	"	75.7	.12120
"	"	79.7	.09879
"	"	66.0	.18141
"	Infected	74.5	.12826
"	"	77.0	.11389
"	"	76.3	.11775
"	"	76.2	.11845
"	"	71.6	.14543
"	"	74.5	.12838

+ Each set of data represents the average nucleic acid concentrations of each individual blackfly.

COMPARISON OF MEANS

Corpora Cardiaca Nucleic Acid Concentrations

P. mixtum/fuscum	Infected vs Control	not significant
S. venustum Complex	Infected vs Control	not significant

TIGHT BINDING

APPENDIX 9

Data of Fat Body Glycogen Level Concentrations of Control and Mermithid Infected Larvae of P. mixtum/fuscum and S. venustum Complexes +

Blackfly Species	Experimental Group	Spectrophotometer Reading (% Transmittance)	Absorbance Values
P. mixtum/fuscum	Control	78.2	.10978
"	"	76.1	.11837
"	"	75.1	.12472
"	"	77.5	.11113
"	"	76.9	.11467
"	"	73.7	.13084
"	"	72.0	.12365
"	Infected	83.3	.08035
"	"	83.2	.07945
"	"	84.2	.07494
"	"	84.4	.07405
"	"	86.8	.06166
"	"	82.4	.08414
"	"	83.1	.08115
S. venustum Complex	Control	76.4	.11742
"	"	80.7	.09331
"	"	82.0	.08678
"	"	83.4	.07931
"	"	80.0	.09707
"	"	80.7	.09359
"	Infected	83.8	.07687
"	"	85.3	.06926
"	"	83.3	.07939
"	"	84.9	.07143
"	"	83.1	.08071

+ Each set of data represents the average fat body glycogen level concentrations of each individual blackfly.

COMPARISON OF MEANS

Fat Body Glycogen Level Concentrations

P. mixtum/fuscum Infected vs Control $t = 10.750$, $p < 0.001$
S. venustum Complex Infected vs Control $t = 3.704$, $p < 0.02$

TIGHT BINDING

APPENDIX 10

Data of Fat Body Nucleic Acid Concentrations of Control and Mermithid Infected Larvae of P. mixtum/fuscum and S. venustum Complexes +

Blackfly Species	Experimental Group	Spectrophotometer Reading (% Transmittance)	Absorbance Values
P. mixtum/fuscum	Control	85.7	.06715
"	"	87.7	.05725
"	"	86.9	.06108
"	"	88.3	.05433
"	"	88.5	.05307
"	"	87.4	.05834
"	Infected	88.0	.05552
"	"	82.9	.08164
"	"	87.8	.05679
"	"	88.6	.05273
"	"	89.6	.04702
S. venustum Complex	Control	89.0	.05063
"	"	86.6	.06273
"	"	88.5	.05311
"	"	89.4	.04880
"	"	89.3	.04923
"	"	89.0	.05063
"	Infected	87.9	.05625
"	"	89.0	.05063
"	"	88.5	.05308
"	"	87.3	.05908
"	"	88.9	.05123
"	"	88.4	.05369

+ Each set of data represents the average fat body nucleic acid concentrations of each individual blackfly.

COMPARISON OF MEANS

Fat Body Nucleic Acid Concentrations

P. mixtum/fuscum	Infected vs Control	not significant
S. venustum Complex	Infected vs Control	not significant

TIGHT BINDING