THE UNIVERSITY OF MANITOBA

GONAD MORPHOGENESIS AND EFFECT OF GROWTH INHIBITORS ON THE FREE LIVING NEMATODE PANAGRELLUS REDIVIVUS (LINN.)

by

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ACKNOWLEDGMENT

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I would also like to thank Dr. Ken Stewart and Dr. Dave Burton for their comprehensive review and valuable criticism of this thesis, and Dr. Harvey Wiseman for being an excellent chairman.

A special thanks to Mr. Wolf Heck for his assistance with some of the photographic work.

To my wife Avrian for her devotion and support these past three years and for her ability as a typist I dedicate this thesis.

To Avrian

CONTENTS

ABSTRACT

The postembryonic development of *P. redivivus* comprises four larval stages and an adult stage. The three free-living larval stages can be recognized on the basis of the degree of development of the reproductive system. A continuous and gradual multiplication of the nuclei of the gonad takes place throughout the larval stages. The sexes can be separated even at the time of the second moult by a lobe of somatic cells in the gonad, directed anteriorly in the males and posteriorly in the females. The lobe in the males reflexes posteriorly at the third moult and at the fourth moult joins the rectum. Sperm are first produced at the late fourth moult. Third moult females have a thickened vaginal primordium and at the fourth moult the spermathecal and uterine primordia are evident. In the adult female the uterus elongates. Eggs are not produced until after copulation and hatch in the uterus. It is concluded that gonad development can be characterized by three stage specific processes. Second stage gonad primordia display active nuclear divisions without much growth, while substantial growth of the primordium begins with the completion of the third moult. The final differentiation of the various gonadal parts takes place only after the fourth moult.

The effects of inhibitors of RNA, protein and DNA synthesis on growth and gonad development during synchronized postembryonic development of P. redivivus was examined. Actinomycin D and Actidione blocked growth and gonad development at each stage but to different degrees, while hydroxyurea had only slightly inhibitory effect on growth but stopped gonad differentiation.

The uncoupling of growth and gonad development as a result of inhibition with AMD and actidione suggests that the macromolecular information for division and proliferation of the gonad is laid down quite early, probably during the third moult or late second stage juvenile, while the informational synthesis for differentiation occurs quite late in the fourth stage larvae. Fourth stage larvae placed in AMD or actidione all failed to undergo gonad differentiation. Hydroxyurea blocked the morphological organization of the gonad but had little if any effect on growth; a high level of cell proliferation is evident while gonad differentiation is never achieved. Renewal of DNA synthesis in the fourth moult is required to complete development.

INTRODUCTION

Nematodes are eutelic, that is they have a fixed number of nuclei that are maintained throughout the entire life of the organism (Hyman, 1951). Growth is therefore due to cell enlargement and not to an increase in cell number. The reproductive system is the principle exception to cell constancy, showing a sequential pattern of growth, proliferation and differentiation of cells. Sin and Pasternak (1971) have shown that the free-living nematode Panagrellus silusiae is not strictly eutelic; showing some proliferation of the muscle nuclei.

Pasternak and Samoiloff (1970), have demonstrated that DNA, RNA and protein synthesis are concomitant and continuous during the growth of the nematode, Panagrellus silusiae. Proteins synthesized in the gonad of P. silusiae are different from those in the somatic tissue (Chow and Pasternak, 1969), suggesting that differentiation of the gonad at the fourth moult is dependent upon a sequence of primary biochemical events involving RNA and protein synthesis. Changing patterns of organelle morphology also occur at this time (Pasternak and Samoiloff, 1972). It can be anticipated therefore that alteration of DNA, RNA and protein synthesis would result in morphological

changes in the gonad.

Inhibition of DNA, RNA and protein synthesis in synchronous cultures of the free-living nematode has permitted analysis of the requirement for macromolecular synthesis during postembryonic growth of P. silusiae (Pasternak and Samoiloff, 1970).

This work was carried out to describe the normal pattern of development of the reproductive system and to discover the patterns of macromolecular synthesis during this development in Panagrellus redivivus (Anderson, pers. comm.).

MATERIALS AND METHODS

Nematode Cultures

P. redivivus was maintained xenically on 4.0% czapek dox agar (BBL) in petri plates at room temperature. Populations in excess of 33,000 animals per ml were obtained after 12-14 days under these conditions.

The first postembryonic stage, *in utero*, is called the L1 stage and the next four postpartum stages are designated L2, L3, L4 and adult respectively. The simplest criterion for recognizing the various stages in the life cycle is the length of the worm (Samoiloff and Pasternak, 1968). The mean lengths of; 338 ± 28μ (standard error), 502 ± 13μ, 662 ± 42μ and 881 ± 109μ respectively define the mid points of L2, L3, L4 and young adult stages of *P. redivivus*.

Isolation Procedure

Mass cultures of nematodes from eight petri plates were freed of agar by passing the contents of each plate through two layers of facial tissue. The worms were passed through a 125 ml separatory funnel packed with a mixture of glass microbeads (0.50 mm & 0.30 mm in diameter 1:1). The first 10 to 20 ml of effluent contained exclusively early second stage

larvae (284 ± 31μ (s.e.)). The larvae were concentrated by centrifugation for 5 minutes at 1400Xg and re-suspended in 40 ml distilled water. They were then distributed into three spot depression plates containing 4 drops of Nigon's Agar (Dougherty, 1960), 2 drops of animals and 6 drops of inhibitor solution or water. Each depression contained approximately 100 nematodes and each experiment consisted of 100 depressions; 60 experimental and 40 control. The cultures were covered and placed in a moist chamber at 27°C. The resulting growth to maturation was synchronous.

Staining Procedure

Samples were taken at 12 hour intervals, washed twice in distilled water, heat killed on a slide and fixed for 10 minutes in Carnoy's Fluid (absolute alcohol; chloroform; glacial acetic acid; 6:3:1). For observation of growth rate and gonad development two different staining procedures were followed.

Temporary preparations were made employing a modification of Mulvey's (1960) technique. The fixed nematodes were air dried and stained with three or four drops of propionic orcein. A cover slip was placed over the drops and the slide was warmed for a few

minutes at 40°C. Excess stain was absorbed and the mount sealed with polish. Slides were examined 1 to 18 hours after staining depending on the stage of gonad development of the nematode sample.

Permanent preparations were made on "subbed" slides stained in 0.5% Azur II in 1% sodium borate for 1 to 2 minutes. The specimens were dehydrated in ethanol, counterstained with 0.5% eosin in ethanol, cleared in xylene and mounted in DPX.

The stained whole mounts were examined and selected gonad development was photographed using a Carl Zeiss automatic photomicroscope.

Chemicals

Actinomycin D and Act-Dione (cyclo-heximide) (Calbiochem) and hydroxyurea (B.D.H.) were used as inhibitors of RNA, protein and DNA respectively. The effective concentrations of each inhibitor on nematode growth and development were previously established (Pasternak and Samoiloff, 1970). Inhibitor solutions were added to synchronously growing populations at various times during the postembryonic period at final concentrations of 20, 10 and 6 µg/ml for Actinomycin D; 200 and 100 µg/ml for Act-Dione; and 600 and 400 µg/ml for hydroxyurea.

RESULTS

Normal Postembryonic Development

The measurements of the different larval stages are presented in Table I. Three larval stages occur between hatching and the adult stage. The first stage is *in utero* and was not examined in the present study.

Second Stage: The genital primordium measures 8.2 ± 1.59μ (s.e.) and is situated near the mid body region of the nematodes. It consists of four cells; two large centrally located germinal nuclei and a smaller somatic nucleus at each end (Fig. 1 and 12). During the moult, division of the somatic nuclei takes place (Fig. 2, 3, 12 and 13), the genital primordium enlarges and a small lobe appears which projects anteriorly in males (Fig. 4 and 5) and posteriorly in females (Fig. 14, 15 and 16).

Third Stage: The third stage is characterized by considerable cell proliferation and growth of the genital primordium, with the gonad finally flexing back on itself directed dorsally in females (Fig. 17, 18 and 19) and ventrally in males (Fig. 6, 7, 8 and 9). The greatest elongation is in the somatic part of the

TABLE I. Morphometrics of the different stages of development of *Panagrellus redivivus*.

STAGE	BODY LENGTH	GONAD LENGTH
L_2	338 ± 46μ (s.e.)	8 ± 2μ (s.e.)
L_3 Female	632 ± 72μ	134 ± 14μ
L_3 Male	630 ± 36μ	60 ± 29μ
L_4 Female	958 ± 138μ	409 ± 13μ
L_4 Male	804 ± 75μ	393 ± 8μ
Adult Female	1341 ± 108μ	1366 ± 134μ
Adult Male	1356 ± 133μ	892 ± 112μ

gonad which lengthens posteriorly until at the end of the third stage it has reached an overall length of 134 ± 14.4μ in females (Fig. 20) and 60 ± 29.1μ in the males. In the third stage male the primordial spicule pouch is visible as a thickening surrounding the rectum, while in females the vaginal primordium is barely detectable.

Fourth Stage: Regional differentiation of the adult gonad is manifested in the gonads of fourth stage larvae. In the females the ovary and uterus begin to differentiate (Fig. 21). The uterus grows considerably and occupies most of the body length. A thickened posterior uterine sac is also visible. The vagina becomes lined with cuticle and the external vulval opening appears. The final fourth stage gonad has a mean overall length of 409 ± 13.3μ (s.e.) (Fig. 22). Fourth stage males show differentiation of vas deferens and testis. The greatest elongation of the gonad is in the growth zone (Fig. 10) which grows posteriorly toward the rectum and finally, after the moult, becomes linked to the rectum to form the cloaca. At the final moult meiosis in the testis has begun and several mature sperm can be seen (Fig. 11). The final fourth stage male gonad reaches a mean overall length of 393 ± 7.5μ (s.e.).

Adult: The adult female gonad is considerably larger than the male gonad (1366 ± 133μ vs 892 ± 112μ (s.e.)). The uterus is thick walled with faint striae marking the epithelial cell boundaries sometimes visible in surface view throughout the length of the uterus. Developing eggs and sperm are often seen in the uterus after copulation. The vagina has muscular walls and a transverse vulva split. The ovary is long, extending sometimes past the post-vulvar sac (Fig. 23). The adult male testis is located in the anterior flexed portion of the reproductive tract. Spermatogonia are located in the anterior half of the gonad. Mature sperm are rarely seen in the vas deferens.

Life Cycle: Reproduction in P. redivivus is amphimictic. The females are normally ovoviviparous. The life cycle from egg to egg requires 120 hours at 25°C on 4% czapek dox agar. Four moults and four larval stages occur. The first and second stage last for 46 hours, the third stage 28 hours, the fourth stage 36 hours and adults produce eggs in 12 hours.

Inhibition of RNA Synthesis

There are two general responses to inhibition of RNA synthesis: (1) normal growth of the worm is inhibited and (2) normal development of the reproductive

system is blocked (Pasternak and Samoiloff, 1970). Actinomycin D (40 µg/ml) was used to induce these changes at three specific intervals in the life cycle of P. redivivus. They were: second juvenile stage (0 hours) when the gonad is undergoing active nuclear division; third juvenile stage (25-40 hours) at which time the gonad is undergoing proliferation, and the fourth juvenile stage (70-80 hours) in which active growth and differentiation characterize gonad development. Second stage juvenile animals allowed to develop in the presence of Actinomycin D for 96 hours (Fig. 42) show that 100% of the animals are still in the second stage. Both body length and gonad development were inhibited to the same degree (Fig. 24 and 25). However, if third stage larvae were placed in Actinomycin D (Fig. 42) 78% had gonads characteristic of fourth stage juveniles, while 79% failed to undergo growth of body length past the third stage (Fig. 26 and 27). Thus gonad development is continuing while animal growth is inhibited. This uncoupling of growth and gonad development can be seen more dramatically with fourth stage larvae (Fig. 42) placed in Actinomycin D. Eighty-four percent show gonad development past the fourth larval stage while only 52% continue to grow. Complete differentiation of the gonad however is never achieved (Fig. 28 and 29). The inhibition was found to

be reversible when the animals were returned to antibiotic free medium except in the case of the second stage juveniles. In all stages growth is disrupted and development begins after an initial lag phase, with 78% of the nematodes reaching sexual maturity.

Inhibition of Protein Synthesis

Previous results obtained with actidione have shown that P. silusiae must maintain protein synthesis for growth (Pasternak and Samoiloff, 1970). Development of P. redivivus placed in actidione (400 µg/ml) as second stage larvae (Fig. 43) was inhibited. Ninety percent showed no growth or gonad development (Fig. 30 and 31) beyond the second stage. Sixty percent of third stage larvae, which were allowed to develop in contact with the inhibitor solution (Fig. 43) showed gonad development past the third stage while 75% grew to the fourth stage, (Fig. 32 and 33). Sixty eight percent of fourth stage larvae which were placed in actidione solution (Fig. 43), showed no gonad development past the fourth juvenile stage, while 100% proceeded to grow to the adult stage. Differentiation of the reproductive system was blocked. (Fig. 34 and 35). It seems that in contrast to RNA synthesis there is a greater requirement for protein synthesis in gonad development than for growth, as gonad development was inhibited to a greater extent than growth.

Animals returned to antibiotic free medium resumed normal growth and development after an initial lag phase in 90% of the animals tested. Only those animals in contact with the inhibitor solution for more than 48 hours failed to recover completely.

Inhibition of DNA Synthesis

In P. silusiae hydroxyurea was found to block RNA and DNA synthesis by about 50% and markedly interferred with protein synthesis, with little effect on growth (Pasternak and Samoiloff, 1970). However, hydroxyurea blocked development of the P. silusiae reproductive system. Three separate experiments were carried out using hydroxyurea (600 µg/ml) to examine this interference with development of the reproductive system. Second stage juvenile animals in inhibitor solution (Fig. 14) showed altered gonad development. Examination revealed that only 22% of treated worms had normal gonads while 60% had incomplete gonads and 18% had gonads not developed past the third juvenile stage (Fig. 36 and 37). A high level of cell proliferation was evident in the incomplete gonads but cell differentiation had been completely blocked (Fig. 41). In most cases the growth zone of the gonad had not matured (Fig. 38, 39 and 40). The worms did not display this variable effect in body growth, with 80% showing lengths equal to the adult stage. Recovery

from hydroxyurea was apparent within a very short time upon return to antibiotic-free medium. In over 96% of the cases, sexual maturity was reached, and copulation occurred.

DISCUSSION

Normal gonad development can be characterized by three stage specific processes. Second stage gonad primordia display active nuclear division without much growth, while substantial growth of the primordium begins with the completion of the third moult. The final differentiation of the various gonadal parts takes place only after the fourth moult (Fig. 45). This is in agreement with a previous study on the gametogenesis, chromosome number and reproduction of P. redivivus (Hechler, 1970). However, this study has denoted the times of differential cell activities and has demonstrated a discrete temporal sequence of events involving nuclear divisions, cell proliferation and differentiation with the onset of meiotic activities during the development of the reproductive system.

Gonad Development and Inhibition of Macromolecular Synthesis

Actinomycin-D (AMD) causes a block in protein synthesis by binding to the guanine bases of DNA, preventing genetic transcription by RNA polymerase, and, therefore inhibiting DNA dependent RNA synthesis, (Reich and Goldberg, 1964; Muller and Crothers, 1968). In this study AMD has been useful in studying gene activity during development of P. redivivus. The fact

that second stage juvenile larvae did not develop any further in the presence of AMD shows that the information, in the form of RNA, for proliferation and differentiation of the gonad is laid down after this stage. When third and fourth stage juvenile larvae are placed in AMD, gonad development occurs to the next moult while growth of the worm is inhibited. This uncoupling of growth and gonad development is surprising. It is expected that the effect of the inhibitor would be an immediate one as in the case of the second stage larvae (T_0 results). The fact that AMD had a greater effect on growth than on gonad development indicates that continued RNA synthesis is relatively more important in growth of the worm than in gonad development. The results further indicate that development of the reproductive system at each moult depends upon bursts of m-RNA, especially during the fourth moult when gonad differentiation takes place. Thus, the effect of AMD is more a function of when it is applied, as the pool of preformed m-RNA is sufficient to continue gonad development to the stage where the next burst of m-RNA is made. Whenever the inhibitor is applied the nematodes will proceed to develop to the next moult, using up the supply of pre-synthesized m-RNA. Whatever macromolecular synthesis is required for morphogenesis, it is required in large

doses at the beginning of each moult to be used for the rest of moult. This concept of pre-synthesized and possibly stored informational RNA for use in protein synthesis is not a new one and has been well documented in the development of sea urchins and amphibian embryos (Gross and Cousinea, 1964; Gross et all, 1964, Gross, 1968 and Brachet, 1968).

Actidione is capable of blocking DNA, RNA and/or protein synthesis in different biological systems (Bennet et all 1964; deKloet, 1966, Siegel and Sisler, 1963). In P. silusiae it inhibits all three (Pasternak and Samoiloff, 1970). The main action of the antibiotic is however inhibition of protein biosynthesis. The transfer of amino acids from t-RNA to the ribosomes is considered to be the primary site of action of actidione. Other effects are probably a secondary result of this interference with protein biosynthesis (Siegel and Sisler, 1964). In P. redivivus protein synthesis must be maintained throughout the entire life cycle for growth. This conforms to previous work with P. silusiae (Pasternak and Samoiloff, 1970). Second stage juvenile larvae allowed to develop in the presence of actidione were inhibited. No growth and gonad development were detected. This in itself suggests that information transfer for growth and further development of the

gonad is laid down later, during the third stage. The uncoupling of growth and gonad development was apparent once more when third and fourth stage larvae were allowed to complete development in actidione solution. This time, however, it was gonad development which was inhibited to a greater degree than growth. There seems to be a greater requirement for protein synthesis in gonad development than for body growth. The effect of the actidione was not immediate in the later stages of the life cycle as expected, instead the animals proceeded to develop to the next moult suggesting that assembly of preformed protein is important in later formation of the reproductive system. Ultrastructural reorganization without obvious synthesis has been demonstrated in spermiogenesis in P. silusiae (Pasternak and Samoiloff, 1972). The similarity in the results obtained with AMD and Actidione suggest very strongly that more than control of transcription occurs in sequential gene activation during development. Presynthesized m-RNA may be stored for later use in protein synthesis.

Table II summarizes the developmental events that take place at each stage in the life cycle of P. redivivus. It can be seen that the informational synthesis for division and proliferation of the gonad

is laid down quite early, probably during the third moult or late second stage juvenile stage, while the informational synthesis for differentiation occurs quite late in the fourth stage larvae. This is shown by the fact that fourth stage larvae placed in AMD or actidione all failed to undergo gonad differentiation.

Hydroxyurea is a potent inhibitor of DNA synthesis (Young and Hodas, 1964). Hydroxyurea blocks morphological organization of the gonad but has little, if any, effect on growth. The morphological effect seemed variable. In all cases a high level of cell proliferation is evident while gonad differentiation is never achieved. This suggests that the complete renewal of DNA synthesis in the fourth stage is required for differentiation of the gonad. The nematode Turbatrix aceti develops only a rudimentary reproductive system in the presence of hydroxyurea (Gershon, 1970). Several explanations are possible. There may not be sufficient DNA for differentiation in the gonad. This could come about if the DNA were to become diminished by failure of complete synthesis of the chromosomes at each division as the cells divide mitotically in the gonad. DNA synthesis is inhibited but cell division continues resulting in an aneuploid nucleus with random parcelling out of the chromosomal

material at each division. Dilution due to inhibition of DNA synthesis would thus be a random event resulting in the variability in abnormal gonad development that is observed. A second explanation is that the information for differentiation is regulated at the level of replication. Pasternak and Samoiloff (1970) showed 50% inhibition of DNA synthesis with hydroxyurea. It is possible that this level of DNA synthesis is sufficient for cell proliferation but not for selected replication of informational DNA (Bell, 1969). This could explain why the animals recover quickly from hydroxyurea and complete gonad development. Bell (1971) has shown that hydroxyurea depresses "I-DNA" synthesis preferentially but does not affect DNA transport between cell compartments. However, one would expect a constant effect in the gonad which is not seen. The variability may reflect the multiplicity of replication dependant events associated with the final differentiation of the reproductive system.

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FIGURES 1-41 - Postembryonic development of the reproductive system in *Panagrellus redivivus*. Propionic-orcein stain, Scale = 10μ.

FIGURE 1 - Genital primordium of first moult larvae. 1000X.

FIGURE 2 and 3 - Second Stage. 1000X.

FIGURE 4 - Late second stage male. 1000X.

FIGURE 5 - Second moult male lobe directed anteriorly. 1000X.

FIGURE 6 - Early third stage male. 250X.

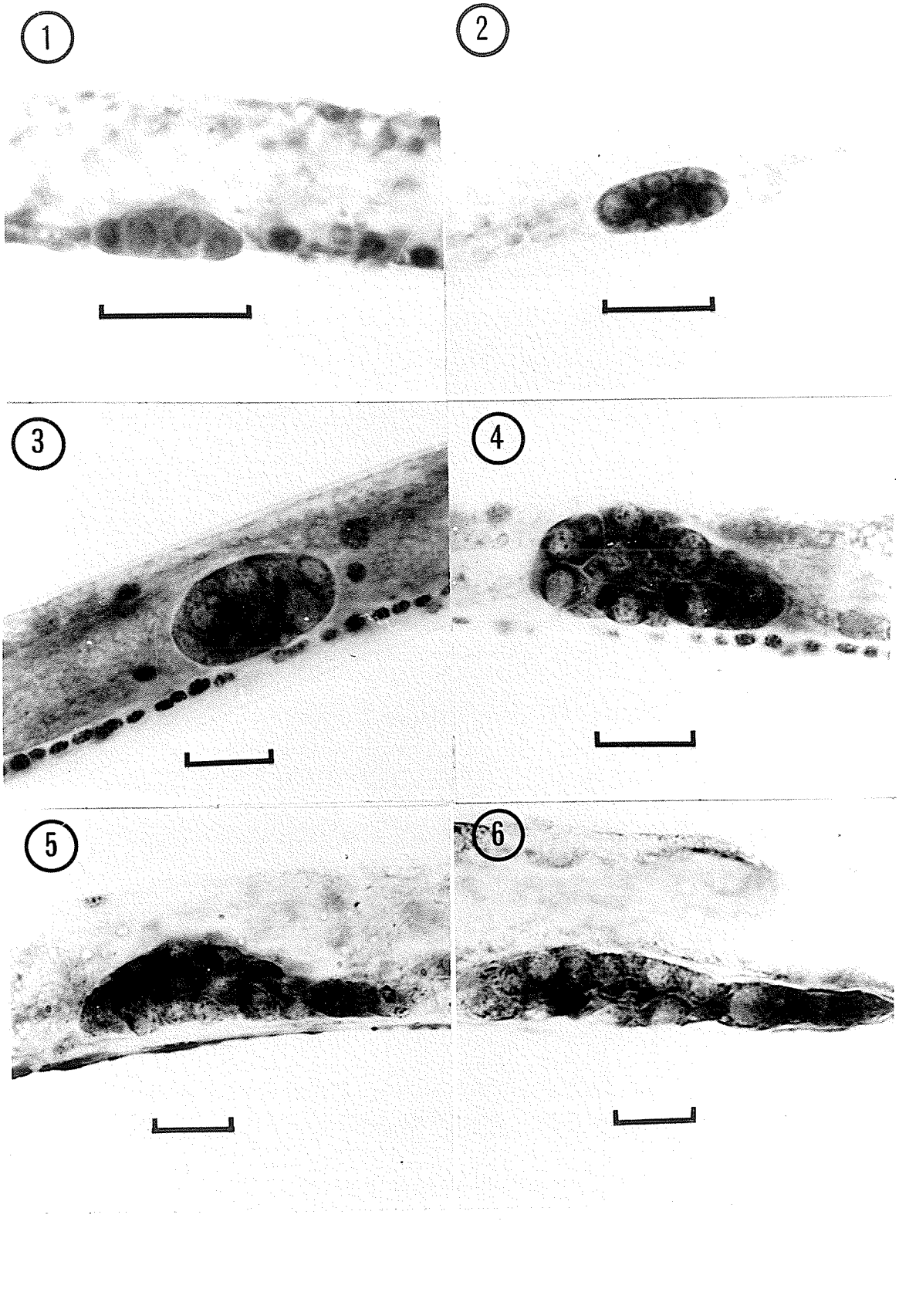
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FIGURE 7 - Third stage male. 250X.

FIGURE 8 - Third moult male. 250X.

FIGURE 9 - Early fourth stage male. Reflex is located ventrally. 250X.

FIGURE 10 - Fourth stage male. 160X

FIGURE 11 - Adult male. 63X.

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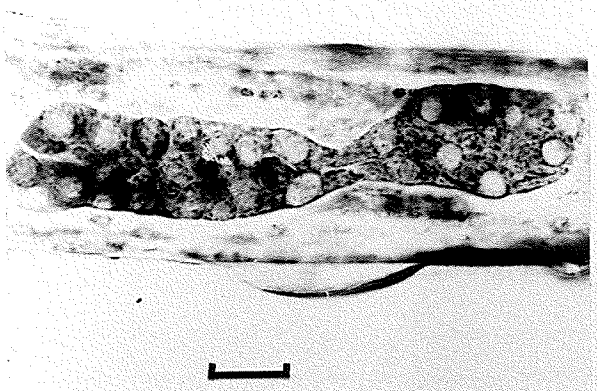

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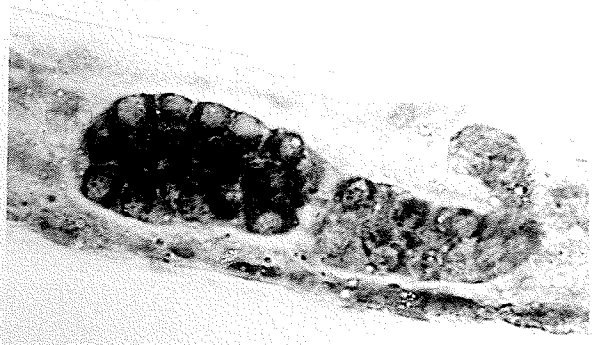

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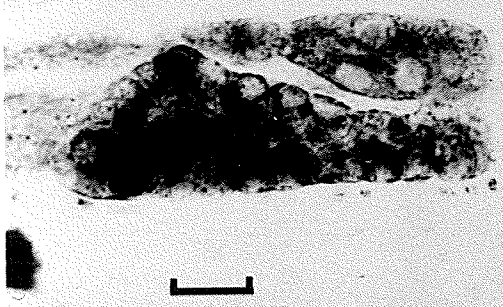

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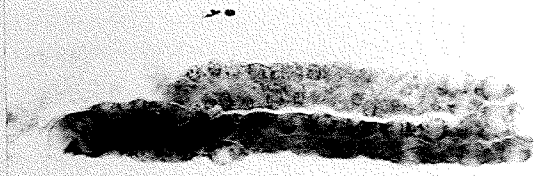

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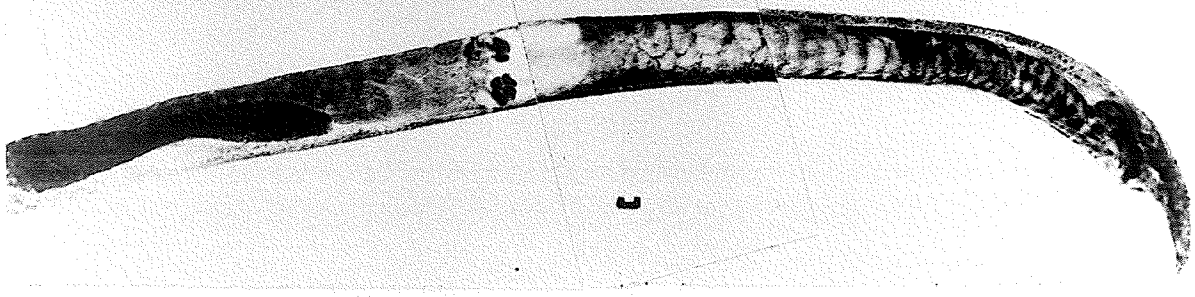


FIGURE 12 - Genital primordium of first moult larva. 1000X.

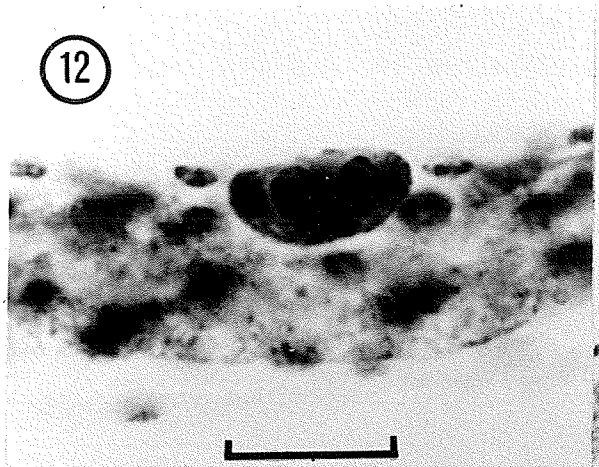
FIGURE 13 - Second stage gonad. 1000X.

FIGURE 14 - Late second stage female. 250X.

FIGURE 15 - Second moult female. Lobe directed posteriorly. 250X.

FIGURE 16 - Third stage female. 160X.

FIGURE 17 - Third moult female. Reflex is dorsally located. 160X.



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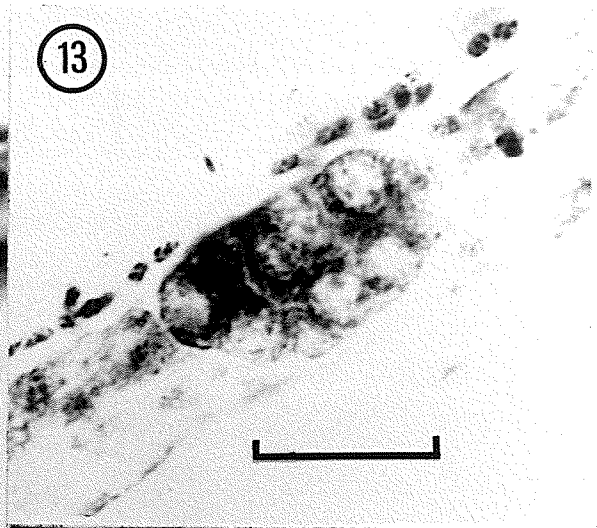

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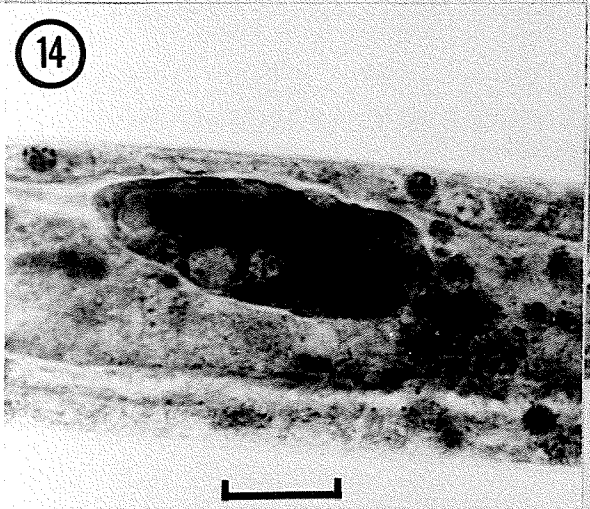

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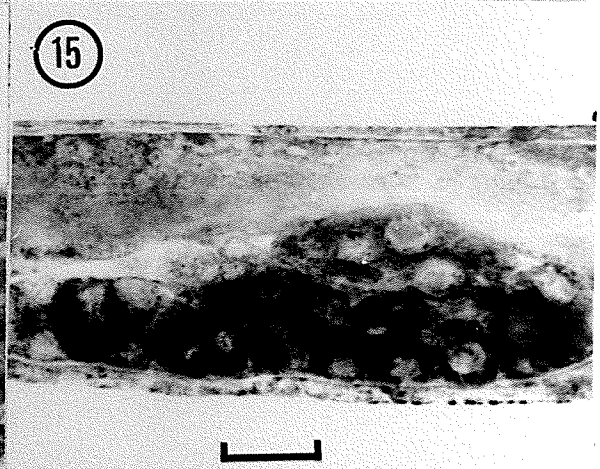

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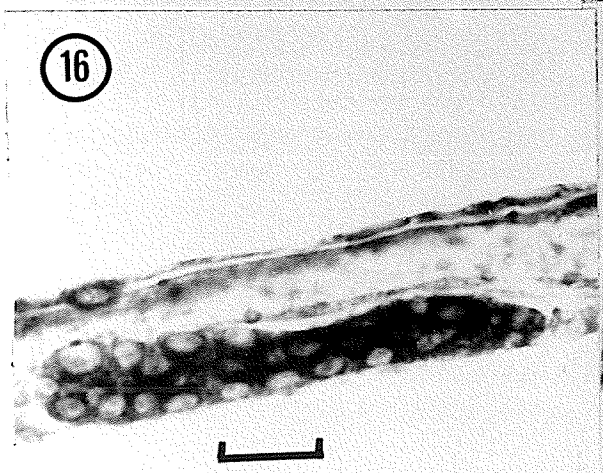

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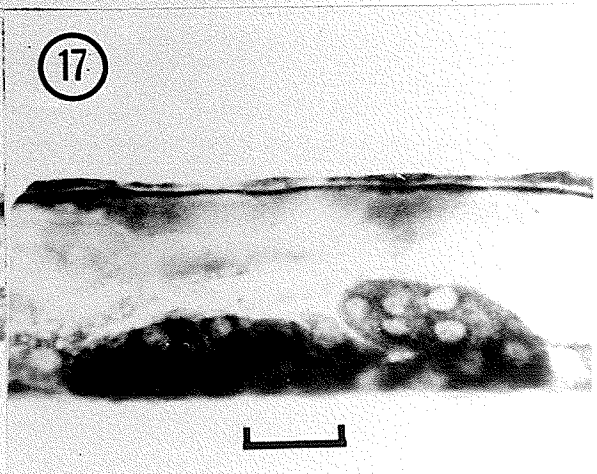

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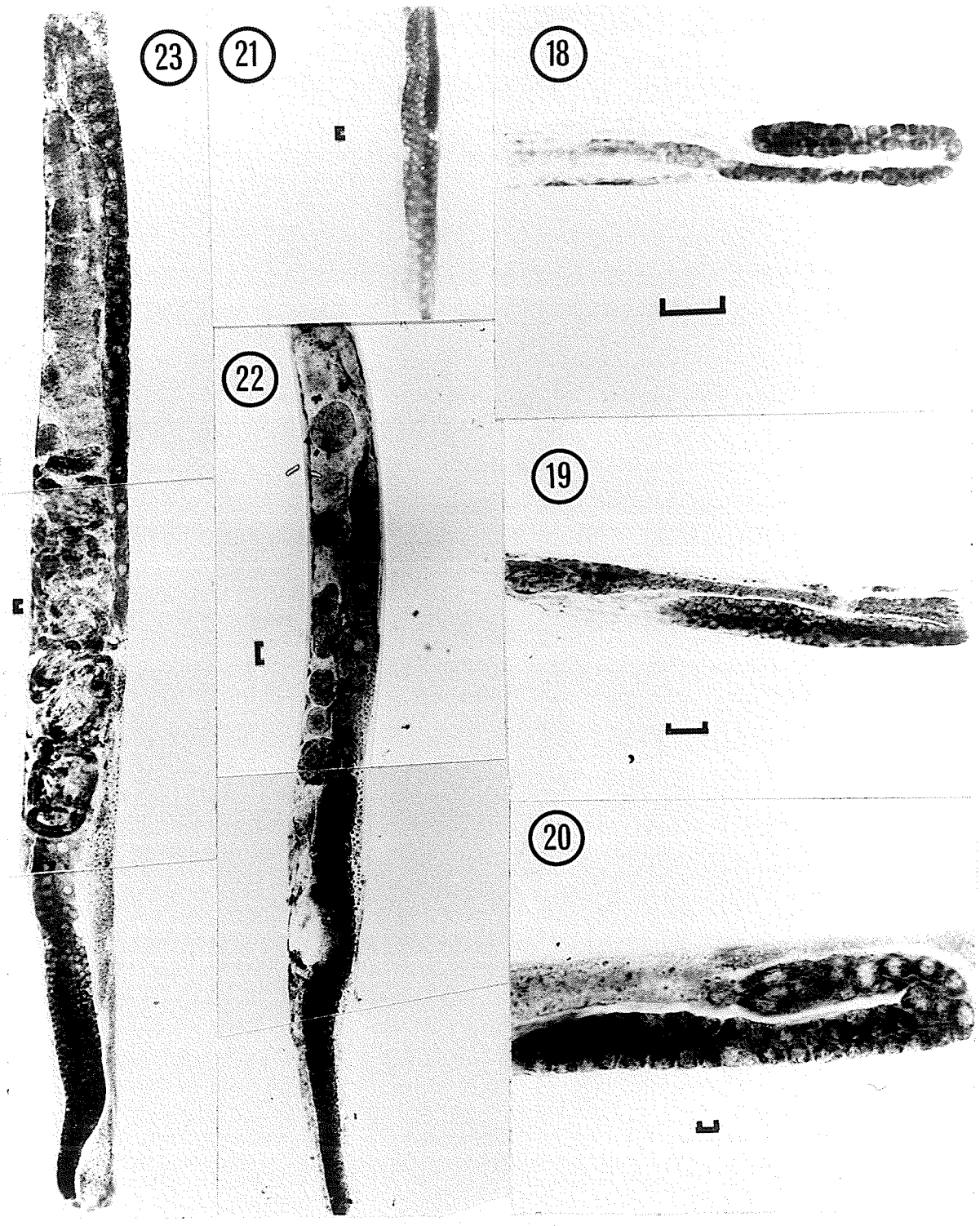
FIGURE 18, 19 and 20 - Fourth stage female. 160X.

FIGURE 21 - Fourth moult female. Beginning of differentiation. 160X.

FIGURE 22 - Virgin adult female with eggs. 63X.

FIGURE 23 - Adult female with first stage larvae in the uterus. 63X.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FIGURES 24-29; Inhibition of normal development of the reproductive system of Actinomycin-D.

FIGURE 24 - First moult gonad at time of T_0 exposure. 1000X.

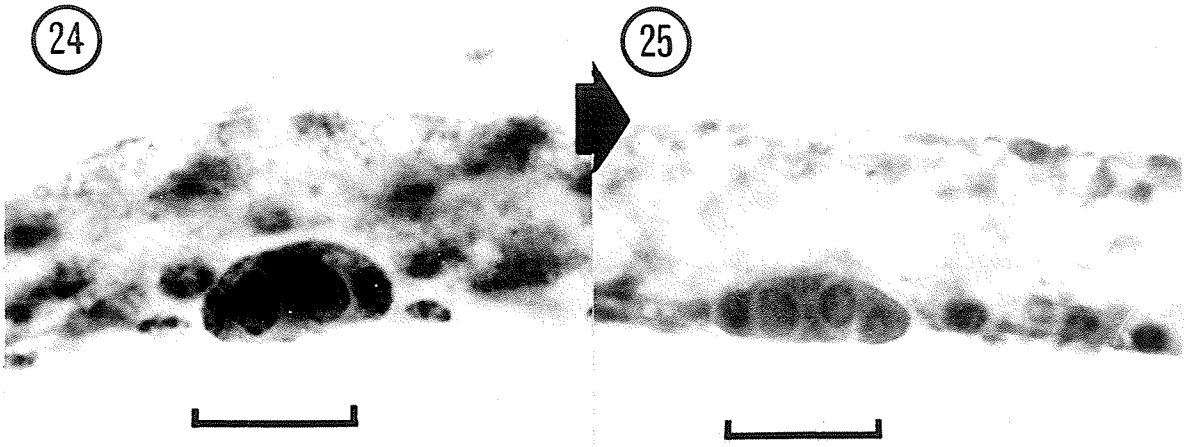
FIGURE 25 - Gonad primordium resulting after 96 hours of Actinomycin-D exposure at T_0. 1000X.

FIGURE 26 - Second moult gonad at time of T_1 exposure. 250X.

FIGURE 27 - Third moult gonad resulting from Actinomycin-D treatment at T_1 showing active proliferation. 250X.

FIGURE 28 - Fourth stage gonad at time of T_2 exposure. 160X.

FIGURE 29 - Late fourth stage gonad resulting from Actinomycin-D treatment at T_2. No differentiation is apparent. 160X.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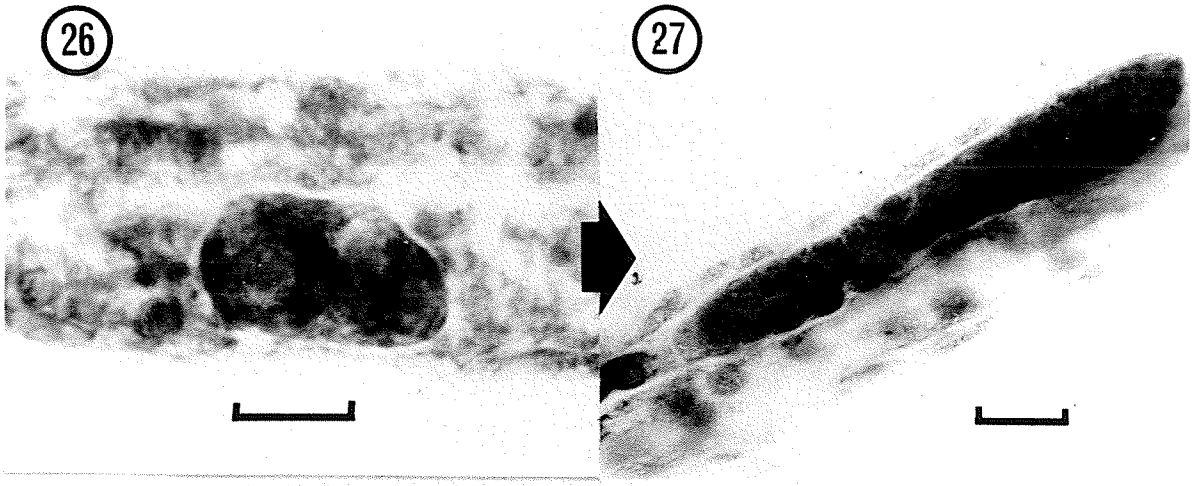
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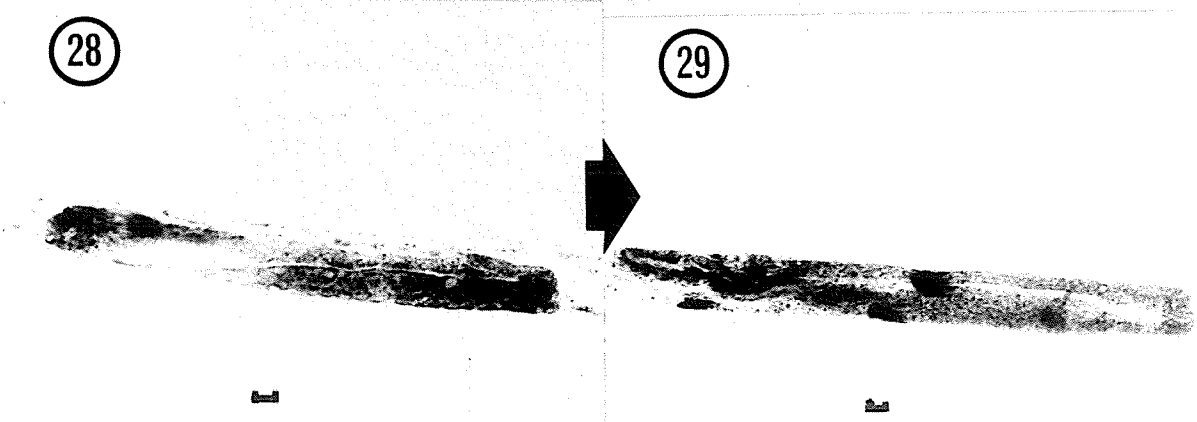
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FIGURES 30 - 35; Inhibition of normal development of the reproductive system by Actidione.

FIGURE 30 - First moult gonad at time of T_0 exposure. 1000X.

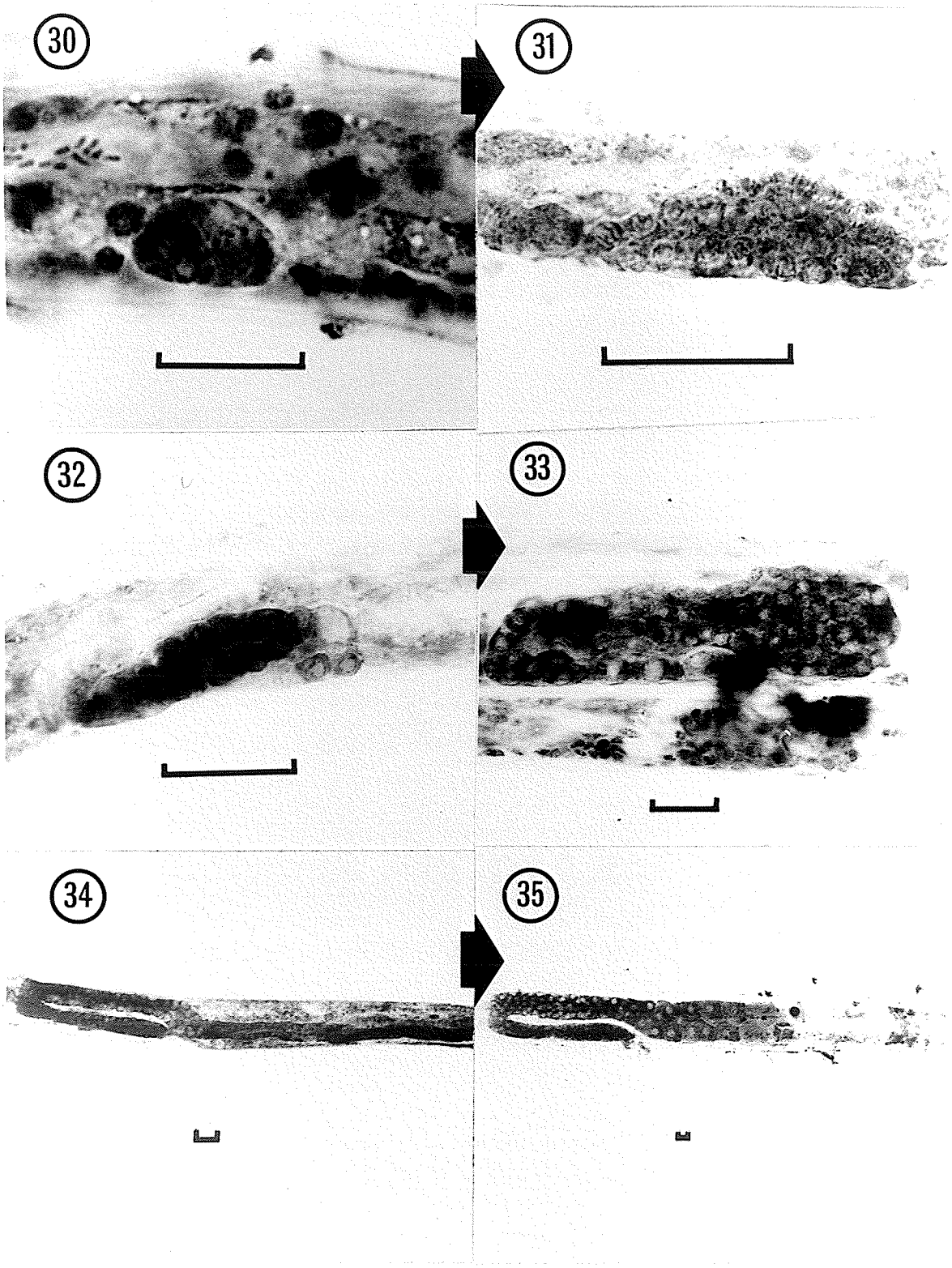
FIGURE 31 - Second stage gonad resulting after 140 hours of Actidione exposure at T_0. 250X.

FIGURE 32 - Third stage gonad at time of T_1 exposure. 250X.

FIGURE 33 - Third moult gonad resulting from Actidione treatment at T_1 showing active proliferation. 250X.

FIGURE 34 - Fourth stage gonad at time of T_2 exposure. 160X

FIGURE 35 - Late fourth stage gonad resulting from Actidione treatment at T_2. No differentiation is apparent. 160X.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FIGURES 36-41; Effect of Hydroxyurea on normal development of the reproductive system demonstrating the variable effect obtained.

FIGURES 36, 38 and 40 - Male reproductive systems.

FIGURE 36 - Incomplete gonad. 250X.

FIGURE 38 - Testis developed, but no vas deferens. 160X.

FIGURE 40 - Gonad differentiation with sperm. Vas deferens not developed. 160X.

FIGURES 37, 39 and 40 - Female reproductive system.

FIGURES 37 and 39 - Incomplete reproductive system. 160X.

FIGURE 41 - A high level of cell proliferation. 160X

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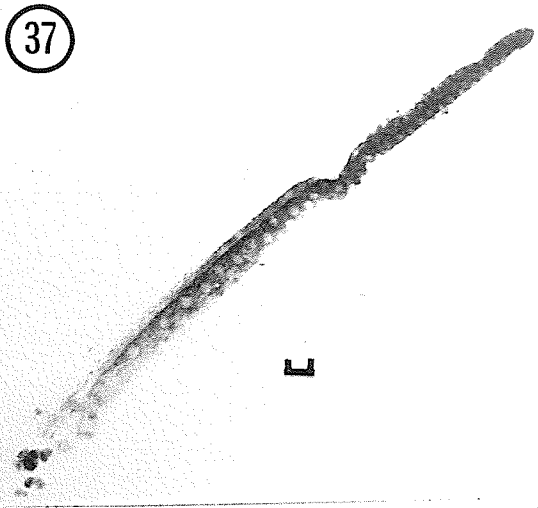

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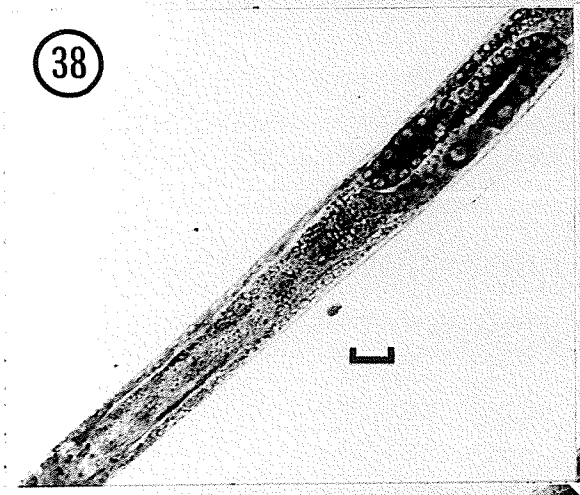

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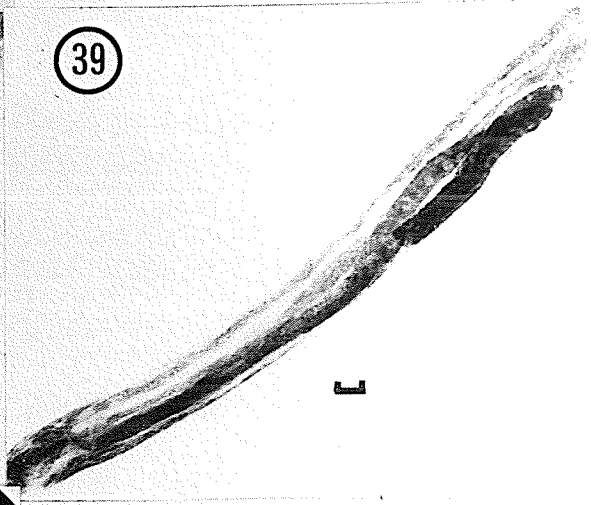

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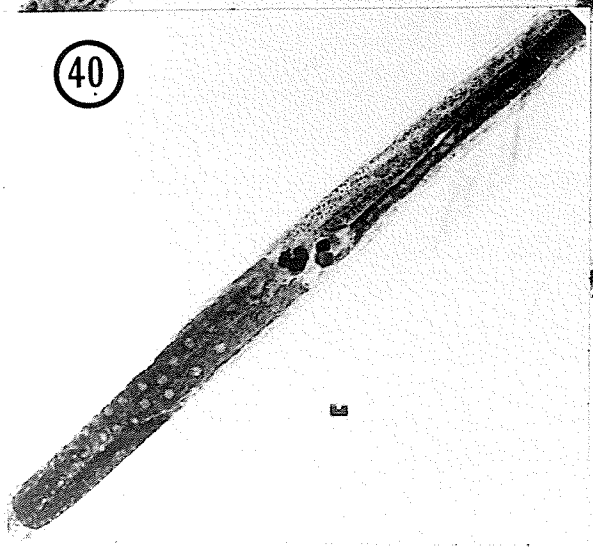

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FIGURE 42 - Effect of the addition of Actinomycin-D on gonad length and body length at three different times during postembryonic development. T_0 (0 hour) second stage, T_1 (25-40 hours) third stage, T_2 (70-80 hours) fourth stage. Arrows denote time of addition.

a - Body length control.
b - Body length treated.
c - Gonad length control.
d - Gonad length treated.

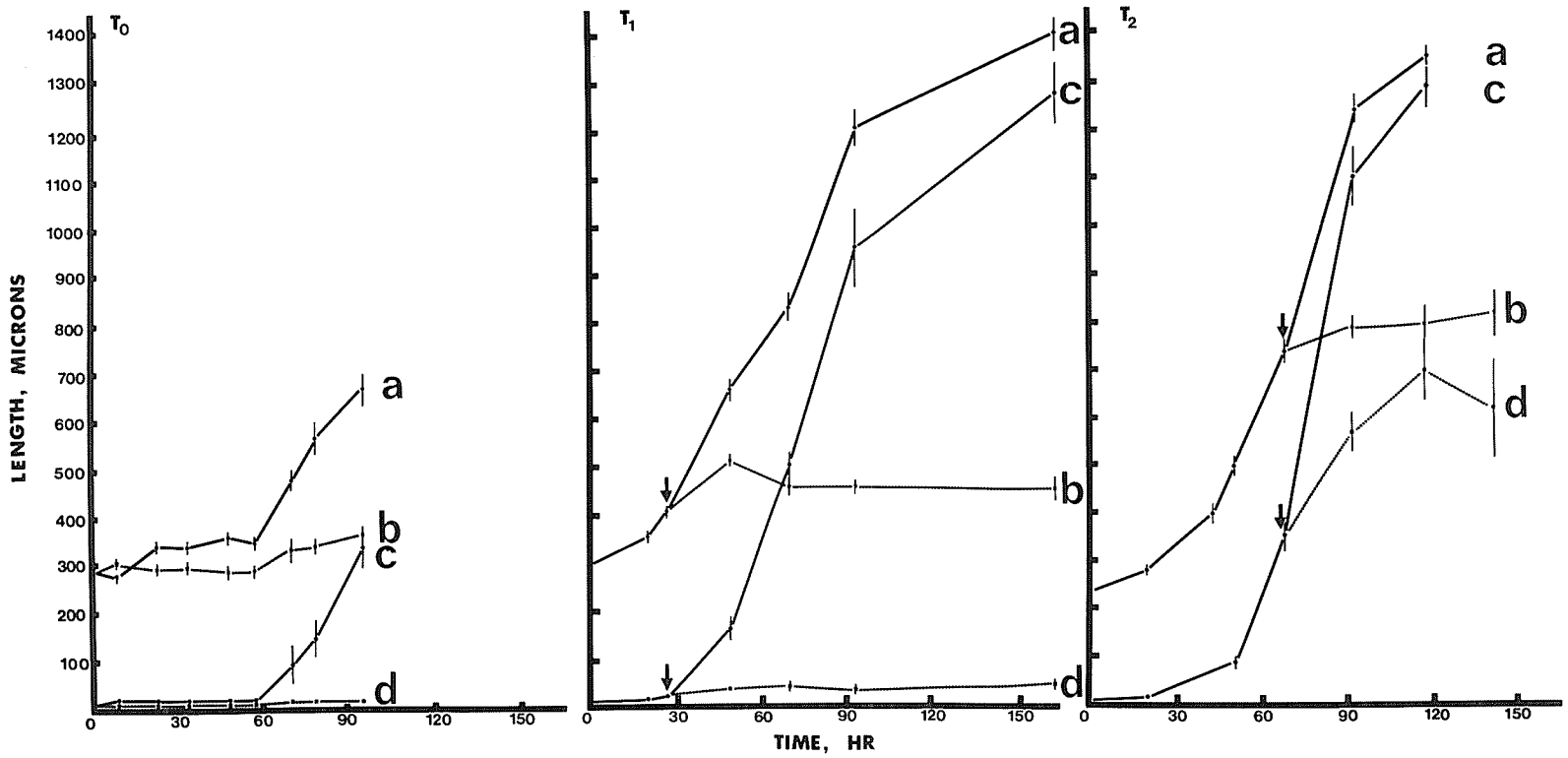
LENGTH, MICRONS
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
T_0
T_1
T_2
a
b
c
d
0
30
60
90
120
150
TIME, HR

FIGURE 43 - The effect of addition of Actidione at three different times on gonad length and body length during postembryonic development. T_0 (0 hour) second stage, T_1 (25-40 hour) third stage, T_2 (70-80 hour) fourth stage. Arrows denote times of addition.

a - Body length control.
b - Body length treated.
c - Gonad length control.
d - Gonad length treated.

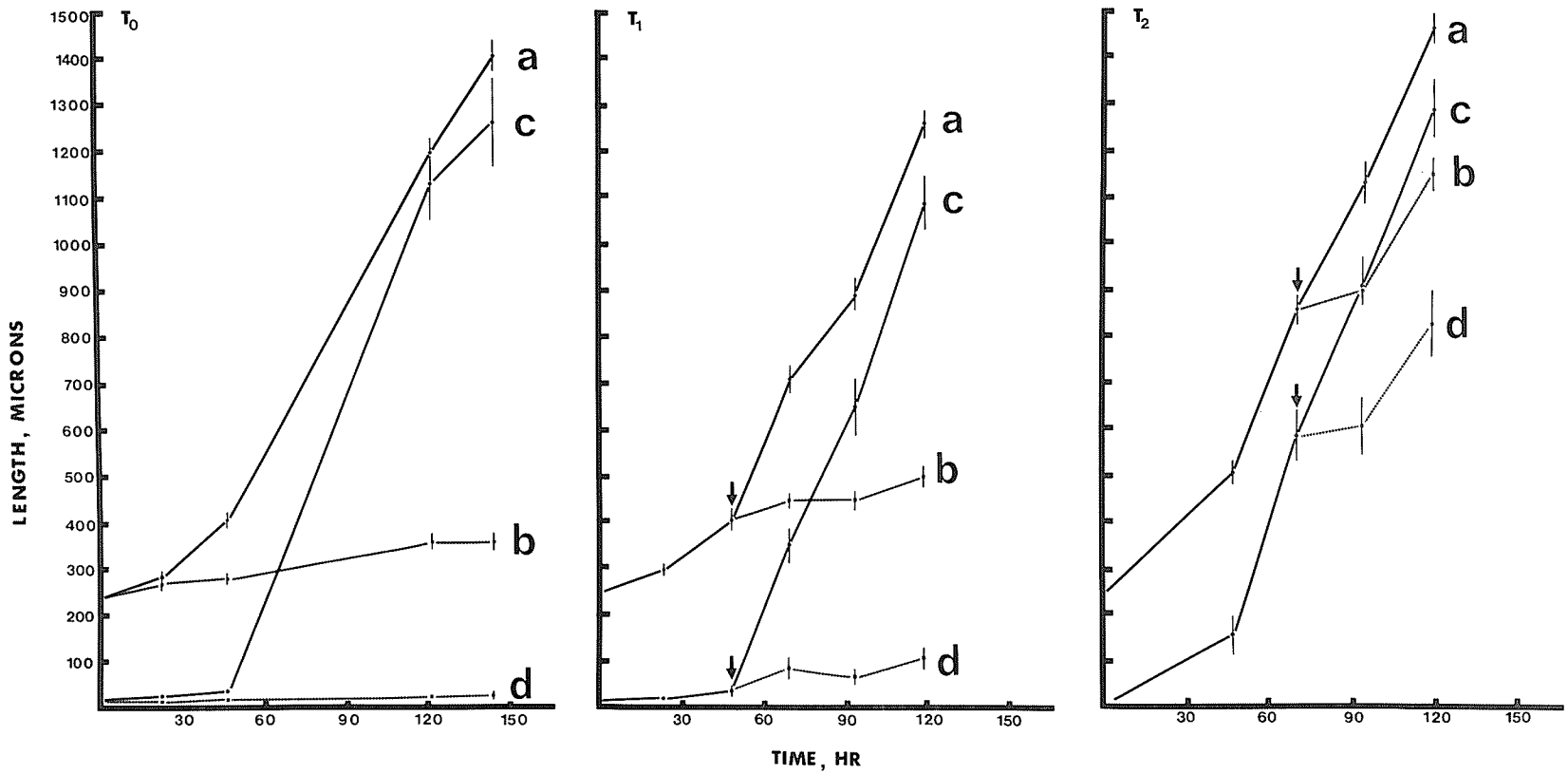
LENGTH, MICRONS
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
T_0
T_1
T_2
a
b
c
d
30
60
90
120
150
TIME, HR

FIGURE 44 - The effect of the addition of hydroxyurea on development of second stage larvae.

a - Body length control.
b - Body length treated.
c - Gonad length control.
d - Gonad length treated.

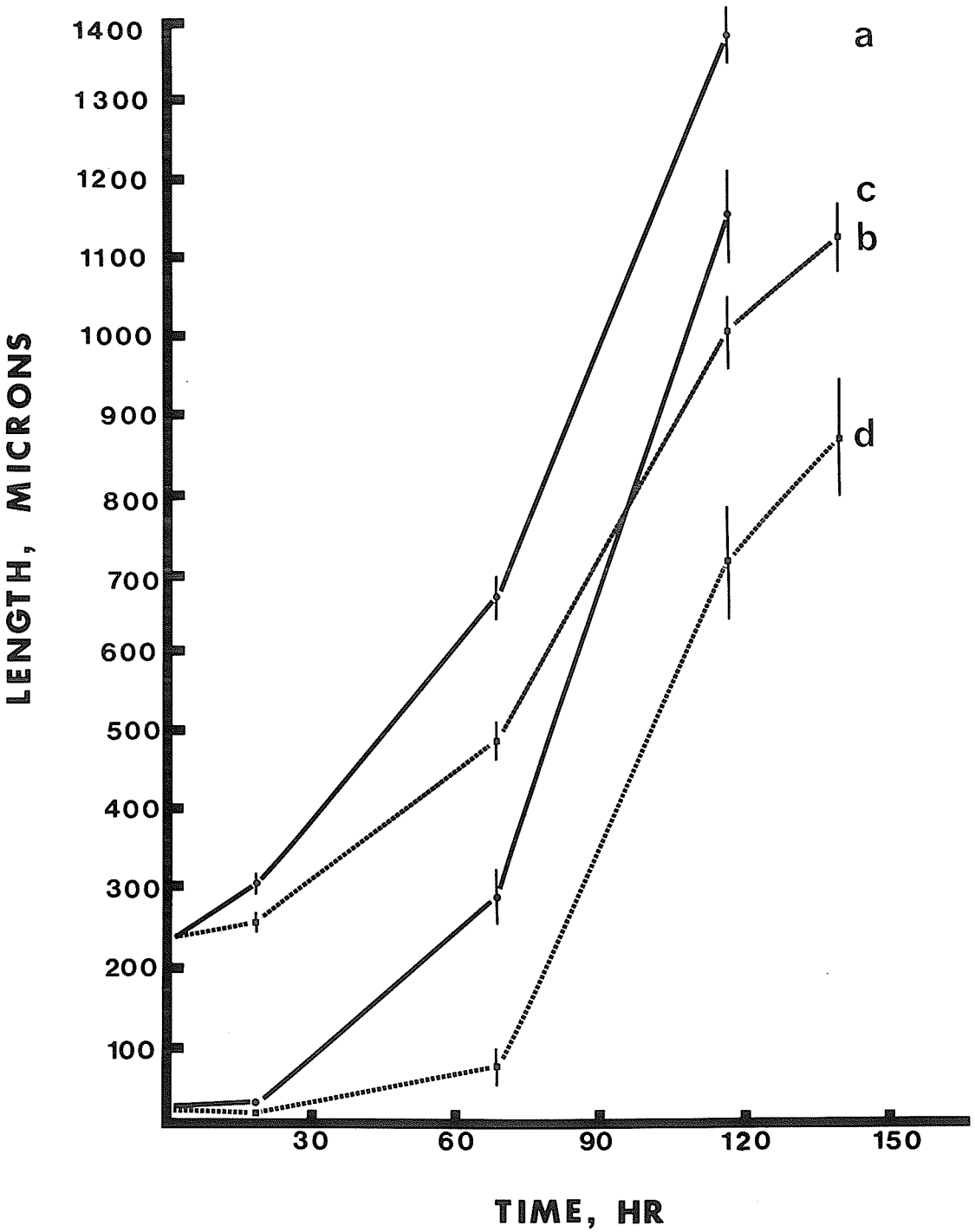
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
LENGTH, MICRONS
30
60
90
120
150
TIME, HR
a
c
b
d

FIGURE 45 - Summary of gonad development in Panagrellus redivivus denoting stage specific events. Arrows show times of second and third moults.

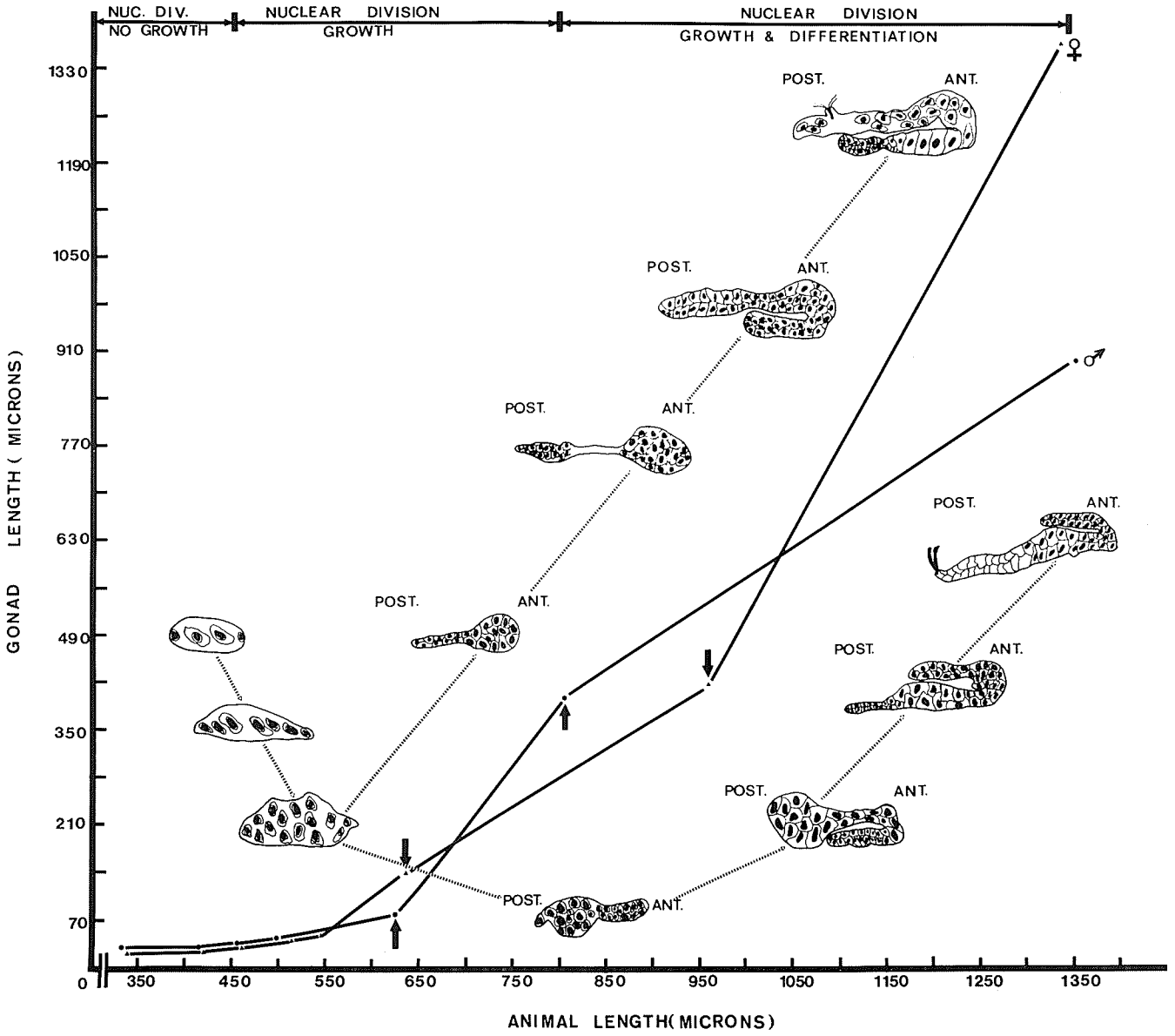
NUC. DIV.
NO GROWTH
NUCLEAR DIVISION
GROWTH
NUCLEAR DIVISION
GROWTH & DIFFERENTIATION
♀
♂
POST.
ANT.
GONAD LENGTH (MICRONS)
1330
1190
1050
910
770
630
490
350
210
70
0
350
450
550
650
750
850
950
1050
1150
1250
1350
ANIMAL LENGTH(MICRONS)

TABLE II. Effects of growth inhibitors on growth and gonad development of Panagrellus redivivus

TREATMENT	BODY LENGTH	GONAD DEVELOPMENT
L_2 in Actinomycin D	100% at L2	100% at L2
L_3 in Actinomycin D	79% at L3 21% at L4	22% at L3 78% at L4
L_4 in Actinomycin D	52% at L4 48% at adult	16% at L4 84% at adult
L_2 in Actidione	90% at L2 10% at L3	90% at L2 10% at L3
L_3 in Actidione	25% at L3 75% at L4	40% at L3 60% at L4
L_4 in Actidione	100% at adult	32% at L4 68% at adult
L_2 in hydroxyurea	20% L4 80% adult	18% at L2 60% incomplete 22% adult
No Treatment	100% adult	100% adult

APPENDIX

The morphology of the reproductive system in nematodes has been well documented by many investigators, including Chitwood (1930, 1949), Chitwood and Chitwood (1950), Filipjev (1929), Hirschmann (1960), Hirschmann and Triantaphyllou (1967), Machin (1936), Thorne (1937), Triantaphyllou and Hirschmann (1960), and Wu (1958).

Nematodes generally are dioecious or bisexual, that is, they exist as separate males and females. Females and males can be distinguished by their primary and secondary sexual characters. Males can be readily separated from females by the presence of a copulatory apparatus and in many cases by their smaller size. The reproductive system is similar in both sexes of all nematodes. It is composed of 1 or 2 tubular gonads which may vary greatly in length and may be straight reflexed or coiled.

In most nematodes the germ cells are proliferated only in the proximal end of the gonad. The proximal end of the gonad in females is occupied by the ovary, a tubular sac in which the germinal cells develop. In general, the ovary can be subdivided into two regions: 1) The germinal zone:

A region in which rapid division of cells takes place; 2) The growth zone: A region of gradual increase in size of the oogonia. The free end of the ovary may be either reflexed or it may be outstretched. The ovary is followed by the oviduct, a functional rather than a structural entity of the female gonad. It is a constricted, thick-walled region between uterus and ovary. The oviduct is attached to the uterus which is a broad tube lined with a flat, cuboidal epithelium and covered by a muscle layer of circular and oblique fibers. The beginning of the uterus usually functions as a spermatheca and fertilization occurs in this region. Females with one genital tube generally possess a post vulva uterine sac, which may function as a spermatheca. The eggs are stored in the uterus and as they pass distally along it they undergo fertilization and maturation and some degree of embryonic development. Distally the uterus or uteri enter a common tube, the vagina which is lined with cuticle and provided with muscles. The vagina is usually quite short and opens through the females gonopore, the vulva.

The testis of nematodes can also be subdivided into a germinal zone and a growth zone. The

maturation of the sperm takes place at the end of the growth zone. The sperm are of different shape in the various nematodes, some being amoeboid or rounded, flagellate or conical. The free end of the testis is often reflexed. The testis is followed by the seminal vesicle which is a dilated portion of the male gonoduct and functions in storage of sperm. The main part of the male gonoduct is the vas deferens. It is usually composed of a tubular and glandular region. It may be provided with a musculature throughout its length or only in its terminal portion.

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