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A Histochemical Study of the Ovary of the Milkweed Bug Oncopeltus fasciatus (Dallas), with Special Reference to the "Intermediate" Cell

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A HISTOCHEMICAL STUDY OF THE OVARY OF THE MILKWEED BUG, ONCOPELTUS FASCIATUS (DALLAS), WITH SPECIAL REFERENCE TO THE "INTERMEDIATE" CELL

(TITLE)

BY

KHIAN KIOE LIEM

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I HEREBY RECOMMEND THIS THESIS BE ACCEPTED AS FULFILLING
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A HISTOCHEMICAL STUDY OF THE OVARY OF THE MILKWEED BUG ONCOPELTUS FASCIATUS (DALLAS) WITH SPECIAL REFERENCE TO THE "INTERMEDIATE CELL"

Presented by

Khian Kioe Liem

a candidate for the degree of Master of Science in Zoology and hereby certify that in their opinion it is acceptable.

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TABLE OF CONTENTS

List of Plates

INTRODUCTION

Since Andre (1934) described the biology of the milkweed bug, Oncopeltus fasciatus (Dallas) and found its ability to live and reproduce normally when feeding on dried milkweed seeds and supplied with water, this insect has become a very desirable experimental animal for entomological research.

Bonhag and Wick (1953) studied the functional anatomy of the male and female reproductive systems of the milkweed bug. They observed the anatomy, histology and certain cytological aspects of the genital organs of the adult and interpreted the architecture of these organs in terms of their functions. Their main intention was to stimulate interest in the functions of the reproductive organs of this insect, thus providing a foundation for further physiological research and giving an adequate background for certain cytochemical or histochemical studies.

Wick and Bonhag (1955) also studied the postembryonic development of the ovaries of this insect, in order to resolve some of the conflicting viewpoints found in the literature. They studied the complete post-embryonic development of the ovary beginning at the time of hatching.

Furthermore Bonhag (1955) made histochemical studies of the ovarian nurse tissues and oocytes of the milkweed bug to investigate the origin and chemical nature of nutritive substances found in the oocytes and to clarify the special role of the nuclei in the syncytial apical trophocyte tissue.

Massner (1968) studied follicular differentiation in ovarioles of Pyrrhocoris apterus (Heteroptera) and found that the oocytes of the

telotrophic ovarioles were nourished by two nutritive systems: the trophocytes and the follicular epithelium. The latter must first differentiate from the prefollicular syncytium, and this differentiation was shown to pass through two periods induced by two different inductors, the activated oocyte as it grew into the prefollicular syncytium, and the juvenile hormone. He also noticed that the resorptive body of the ovariole was overgrown by the interfollicular tissue, which still retained a great developmental capacity and that the free cells found in the material between the resorptive body and peritoneal epithelium originated from the isolated cellular patches of the tissue of the inner envelope, arising in the larval ovarioles.

The nature of the intermediate cell itself had been studied and Wigglesworth (1959) speculated that intermediate cells were sedentary hemocytes which had adhered to the body wall and the surface of organs.

Sastrodihardjo (1967) studied the competence of the ovary of Hyalophora cecropia (Lepidoptera) with special reference to the intermediate cells and found that migrating intermediate cells were morphologically different from hemocytes of the same animal, that these cells appeared in the female gonad of the fourth instar larvae, and that they were closely related to prefollicular cells of the gonad.

The function of intermediate cells from milkweed bug ovaries is still unknown. Therefore, to elucidate this problem, the ovaries of this insect were subjected to several histochemical tests. It was mentioned by Sastrodihardjo (1967) that intermediate cells appeared in small numbers for the first time in the fourth instar larvae of the Hyalophora cecropia and that they occupied the space between the basement

membranes of the germinal cells and ovariole sheath. The intermediate cells are round loosely arranged cells each with a large nucleus containing distinct chromatin granules and one or more nucleoli.

It is known that the telotrophic ovary of Oncopeltus fasciatus consists of seven ovarioles and each ovariole comprises a terminal filament, germarium, vitellarium and pedicel (Bonhag, 1958). It is further known from the work of Bonhag (1958) that two sheaths cover the ovariole, an outer epithelial sheath and the inner envelope. Also that this inner envelope consists of a single layer of cells enclosed by two membranes and that in the region of the large follicles, the outer epithelial sheath is reduced to a thin membrane and the inner envelope is reduced to a few loose cells found mostly in the constrictions between follicles.

Due to the small size of the ovaries, it was impossible to dissect these organs in the first through third nymphal stadia. Therefore it was decided to study ovaries taken from the fourth, fifth and adult stages and to observe the differences in the distribution of the PAS+ and PAF+ granules in the intermediate cells and also the activity of the enzyme-producing lysosomes, if any.

The PAS+ granules in the cytoplasm of the oocytes have been characterized as a carbohydrate-protein complex, possibly muco- or glycoprotein and no glycogen was detected in the oocytes (Bonhag, 1953).

Bonhag (1955) also mentioned that the membranes of the outer sheaths of the ovariole react quite intensively, especially the inner lamella of the inner envelope and that the cytoplasm of the intermediate cells reacts more strongly than that of the nurse tissues and the outer

epithelial sheath. However he did not mention the distribution of these PAS+ granules in various stages of development.

To elucidate the possibility of these intermediate cells being neurosecretory cells, a test to detect neurosecretory substances was carried out. In addition, a method for phosphomonoesterase II was used to determine whether these intermediate cells have some enzymatic activities.

MATERIAL AND METHODS

Stock cultures of the milkweed bug, Oncopeltus fasciatus were obtained from Dr. Frank Fraembs, Department of Zoology, Eastern Illinois University and from Dr. Judith Willis, Department of Entomology, University of Illinois. These cultures were maintained in the laboratory in glass jars where they were fed dried milkweed seeds and provided with water. The breeding was carried out according to Wick and Bonhag (1955) within a temperature range of 80-86° F. The humidity was not controlled.

The milkweed bugs studied were females of the fourth and fifth instar and the adult stage. The ovaries of these female bugs were exposed by vivisection before fixation. The insects were immobilized for vivisection according to Wick and Bonhag (1955). The insects were then covered with Ephrussi and Beadle insect Ringer's (Roeder, 1953) and the wings and tergites of the thorax and abdomen were removed with scissors and jeweler's forceps. The whole dissecting process was done under a binocular microscope and the fat bodies and digestive tracts were removed to expose the ovaries.

Ovaries used in this study were fixed in aqueous Bouin (Baker, 1958) for about 6-12 hours or overnight. This fixative was compatible with both the periodic acid-Schiff and Gomori's paraldehyde fuchsin staining method. However for Gomori's acid phosphatase method cold acetone (0-5° C) was used. The fixation time was twenty-four hours or overnight.

After the ovaries were fixed in aqueous Bouin, washing of the tissues were done with 80% ethanol and the ethanol was changed three to four times until most of the yellow color was gone. The tissue was

then placed in ethyl-cellosolve for eight hours or more and clearing was done with methyl-benzoate until the tissue sank to the bottom or left overnight, then passed into benzene in two changes, one hour each. The material was then allowed to remain in a mixture of paraplast-benzene (1:1) for one hour at a temperature of 58° C in a warming oven. An adequate infiltration by pure paraplast was accomplished at oven temperature 58° C for one hour. The pure paraplast was changed three times during this hour to remove all the benzene from the tissue and finally it was embedded in pure paraplast. Handling of the tissue must be done very carefully due to its tiny and fragile structure. Transferring the tissue from one reagent to another was done by placing the tissue into a small basket made of foil paper with very tiny holes at the bottom of it. Sections were cut seven microns thick.

Three histochemical methods were used for determining the possible function of the intermediate cells of the ovary: the periodic acid-Schiff method for mucopolysaccharides (MPS) and glycogen, modified after Hotchkiss (1948), Gomori's paraldehyde fuchsin (PAF) for neuro-secretory substances (Halmi, 1952) and Gomori's acid phosphatase method for lysosomes (Pearse, 1960).

Periodic acid-reactive carbohydrates

Serial sections were cut at seven microns, deparaffinized in xylene and brought to water or hydrated in graded series of alcohols. Sections were then placed in 1% solution of periodic acid for ten minutes at room temperature, washed for five minutes in running water and stained

for ten minutes in Schiff's reagent. After this treatment, the sections were transferred to three changes (two minutes each) of a solution consisting of 0.52% $NaHSO_3$, then washed in running water for ten minutes, counterstained in Light green or Harris' Haematoxylin, dehydrated with graded alcohols to xylene and finally mounted with H.S.R. mounting medium. A control was used to indicate whether the periodic acid-reactive carbohydrate were mucopolysaccharides or glycogen. Adjacent sections were pretreated with 0.1% malt diastase solution at 37° C for one hour.

Neurosecretory substances

Gomori's paraldehyde fuchsin staining method for neurosecretory substances was used as follows: serial sections were cut at seven microns, mounted on slides and deparaffinized in two changes of xylene, ten and three minutes, then hydrated with graded series of alcohols to distilled water. Hydrolysis took place in 0.3% $KMnO_4$ containing 0.3% sulphuric acid at room temperature. The time of hydrolysis was 3-4 minutes and adjacent sections were placed in 2.5% $NaHSO_3$ for one minute, then rinsed in running tap water for five minutes, dehydrated in 35% and 50% ethanol for one minute each, stained in PAF reagent for three to four minutes and washed out in three changes of 95% ethanol, one minute, one minute and ten minutes. Sections were then hydrated with graded series of alcohols to distilled water and counterstained in light green for one minute, washed in 95% ethanol with two drops of glacial acetic acid for two minutes, dehydrated in graded series of alcohols, cleared with xylene and finally mounted in HSR medium. As a

control to detect whether these PAF+ granules contained protein, the enzyme trypsin was used. Adjacent sections were pretreated with one gram trypsin per 100 ml 0.01N HCl at 37° C for one hour.

Enzymes

Gomori's acid phosphatase method was used for localizing phosphomonoesterase II. The fixative used for this method was cold acetone (0-5° C) overnight or twenty-four hours, then hardening in absolute ethanol for twenty-four hours. The tissue was then cleared in xylene, two changes, one hour each and embedded in pure paraplast. Serial sections were cut at seven microns, mounted on glass slides and deparaffinized in xylene and hydrated in graded series of alcohols to distilled water. The sections were then incubated in a substrate containing five ml 1M Na-acetate buffer, pH = 4.7, two ml 5% lead-nitrate ($PbNO_3$), 87 ml distilled water, and 6 ml 3.2% B-glycerol-phosphate. The time of incubation was one hour. After incubation, the sections were rinsed in distilled water, then placed in 1% acetic acid solution for two minutes, rinsed again in distilled water and placed in 2% yellow ammonium sulfide for two minutes. The sections were washed again in three changes of distilled water, dehydrated to 70% ethanol and counterstained in eosin for one minute. The sections were then further dehydrated, cleared in xylene and finally mounted in HSR medium. Control slides were incubated only in the buffer systems without B-glycerol-phosphate.

RESULTS

Periodic acid-reactive carbohydrates

PAS+ granules were detected in the intermediate cells of the ovary of all three developmental stages. The cytoplasm of these cells reacts quite strongly and the data on the distribution of PAS+ granules are presented in Table I. Intermediate cells in very small number appear in the fourth instar nymph and in most of the sections are hard to find. The ovary of the fifth instar nymph is almost fully developed and an increase in the number of intermediate cells is obvious. Furthermore the number of intermediate cells in the adult animal varies from three to six intermediate cells per unit area. A unit area is an area mostly in the constriction between two follicles (or between the germarium and a follicle) and the outer epithelial sheath of an ovariole. However in the fourth instar nymph a unit area is any area where one or two at the most intermediate cells are found between the inner and outer lamella of the inner envelope or even the cells that are found outside the inner envelope due to improper cutting with the microtome. The number of areas observed are five areas for each ovary randomly taken from five milk-weed bugs of the three developmental stages mentioned above. The number of cells in five unit areas is the summation of the cells found in those five areas of one ovary. The number of granules in one cell is the mean of the number of granules found in one cell of all the cells in the cells in the five areas observed. The counting of the granules was done under high magnification using the oil immersion lens (1000x). The PAS reaction is histochemically

specific, but since many different components of tissues give a positive reaction it is not diagnostic of any single tissue component or group of substances. To improve the value of the PAS reaction for identification of tissue structures it is used in conjunction with enzymic or chemical controls.

Control slides pretreated with 0.1% malt diastase solution at 37° C showed no differences at all compared to those that were not treated (Table II). Malt diastase is an enzyme used to identify the presence of glycogen. The PAS reaction will demonstrate the sites of glycogen and many other CHO substances in sections, and by the removal of glycogen from a control section with diastase it is possible to determine the site and approximate quantity of glycogen in the tissue by comparison of the two sections.

TABLE I

Number of insects & stadium	glycogen	Muco-polysaccharide	Number of cells per five areas	Number of granules per cell
1 IVth instar	+	+	4 cells	8 granules
2 "	+	+	3 cells	8 granules
3 "	+	+	3 cells	8 granules
4 "	+	+	2 cells	7 granules
5 "	+	+	2 cells	8 granules
1 Vth instar	+	+	12 cells	10 granules
2 "	+	+	10 cells	9 granules
3 "	+	+	14 cells	10 granules
4 "	+	+	15 cells	10 granules
5 "	+	+	14 cells	9 granules

Table I, continued

1	Adult	+	+	15 cells	11 granules
2	Adult	+	+	18 cells	10 granules
3	Adult	+	+	14 cells	12 granules
4	Adult	+	+	17 cells	11 granules
5	Adult	+	+	16 cells	12 granules

TABLE II

	PAS	Diastase & PAS
Polysaccharide = Glycogen	+	-
Mucopolysaccharide (MPS)	+	+

Neurosecretory substances

The intermediate cells of the ovary of all three developmental stages gave positive reactions to Gomori's paraldehyde fuchsin staining method. Results showed that the number of intermediate cells per five areas observed was very low in the fourth instar nymph, increased obviously in the fifth instar nymph and adult stage. The method of counting the number of cells in an ovary of one insect and furthermore of the PAF+ granules was exactly the same as for the periodic acid-reactive carbohydrates. The counting of the relatively smaller PAF+ granules was done under high magnification using the oil immersion lens (1000x). Data on the distribution of the intermediate cells and the

PAF+ granules found in the ovary of the three developmental stages are presented in Table III.

Control slides pretreated with the enzyme trypsin showed the same results as the ones that were not treated (Table III).

The PAF reaction is histochemically non-specific since it does not only identify neurosecretory substances in neurons possessing features associated with glandular activity or related to the production of chemical agents with measurable physiologic effects, agents definable as hormones, but also to proteins rich in cysteine and cystine (Schreiner, 1966).

TABLE III

Number of insect	Stadium	PAF	Enzyme trypsin & PAF	Number of cells per 5 areas	Number of granules per cell
1	IVth instar	+	+	5 cells	9 granules
2	"	+	+	6 "	8 "
3	"	+	+	5 "	9 "
4	"	+	+	4 "	10 "
5	"	+	+	6 "	9 "
1	Vth instar	+	+	15 cells	12 granules
2	"	+	+	14 "	11 "
3	"	+	+	15 "	12 "
4	"	+	+	12 "	12 "
5	"	+	+	14 "	12 "
1	Adult	+	+	15 cells	14 granules
2	"	+	+	18 "	13 "
3	"	+	+	16 "	15 "
4	"	+	+	18 "	14 "
5	"	+	+	19 "	14 "

Acid phosphatase

Acid phosphatase or phosphomonoesterase II splits mono-orthophosphate esters in an acidic medium (pH = 4.7). The enzyme acts upon a substrate of organic phosphate, Na-B-glycerophosphate, in an incubating medium containing lead-nitrate ($PbNO_3$). The phosphate that is produced forms lead-phosphate as it is liberated and this is subsequently converted by ammonium-sulphide into a black deposit of opaque lead-sulphide (PbS).

Insignificant results of this enzyme activity was found in the fourth instar nymph ovary, while a few black spots of the lead-sulphide precipitation were detected in the fifth and adult stages, showing the enzyme activity of these cells. Table IV shows the results of the treatment detecting the presence of acid phosphatase or phosphomonoesterase II.

Control slides incubated in the buffer systems without Na-B-glycerophosphate showed negative results in all the three developmental stages.

TABLE IV

	Number of insect	Stadium	Buffer systems with Na-B-glycero-PO_4	Buffer systems without Na-B-glycero-PO_4
Acid-P-ase	1	IVth instar	±	-
	2	"	±	-
	3	"	±	-
	1	Vth instar	+	-
	2	"	+	-
	3	"	+	-
	1	Adult	+	-
	2	"	+	-
	3	"	+	-

DISCUSSION

It is obvious from this study that the number of intermediate cells appears to be very low in the fourth instar nymphal stadium and increases in the fifth nymphal and adult stages (Table I, III, and IV). This increase in number may be due to the consequence of growth and development of the ovaries to maturity. According to Wick and Bonhag (1955) the inner envelope of the ovary of the fourth instar nymph extends from the anterior end of the pedicel to the basal body of the ovarian strand; they also found that mitotic activity occurs frequently in these cells and that they have distinct boundaries and appear separated from each other. There are no significant changes in the histological organization of the outer epithelial sheath and inner envelope of the ovary in the fifth instar nymph except an increase in size and number of cells due to growth and mitosis. Mitotic activity continues at a rapid rate in all the follicular tissues during the fifth instar stadium (Wick and Bonhag, 1955). The ovariole of the early adult is fully grown and an increase in over-all dimensions of the ovariole as compared to the early fifth instar nymph is apparent. The inner envelope has increased in length in proportion to the increase in size of the ovariole; however it remains as a single layer of separate cells enclosed between two membranous lamellae, the inner and outer lamellae (Figure 2).

Complex carbohydrates

Results of the PAS test (Table I) showed that the cytoplasm of the intermediate cells reacted quite strongly. PAS+ granules were found scattered in the cytoplasm of these cells. A slight difference in the

distribution of PAS+ granules between the fourth, fifth, and adult stages was detected. This slight increase in the number of granules from one instar to the next may be due to the growth and size of the cell. Since the whole ovary tissue is constantly growing through mitotic activity from one developmental stage to the next until it reaches maturity in the adult stage, this might be the reason for the slight increase in the number of PAS+ granules.

No differences were observed in the tissue after it was pretreated with 0.1% malt diastase solution for one hour at 37° C. Malt diastase is an enzyme that digests glycogen if present, thus making the PAS reaction specific for mucopolysaccharides only. A conclusion could be drawn from this control test that there was no glycogen present in the intermediate cells and that PAS+ granules were mucopolysaccharides. This is in agreement with the result of Bonhag (1955) who made a histochemical study of the ovaries of Oncopeltus fasciatus using the salivary ptyalin enzyme to detect the presence of glycogen and found that this pretreatment did not reduce the intensity or localization of the PAS stain. The size, distribution and staining properties of these granules are consistent with the possibility that they are lysosomes.

Paraldehyde fuchsin (PAF) positive granules

The PAF+ granules or neurosecretory material found in the cytoplasm of the intermediate cells suggests that these cells are possibly neurosecretory cells. The generally accepted concept of the neurosecretory cell is that of a nerve cell possessing features associated with glandular activity in addition to ordinary neuronal characteristics (Bern, 1962).

Bern (1962) in his most useful definition concluded that "the finding of stainable materials in neurons, cytoplasmic inclusions in the form of droplets, granules or vacuoles, regardless of the stain employed, only allows the conclusion that such neurons are possibly neurosecretory; and if the signs of secretory activity can be related to the production of chemical agents with measurable physiologic effects, agents definable as hormones, then it can be concluded that such cells are definitely neurosecretory. If on the other hand, evidence of a secretory cycle can be adduced, based on the same kind of cytologic criteria used in judging the secretory activity of an epithelial cell, then it is possible to conclude that such neurons are probably, but not necessarily neurosecretory."

Since the PAF reaction is histochemically non-specific and does react to both hormones and proteins rich in cysteine and cystine or sulphur containing proteins it is impossible to define whether these intermediate cells are definitely neurosecretory or possibly neurosecretory.

Control slides pretreated with the enzyme trypsin still showed PAF positive granules and this result is in agreement with Schreiner (1966) who studied the histochemistry of the A cell neurosecretory material of the brain of Oncopeltus fasciatus. He found through histochemical tests that the neurosecretory material is a protein rich in cystine and cysteine but found it impossible to split the material with the enzymes pepsin and trypsin. Pepsin and trypsin are classified as proteinases, since they cause the degradation of nearly all proteins. Schreiner (1966) suggests that although pepsin and trypsin are proteinases, they show some specificity, for pepsin favors the hydrolysis of peptide linkages in which L-tyrosine or L-phenylalanine provides the

amino group for the sensitive peptide bond, while trypsin acts at the CO-NH linkages that involve the carbonyl group of L-lysine or L-arginine. He assumed that these special peptide linkages are present in the neurosecretory material of insects, but they in some way are not accessible to the enzymes.

Slight differences were also observed in the distribution of PAF positive granules in the intermediate cells of the ovary of the three developmental stages. Again, this might be due to the relative size and growth activity of the cell itself, for growth activity parallels neurosecretory activity of cells in many tissues.

Acid phosphatase positive granules

Results of this study showed that enzymes were present in the intermediate cells (Table IV). The detection of these enzymes is somewhat borderline in the intermediate cells of the fourth instar ovary, but in the fifth and adult stages it was quite obvious but there were fewer granules than with either of the other two methods. The ambiguous result in the fourth instar ovary was probably due to the small quantity of the enzyme present in this developmental stage. Further discussion of these acid phosphatase positive granules is premature because the technique was not perfected for the use of frozen sections could give better results.

SUMMARY

1. The number of intermediate cells appears to be very low in the fourth instar nymphal stadium and evidently increases in the fifth and adult stages.

2. PAS+ granules were found scattered in the cytoplasm of the intermediate cells of all three developmental stages with a slight increase in the distribution from one instar to the other which might be due to the relative growth and size of the cell itself.

3. The number of PAS+ granules was less in the fourth instar nymph and slightly increased in the fifth and adult stadia.

4. There was no glycogen present in the intermediate cells; these PAS+ granules were mucopolysaccharides (MPS).

5. PAF+ granules were detected in the cytoplasm of the intermediate cells with a slight increase observed from one instar to the other. This slight increase in the number of granules from the fourth to the fifth and adult stages might be due to the growth and mitotic activity of these cells in the referring stadia.

6. Since the PAF reaction is histochemically non-specific and does demonstrate both hormones and proteins rich in cysteine and cystine, it is impossible to define whether these intermediate cells are definitely or possibly neurosecretory cells.

7. Evidence showed that enzyme phosphatases were present in the intermediate cells of all three developmental stages and the distribution of these enzymes were again less in the fourth instar nymphal stadia. The enzymic activity of these cells became more evident in the fifth and adult stadia.

8. These granules share some of the properties which have been used to characterize lysosomes.

BIBLIOGRAPHY

Andre, F. 1934. Notes on the biology of Oncopeltus fasciatus (Dallas). Iowa State College. J. Sci. 9, 73-87.

Baker, J.R. 1958. Principles of biological microtechnique. London.

Bern, H.A. 1962. The properties of neurosecretory cells. Gen. and Comp. Endocrin. 1, 117-132.

Bonhag, P.F. and J.R. Wick. 1953. The functional anatomy of the male and female reproductive systems of the milkweed bug, Oncopeltus fasciatus (Dallas) Heteroptera:Lygaeidae. J. Morph. 93, 177-230.

Bonhag, P.F. 1955. Histochemical studies of the ovarian nurse tissues and oocytes of the milkweed bug, Oncopeltus fasciatus (Dallas). J. Morph. 96, 381-413.

Bonhag, P.F. 1958. Ovarian structure and vitellogenesis in insects. Ann. Rev. Ent. 3, 137-160.

Drury, R.A.B. and E.A. Wallington. 1967. Carleton histological technique Oxford University Press, New York-Toronto. p. 245-246.

Halmi, N.S. 1952. Differentiation of two types of basophils in the adenohypophysis of the rat and the mouse. Stain Technol. 27, 61-64.

Hotchkiss, R.D. 1948. A microchemical reaction resulting in the staining of polysaccharide structures in the fixed tissue preparation. Arch. Biochem. 16, 131-141.

Massner, P. 1968. The inductors of differentiation of prefollicular tissue and the follicular epithelium in ovarioles of Pyrrhocoris apterus (Heteroptera). J. Embryol. Exp. Morph. 20, 1-13.

McManus, J.F.A. 1946. Histological demonstration of mucin after periodic acid. Nature (London), 158, 202.

Pearse, A.G.E. 1960. Histochemistry theoretical and applied. 2nd Ed. London. Y.Z.A. Churchill.

Roeder, K.D. 1953. Insect Physiology. John Wiley and Sons, Inc. New York, Chapman and Hall, Ltd, London. p. 161.

Sastrodihardjo, S. 1967. The competence of the ovary of Hyalophora cecropia (Lepidoptera) in vitro, with special reference to the "intermediate" cell. Doctoral thesis, University of Illinois, Urbana, Illinois.

Schreiner, B. 1966. Histochemistry of the A cell neurosecretory material in the milkweed bug, Oncopeltus fasciatus Dallas (Heteroptera: Lygaeidae) with a discussion of the neurosecretory material and carrier substance problem. Gen. and Comp. Endocrin. 6, 388-400.

Wick, J.R. and P.F. Bonhag. 1955. Post-embryonic development of the ovaries of Oncopeltus fasciatus (Dallas). J. Morph. 96, 31-60.

Wigglesworth, V.B. 1959. Insect blood cells. Ann. Rev. Ent. 4, 1-16.

KEY TO ABBREVIATIONS USED IN FIGURES

C = Calyx

e.p. = epithelial plug

f.e. = follicular epithelium

G = Germarium

g.v. = germinal vesicle

i.c. = intermediate cell

i.t. = interfollicular tissue

l.o. = lateral oviduct

n.c. = nutritive cord

o = oocyte

o.e.s.= outer epithelial sheath

P = Pedicel

p.t. = prefollicular tissue

t.f. = terminal filament

tr. = trophocytes

tr.c = trophic core

V = Vitellarium

Y = Yolk

y.o = young oocyte

Plate I

Dorsal dissection of an ovary of Oncopeltus fasciatus (Dallas). Young adult stadium (fig. 1). 15X.

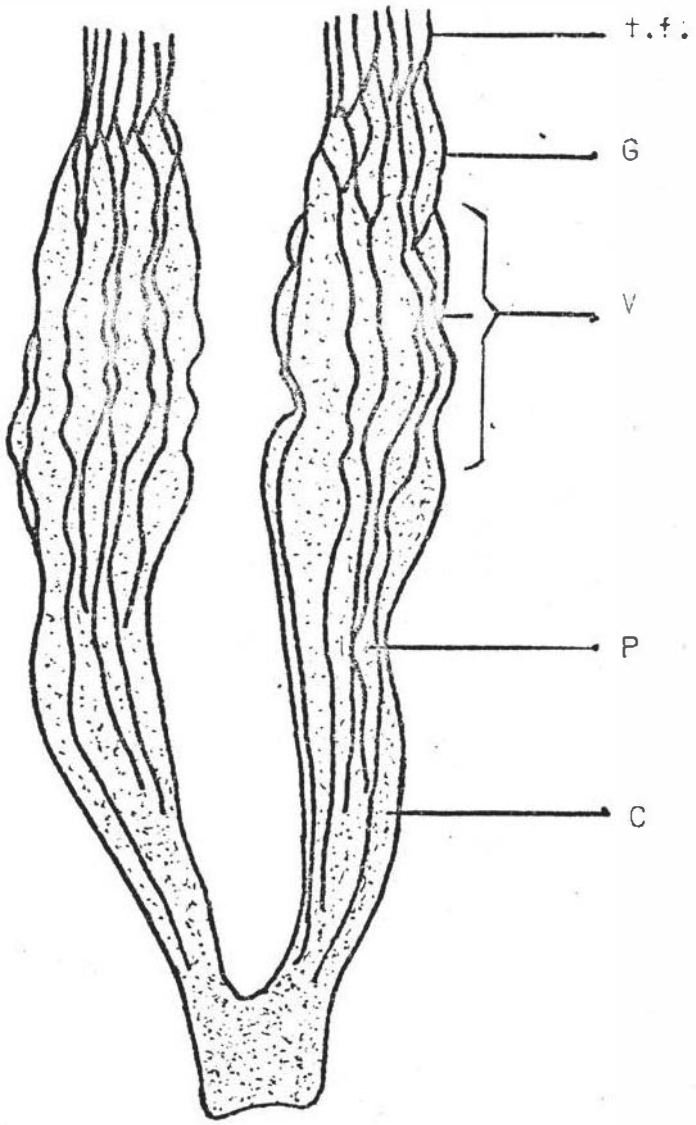

Fig. 1

Plate 2

Longitudinal section of anterior end of ovariole of Oncopeltus fasciatus (Dallas). Young adult stadium (fig. 2). 100X.

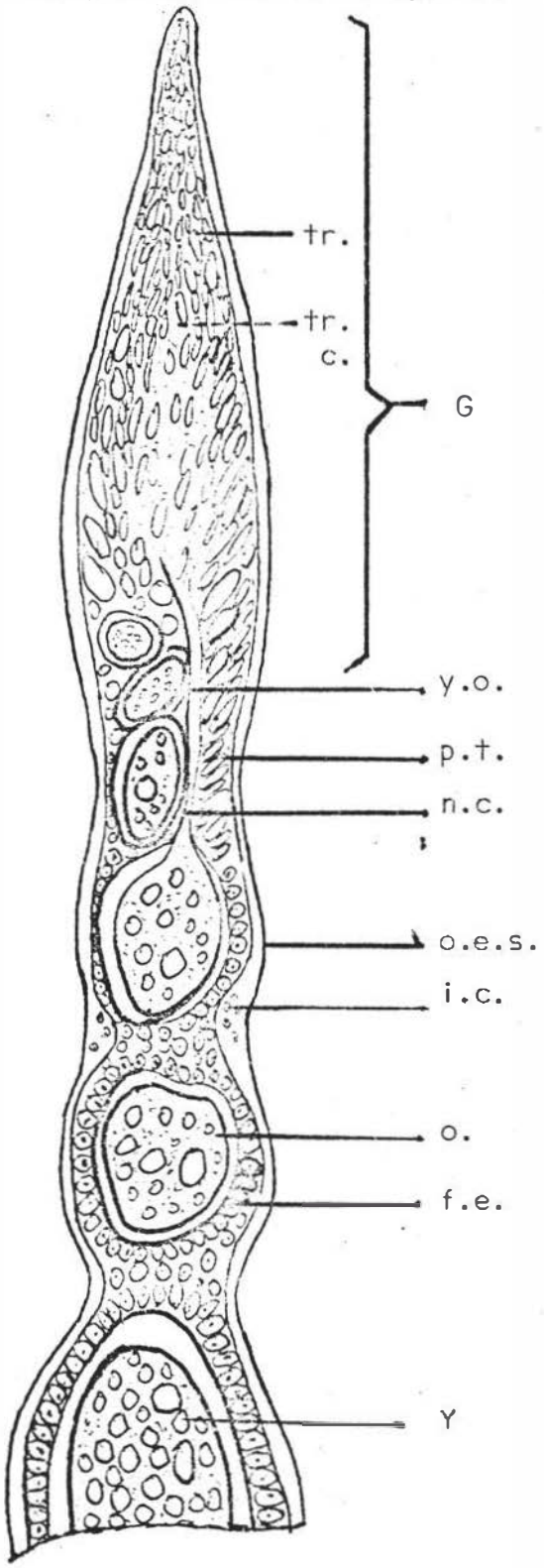

Fig. 2

Plate 3

Sagittal section of a follicle of Oncopeltus fasciatus (Dallas).

Adult stadium (fig. 3). 450X.

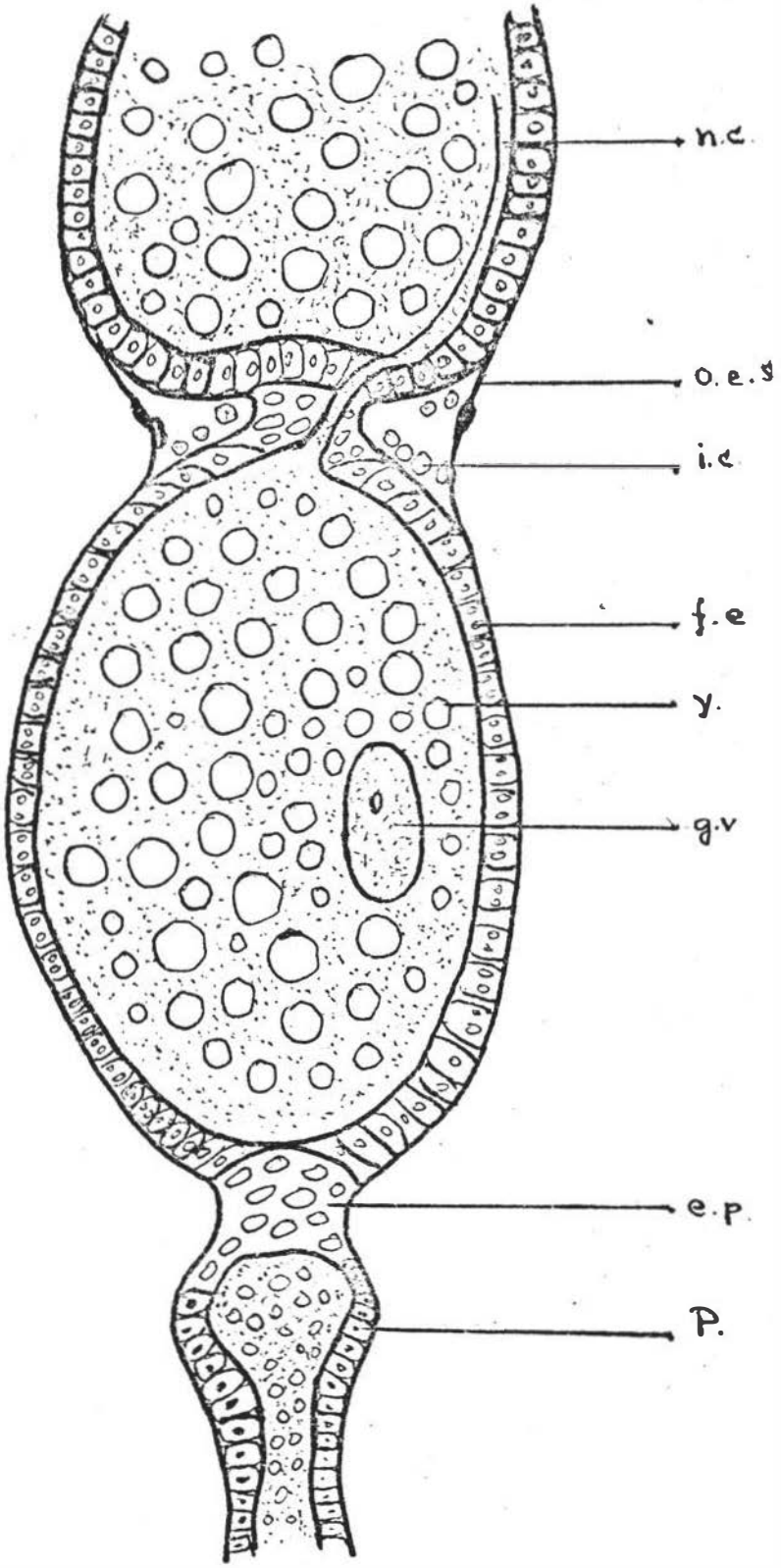

Fig. 3

Plate 4

PAS staining reaction for mucopolysaccharides using Harris' haematoxylin as counterstain. Note the purplish-pink granules in the cytoplasm of the intermediate cells.

Fig. 4 ovary of the fourth instar nymphal stadium 250X

Fig. 5 ovary of the fourth instar nymphal stadium 400X

Fig. 6 ovary of the fifth instar nymph 400X

Fig. 7 ovary of the adult stadium 400X

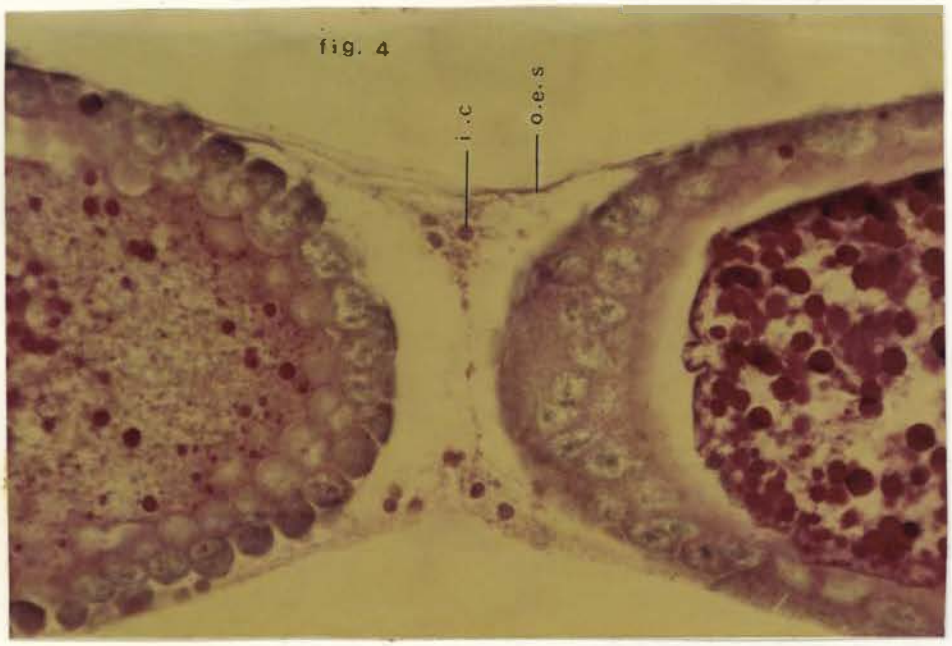
fig. 4
i.c
o.e.s

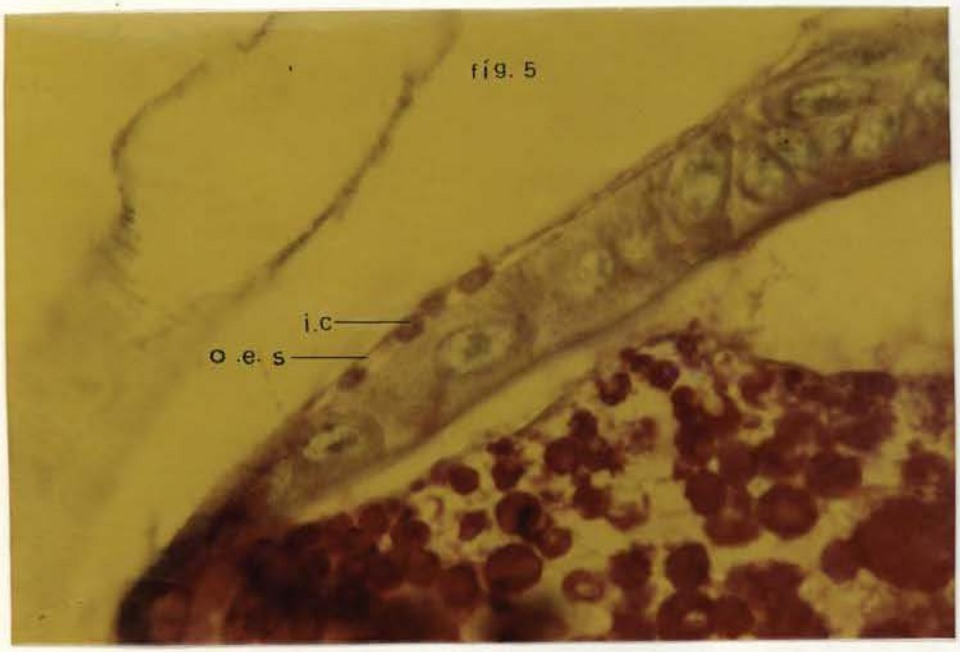
fig. 5
i.c
o.e.s

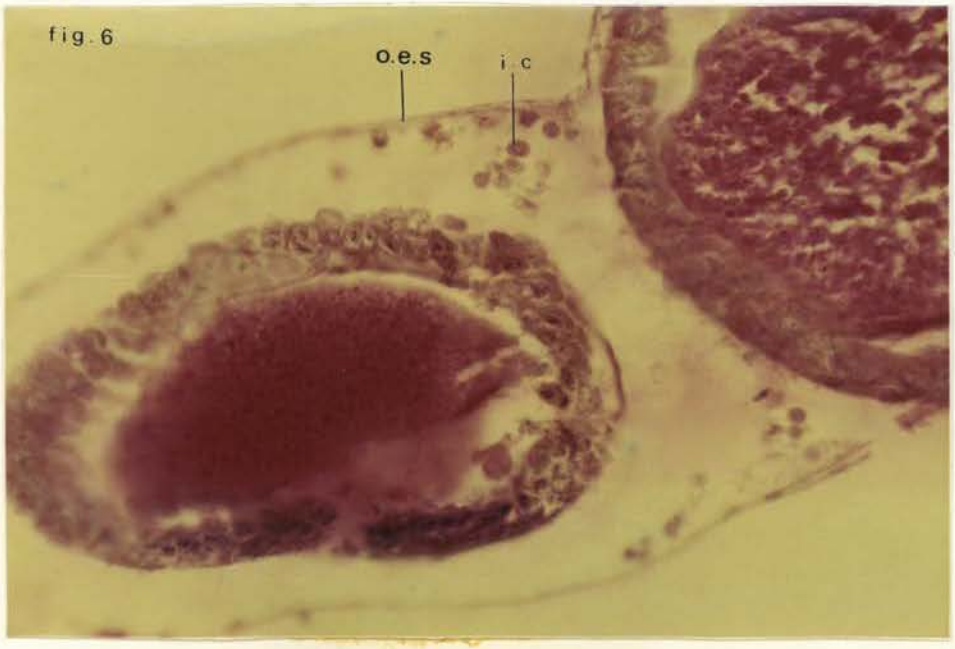
fig. 6
o.e.s
i.c

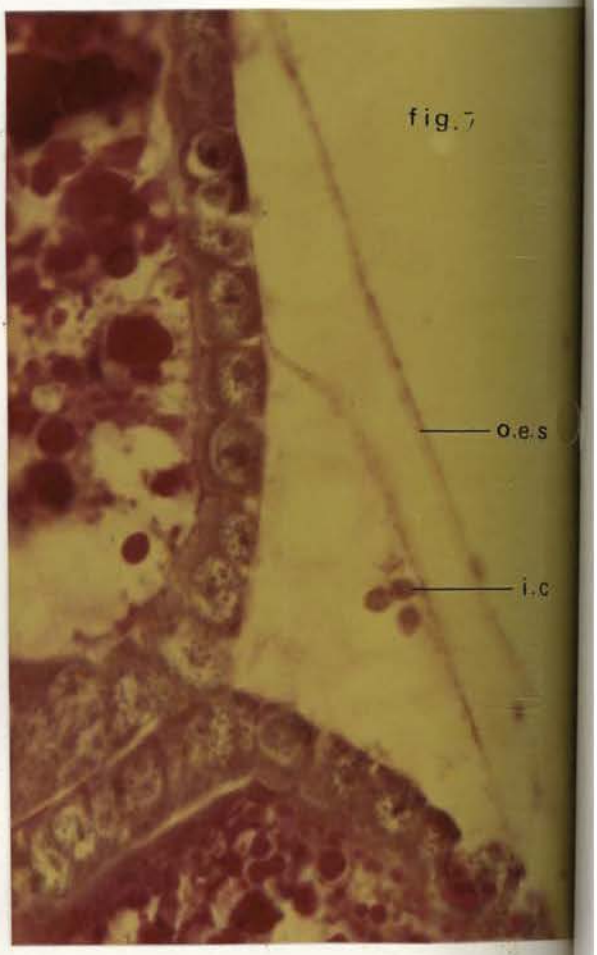
fig. 7
o.e.s
i.c

Plate 5

PAS staining reaction for mucopolysaccharides using light green as counterstain. Note the purplish-pink granules of the intermediate cells.

Fig. 8 ovary of the fourth instar nymph 400X

Fig. 9 ovary of the fifth instar nymph 400X

Fig. 10 ovary of the adult stadium 400X

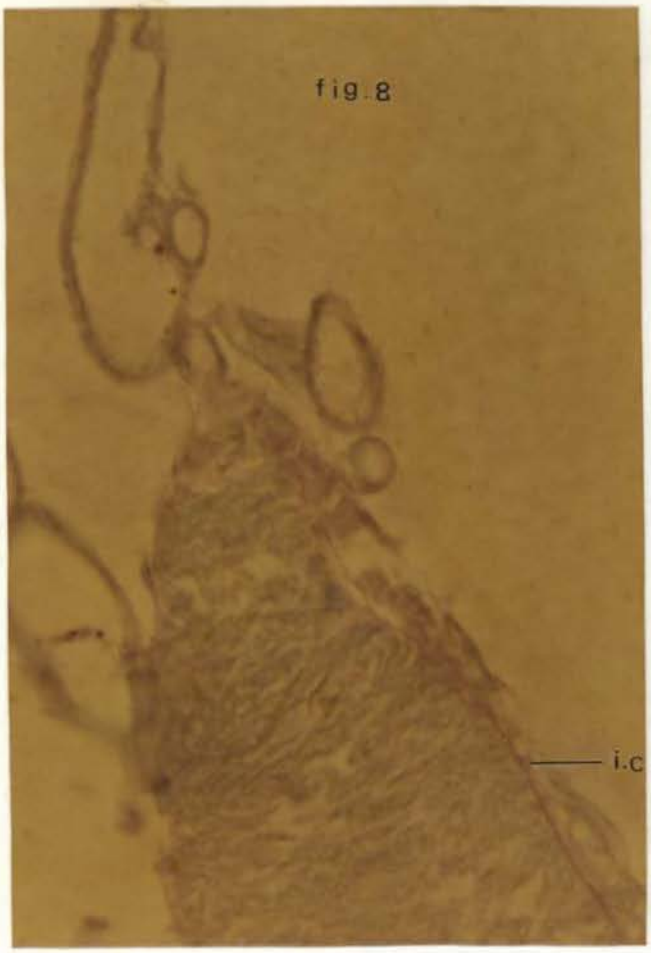

fig.8

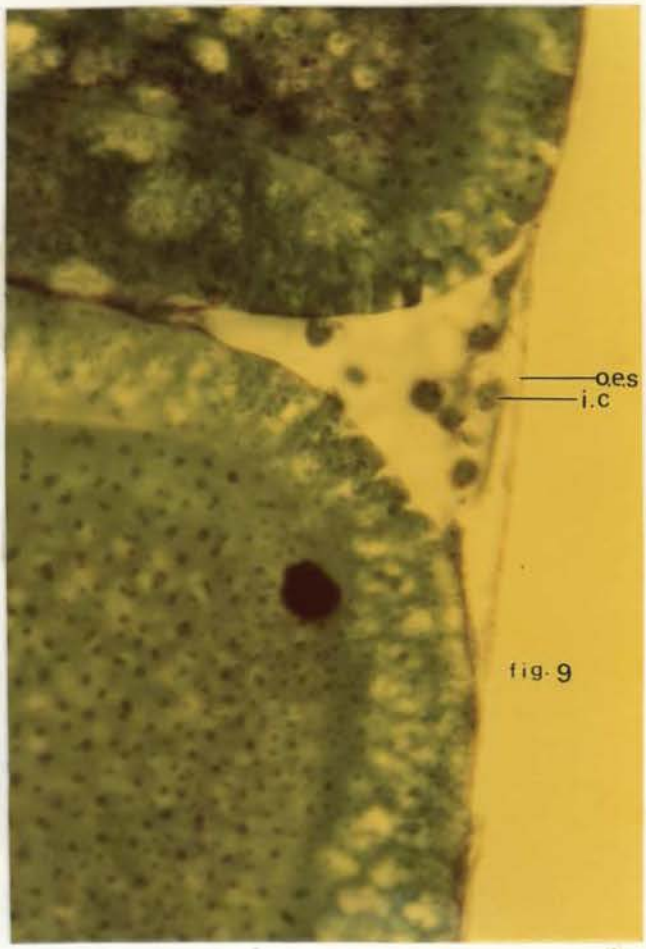

fig.9

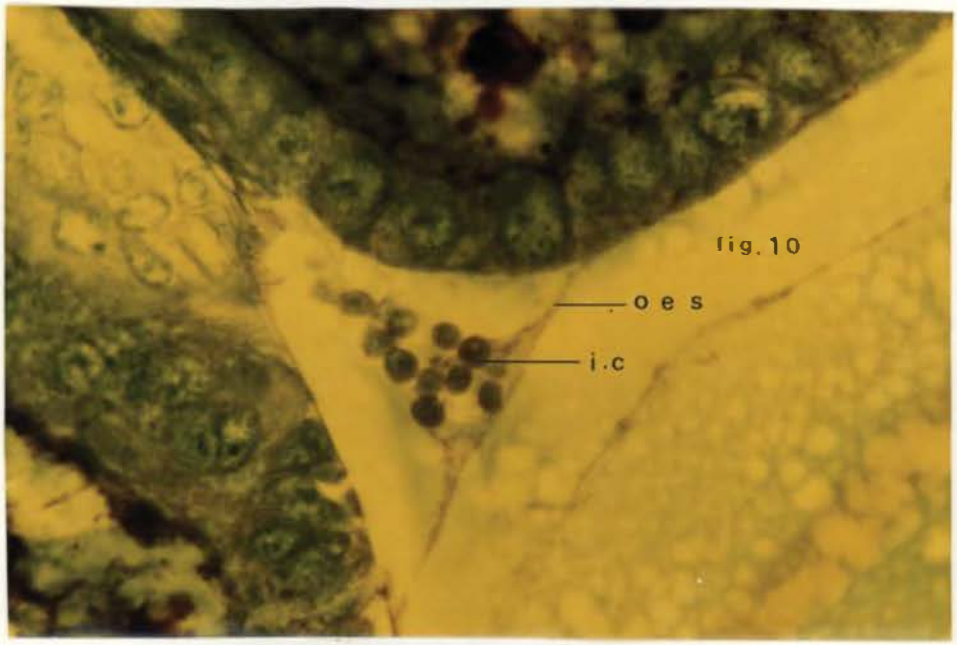

fig.10

Plate 6

PAS staining reaction on control slides pretreated with 0.1% malt diastase solution at 37° C

Fig. 11 ovary of the adult stadium
PAS-Harris' haematoxylin

Fig. 12 ovary of the adult stadium
PAS-light green

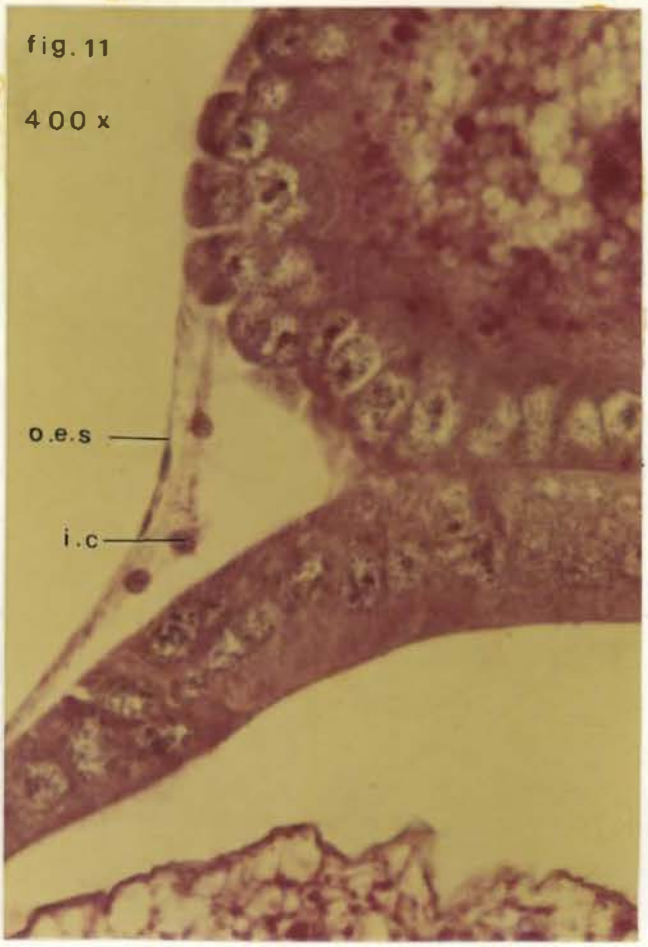
fig. 11
400 x
o.e.s
i.c

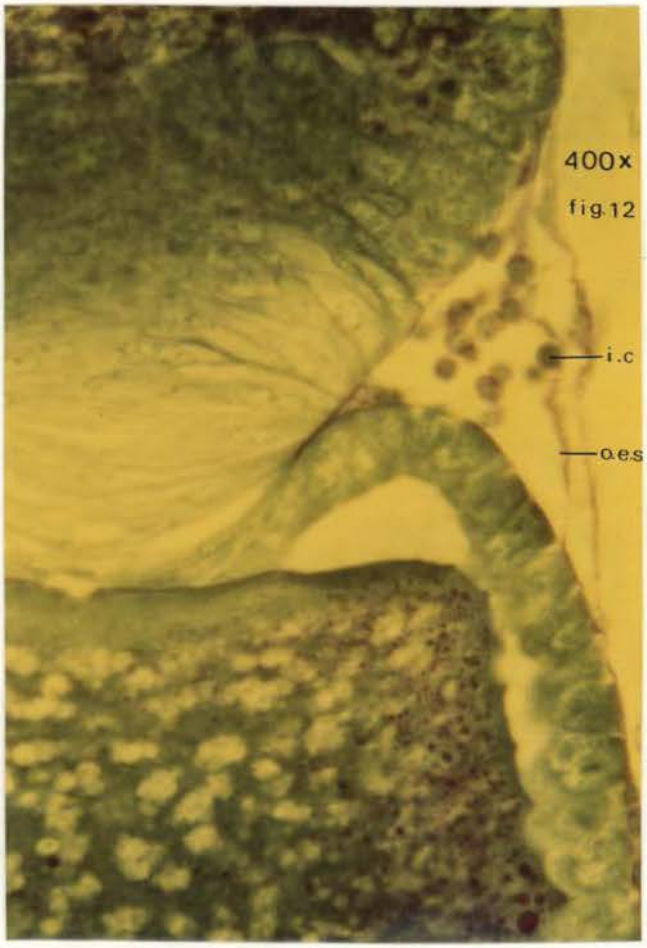
400x
fig.12
i.c
o.e.s

Plate 7

Gomori's paraldehyde fuchsin staining reaction for neurosecretory substances. Note the blue-purplish granules in the cytoplasm of the intermediate cells. Light green was used as counterstain.

Fig. 13 ovary of the fourth instar nymph 400X

Fig. 14 ovary of the fifth instar nymph 400X

Fig. 15 ovary of the adult stadium 400X

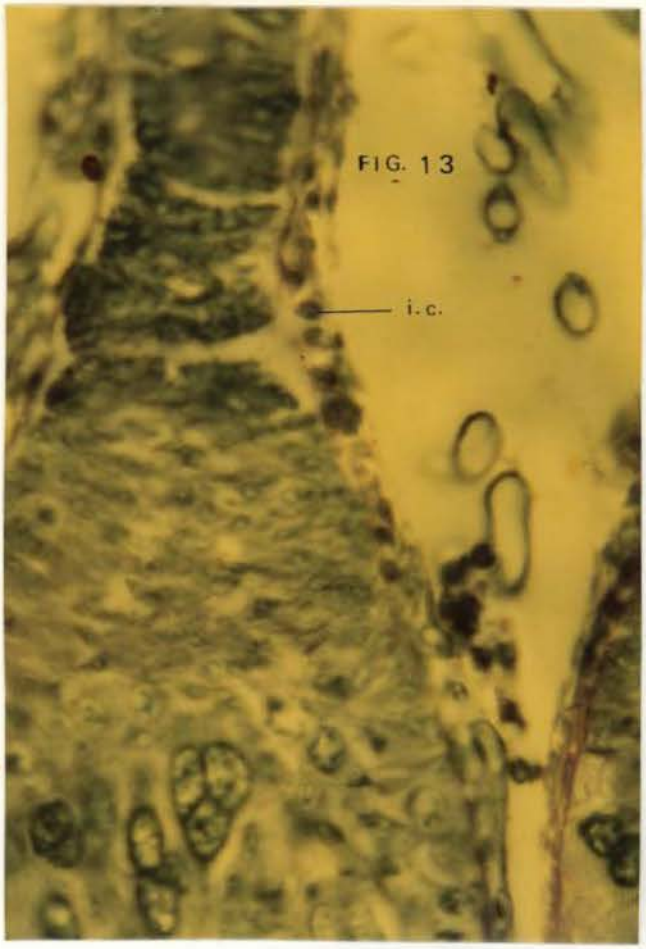

FIG. 13

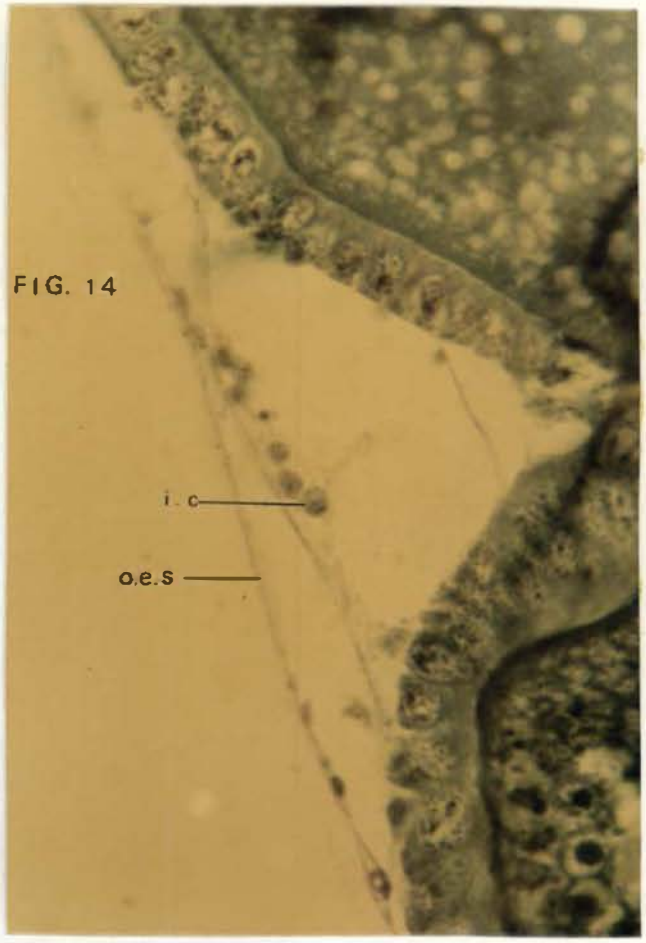

FIG. 14

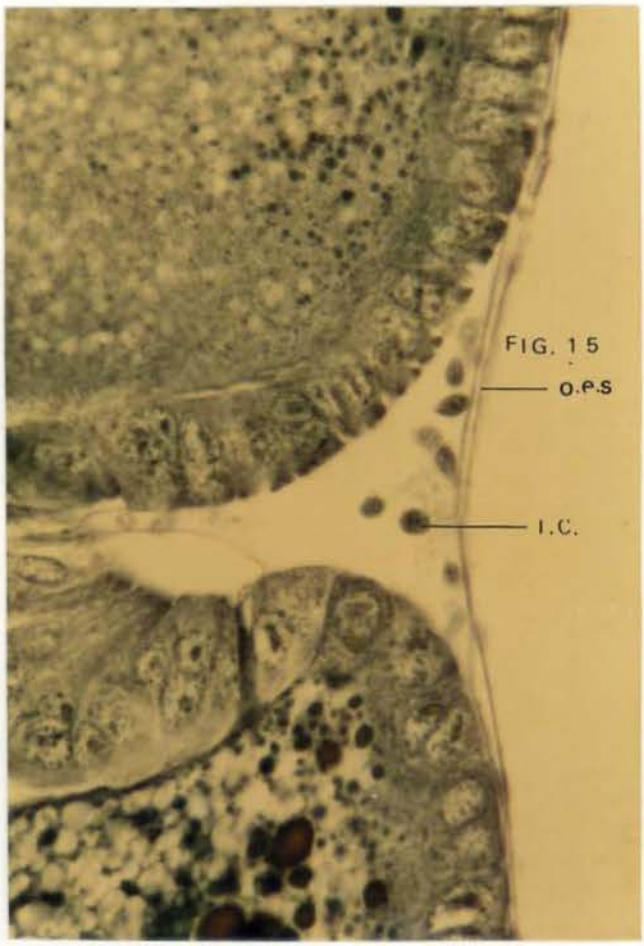

FIG. 15

Plate 8

PAF-light green on control slides pretreated with 1 gram trypsin per 100 ml 0.01N HCl at 37° C

Fig. 16 ovary of the fourth instar nymph 400X

Fig. 17 ovary of the fifth instar nymph 400X

Fig. 18 ovary of the adult stadium 400X

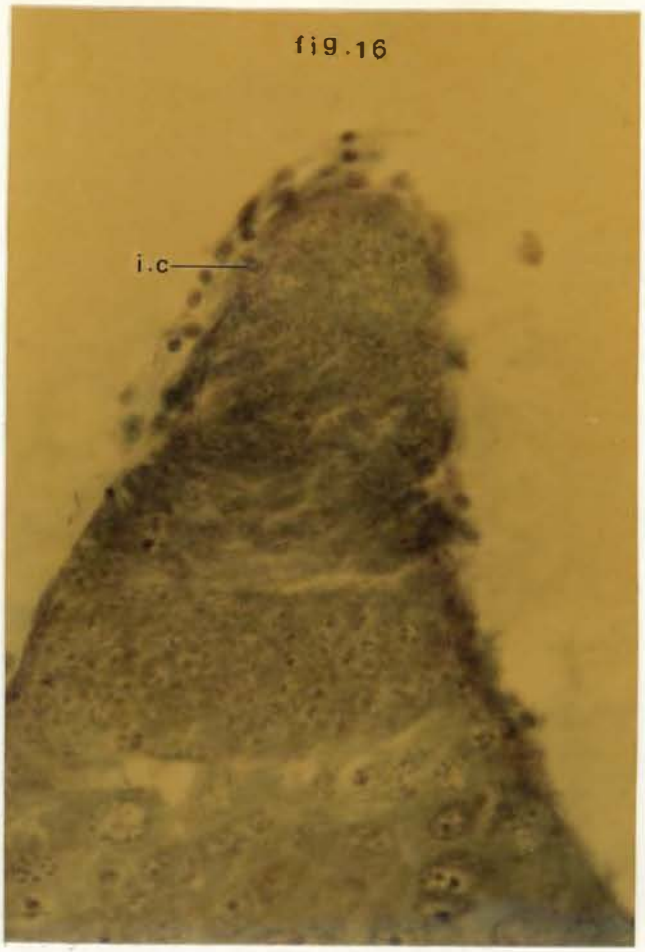

fig.16

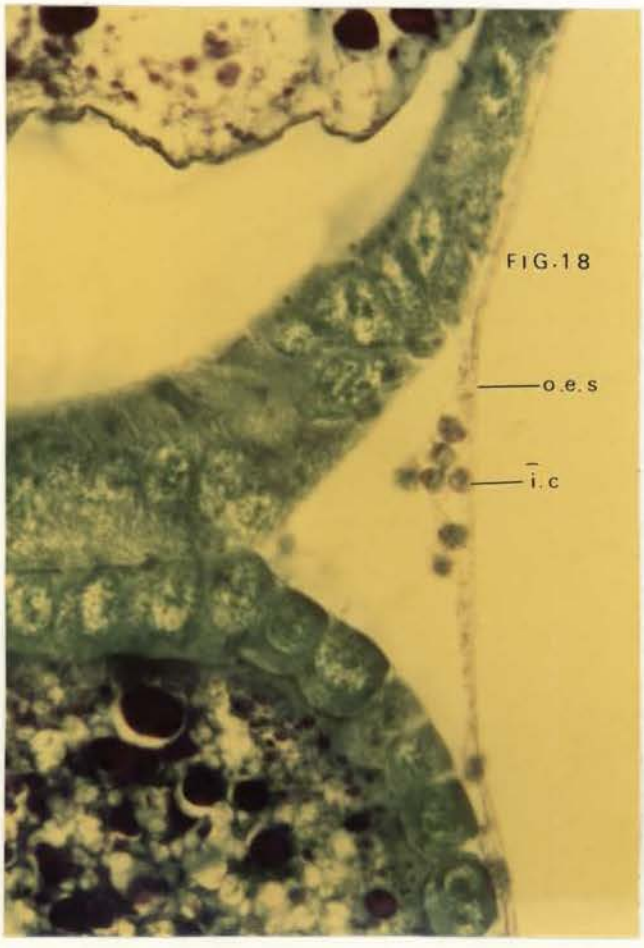

FIG.18

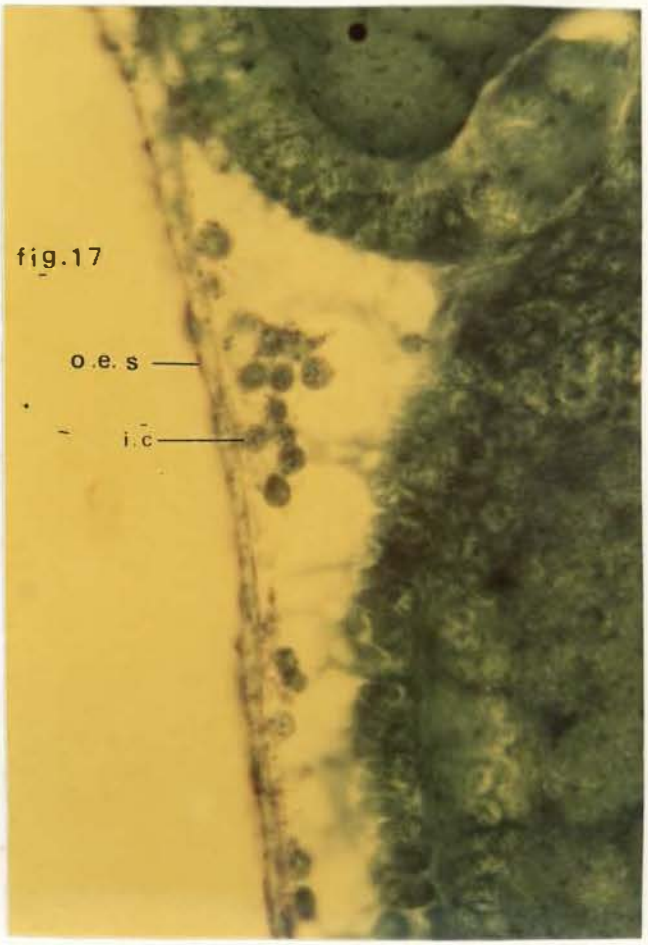

fig.17

Plate 9

Gomori's acid-phosphatase staining reaction for phosphomonesterase II. Note the few black spots in the intermediate cells.

Fig. 19 ovary of the fifth instar nymph 400X

Fig. 20 ovary of the adult stadium 400X

FIG. 19

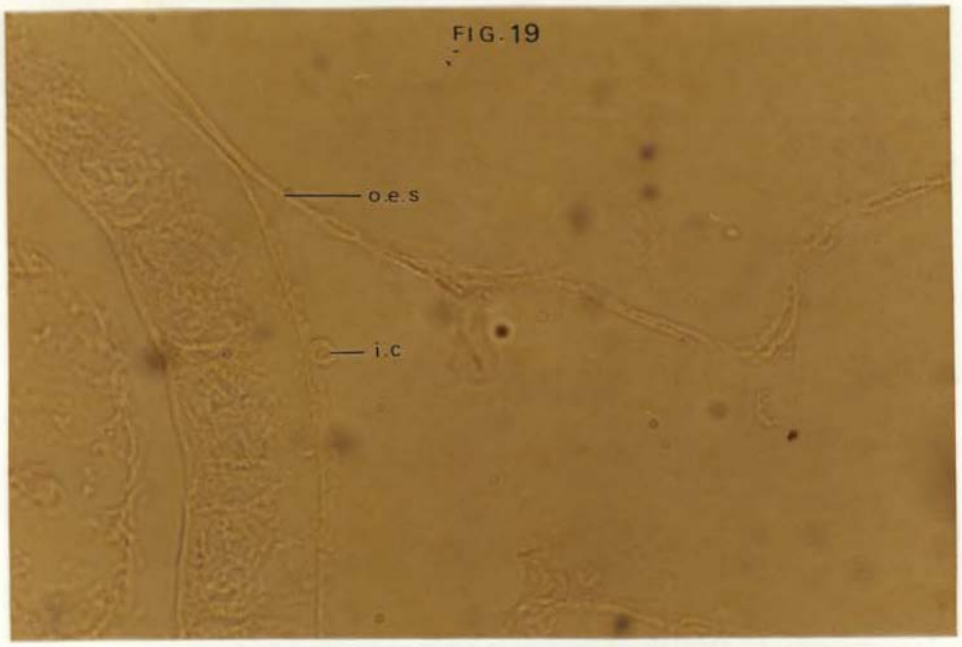

fig. 20

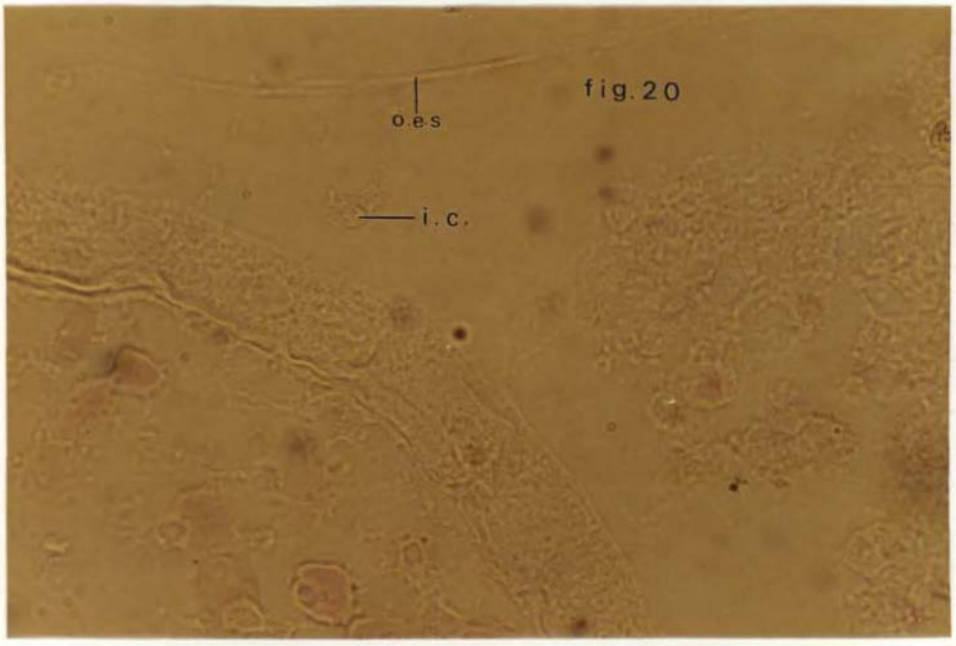

Plate 10

Control slides incubated only in the buffer system without Na-B-glycerophosphate.

Fig. 21 ovary of the fifth instar nymph 400X

Fig. 22 ovary of the adult stadium 400X

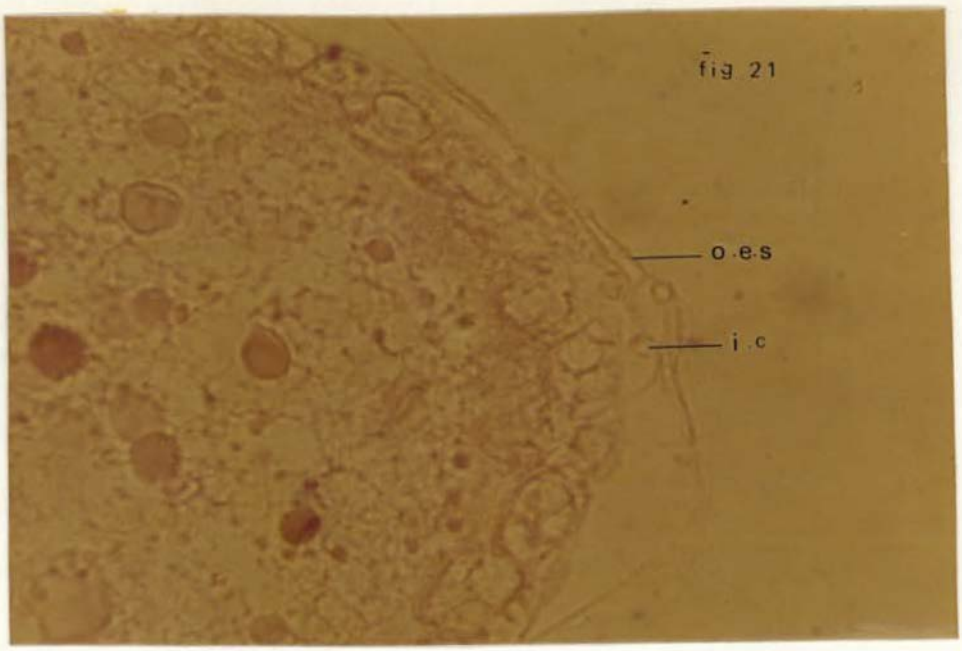
fig. 21
o.e.s
i.c

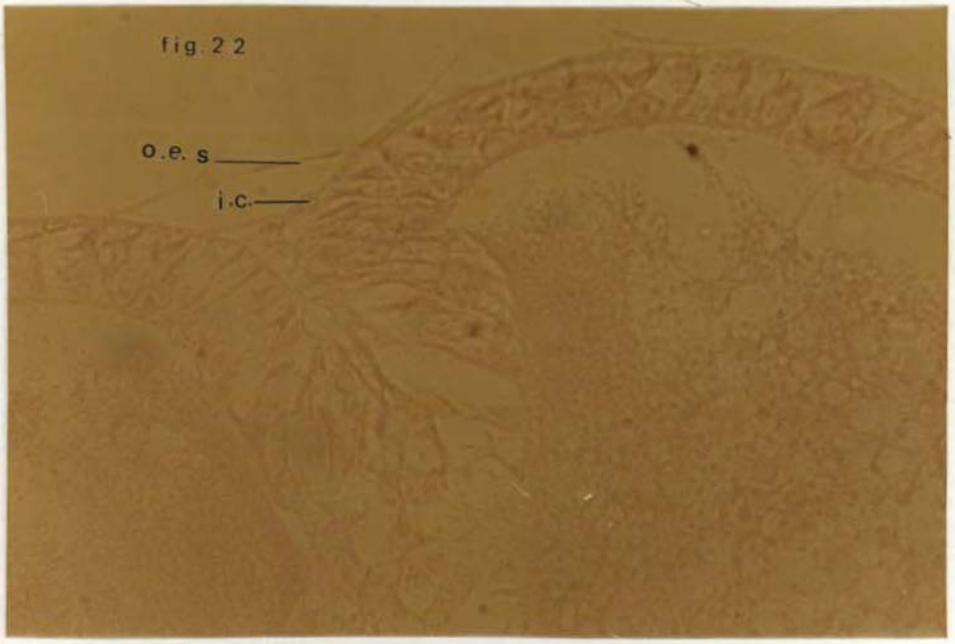
fig. 22
o.e. s
i.c.