University of New Hampshire
University of New Hampshire Scholars' Repository

Doctoral Dissertations Student Scholarship

Fall 1984

OOGENESIS IN TUBULARIA LARYNX AND TUBULARIA INDIVISA (HYDROZOA, ATHECATA)

BARRY W. SPRACKLIN
University of New Hampshire, Durham

Follow this and additional works at: https://scholars.unh.edu/dissertation

Recommended Citation

SPRACKLIN, BARRY W., "OOGENESIS IN TUBULARIA LARYNX AND TUBULARIA INDIVISA (HYDROZOA, ATHECATA)" (1984). *Doctoral Dissertations*. 1436.
https://scholars.unh.edu/dissertation/1436

This Dissertation is brought to you for free and open access by the Student Scholarship at University of New Hampshire Scholars' Repository. It has been accepted for inclusion in Doctoral Dissertations by an authorized administrator of University of New Hampshire Scholars' Repository. For more information, please contact nicole.hentz@unh.edu.

INFORMATION TO USERS

This reproduction was made from a copy of a document sent to us for microfilming. While the most advanced technology has been used to photograph and reproduce this document, the quality of the reproduction is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help clarify markings or notations which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure complete continuity.

2. When an image on the film is obliterated with a round black mark, it is an indication of either blurred copy because of movement during exposure, duplicate copy, or copyrighted materials that should not have been filmed. For blurred pages, a good image of the page can be found in the adjacent frame. If copyrighted materials were deleted, a target note will appear listing the pages in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being photographed, a definite method of "sectioning" the material has been followed. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again—beginning below the first row and continuing on until complete.

4. For illustrations that cannot be satisfactorily reproduced by xerographic means, photographic prints can be purchased at additional cost and inserted into your xerographic copy. These prints are available upon request from the Dissertations Customer Services Department.

5. Some pages in any document may have indistinct print. In all cases the best available copy has been filmed.

University Microfilms International
300 N. Zeeb Road
Ann Arbor, MI 48106

8428160

Spracklin, Barry W.

OOGENESIS IN TUBULARIA LARYNX AND TUBULARIA INDIVISA (HYDROZOA, ATHECATA)

University of New Hampshire PH.D. 1984

University
Microfilms
International 300 N. Zeeb Road, Ann Arbor, MI 48106

PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark ✓.

1. Glossy photographs or pages ✓
2. Colored illustrations, paper or print _____
3. Photographs with dark background ✓
4. Illustrations are poor copy _____
5. Pages with black marks, not original copy _____
6. Print shows through as there is text on both sides of page _____
7. Indistinct, broken or small print on several pages ✓
8. Print exceeds margin requirements _____
9. Tightly bound copy with print lost in spine _____
10. Computer printout pages with indistinct print _____
11. Page(s) __________ lacking when material received, and not available from school or author.
12. Page(s) __________ seem to be missing in numbering only as text follows.
13. Two pages numbered __________. Text follows.
14. Curling and wrinkled pages _____
15. Other ______________________________

University
Microfilms
International

OOGENESIS IN *TUBULARIA LARYNX* AND *TUBULARIA INDIVISA* (HYDROZOA, ATHECATA)

BY

Barry W. Spracklin
B.A., Noreastern University, 1969
M.S., Northeastern University, 1974

DISSERTATION

Submitted to the University of New Hampshire
in Partial Fulfillment of
the Requirements for the Degree of

Doctor of Philosophy
in
Zoology

September, 1984

This dissertation has been examined and approved.

John E. Foret

Dissertation director, John E. Foret
Associate Professor of Zoology

Larry G. Harris

Larry G. Harris, Associate Professor of Zoology

Nathan W. Riser

Nathan W. Riser, Professor of Marine Biology
Marine Science and Maritime Studies Center
Northeastern University

John J. Sasner Jr

John J. Sasner, Jr., Professor of Zoology

E. Tillinghast

Edward K. Tillinghast, Professor of Zoology

7/19/84

Date

ACKNOWLEDGEMENTS

I am grateful for the support, assistance, and encouragement of my doctoral committee, Drs. John E. Foret, Larry G. Harris, John J. Sasner, Jr., Nathan W. Riser, and Edward K. Tillinghast. I thank Dr. Charles W. Walker who reviewed and commented on many parts of this dissertaion and for the use of his lab and research equipment. I am indebted to Eileen Wong for her excellent typing and also her patience. Thanks also to the many graduate students who have assisted in the collecting of specimens and who often returned from diving and collecting trips with hydroids.

I gratefully acknowledge financial support of this research by the Central University Research Fund grant number S74 and a research assistantship from Dr. Charles W. Walker and the Hubbard Farms Fund.

I especially thank my family and friends for their encouragement and support during my graduate studies.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS.. iii

LIST OF FIGURES.. v

ABSTRACT.. vi

SECTION PAGE

INTRODUCTION.. 1

Oogenesis.. 1

Hydroids.. 4

Gonophores and Germ Cells.. 7

Microscopy and Autoradiography.. 15

MATERIALS AND METHODS.. 21

Light Microscopy.. 21

Autoradiography.. 22

Electron Microscopy.. 24

RESULTS.. 26

Light Microscopy.. 26

Autoradiography.. 32

Electron Microscopy.. 76

DISCUSSION.. 112

LIST OF REFERENCES.. 136

LIST OF ILLUSTRATIONS

PAGE

1. _T. larynx_. Underwater photograph (20 feet)................. 34
2. _Clava leptostyla_. Light microscopy photograph of gonophore.. 36
3. _T. larynx_. Light microscopy photograph of small germ cell mass... 38
4. _T. larynx_. Light microscopy photograph of large germ cell mass... 40
5. _T. larynx_. Light microscopy photograph of early stages of oogenesis.. 42
6. _T. indivisa_. Light microscopy photograph of developing oocytes... 44
7. _T. larynx_. Light microscopy photograph of intercellular bridges.. 46
8. _T. indivisa_. Light microscopy photograph of intercellular bridges.. 48
9. _T. indivisa_. Light microscopy photograph of oocyte with two nuclei... 50
10. _T. indivisa_. Light microscopy photograph of oocytes......... 52
11. _T. larynx_. Light microscopy photograph of an actinula....... 54
12. _T. indivisa_. Light microscopy photograph of oocyte-nurse cell complex... 56
13. _T. larynx_. Light microscopy photograph of mature gonophore.. 58
14. _T. indivisa_. Light microscopy photograph of oocyte nucleus.. 60
15. _T. larynx_. Light microscopy photograph of spent gonophore... 62
16. _T. larynx_. Light microscopy photograph of hermaphroditic polyp.. 64
17. _T. indivisa_. Line drawing of oocyte-nurse cell complex...... 66
18. _T. indivisa_. Line drawing of oocyte-nurse cell complex...... 68
19. _T. indivisa_. Line drawing of oocyte-nurse cell complex...... 70

20. *T. indivisa*. Line drawing of oocyte-nurse cell complex...... 72

21. *T. indivisa*. Line drawing of two oocyte-nurse cell complexes.. 74

22. *T. larynx*. Autoradiograph.................................. 78

23. *T. indivisa*. Autoradiograph............................... 80

24. *T. indivisa*. Autoradiograph............................... 82

25. *T. larynx*. Transmission electron micrograph of spadix....... 89

26. *T. larynx*. Transmission electron micrograph of secondary mesolamella.. 91

27. *T. larynx*. Transmission electron micrograph of nutrient vesicles... 93

28. *T. larynx*. Transmission electron micrograph of four intercellular bridges...................................... 95

29. *T. larynx*. Transmission electron micrograph of intercellular bridges.. 97

30. *T. larynx*. Transmission electron micrograph of intercellular bridges.. 99

31. *T. indivisa*. Plastic section of vitellogenic germ cells..... 101

32. *T. larynx*. Transmission electron micrograph of vitellogenic germ cells... 103

33. *T. larynx*. Transmission electron micrograph of shrunken cells.. 105

34. *T. larynx*. Transmission electron micrograph of spadix....... 107

35. *T. larynx*. Transmission electron micrograph of vitellogenic germ cells... 109

36. *T. larynx*. Transmission electron micrograph of yolk......... 111

ABSTRACT

OOGENESIS IN *TUBULARIA LARYNX* AND *TUBULARIA INDIVISA* (HYDROZOA, ATHECATA)

by

BARRY W. SPRACKLIN

University of New Hampshire, September, 1984

In *Tubularia larynx* and *T. indivisa*, interstitial cells proliferate in the ectoderm of the gonophore stalk and peduncle of the raceme and migrate in the endoderm to the apex of the young gonophore buds where they proliferate in the endoderm to form the entocodon and germ cell mass. Eggs are produced sequentially in the gonophores of both species. Each egg is the result of fusion of 1200-2000 cells interconnected by cytoplasmic bridges into a large complex. At the center of each complex are 15-18 morphologically similar cells, all of which begin meiosis and form germinal vesicles and are considered oocytes. Toward the end of vitellogenesis, the oocytes fuse to form a single large oocyte with only one germinal vesicle surviving and completing meiosis. The remaining cells of each complex enlarge and produce yolk, but do not develop a germinal vesicle. These cells are true nurse cells, connected by cytoplasmic bridges to the oocytes. The nurse cells are engulfed by the oocytes and transformed into shrunken cells as their cytoplasm is incorporated by the oocytes. The organization of the oocyte-nurse cell complexes is variable. As many as three oocytes in each complex have four cytoplasmic bridges. Cells

with four bridges may be the result of cellular fusion as both oocytes and nurse cells in many complexes contain two nuclei. An oocyte-nurse cell complex is produced from one initial oogonium by mitotic proliferation and the subsequent complexes in each gonophore are probably from other oogonia. Older polyps of *T. larynx* are frequently hermaphroditic while *T. indivisa* is strictly dioecious.

INTRODUCTION

Oogenesis

Oogenesis in long lived animals such as vertebrates is a complex process, synchronized by hormones, and involving the cooperative interaction of many organ systems. However, even with all of the complexity and capabilities of the vertebrate body, oogenesis can be a rather slow process. In certain frogs each oocyte requires three consecutive years to complete oogenesis. Human oocytes are arrested in diplotene of the first meiotic division in the fourth month of fetal life (Baker, 1963), while the first egg is not normally ready for fertilization for 12 to 14 more years. Postlethwait and Shirk (1981) observed that "people invest nearly two decades in providing sustenance and instruction to their offspring, but insects often spend only a few days in equipping their eggs with nutrient reserves and developmental instructions sufficient to sustain life and program development." In addition to insects, species in the class Hydrozoa, most of which have a brief seasonal existence and a relatively simple level of tissue organization, also manage to accomplish the entire oogenetic process in a relatively short period. _Tubularia larynx_ polyps can release actinulae within 24 days of larval settlement (Pyefinch and Downing, 1949) and _T. crocea_ releases actinulae only six to eight days after gonophores first appear (Mackie, 1966). Gametogenesis in _Hydra carnea_ is completed in only four days (Honegger, 1981). To accomplish this rapid development hydroids employ a number of modes of germ cell formation, oocyte nutrition, and larval development. Considering the varied developmental strategies, range of body forms, photoperiod control, local abundance, and ease of collection of many species, hydrozoans are, in many ways, excellent subjects for gametogenetic and embryogenetic studies.

The result of oogenesis is a highly specialized cell, the egg, which is developmentally totipotent and has the capacity to produce a multicellular organism formed of varied cellular types. However, with all of its latent capacity and raw materials, the haploid egg is normally incapable of further development and is merely a transitional stage between two generations. Except in parthenogenetic species, sperm penetration and pronuclear fusion are required before the egg can begin mitosis and express its developmental potential during subsequent development. Since sperm contribute only chromosomes and often a pair of centrioles, the egg is essentially a self-contained unit. Sufficient yolk, RNA, and other materials must be produced and stored during oogenesis to last until embryogenesis proceeds to the point at which endogenous production commences and a functional feeding stage can utilize an exogenous supply of food. In most species, the process of yolk production and ribosomal synthesis requires a significant expenditure of energy and nutrients by the parent organism. Vitellogenesis, the production and storage of yolk, is thus one of the major parts of oogenesis, except in mammals and other organisms where placental nutrition sustains development from essentially yolkless eggs.

Vitellogenesis occurs in different species of animals by one of three methods. These include, autosynthesis, in which the oocyte produces its own yolk from basic nutrients processed by the somatic cells of the organism; heterosynthesis, in which the yolk materials are synthesized by somatic cells or other germ-line cells and then transported to the oocyte; and by a combination of the two methods referred to as heterosynthesis (Schechtman, 1955; Anderson, 1974). There is a large amount of variation in each of these three basic methods in the type and amount of yolk produced by different animals. Anderson (1974, pg. 39) observed "that nature has richly elaborated on, and in diverse ways, the mechanisms of vitellogensis," and that there has not appeared "some common pattern that would make it an easy task to formulate a classification of vitellogenesis from an evolutionary point of view." There are examples of autosynthesis from a number of phyla including the Cnidaria, and according to Hisaw (1963) this was probably the primitive situation. However, early in the evolutionary development of many animals, the vitellogenic requirements exceeded the capacity of a single oocyte during a breeding season. Berrill and Karp (1976, pg. 100) report that "in most animals - though many exceptions exist - the development of the oocyte takes place with the aid of another type of cell, an accessory cell."

A large number of different terms are used to refer to the various types of cells associated with oocytes during vitellogenesis; all of these are included under the term, accessory cell. This term embraces both accessory cells of somatic origin, called follicle cells, and accessory cells of germ-line origin, called nurse cells (Berrill and Karp, 1976; Browder, 1980). Following Anderson (1974) I will use the term nurse cell to include only those cells derived along with the successful oocyte from a single oogonium. All such cells are interconnected by cytoplasmic bridges that result from incomplete cytokinesis. In hydroids, germ-line accessory cells that lack cytoplasmic bridges are termed nutritive oocytes. Examples of nutritive germ-line cells that are active during oogenesis are known from many invertebrate phyla and nurse cells, in particular, appear to have evolved independently many times. The "nurse cells" reported in sponges by Fell (1969, 1974) are not connected by cytoplasmic bridges to the oocyte and are, in different species, amoebocytes, choanocytes, or cells derived from choanocytes. With the discovery of nurse cells in *Tubularia* during the course of this investigation, the class Hydrozoa of the phylum Cnidaria is now the evolutionarily most primitive grade in which nurse cells have been seen and may be significant to our understanding of the evolution and function of nurse cells.

Hydroids

Within the class Hydrozoa there is a wide range of variation in the type of life cycle, the size, and the life span exhibited by different species. Some species are strictly polypoid, others strictly medusoid, and some species have both polypoid and medusoid phases in their life history. Included in the class are the interstitial actinulids, chondrophores, trachylines, and the polymorphic polypoid-medusoid siphonophores. Among the polyps are included the small hydras and the seven foot tall *Brachiocerianthus imperator*; the medusae include the small, short lived medusae of *Millepora* which survive for only a few hours after release (Hyman, 1940), and the medusae of *Aquorea aquorea* which may live six or seven months in the plankton and grow to 20 cm in diameter (Russell, 1953).

While hydrozoans are structurally simple, with only a tissue level of organization and two cellular layers, a wide range of diversity is seen in the development of hydrozoans and the involvement of accessory cells supporting the growth of the oocyte during vitellogenesis. Mergner (1971) lists five different cleavage types and six methods of germ layer formation. In many hydroids, and in cnidarians in general, accessory cells are not involved during gametogenesis (Campbell, 1974) and vitellogenesis is entirely autosynthetic. At the other extreme are the species in which thousands of germ-line accessory cells support the production of a few or even a single successful or definitive oocyte.

Species in the orders Anthomedusae (Athecata) and Leptomedusae (Thecata) are commonly referred to as hydroids (athecate and thecate) and are the most generally representative groups in the class Hydrozoa which also contains the siphonophores, trachylines, and the interstitial actinulids. Many species of athecate and thecate hydroids exhibit the classic alternation between polypoid and medusoid phases in their life history. While the thecate hydroids are all colonial and have only a planula larva, the athecte hydroids are much more variable. Both solitary and colonial species are present in the Athecata and while most species have a planula larva typical of the phylum Cnidaria, many species develop an actinula larva. There are even species in the athecate family Margelopsidae with pelagic polyps that have no attached phase in their life history.

Most studies of hydrozoan germ cell development and embryogenesis have been accomplished using species with attached gonophores. This is partly because they are usually easier to collect in a reproductive condition in the field or easier to maintain in the laboratory. However, the majority of species of hydroids have no free swimming medusoid phase with feeding tentacles, a functional mouth, and sensory organs, but instead develop attached reproductive structures called gonophores which do not feed and have no sensory structures. The gonophores have been considered by most investigators to represent stages in the reduction of medusae that no longer detach (Kuhn, 1910, 1913; England, 1926; Hyman, 1940). There are, however, no basic developmental differences between them and the species which release free medusae.

Tubularia, a genus of athecate hydroids that lacks a free medusa in its life history, is a member of the superfamily Tubularoidea (Rees, 1957) which includes *Hybocodon*, *Acaulis*, *Corymorpha*, *Euphysa*, *Margelopsis*, *Branchiocerianthus*, and *Pelagohydra*. *Tubularia* is the best known genus in the superfamily and *T. indivisa* Linneus, 1758 is the type species of the family Tubulariidae Allman, 1864. *Tubularia* is one of the most thoroughly investigated genera of marine hydroids and the fresh water hydras may be the only hydroids to have received more attention. Abundance and ease of collecton are among the factors that have led many investigators to study different species of *Tubularia* and the polyps have been described by Agassiz (1862), Allman (1871), Kingsly (1910), Fraser (1944), and Calder (1974). Studies on various aspects of the biology of *Tubularia* have included autotomy of hydranths (Morse, 1909), ecology (Fenchel, 1905; Pyefinch and Downing, 1949), behavioral physiology (Josephson, 1965; Josephson and Mackie, 1965), growth and culture (Mackie, 1966), and regeneration (Barth, 1940; Berrill, 1948; Davidson and Berrill, 1948; Fulton, 1959; Tardent, 1963; Rose, 1974; Tweedell, 1974).

Species of *Tubularia* (Fig. 1) form large, colorful colonies on floats, buoys, and boat hulls along the New England coast and in many parts of the world they are important fouling organisms highly resistant to copper poisons (Barnes, 1948). Polyps of *T. indivisa* Linnaeus, 1758, one of the larger species, may reach a height of 12 inches (Hincks, 1868) and have 40 filiformm tentacles in the proximal whorl and over 40 in the distal whorl (Fraser, 1944). *T. larynx* Ellis and Solander, 1786 is normally 2-3 inches in height with about 20 tentacles in each whorl (Fraser, 1944). Sexually mature polyps of *Tubularia* bear a ring of racemes of reproductive buds or gonophores between the two whorls of tentacles. Fenchel (1905) reported a normal maximum of about 16 racemes on each polyp while Allman (1871) reported over 30 racemes on some polyps of *T. larynx*. Most of the polyps collected for this research had 6-8 fully developed racemes containing mature gonophores and a variable number of immature racemes. Polyps of *T. larynx* usually possessed 100-300 gonophores while the polyps of *T. indivisa* had 400-800. The oldest and most mature gonophores are located at the distal end of a raceme which elongates as new gonophores form at the base of the stalk or peduncle of the raceme. All of the polyps of *T. indivisa* examined were strictly dioecious while older female gonophores of *T. larynx* were often protogynic, which was reported in *T. crocea* by Liu and Berrill (1948).

Gonophores and Germ Cells

The female gonophores (Figs. 3, 4) of both *T. larynx* and *T. indivisa* are eumedusoid (Rees, 1957) which means that they develop all of the cellular layers of a free medusa (Broch, 1915; Teissier, 1926), but fail to form tentacles, sensory organs, or a functional mouth. In *Tubularia* the germ cells segregate at a very early stage from the cells of the entocodon (Liu and Berrill, 1948) which forms the inner ectoderm in a eumedusoid gonophore or the subumbrellar and manubrial ectodermal layers in a free medusa (Hyman, 1940). The spadix is a finger-like projection of endodermal cells into the gonophore and the lumen of the spadix is continuous with the enteron of the raceme and polyp. In mature gonophores the germ cells are tightly packed into a horseshoe-shaped mass (Fig. 4), the germ cell mass (Liu and Berrill, 1948), that surrounds the spadix. Septal cells are scattered within the germ cell mass and interconnect the manubrial layer of the inner ectoderm with the spadix (Liu and Berrill, 1948). When the first egg is produced within a gonophore it ruptures the manubrial layer of the inner ectoderm and simultaneously moves into and creates a space between the manubrial and subumbrellar layers (Boelsterli, 1975). This space, where fertilization and embryonic development to an actinula larva occur, is the subumbrellar cavity (Boelsterli, 1975) and is equivalent to the subumbrellar space in a free medusa (Fig. 13).

An unusual aspect of gametogenesis in the female gonophores of Tubularia is the sequential production and maturation of separate clusters or generations of oocytes (Figs. 12, 13, 15, 16). Before the first egg has been produced from the germ cell mass another cluster of oocytes begins to enlarge to form a second egg (Liu and Berrill, 1948). As the eggs mature they are fertilized and develop within the gonophore which results in the release of a series of actinulae from each gonophore. The total number of eggs and actinulae normally produced by each gonophore is not known.

Many of the characteristics of Tubularia are advantageous during studies of gonophore and germ cell development. Colonies of Tubularia can be raised in the laboratory (Mackie, 1966), the polyps grow and quickly begin to produce gonophores and germ cells (Pyefinch and

Downing, 1949; Mackie, 1966), each polyp has numerous gonophores at various stages of development, one or more species are abundant at many laocation, and at all times at least some of the polyps of a colony are reproductive (Pyefinch and Downing, 1949). However, other features make Tubularia a difficult research animal for developmental studies. One of these features is that it is not an easy animal to maintain in the laboratory and has been used as a water quality indicator in closed seawater systems. West and Renshaw (1970) found that T. crocea would shed its hydranths before other hydroids were affected by poor water quality. This has meant that most developmental studies have relied on freshly collected material, not colonies that have been under long term observation. The large number of gonophores at different developmental stages makes it difficult to follow the progress of individual gonophores and the wall of the gonophore and the large mass of germ cells in each gonphore obscures the observations of germ cells in living animals. Serial sections of fixed material also have disadvantages including the problems associated with deciphering continuous, dynamic processes such as growth, engulfment, or fusion from preparations of different gonophores and different animals. The large number of germ cells in each female gonophore, consisting of groups at different developmental stages, also add to the confusion, not to mention the problem of hermaphroditic gonophores. As a result, even though many investigators have studied the reproductive biology of species of Tubularia, much confusion and controversy remains and interpretations, even within the same species, differ at least somewhat.

Most of the work on germ cell development and gonophore formation of Tubularia has been reviewed by Lowe (1926) and Liu and Berrill (1948) while the literature concerning larval development was summarized by Nagao (1965). There is still no complete agreement on the origin of the entocodon, the site of the initial differentiation of the germ cells, nor the cellular type from which the germ cells of Tubularia develop. This is in addition to the confusion about various aspects of the formation of a mass of about 2000 cells in each female gonophore and their subsequent fusion into a single oocyte.

Since the work by Agassiz (1860) most investigators, including Weismann (1880), Hargitt (1904), Allen (1900), and Hargitt (1909), have considered that the entocodon forms in the ectoderm of the gonophores of Tubularia by the proliferation of local ectodermal cells. They also considered the germ cells to develop from the inner layer of the entocodon without cellular migration. Benoit (1925) and Liu and Berrill (1948) also found no evidence of cellular migration, but reported the entocodon to form by the proliferation of endodermal cells at the apex of the evaginating gonophore. According to Benoit, the entire entocodon formed by the proliferation of a single interstitial cell (I-cell). There have also been advocates for a migration of the germ cells to their final position in the gonophore. Brauer (1891) and Lowe (1926) concluded that the cells of the entocodon, including the germ cells, originate from I-cells that have migrated from the ectoderm of the stalk of the gonophore. Dupont (1942) considered the germ cells and entocodon to have independent origins and according to Campbell (1974, pg. 145), the germ cells "migrate into or with the entocodon" and (pg. 148) "in well-developed medusae, gametogenesis does not occur for some time after medusa liberation." Even allowing for the probability that the development of germ cells and gonophores is not identical in different species of *Tubularia*, the variation in the opinions of different investigators is, at the least, distracting. Liu and Berrill wrote (1948, pg. 39), "Inasmuch as the conclusions in the present paper are in marked disagreement with the more important conclusions both of Dupont and of Benoit ('25), whom Dupont also criticizes, it is optimistic to hope that this will be the last." Boelsterli (1975), the latest investigator of the development of germ cells in *Tubularia*, avoided the problem by starting with oogonia already present in the gonophore and ignoring the problem of earlier developmental stages.

While reviewing the literature on hydrozoan oogenesis it is apparent that hydroids (athecate and thecate) may be separated into two groups based on the proportion of female germ-line cells that complete development or the number of accessory cells supporting the growth of each oocyte. In the first group of hydroids there is no real development of accessory cells and most oocytes complete development, while in the second group a tremendous overproduction of germ cells occurs and these are utilized as accessory cells. Somewhat similar conclusions were obtained by Van de Vyver (1967, 1968a,b) who separated the athecate hydroids into three groups, the Filifera, corynid Capitata, and tubularid Capitata. These groups differed in the type of larva, amount of yolk in the eggs, and the number of excess germ cells.

The first of my two groups of hydrozoans includes the majority of hydrozoan species, none of which are strictly solitary, and includes the Filifera and some of the corynoid Capitata of Van de Vyver. In this group, all, or at least a large proportion, of the female germ-line cells that differentiate, complete oogenesis and there is little or only moderate overproduction of oocytes. According to Berrill (1961) all of the oogonia that differentiate in the gonophores of Hydractinia echinata and Aselomaris mitchili develop into oocytes without proliferation. All of these oocytes complete oogenesis and no accessory cells are present. Vitellogenesis in these species is probably autosynthetic as the nutrients resulting from extracellular digestion of exogenous food resources are carried along the continuous enteron to the gonophores and passed across the mesoglea and the endodermal cells of the spadix to the oocytes. Josephson and Mackie (1965) and Lenhoff (1968) described the pressure changes that move food particles to the gonophores in the polyps of Tubularia. In species that do not release free medusae, the nutritive spadix is resorbed at the end of oogenesis while the embryo is brooded.

Species in which there is a moderate overproduction of oocytes are also included in the first group of hydroids. According to Campbell (1974), while only a few eggs develop in each gonophore, about six or seven oocytes, all of which are capable of completing development, are produced for each egg that actually matures. He reported that (pg. 161), "Most hydrozoan gonads begin with more oocytes than will eventually ripen, and frequently only a single ovum develops in each gonad. The other oocytes are expended in the process of feeding the successful oocyte." Some of these oocytes remain where they originally differentiate and later degenerate, while the rest migrate into the gonophores. In most of these hydroids there is little growth of any but the successful or definitive oocyte which Van de Vyver (1968a) calls "l'oocyte privlegie." The excess oocytes may serve as a partial source of food for the definitive oocytes (Mergner, 1957). However, except for the degeneration and possible resorption of these relatively few excess oocytes, vitellogenesis is still basically autosynthetic. The eggs of the species, including Clava leptostyla (Fig. 2), which produce few excess oocytes are relatively small. The largest eggs reported in these species were produced by Hydractinia echinata and were 168 um in diameter.

A more complex and interesting system of oogenesis is found in the second group of hydroids which includes the tubularid Capitata and most of the corynid Capitata of Van de Vyver. In this group there is a tremendous overproduction of germ cells. The large, solitary athecate hydroids and some colonial species produce large, yolky eggs and many brood the resulting embryo to an advanced larval stage, the actinula (Fig. 11). In gonophores of these hydroids, which include Tubularia, Hydra, and Pennaria, hundreds to thousands of excess or nutritive oocytes are produced and utilized as food in the development of relatively few definitive oocytes. In the species that produce an actinula larva, usually only one is present in each gonophore at a time, although a sequence of actinulae may be brooded and released. Benoit (1925), working with T. mesembryanthemum, and Boelsterli (1975), working with T. crocea, have reported two or three eggs or early embryos in a single gonophore, but there are no reports of two or more actinulae simultaneously present in a single gonophore. The nutritive oocytes may be directly engulfed or may disintegrate; in any case, the resulting nutrients are absorbed by the definitive oocytes. Before being engulfed, the nutritive oocytes in Tubularia species, Hydra (Zihler, 1972), and Pennaria (Cowden, 1964) produce yolk and ribosomes that will be incorporated by the definitive oocytes.

There is a large amount of variation in the size of the oocytes and germinal vesicles of different hydroids and two earlier investigators, Jorgensen (1913) and Hargitt (1919), attempted to correlate the different methods of oocyte nutrition with relative nuclear size. Jorgensen proposed that oocytes that absorb their food directly from the surrounding fluid have relatively large nuclei; oocytes which are fed by special nurse cells or follicle cells or engulf other cells have relatively small nuclei. He believed that the nuclei of the accessory cells governed the growth of the oocyte and that the nuclei of the oocytes remained relatively inactive until the end of the prophase of meiosis one. An extreme example of this type of oocyte nutrition was reported by Pianka (1974) in the ctenophore, _Bolinopsis microptera_, in which the oocyte nucleus does not enlarge at all during vitellogenesis.

Hargitt (1919) observed that in hydrozoans there are two different methods of oocyte nutrition which seem to overlap in certain species. In some hydroids, food is absorbed directly by oocytes from the enteron or from the endodermal cells of the spadix, while in others the oocytes absorb or engulf other cells, usually extra or nutritive oocytes. In an attempt to quantify Jorgensen's proposal, Hargitt made measurements of the oocytes of 14 hydrozoans and obtained results consistent with Jorgensen's predictions. In species that are nourished directly from the enteron, the ratio of oocyte volume to nuclear volume was found to range from 16:1 to 73:1. In species with oocytes that engulf other cells or have some form of nutritive oocytes, the ratio ranged from about 284:1 for _Corymorpha pendula_ to about 6000:1 for _T. crocea_. In all of these species, the germinal vesicle of the definitive oocyte enlarges during oocyte growth. This connection between oocyte-nuclear volume and the mode of oocyte nutrition is presumably related to the extent of participation of the oocyte nucleus in vitellogenesis.

While the entire process of oogenesis is rapid in *Tubularia*, *Hydra*, and other hydroids with nutritive oocytes, in many animals of different phyla, the meiotic prophase has a long duration. The insects which possess nurse cells (meroistic oogenesis) also have a short meiotic prophase. Davidson (1968) hypothesized that there must be some rate limited synthetic activity that can be accomplished in a few days by the concerted efforts of polyploid nurse cells of insects, but which normally takes a solitary oocyte considerably longer. According to Davidson (pg. 183), "the molecules in question cannot be yolk, since yolk and other such products are typically accumulated in the course of a relatively brief period of the diplotene stage." Work on *Xenopus* by Perkowaka *et al*. (1968) revealed that the limiting factor in the rate of oogenesis is not the production of yolk, but the production of ribosomes. The nuclei of *Xenopus laevis* oocytes contain 1400 to 1600 nucleoli and about 5200 nucleolar DNA cores or rings while the normal number of nucleoli of somatic cells in *Xenopus* is two. Calculations indicate that without amplification of the ribosomal cistrons and nucleoli, about 1.7×10^5 days or about 466 years would be required for the oocytes to synthesize an equivalent number of ribosomes on their own. While there are no reports of gene amplification in the Cnidaria, the same result is achieved in the second group of hydroids in which many germ-line cells, each with genes for ribosome RNA, produce materials that are eventually incorporated by the definitive oocytes.

Microscopy and Autoradiography

A large number of light microscopical observations have been made on hydrozoan oogenesis, including ivestigations of species that produce large numbers of excess germ cells. Van de Vyver (1968) found that oogenesis in Arum cocksi involves the growth of one "cellule privilegie" by the engulfment of the other oocytes in the gonophore. While this is the situation in many hydroids with nutritive oocytes, it is readily apparent that there is much variation in the oogenetic process in these hydrozoans. In addition to the absorption of the nutritive oocytes, Hargitt (1917) reported that there were indications of fusion of the oocytes in Hybocodon prolifer. Mangan (1909) suggested that in Millepora the cell membranes between the definitive oocyte and the surrounding nutritive oocytes dissolve and a plasmodium forms. In Eudendrium hargitti, a species with a large oocyte-nuclear volume ratio, but without nutritive oocytes, Congdon (1906) reported engulfment of somatic ectodermal cells by the oocytes. True nurse cells, with direct cytoplasmic connections to the definitive oocytes, are also present in hydroids and have been found in Tublaria larynx and T. indivisa by Spracklin (1978, 1980). It is entirely possible that the excess germ cells of other hydroids are also nurse cells, but where cytoplasmic bridges do not exist, or have not yet been found, these cells are appropriately called nutritive or abortive oocytes.

More extensive investigations of oogenesis involving hydroids with nutritive oocytes have been done on species in a number of genera including Pennaria, Hydra, and Tubularia. Oogenesis in Pennaria tiarella has been investigated by Smallwood (1899), Hargitt (1900), Berrill (1961), and Cowden (1964). In this species the primordial germ cells proliferate to produce several thousand oocytes in each gonophore. Approximately 10 oocytes in each gonophore grow at an accelerated rate and consume the remaining nutritive oocytes. The nuclei of the consumed cells persist until the larval ectoderm develops.

Even though it is sometimes difficult to induce sexuality in *Hydra* held in the laboratory, the ease of maintaining this fresh water hydroid and its availability have led many investigators to use it extensively for investigations of oogenesis including Brauer (1891), Brien (1950a,b, 1961, 1963), Brien and Reniers-Decoen (1949, 1951), Downing (1909), Korotneff (1878), McConnell (1933), Tannreuther (1908, 1909), and Wagner (1909). Zihler (1972), who was the first to report ultrastructural observations of oogenesis in this genus, divided oogenesis in *Hydra attenuata* and *H. circumcinta* into five stages. In stage I, groups of oogonia develop from interstitial cells and proliferate in the ectoderm of the polyp. In stage II, after the oogonia I have grown somewhat, one oogonia in each group develops into an oocyte. During stage III, the ooctye in each group engulfs the remaining oogonia. The remains of the engulfed oogonia are very distinctive structures called "pseudozellen" or "shrunken cells". In stage IV, the large oocytes from the original groups fuse to form a single large oocyte which matures to form the egg in stage V.

Since Agassiz (1860), oogenesis and gonophore development in the genus *Tubularia* have been investigated by numerous researchers including, most recently, Liu and Berrill (1948), Nagao (1965), Van de Vyver (1968), and Boelsterli (1965). Liu and Berrill reported that a germ cell mass of several thousand cells was formed by a rapid proliferation of the primordial germ cells and Boelsterli found that, as in *Hydra*, a small number of oocytes begin to grow and subsequently engulf the surrounding nutritive oocytes. In each gonophore, the growing, or definitive, oocytes fuse to form a single large oocyte that completes meiosis.

The remains of the engulfed nutritive oocytes, the pseudocells, persisted in the oocytes of both Tubularia (Figs. 10, 11, 33) and Hydra. Wager (1909) realized that at least some of the pseudocells were the shrunken remains of whole cells and Zihler (1971) introduced the more descriptive term, shrunken cells. Boelsterli (1975) was the first to demonstrate that the pseudocells in Tubularia oocytes were not degnerating nuclei, but the shrunken remains of the engulfed nutritive oocytes, complete with a small amount of cytoplasm, membranes, yolk, and degenerating mitochondria. According to Nagao (1965), most of the shrunken cells persist for several days after fertilization in Tubularia and a few can still be found at the basal part of the hypostome in the settled actinula six days after fertilization.

While light microscopy has been used extensively in the investigation of germ cell development in the Hydrozoa, only a small number of papers have appeared on the ultrastructure of hydrozoan oogenesis. Very little additional work has been published since Boelsterli (1977, pg. 267) wrote, "Whereas light microscopy studies of oogenesis in hydroids and hydromedusae are quite numerous, relatively little information is available on the ultrastructural aspects of gametogenesis." The only additions I have to add to Beolsterli's list of ultrastructural papers are Van de Vyver (1964, 1967, 1968), Tucker and Wyttenback (1974), and Honegger (1981). Van de Vyver's papers were largely concerned with light microscopy and TEM of the embryological development of five athecate hydroids, but did include some information on gametogenesis. Tucker and Wyttenback included some information on the primoridal egg cells, and Honegger's work involved scanning electron microscopy (SEM) of sperm and egg release and the embryotheca, and the transmission electron microscopy (TEM) of fertilization in *Hydra carnea*. This small collection of ultrastructural papers, some of which are extremely limited in scope and contain minimal ultrastructural information, has produced only a superficial picture of hydrozoan oogenesis at the ultrastrucutral level. In addition, these papers consider only a few of the many genera and species with nutritive ooctyes. Van de Vyver (1968a) published a single micrograph of *T. ceratogyne*; Boelsterli (1975) worked with *T. crocea*; Zihler (1972) investigated *Hydra attenuata* and *H. circumcincta*; and Honegger (1981) observed *H. carnea*.

While the ultrastructural information on hydrozoan oogenesis in the literature is sparse, autoradiographic information is non-existent. Autoradiographic techniques, which have proven valuable research tools in the study of oogenesis in other organisms, have not been used to study oogenesis in hydroids. In other animals autoradiography has been used, with great success, to follow events such as the amplification of nucleolar RNA in *Xenopus laevis* oocytes (Perkouska, Macgregor and Bernstiel, 1968) and to identify the sites of synthesis of the various yolk proteins packaged in the eggs of *Drosophila melanogaster* (Gutzeit, 1980). Isotopically labeled compounds have been especially useful in understanding the function of the nurse cells in the meroistic ovaries of insects. Bier (1963, 1965) produced striking demonstrations of the production of proteins and RNA by the nurse cells of *Drosophila* and subsequent transfer of these products to the oocytes. Autoradiographic studies with hydroids has been concerned with the incorporation of labeled compounds (Burnett et al., 1962), hydrozoan development (Haynes, Summers, and Kessler, 1974), DNA synthesis and cell movement during regeneration (Tweedell, 1974), cellular proliferation (Campbell, 1965), the synthesis of nucleic acids and proteins (Clarkson, 1969), and the symbiotic relationship between algae and hydroids (Muscatine and Lenhoff, 1963; Sznant,1971; Eisenstadt, 1971).

In the present study I examine the organization and ultrastructure of the oocyte-nurse cell complexes in *T. larynx* and *T. indivisa,* propose a method of formation of the complexes, and explore hydrozoan oocyte nutrition.

MATERIALS AND METHODS

Light Microscopy

Colonies of Tubularia larynx and T. indivisa were collected from floats located at the mouth of the Piscataqua River in Portsmouth, New Hampshire; from tide pools at East Point, Nahant, Massachusetts, and Eastport, Maine; and from the subtidal at the Isle of Shoals, New Hampshire. For routine light microscopical histology most specimens were fixed in Bouin's fixative, although Helly's, Zenker's, Orth's, Susa's, AFA, Sanfelice's, and 10% formalin-sea water fixatives were also used. During the water rinse racemes were removed from the fixed polyps with microdissecting scissors and separated into individual gonophores or small clusters of gonophores. To observe very young gonophores, small polyps were fixed in Bouin's fixative, the tentacles removed, and the remainder of the polyp cut into two or four pieces. The gonophores and pieces of polyps were dehydrated in a graded series of ethanol (two 10 minute rinses each of 70%, 85%, 95% and 100%) and, after an overnight infiltration at 37°C or 42°C, were embedded in polyethylene glycol 400 or 600 distearate mixed 10:1 with cetyl alcohol. Specimens were sectioned at 2, 4, 6, 8, 10, 12, 15, or 20 µm. Slides were stained with Mallory-Heidenhain Rapid Stain (Humanson, 1972), Heidenhain Iron Hematoxylin, Thionin-Methyl Green, or Azan, and mounted with Permount containing 10% terpineol to reduce crazing. Gonophores fixed in Zenker's fixative were sectioned and treated with the Feulgen and Periodic Acid-Schiff (PAS) reactions. Whole mounts were made of gonophores or racemes from polyps fixed in Bouin's or Zenker's fixatives and were stained with Lynch's precipitated borax carmine or Proecher's coelestine blue B-lake (Riser, 1950). Approximately 16,000 gonophores of T. larynx and 4,000 gonophores of T. indivisa were serially sectioned and approximately 500 whole mounts were made of gonophores of each species.

Various techniques were tried to produce shrinkage or swelling to permit better observation of the intercellular bridges and the oocyte-nurse cell complexes in the gonophores of both species by spreading out the cells of the complexes. Gonophores were fixed in Bouin's fixative lacking acetic acid, in saturated aqueous picric acid, in 70% and 90% ethyl alcohol, and in 85% ethyl alcohol mixed 5:1 with a solution of saturated aqueous picric acid, and were sectioned on a rotary microtome at 10 µm. Squash preparations with fast green were made of untreated gonophores and gonophores that were opened and then partially digested with trypsin or papain. Whole gonophores and those broken open with dissecting needles were also subjected to the maceration techniques of David (1972) and Schmid et al. (1981).

To provide specimens to compare with Tubularia, a small number of reproductive, female polyps of Acaulis primaris, Rhizogeton fusiformis, Eudendrium ramosum and Clava leptostyla were collected and fixed in Bouin's fixative. The gonophores were embedded in polyethylene glycol 400 distearate, sectioned at 10 um and stained with Mallory-Heidenhain Rapid stain.

Autoradiography

The volumetric capacity of the polyps was determined by injecting a solution of methylene blue sea water (0.1%) directly through the mouth of the polyp into the enteron with a number 33 needle (0.008 inch

O.D.) and a 100 µl syringe. Polyps of T. larynx could retain about 15 ul of stain without significant leakage while the larger polyps of T. indivisa could retain about 100 µl. Ten hydranths of each species were removed, blotted partially dry, and weighed to allow calculation of the amount of isotope required for a dosage of 2 µCi/gm wet weight. This amount was then doubled to allow for label lost to the stems and stolons. The variable length of the stems and the encrusting material on the stolons made it impossible to accurately weigh these parts of the colonies.

For each experiment using autoradiography, large colonies of T. larynx and T. indivisa were collected from Portsmouth Harbor. Portions of female colonies, each consisting of about 20 polyps, were kept in finger bowls. The solutions of ^{3}H-labeled uridine (5,6-^{3}H) and thymidine (methyl-^{3}H) were diluted to 0.0625 µCi/ul with sterile, Millipore filtered (0.22 µm) sea water. Under a dissecting microscope, ^{3}H-labeled uridine or thymidine was injected directly into the enteron through the mouth of each polyp. Injections were initiated about one hour after collection of the colonies.

Polyps of T. larynx received 4 µl (0.25 µCi) of solutions of uridine or thymidine while polyps of T. indivisa received 10 µl (0.625 uCi) of uridine. Uninjected control polyps from the original large colonies were placed in Bouin's fixative at the completion of the injections. Injected polyps were fixed after 2, 5, 12, 24, and 45 hours of incubation.

The first series of polyps was incubated on February 10, 1980 at 4°C. A second series of polyps, that did not include a 45 hour sample, was incubated on July 7, 1980 at 8°C (to match the warmer water temperature at the collection site). Racemes were removed from the fixed polyps and cut into either small clusters of young gonophores or individual large gonophores. The gonophores were embedded in polyethylene glycol 400 distearate (mixed 10:1 with cetyl alcohol). Specimens were sectioned at 4 um or 10 um, mounted on glass slides, and were coated with Kodak NTB-3 Nuclear Track Emulsion. Resulting slides were exposed for 12 days for the first incubation and 30 days for the second incubation, developed for two minutes in Kodak D-19 at 19°C, and counterstained for two minutes with Mallory-Heidenhain Rapid stain.

Electron Microscopy

Small portions of colonies of *T. larynx* and *T. indivisa* were collected from floats in Portsmouth Harbor. Male and female polyps of both species were immediately placed in 2.5% phosphate buffered glutaraldehyde for two hours followed by post fixation in 2% osmium tetroxide for one hour (Cloney and Florey, 1968). Fixation was carried out at pH 7.4, 7.6, or 7.8 at room temperature (20-25°C) and at 4°C. During the first buffer wash racemes were removed and cut into small clusters of young gonophores or individual large gonophores. Specimens were embedded in Spurr's medium (Spurr, 1969; Ladd Research Industries, Inc.), Epon 812 (Ladd), and Epon 812-Araldite 506 (Mollenhauer, 1963; Polysciences). DMP-30 was the catalyst normally used with Epon 812, although DMP-10 was also used along with prolonged infiltration to improve the infiltration of yolk and shrunken cells in the large oocytes and embryos. Sectioning was carried out using glass knives on a Sorvall MT-1 or a Reichert OMU3 ultramicrotome. Some sections were mounted on formvar coated, 100 mesh, copper grids and stained 8 minutes with saturated aqueous uranyl acetate and 8 minutes with Reynold's lead citrate (1963). Other sections were mounted on 200 and 300 mesh grids and stained 8 minutes with saturated, aqueous uranyl acetate and 10 minutes with vanadium molybdate (Callahan and Horner, 1964). All sections were examined with a Philips 200 transmission electron microscope. One micrometer thick plastic sections were stained with Richardson's stain (Richardson *et al.*, 1960), toluidine blue (1% solution in 1% aqueous borax), or Azure II (1% aqueous). Sections were mounted in immersion oil and the cover slips rimmed with clear nail polish to yield permanent preparations.

RESULTS

Light Microscopy

Mallory-Heidenhain rapid stain and Bouin's fixative produced the best overall results and provided the most information for routine light microscopy, including observations of the initial germ cells, gonophore development, and the oocyte-nurse cell complexes. These techniques were especially useful in observations of very young gonophores. Mitotic figures were commonly found in the ectodermal I-cells in sections of both species from the base of racemes where the new gonophores form. Identical cells, some with mitotic figures, were found among the endodermal cells of the stalk and in a cluster at the apex of very young gonophore buds (Figs. 2,3). The smallest apical cluster of I-cells consisted of 17 cells, 2 of which had mitotic figures. Numerous other gonophores were found with 20-25 apical I-cells and 1 or 2 mitotic figures, but no oocytes. At this stage in gonophore development, there was no visible distinction between the distal cells of the apical cluster that would form the entocodon and inner ectoderm and the proximal cells that would develop into the germ cells. The cells of the entocodon initially form a blastula-like ball which flattens out to form the two layers of the inner ectoderm. The space or glockenhohle within the entocodon is usually occluded when the entocodon flattens out. The opening reappears (Fig. 13) when the first egg forces the layers of the inner ectoderm apart creating the subumbrellar space. The smallest gonophore in which an oocyte was found contained only 26 cells in the apical mass.

In older, larger gonophore buds that contain about 200 apical cells, there are 60-75 oogonia with nuclei 7.5-8.0 µm in diameter, while the remaining apical cells, which are cells of the entocodon, have nuclei 6.0-7.5 µm in diameter. In a gonophore of this size as many as 5 oocytes (Fig. 5) have been counted. Oocytes at this time have nuclei 9.0-10.0 µm in diameter. There are also at least 2 and as many as 5 mitotic figures among the germ cells which are now organized into a relatively distinct structure, termed the germ cell mass.

The number of oocytes increases as the cells of the germ cell mass proliferate mitotically and gonophores enlarge (Figs. 6, 10). Different gonophores have been found to contain a maximum of 15-18 oocytes in the first germ cell mass. Eighteen oocytes were counted in a germ cell mass as small as 240 cells. Only the oocytes develop the chromatin network indicating the start of meiosis and develop distinct germinal vesicles. The peripheral germ cells or oogonia continue to proliferate mitotically and form a germ cell mass of 1200-2000 cells. The nuclei of the peripheral cells enlarge and appear similar to a small germinal vesicle. However, their nuclei show no signs of the chromatin organization seen in the oocytes as they begin prophase of meiosis I. These peripheral germ cells are nurse cells that can be shown with TEM to be interconnected by intercellular bridges (Figs 8, 28).

Before the first egg has been produced in a gonophore, a second group of 15-18 oocytes and their associated nurse cells begin to develop (Fig. 12) in the distal portion of the germ cell mass. The intercellular bridges in this second group or generation of germ cells are too small (0.55-0.70 µm) to be resolved with light microscopy, but they have been observed with TEM. A single gonophore of *T. larynx* may contain up to four separate groups of germ cells or embryos at different developmental stages: proliferating oogonia, vitellogenic oocytes, early cleavage, actinula. The much larger gonophores of *T. indivisa* have been found to contain as many as five different developmental stages: proliferating oogonia, small vitellogenic oocytes, large vitellogenic oocytes, early cleavage, actinula. Numerous observations of whole mounts and living animals have revealed no gonophores of either species containing more than one actinula. Many gonophores of *T. indivisa* contain two eggs and one had three.

Nurse cell nuclei appear to have a dense layer of material (Figs. 6, 14) on the inside of the nuclear membrane when they are fixed and sectioned for light microscopy. This dense layer, which is not present in the oocytes (Figs. 9, 14) or proliferating oogonia, was thought to represent the early formation of heterochromatin which is abundant in the shrunken cells, but it did not stain with the Feulgen reaction. The dense layer was prominent in the nurse cells of both *T. larynx* and *T. indivisa* when Bouin's and Zenker's fixatives were used and was distinguishable with the other fixatives for light microscopy, except Orth's. No layer was seen on the inside of the nuclear membrane of the nurse cells that were fixed for TEM, either in the micrographs or in one micrometer thick plastic sections (Figs. 31, 32).

When the first group of oocytes complete vitellogenesis and commence to fuse into a single large oocyte, the centrally located nurse cells close to the oocytes have nuclei measuring about 15 µm in diameter. The smaller, peripheral nurse cells have nuclei 9-10 µm in diameter. Before suspension of mitosis in the second group of germ cells in a gonophore, there is no condensed layer on the inside of the nuclear membranes of the peripheral nurse cells and their nuclei are approximately 7.5 µm in diameter. These characteristics allow the differentiation and counting of the cells of adjacent groups in the germ cell mass of a gonophore. No special intervening layer or cytoplasmic bridges were found between adjacent groups of germ cells at different stages of development.

During growth of the first genetic cohort of germ cells within a gonophore, the oocytes are usually situated adjacent to the spadix. However, in the second and subsequent groups to develop in a gonophore, oocytes are dispersed among their nurse cells and numerous nurse cells are positioned between the oocytes and the spadix. As the oocytes of each cohort of germ cells enlarge during vitellogenesis, they force nurse cells away from and to the opposite side of the spadix. As a result, it appears that there are fewer cells in the germ cell mass on the side of the spadix where the oocytes develop because the oocytes are larger than the nurse cells. The oocytes end up adjacent to the spadix which is presumably the source of nutrients while the nurse cells are moved away from direct contact with the spadix (Fig. 31).

Near completion of the vitellogenic phase of oogenesis several processes occur more or less simultaneously in each oocyte-nurse cell complex, although their initiation, duration, and conclusion are somewhat variable. Two of these processes are the engulfment of nurse cells and transformation of nurse cells into shrunken cells as they discharge their cytoplasm, presumably into the oocytes. These processes usually occur simultaneously, but in a few gonophores of polyps of both species 20-25% of the nurse cells discharged their cytoplasm before engulfment. This has only been observed in the periphperal nurse cells. Engulfment of nurse cells (Fig. 10) usually commences after vitellogenesis is complete and the oocytes have started to fuse into a single oocyte. In both species, however, shrunken cells have been observed in vitellogenic oocytes before there are any indications of oocyte fusion. In gonophores of T. larynx, vitually all of the nurse cells appear to be engulfed by the oocytes. Those not engulfed presumably do not persist long enough to be detected in sectioned gonophores. In T. indivisa most of the nurse cells are engulfed, but the excess nurse cells from several generations of oocyte-nurse cell complexes accumulate within the gonophore at the base of the spadix. The third simultaneously occurring process is the fusion of the 15-18 oocytes of the complex into one large oocyte containing a single germinal vesicle. When the oocytes begin to fuse, the intercellular bridges (Fig. 7), which reach 5-7 µm in diameter during vitellogenesis, expand and gradually disappear. When the fused oocyte of T. indivisa ruptures through the inner ectoderm into the subumbrellar space, about 100-150 shrunken nurse cells remain at the base of the spadix along with the septal cells that were intermixed with the germ cells of that complex. In some gonophores of T. indivisa there are also one or two large anucleate cellular fragments (30-55 µm diameter) that may represent portions of oocytes that did not successfully fuse.

At the base of the spadix in some gonophores of T. indivisa, there are what appear to be the remains of excess oocyte nuclei, which are surrounded by a small amount of cytoplasm. In Tubularia only one of the nuclei of the fusing oocytes survives to complete meiosis. The germinal vesicles of the fusing oocytes seem to disappear gradually and only 5 or 6 of the original 15-18 are found in serial sections of oocytes that have almost completed fusion. No trace of abortive germinal vesicles has been found in the gonophores of *T. larynx*. However, up to 12 of what appear to be voided germinal vesicles have been found at the base of the spadix in gonophores of *T. indivisa*. These "germinal vesicles" are not found in gonophores in which the latest embryo has developed to an actinula although the discarded spetal cells and nurse cells from several oocyte-nurse cell complexes may be found at the base of the spadix.

Treatments used in attempts to increase the visibility of the intercellular bridges in the oocyte-nurse cell complexes were marginally successful in *T. indivisa* (Fig. 8), but failed with the gonophores of *T. larynx*. Rapid dehydration (directly from water to 95% ethyl alcohol) sometimes highlighted small sections of the peripheral nurse cells if the gonophores were opened before transfer to alcohol. The germ cells are tightly packed in the gonophores of *T. larynx* and only short sections of 3-4 oocytes could be accurately mapped, even in serial sections. The germ cells are much more loosely packed in the gonophores of *T. indivisa* which are much larger than those of *T. larynx*. The largest ooctye-nurse cell complexes mapped in *T. indivisa* contained 20, 22, and 45 cells although many smaller sections of other complexes were mapped (Figs. 18-21).

In most of the oocyte-nurse cell complexes each of the ooctyes and central nurse cells were connected by intercellular bridges to 3 other cells. In one instance 2 of the oocytes, not connected to each other, were each connected to 4 other oocytes. In a few other instances, one oocyte with 4 intercellular bridges was found in each complex, but in most cases the center most and largest oocyte of a complex had only 3 intercellular bridges. In some of the complexes that were mapped, oocytes and nurse cells were found that contained 2 nuclei. Nurse cells that are directly connected to oocytes have larger nuclei than peripheral nurse cells and in some cases were difficult to distinguish from oocyte nuclei. The largest nuclei were usually found in oocytes in the center of an oocyte-nurse cell complex.

The oocyte-nurse cell complexes of both species of Tubularia contained approximately 1200-2000 cells. Since each oocyte or nurse cell normally had only one nucleolus, counts were made of the nucleoli in 10 µm serial sections of 6 vitellogenic oocyte-nurse cell complexes in each species. In T. larynx, complexes contained 1233-1920 nucleoli (avg. 1521) and in T. indivisa, complexes contained 1293-1965 nucleoli (avg. 1570). The early embryos (20 cells or less) that were observed contained a smaller number of shrunken cells. Seven embryos of T. larynx contained 798-2054 shrunken cells (avg. 1285) and the 6 embryos of T. indivisa contained 989-1532 shrunken cells (avg. 1287).

Autoradiography

The movement of labeled molecules from the enteron of injected polyps to germ cells was much slower than expected and was highly irregular in both of the experiments utilizing tritium labeled nucleotides. Two hours after injection, none of the labeled nucleotides had reached the racemes. At subsequent fixation times some racemes still showed little or no evidence of radioiactive molecules while others were heavily labeled. The irregular movement of labeled molecules is partially consistent with the results from the practice

Figure 1. *T. larynx*. Underwater photograph (20 feet) of small colony. (X 1.5)

Figure 2. *Clava leptostyla*. Light microscopy photograph of a gonophore containing a single oocyte. (X 3300)

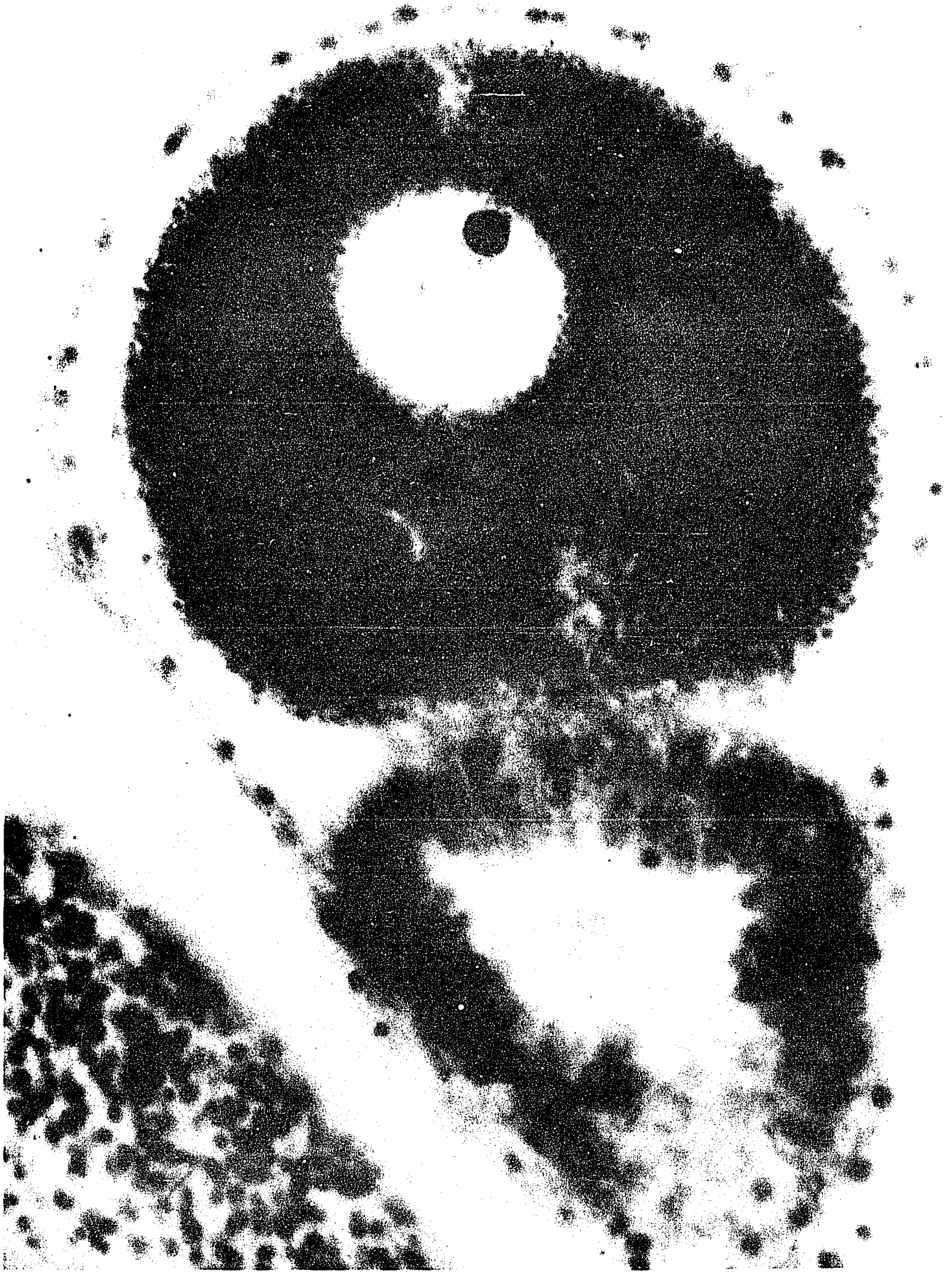


Figure 3. *T. larynx*. Light microscopy photograph of a young gonophore containing a small germ cell mass (GCM), endoderm lamella (EL), and inner ectoderm (IE) with a migrating I-cell in the endoderm. The spadix has not yet pushed into the germ cell mass. (X 600)

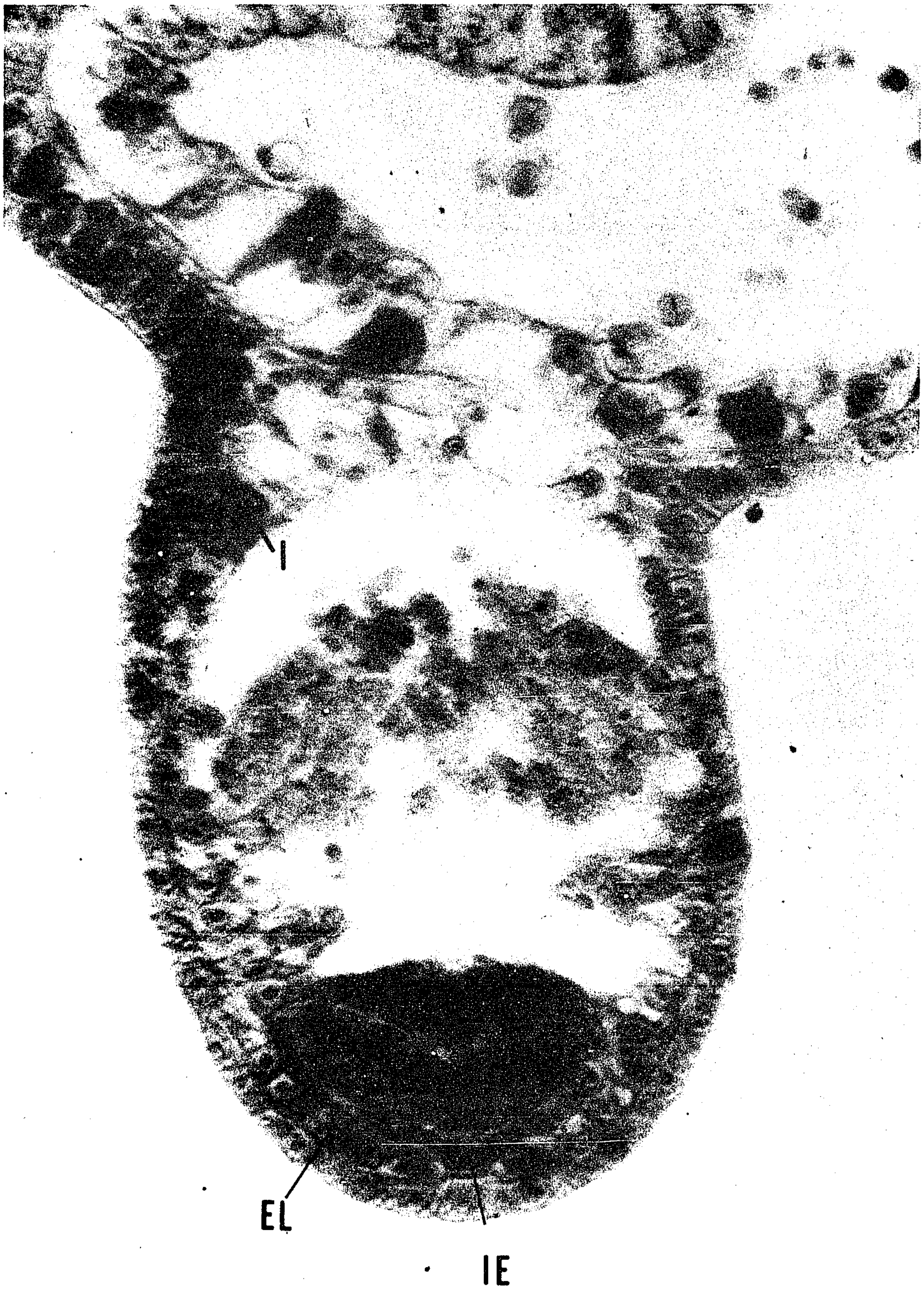

I
EL
IE

Figure 4. *T. larynx*. Light microscopy photograph of a gonophore with a horseshoe-shaped germ cell mass (GCM) and spadix (SP). (X 220)

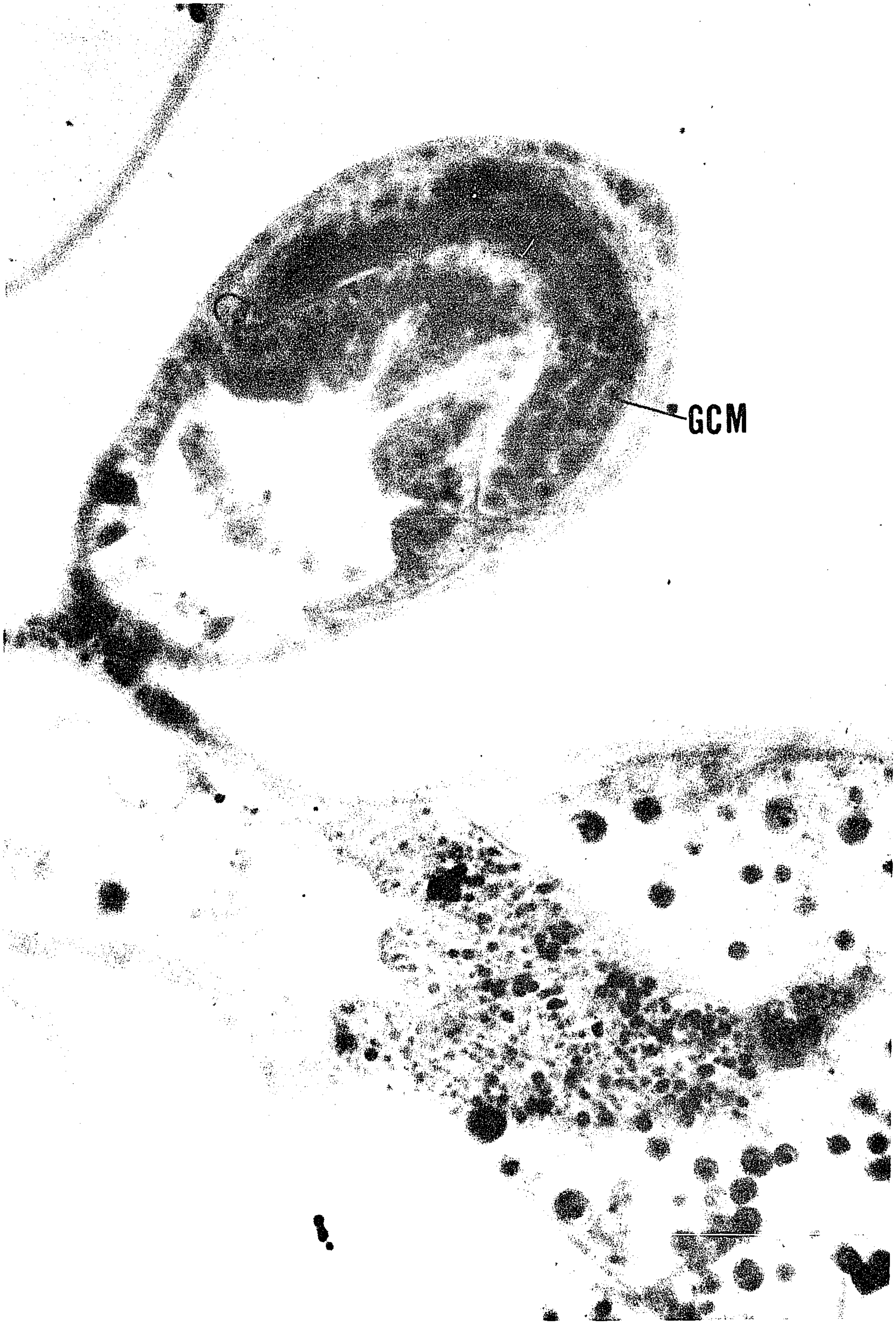
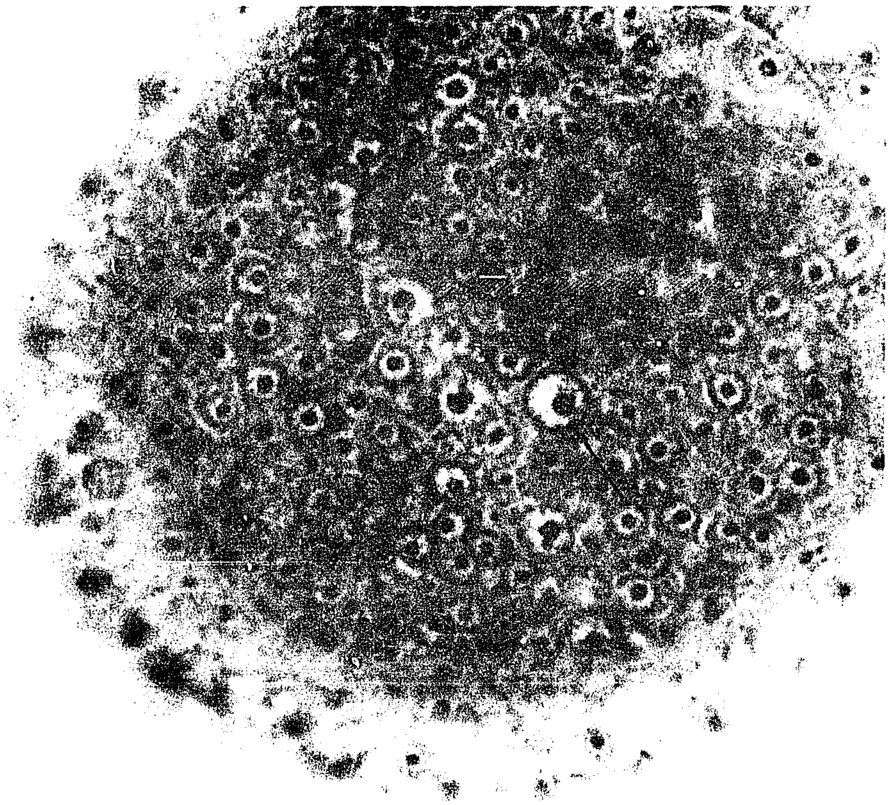

GCM

Figure 5. *T. larynx*. Light microscopy photograph of two young gonophores; one with several oocytes in the early prophase of meiosis (arrows) and the other with a small germinal veiscle. (X 670)



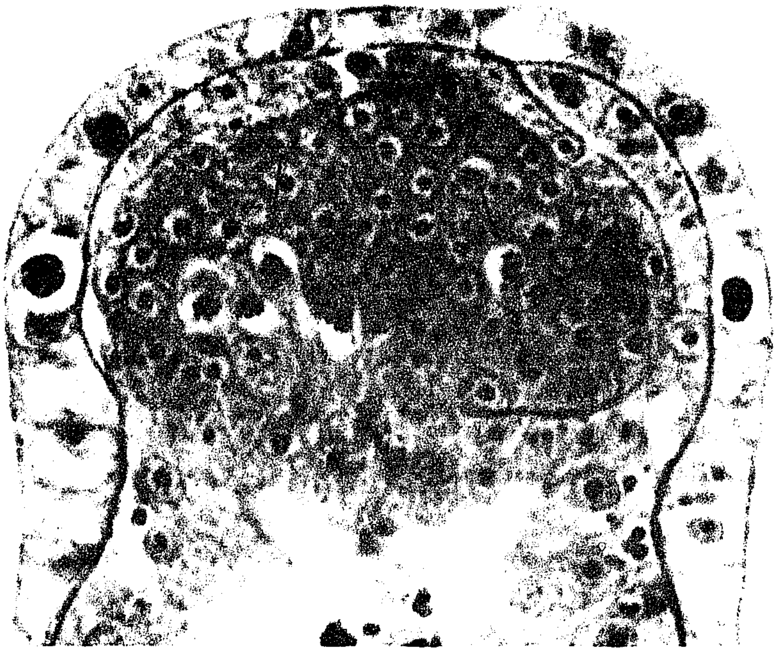


Figure 6. T. *indivisa*. Light microscopy photograph of an oocyte-nurse cell complex that contains a single large oocyte (O), smaller oocytes (SO), and numerous nurse cells (NC). A dense layer (arrow) is seen on the inside of the nuclear membrane. (X 730)

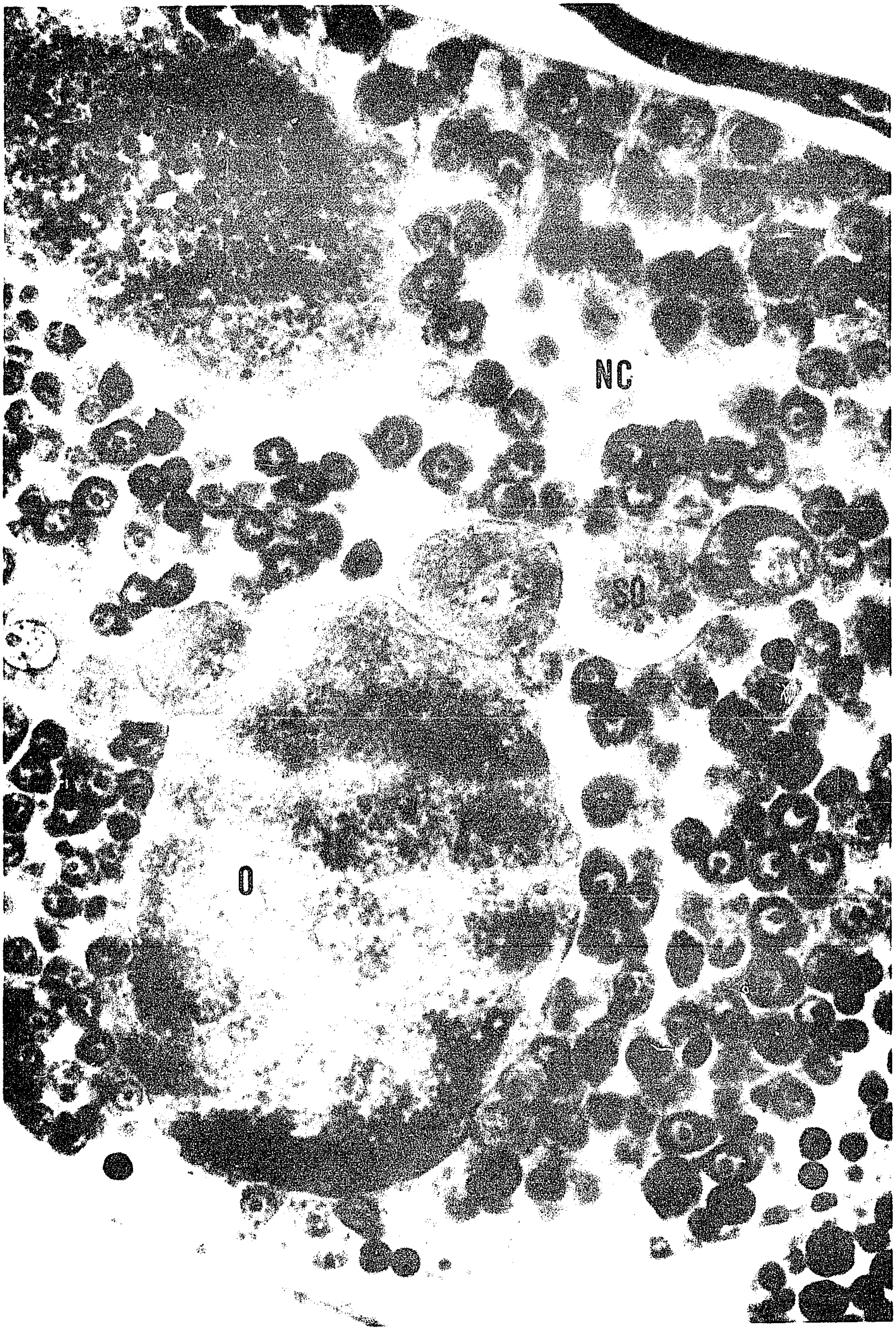

NC
SO
O

Figure 7. *T. larynx*. Light microscopy photograph of an intercellular bridge between two oocytes. (X 800)

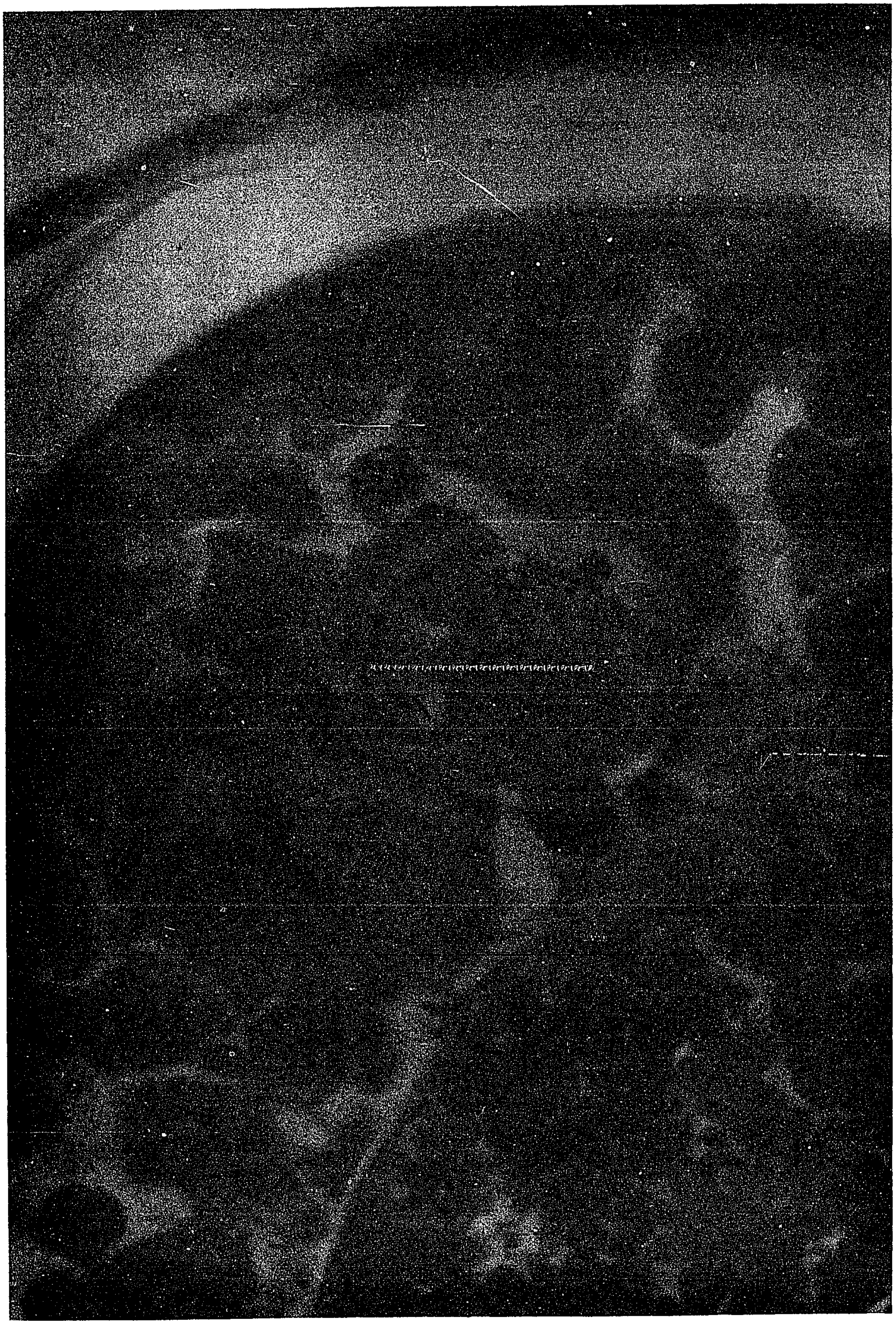


Figure 8. *T. indivisa*. Light microscopy photograph of a gonophore in which shrinkage has highlighted the intercellular bridges (arrows) between nurse cells. (X 980)

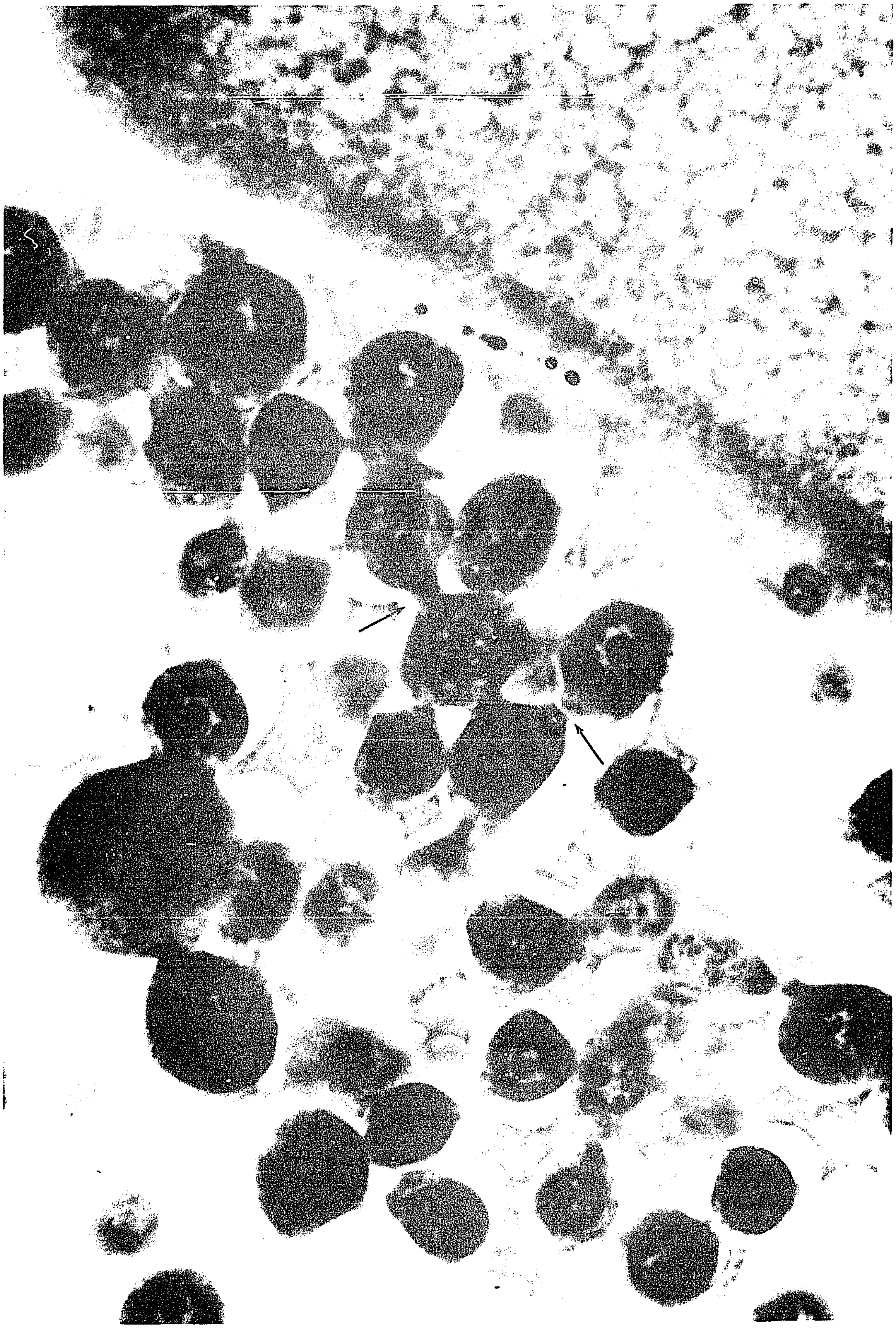


Figure 9. *T. indivisa*. Light microscopy photograph of an oocyte with two nuclei (arrow). (X 620)

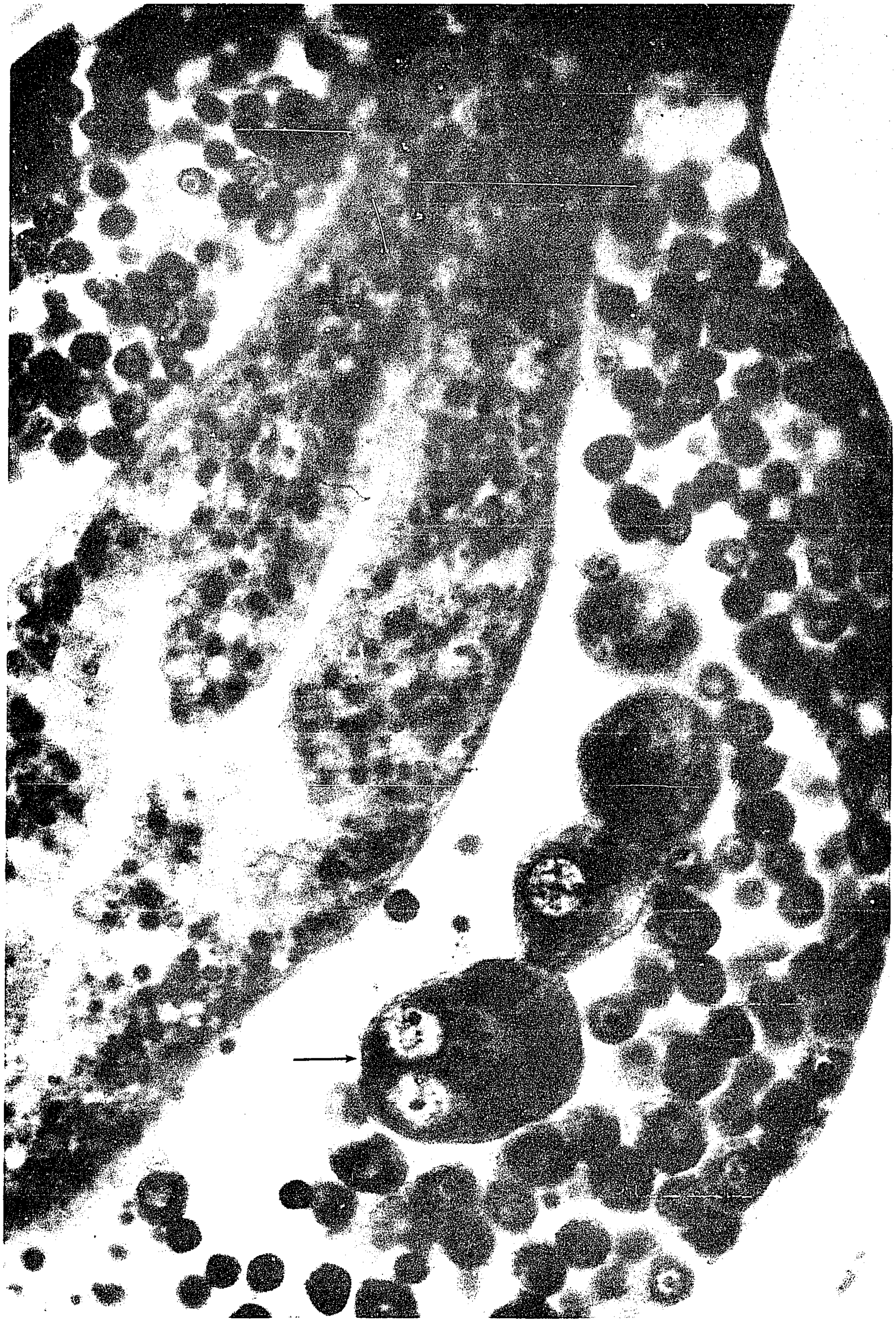


Figure 10. *T. larynx*. Light microscopy photograph of a gonophore engulfment of the shrunken cells (arrow) by the oocytes has begun. (X 300)

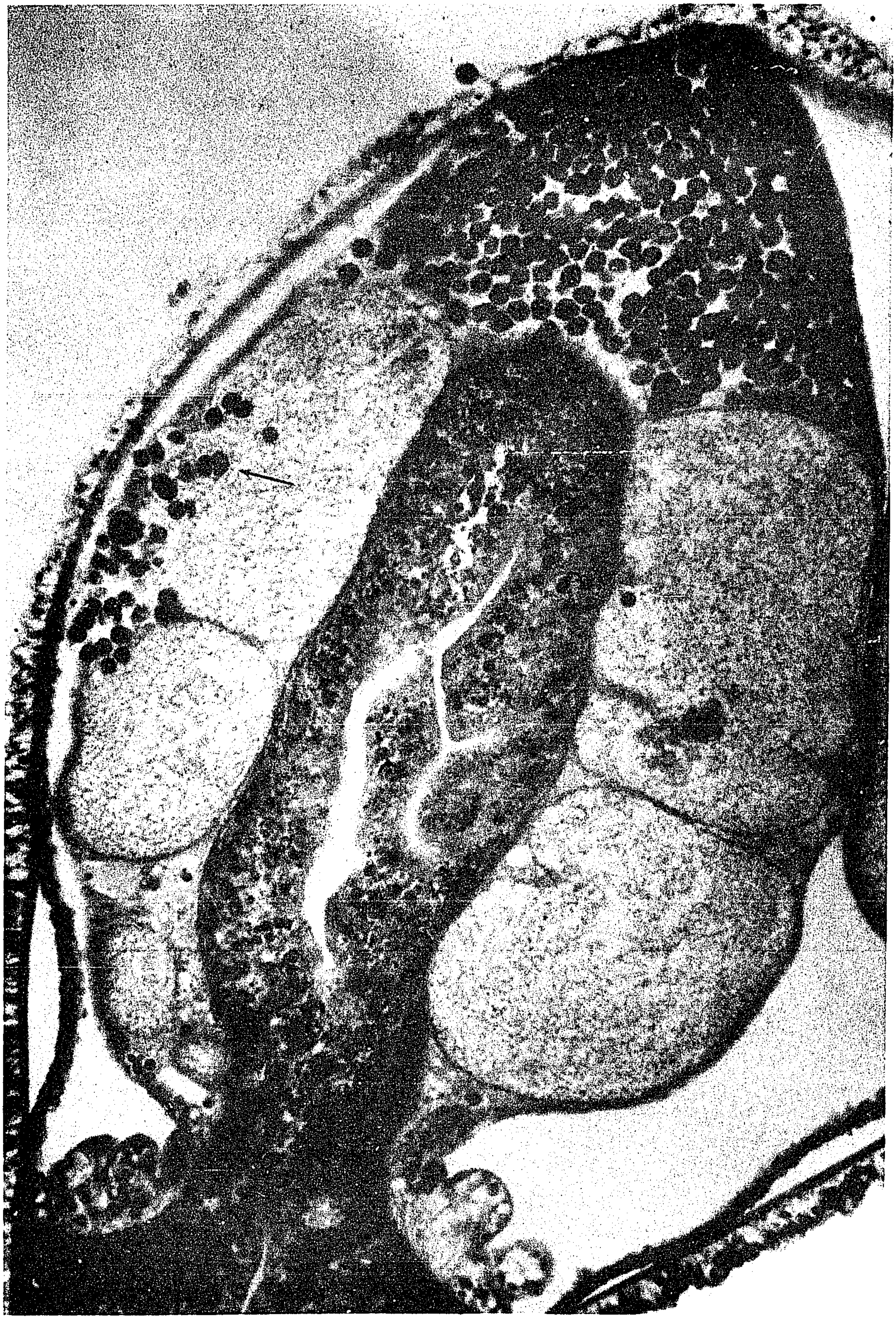


Figure 11. *T. larynx*. Actinula larva containing numerous shrunken cells (arrow) within the endoderm. (X 260)

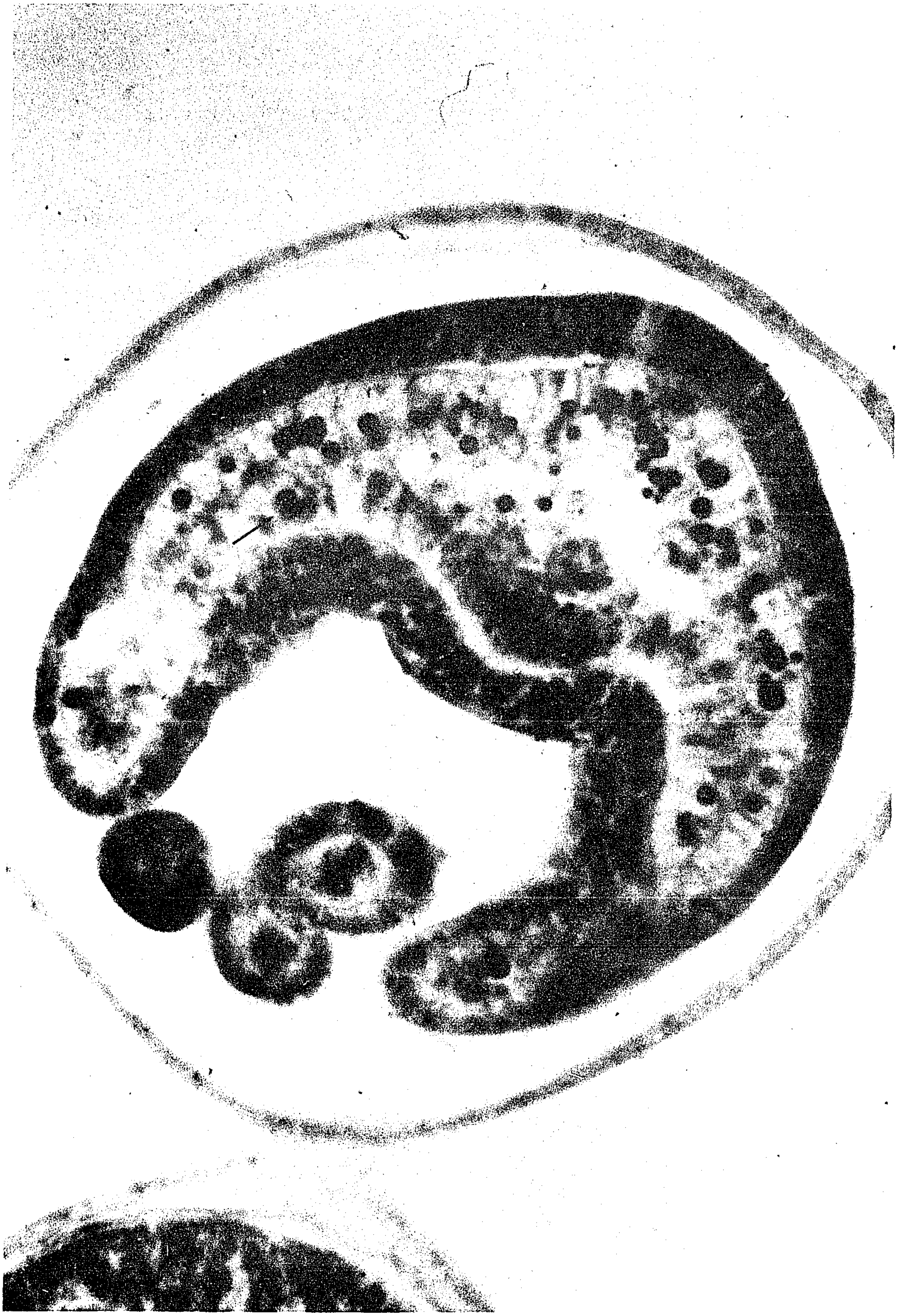


Figure 12. T. *indivisa*. Light microscopy photograph of a gonophore containing a vitellogenic oocyte-nurse cell complex (ONC) and two oocytes of a young oocyte-nurse cell complex. (X 500)

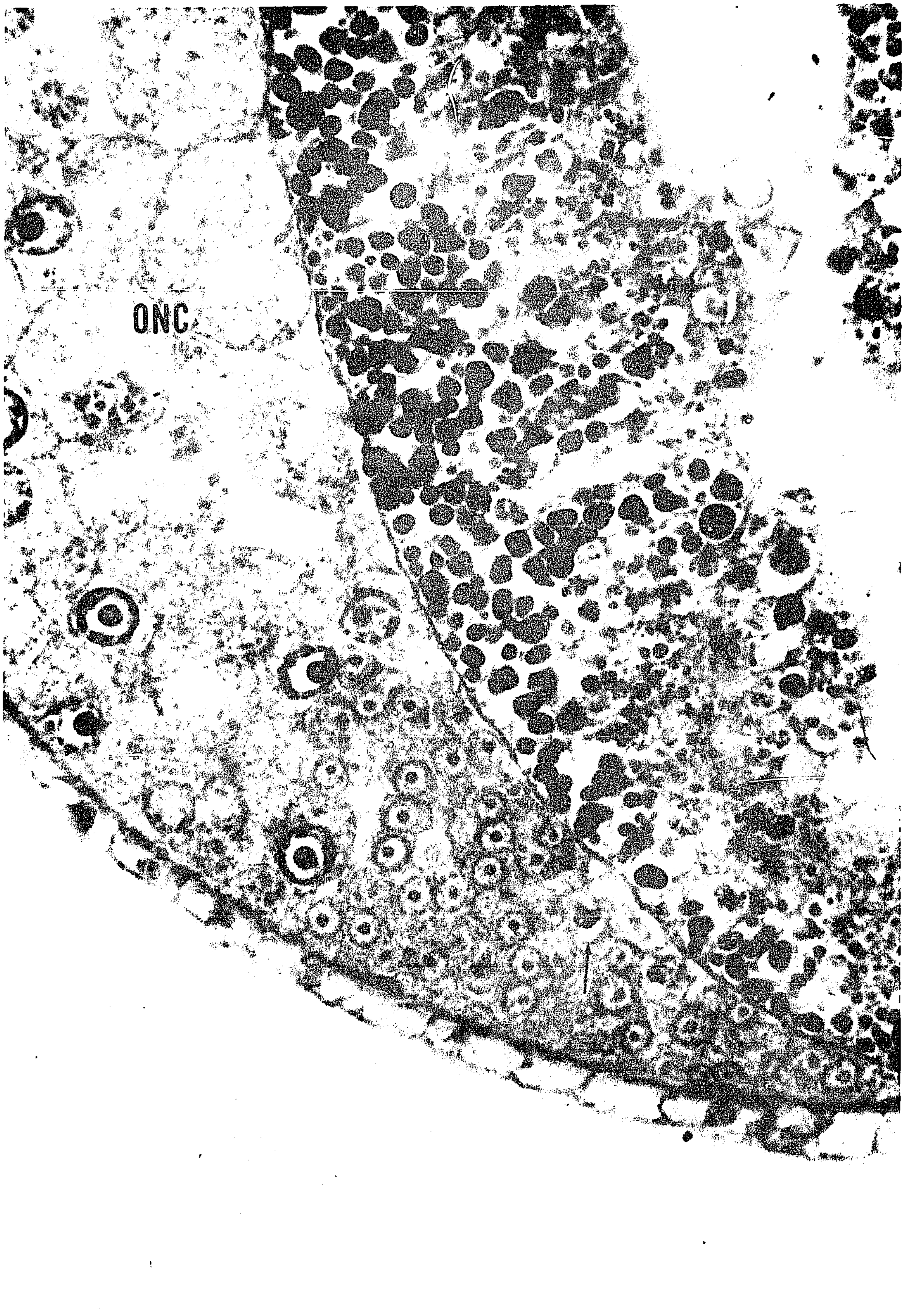

ONC

Figure 13. *T. larynx*. Light microscopy photograph of a gonophore containing an actinula (A) and egg (E) within the subumbrellar space (SC) and a developing oocyte-nurse cell complex (ONC) on the spadix. (X 166)

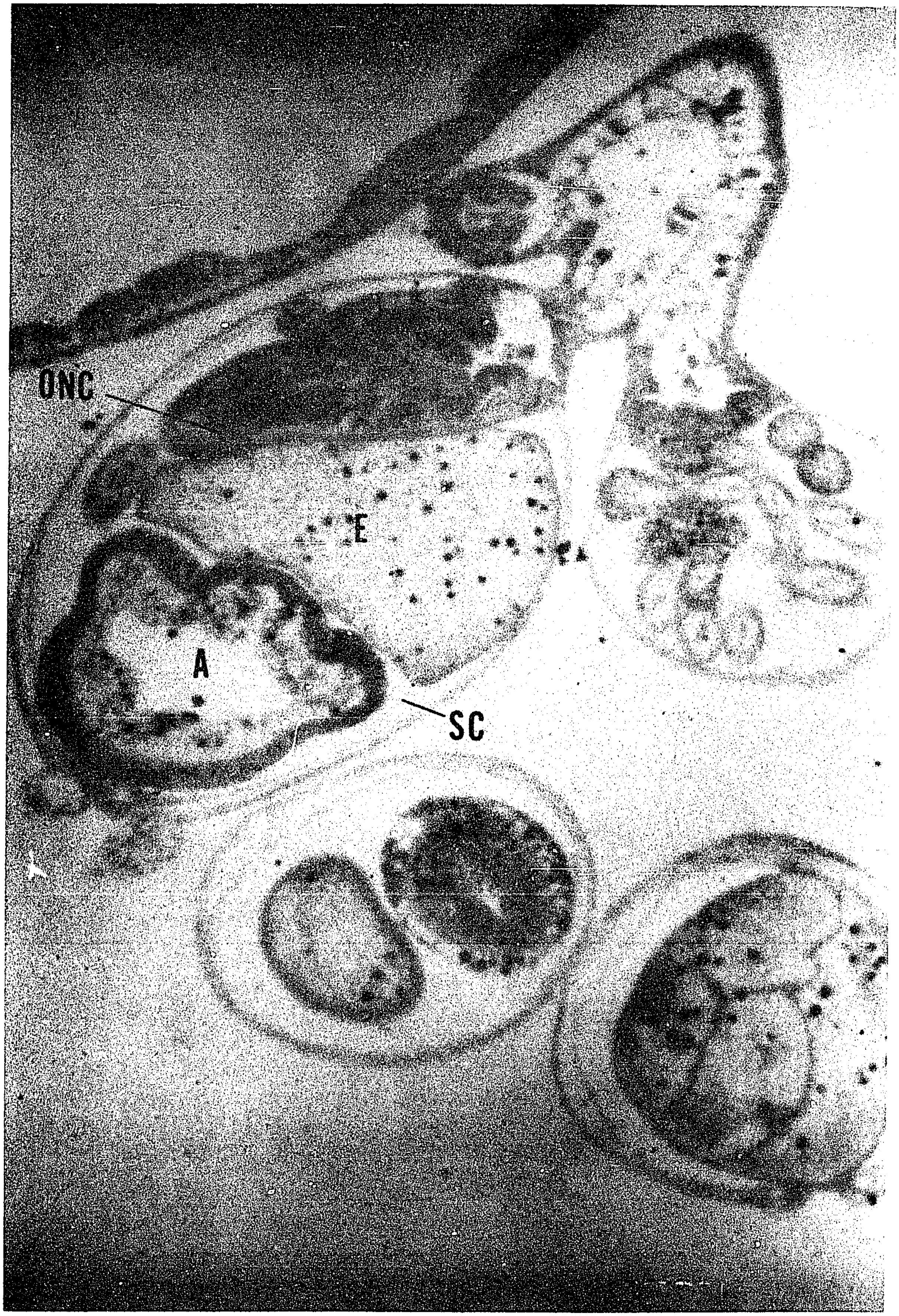

ONC
E
A
SC

Figure 14. *T. indivisa*. Light microscopy photograph of a germinal vesicle (GV) at periphery of an oocyte. The layer of material on the inside of the nuclear membrane of the nurse cells (NC) is not seen in the germinal vesicles. (X 800)

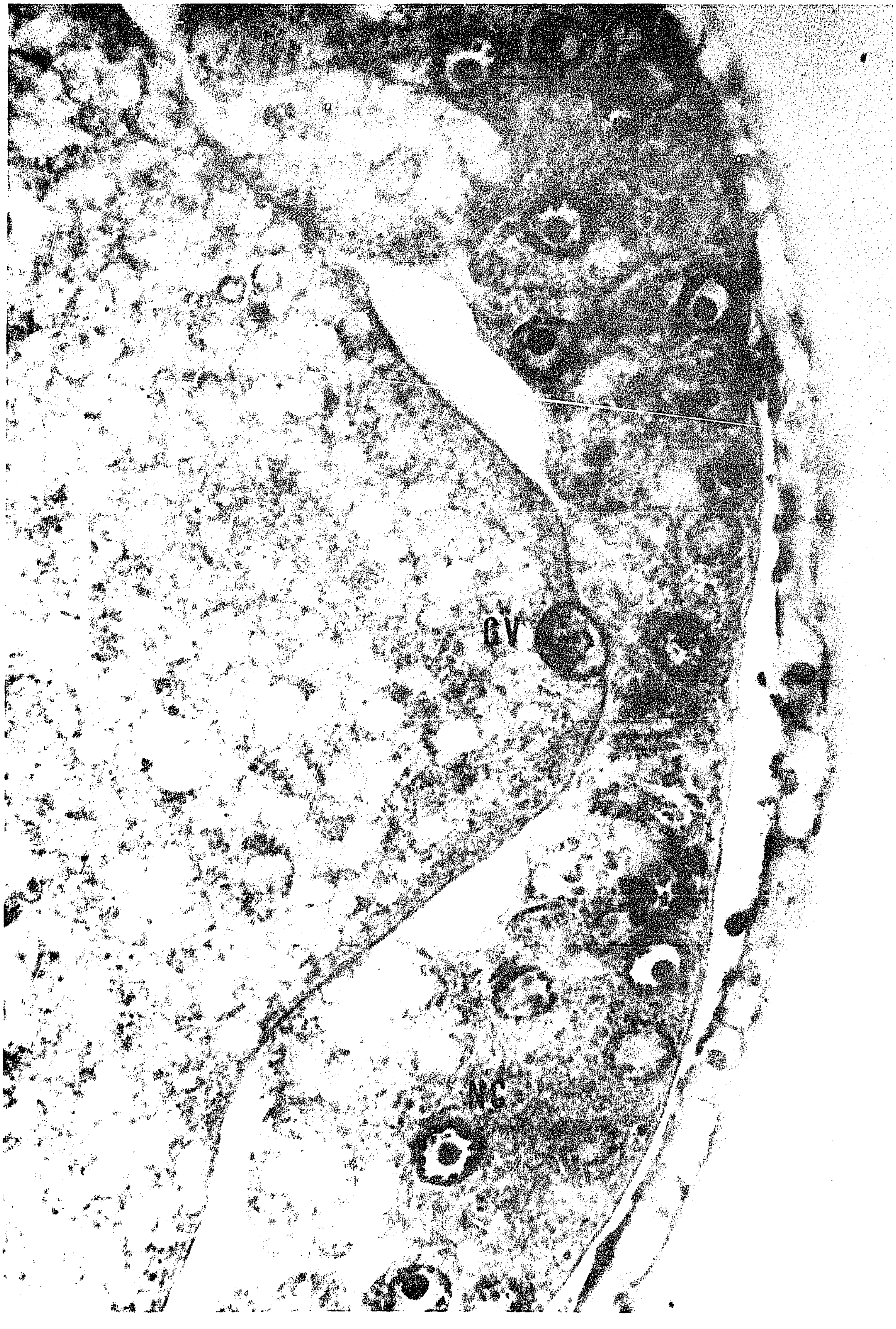

GV
NC

Figure 15. *T. larynx*. Light microscopy photograph of a gonophore that has ceased production of oocyte-nurse cell complexes. Septal cells and unengulfed nurse cells remain on the spadix. (X 700)

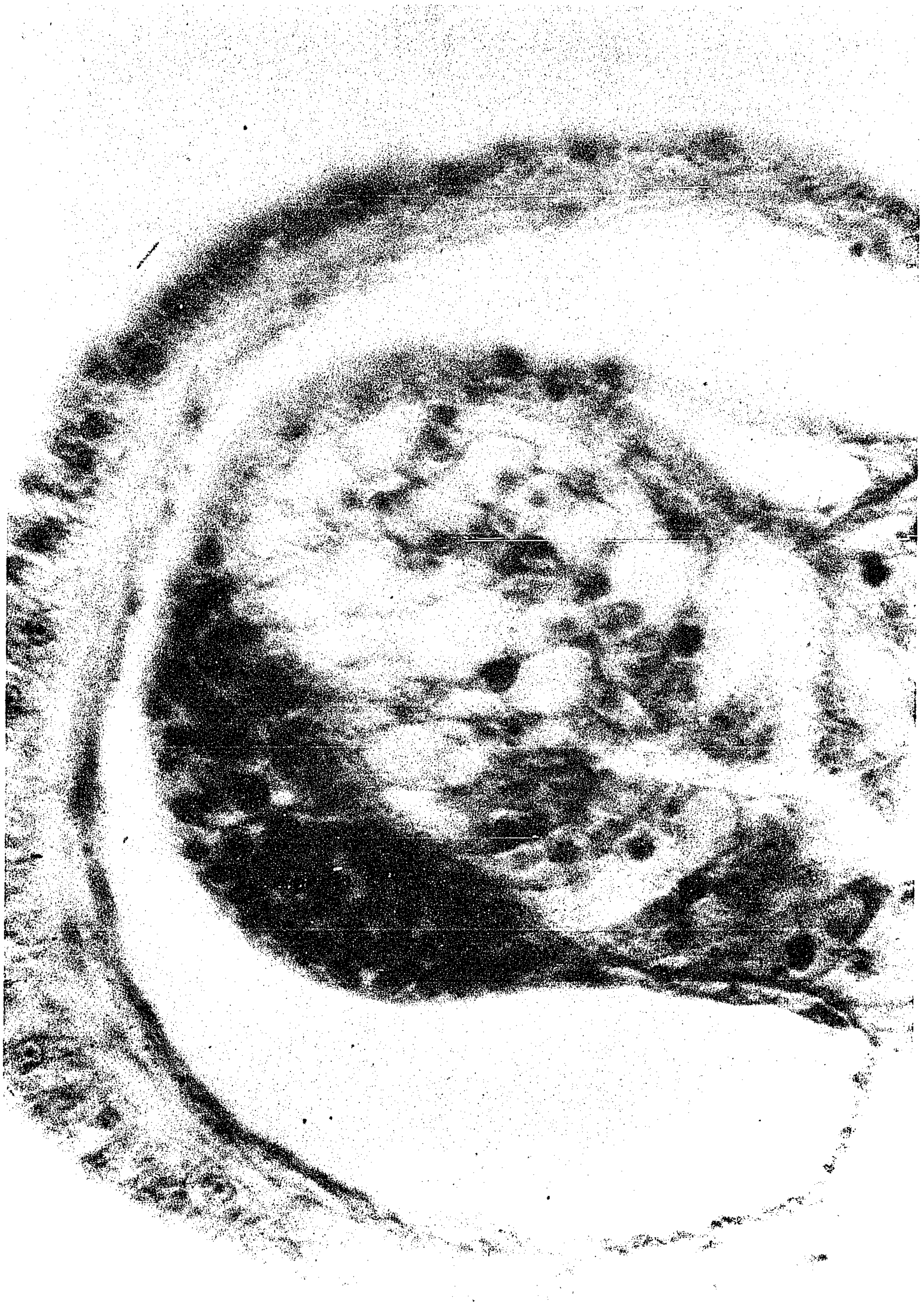


Figure 16. *T. larynx*. Light microscopy photograph of the male (M) and female gonophores of a hermaphroditic polyp. (X 130)

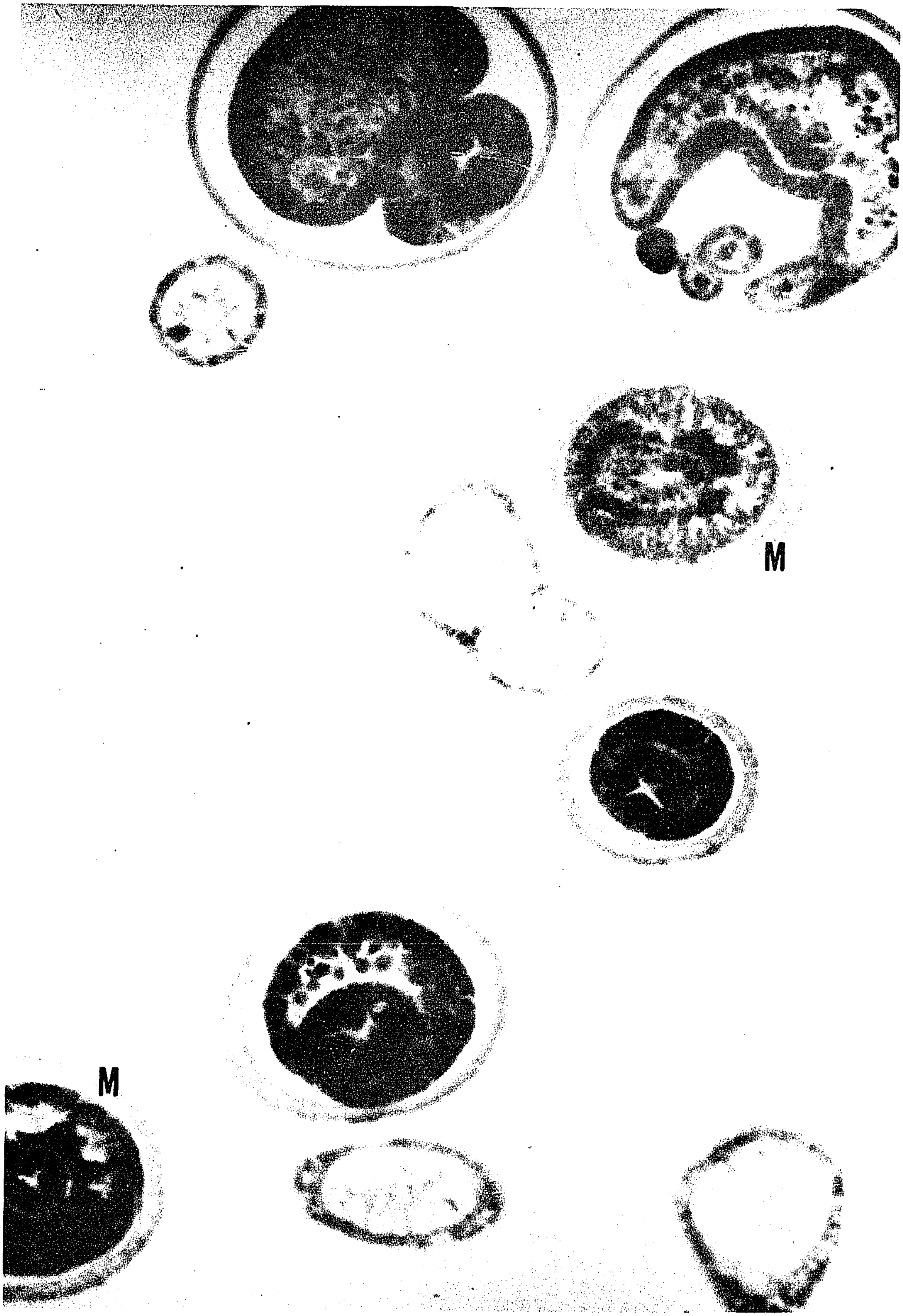

M
M

Figure 17. T. *indivisa*. Line drawing of 43 cells of an oocyte-nurse cell complex. Three cells contained two nuclei and three cells had four intercellular bridges.

(In each line drawing the sizes of the nuclei are in micrometers. The cells are drawn to show approximate relative cellular volume.)

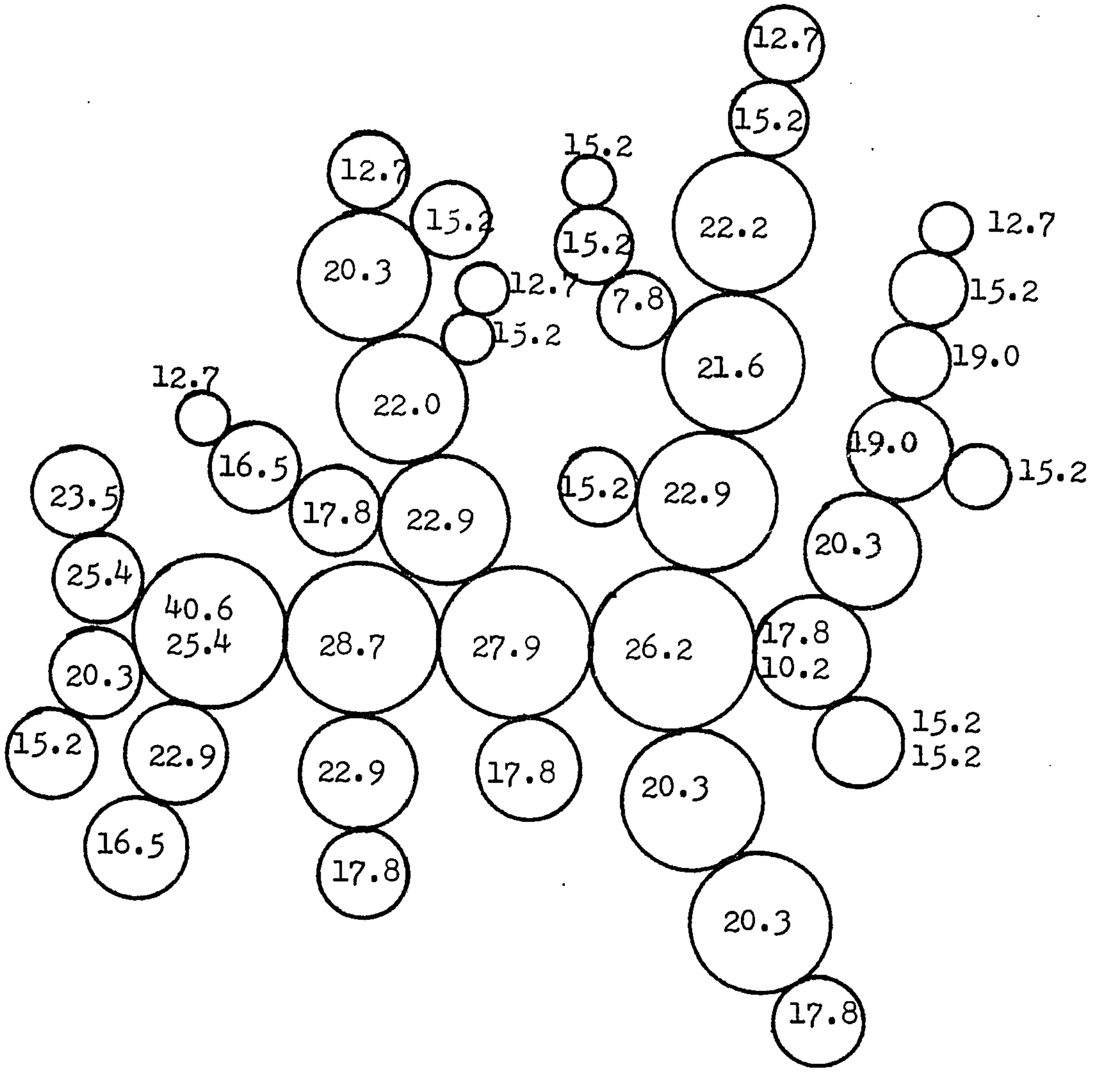

12.7
15.2
15.2
12.7
15.2
22.2
15.2
12.7
20.3
12.7
15.2
7.8
15.2
21.6
19.0
12.7
22.0
19.0
15.2
16.5
23.5
17.8
22.9
15.2
22.9
20.3
25.4
40.6
25.4
28.7
27.9
26.2
17.8
10.2
20.3
15.2
15.2
15.2
22.9
22.9
17.8
20.3
16.5
17.8
20.3
17.8

Figure 18. *T. indivisa*. Line drawing of 22 cells of an oocyte-nurse cell complex.

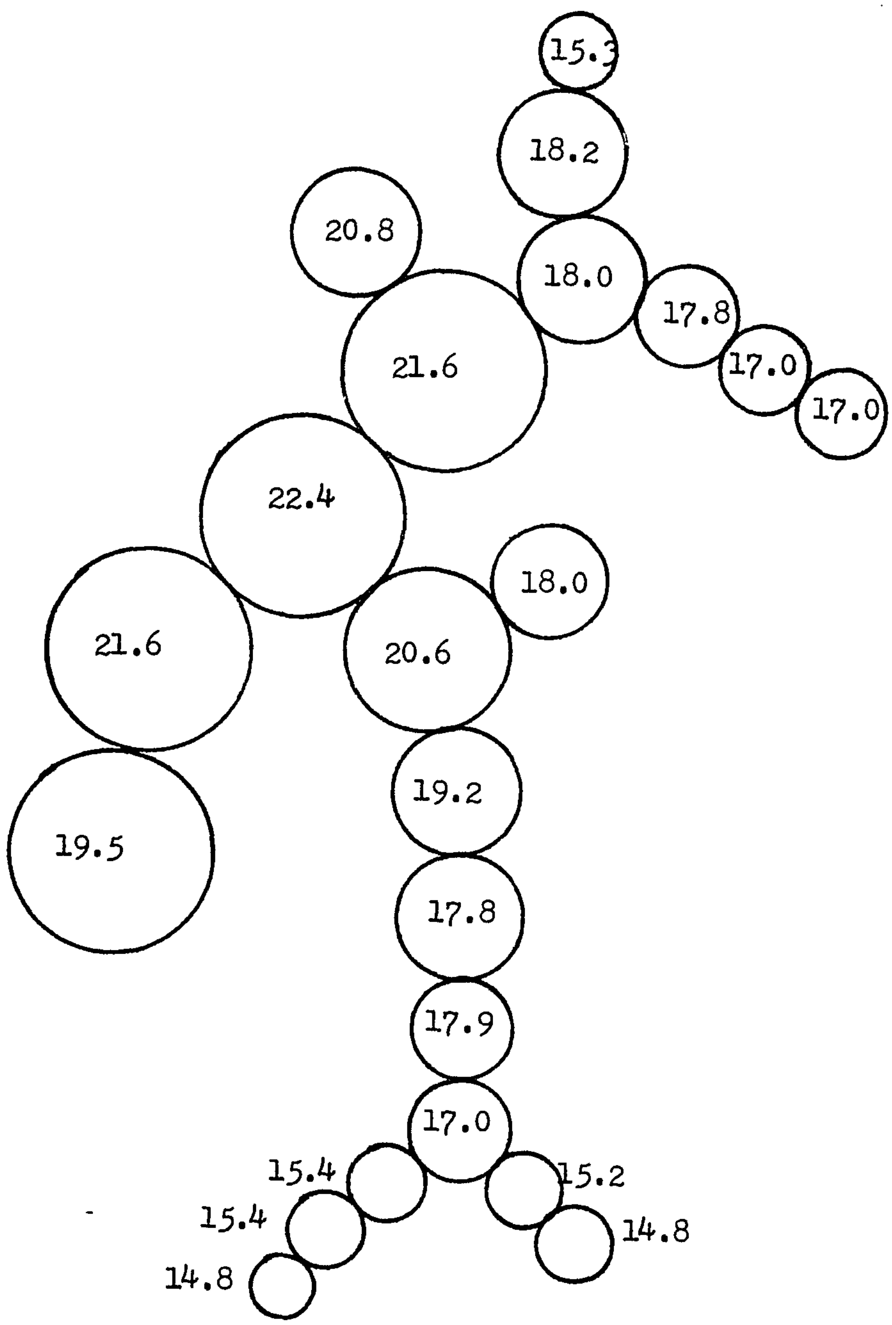

15.3
18.2
20.8
18.0
17.8
17.0
17.0
21.6
22.4
18.0
21.6
20.6
19.2
19.5
17.8
17.9
17.0
15.4
15.2
15.4
14.8
14.8
14.8

Figure 19. *T. indivisa*. Line drawing of 20 cells of an oocyte-nurse cell complex.

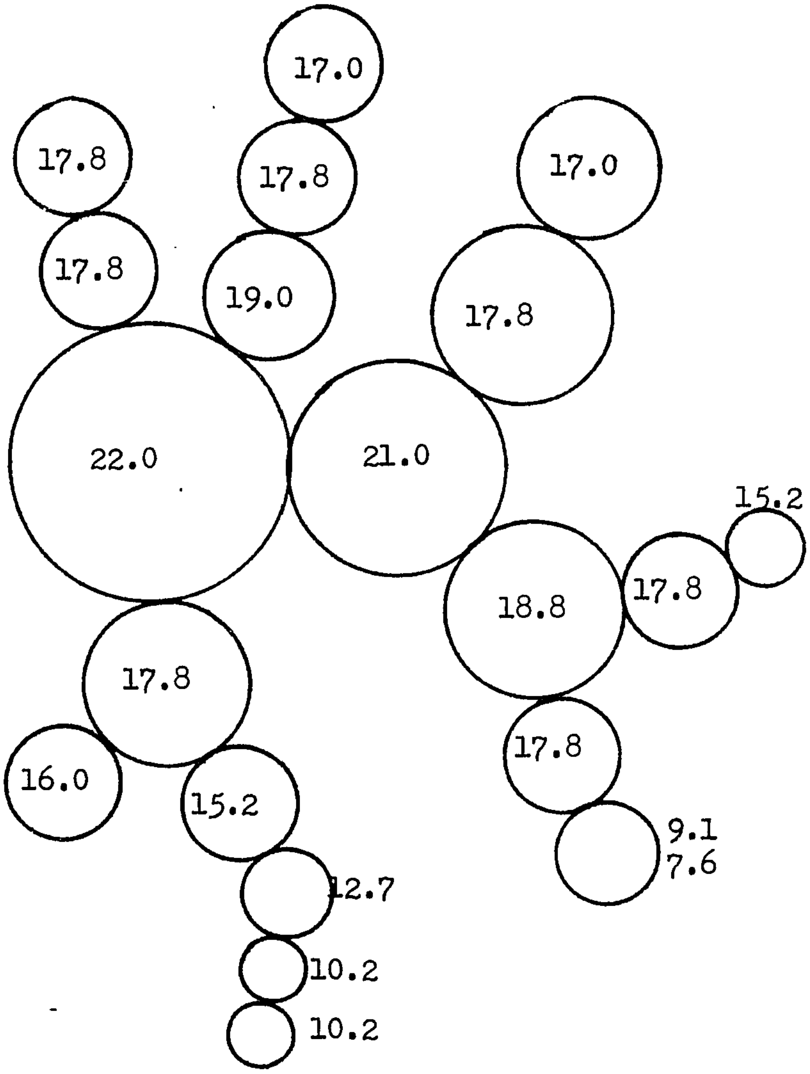

17.0
17.8
17.8
17.0
17.8
19.0
17.8
22.0
21.0
15.2
18.8
17.8
17.8
17.8
16.0
15.2
9.1
7.6
12.7
10.2
10.2

Figure 20. *T. indivisa*. Line drawing of 17 cells of an oocyte-nurse cell complex.

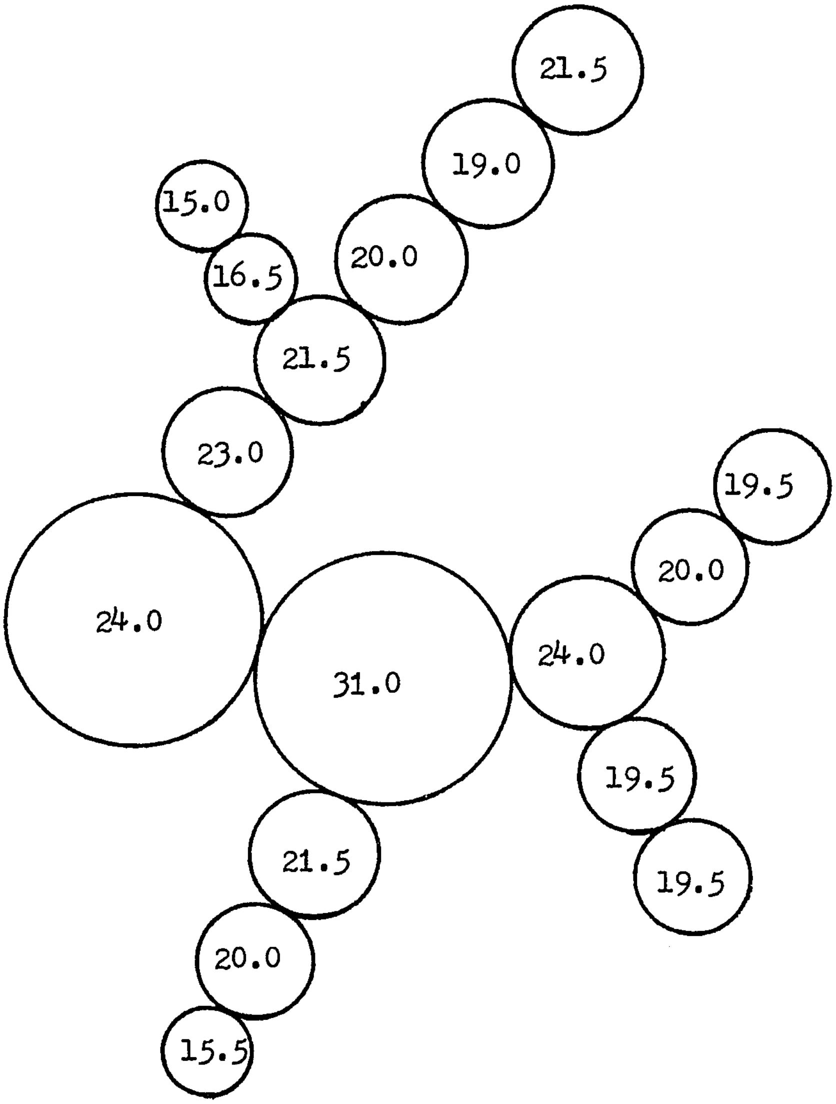
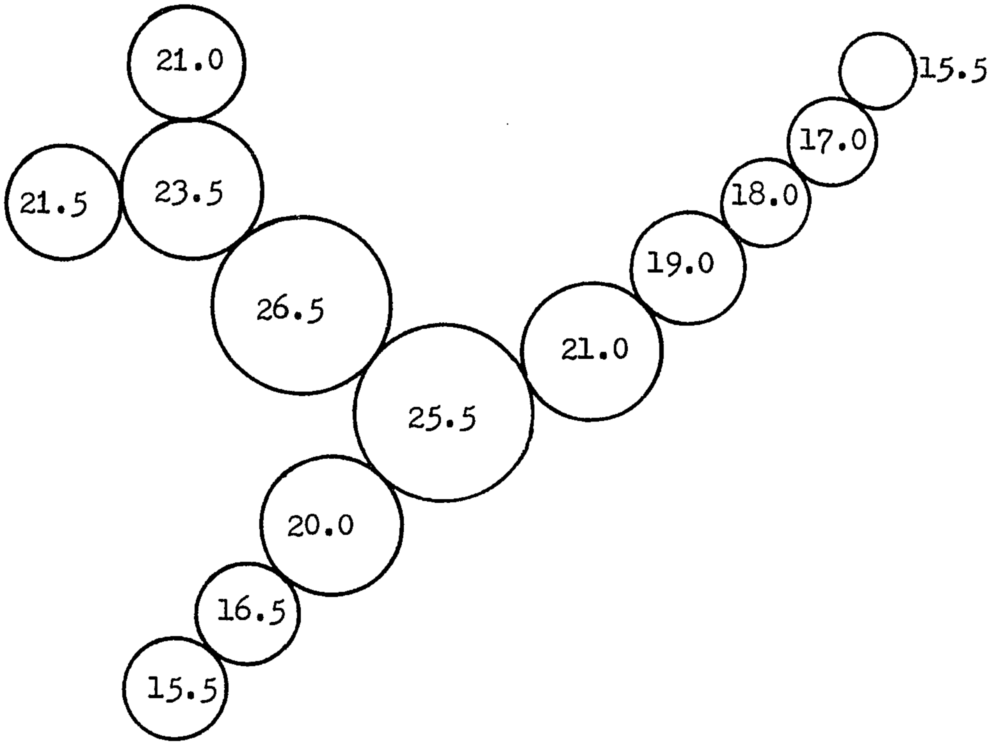
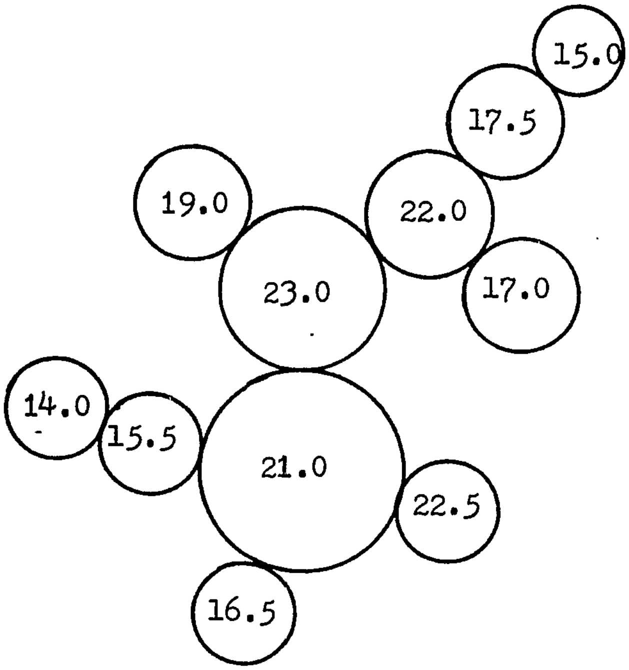

21.5
19.0
15.0
20.0
16.5
21.5
23.0
19.5
20.0
24.0
24.0
31.0
19.5
19.5
21.5
20.0
15.5

Figure 21. *T. indivisa*. Line drawing of 13 and 11 cells of oocyte-nurse cell complexes.


21.0
21.5
23.5
26.5
25.5
21.0
19.0
18.0
17.0
15.5
20.0
16.5
15.5


15.0
17.5
19.0
22.0
23.0
17.0
14.0
15.5
21.0
22.5
16.5

injections of methylene blue-sea water during which some racemes were unstained while others were heavily stained. However, during practice injections, methylene blue usually reached some of the racemes of each polyp within 20 minutes. There were no noticeable differences in results of the two labeling experiments run at different temperatures; observations from both experiments have been combined.

Movement of the ^{3}H-labeled uridine to the gonophores and germ cells was extremely slow. No labeled uridine was detected in any of the racemes 2 hours after injection. After 5 hours of incubation, most radioactivity was localized in the endoderm of the stalk and the spadix of the gonophore. Labeled material appeared to be localized in the large vesicles of food and in the cytoplasm of the endodermal cells. In a few gonophores terminated after 5 hours of incubation, radioactivity was detectable in the cytoplasm of the first generation of germ cells of younger gonophores and in the vitellogenic generation of germ cells of older gonophores. No detectable differences were noted between the gonophores terminated after 12 and 24 hours of incubation. At both sample periods some of the gonophores were labeled while others were not. A small amount of label was still present in the spadix of the labeled gonophores while most label was in the germ cells. Labeled uridine became detectable in the oocytes and nurse cells at the same time and no radioactivity was detected in the shrunken cells.

Some of the gonophores fixed after 45 hours of incubation were sectioned and stained for routine histology, but were not coated with emulsion. These polyps were fed freshly hatched brine shrimp after 24 hours of incubation, but were in poor condition after 45 hours. The gonophores that were sectioned showed signs of degeneration.

As in the case of uridine, no ^{3}H-thymidine was detected in the gonophores of polyps fixed after 2 hours of incubation. A large amount of labeled nucleotide had reached the gonophore after 5 hours of incubation, but most of it was still in the spadix. After 5, 8, 12, and 24 hours of incubation, nuclei of the proliferating germ cells in young gonophores forming the first oocyte-nurse cell complex (Figs. 22, 23) were heavily labeled. In the older gonophores, only the nuclei of the proliferating, pre-vitellogenic generation were labeled. No differences were noted between the labeling of the nuclei of the nurse cells and oocytes and no labeling was detected in the shrunken cells. Label was detected in the eggs and early cleavage stages of _T. indivisa_, but it was not possible to determine if the label was on the surface membrane of the densely packed vesicles of yolk or in the cytoplasm surrounding the vesicles (Fig. 24).

Transmission Electron Microscopy

Modifications in the fixation, embedding, and staining procedures produced some variations in the ultrastructural appearance of the gonophores and germ cells. Best overall results were obtained with gonophores that were fixed at pH 7.4 or 7.6 and embedded in EPON 812 using DMP-10 as the accelerator. The cytoplasm was much less granular and better preserved at pH 7.4 or 7.6; the matrix between the cristae in mitochondria was usually better preserved at pH 7.8. Infiltration of shrunken cells and yolk was generally poor with the EPON 812-Araldite 506 mixture and the lumen of the spadix was often empty of plastic. Spurr's embedding medium, which was expected to yield the most rapid penetration of tissue as a result of its low viscosity, penetrated poorly and provided the worst support. Best infiltration and tissue

Figure 22. *T. larynx*. Autoradiograph of a young gonophore from a polyp injected with H^3-thymidine. The proliferating germ cells were labeled (arrow). (X 440)

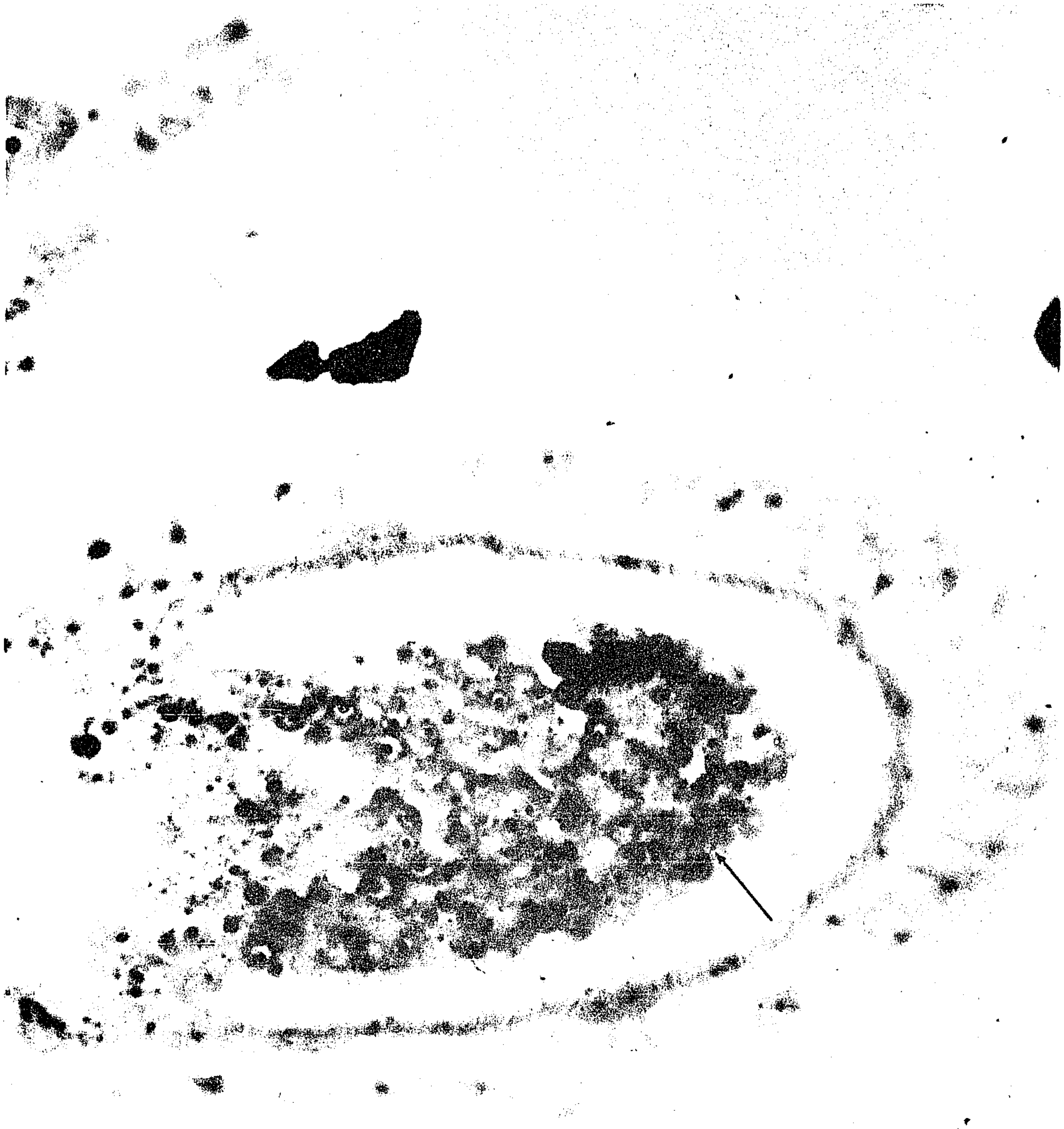
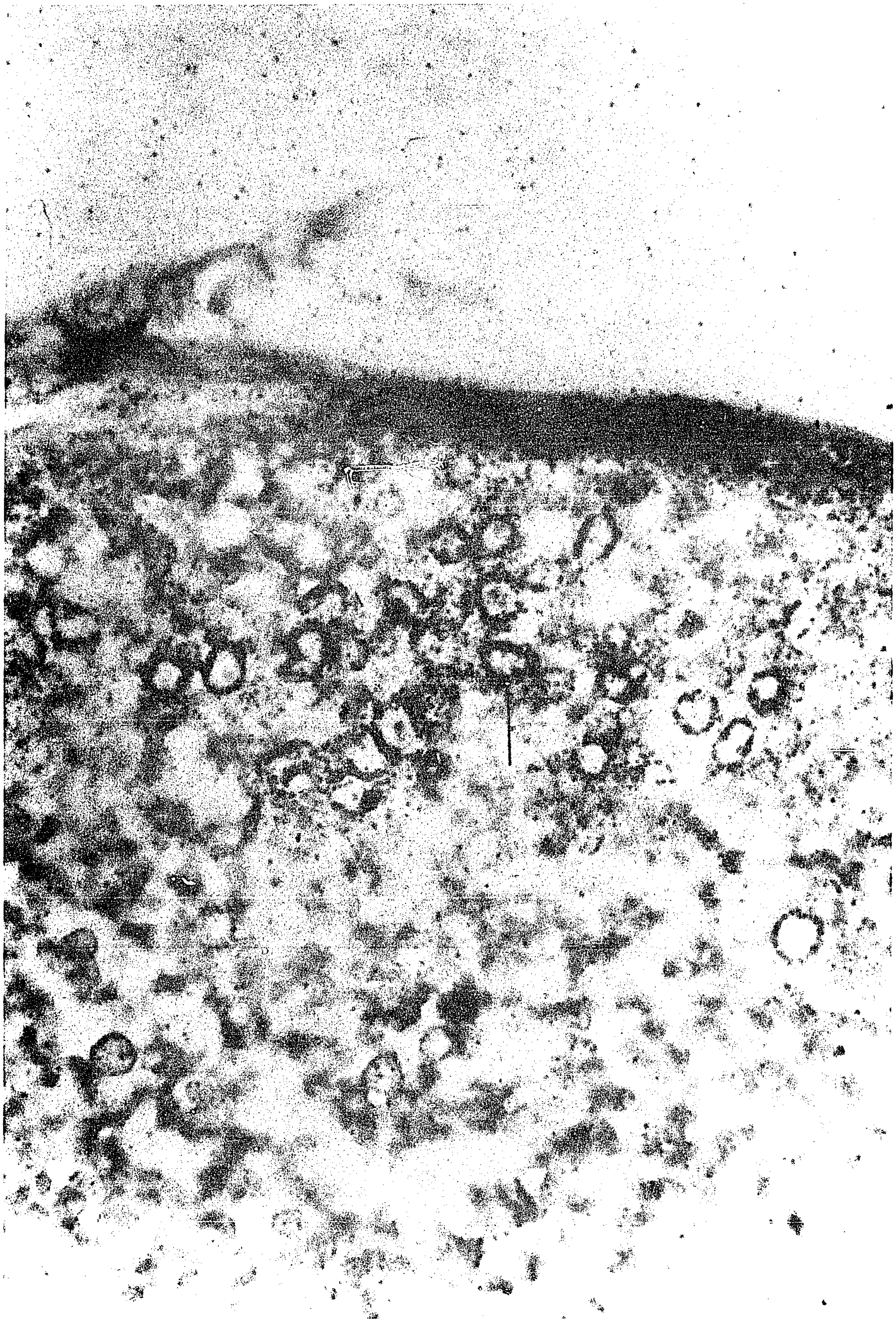


Figure 23. *T. indivisa*. Autoradiograph of a gonophore from a polyp injected with H^3-thymidine. The proliferating cells of a young oocyte-nurse cell complex were labeled (arrow). (X 680)

Figure 24. *T. indivisa*. Autoradiograph of an egg from a polyp labeled with H^3-thymidine. Label (arrow) is associated with yolk vesicles. (X 730)



support was obtained with EPON 812 and prolonged infiltration using DMP-10, although the shrunken cells often moved slightly during sectioning. No differences between fixation at 4°C and room temperature (20-25°C) were noted.

Membrane bound droplets 2.0-8.5 µm in diameter, assumed to be nutrient material, were found in the enteron (Fig. 25) of the stalk of the gonophore and spadix. The cells of the spadix are filled with these vesicles and many vesicles are found partially engulfed (or partially ejected). The luminal surface of the cells of the spadix have numerous microvilli (0.5-2.0 µm long) and flagella are occasionally present (Fig. 34). The luminal ends of adjacent endodermal cells are connected by a junctional complex. Discoidal coated vesicles are abundant in the cytoplasm of the endodermal cells. A homogeneous layer of cytoplasm is usually present in the base of the cells of the spadix adjacent to the secondary mesolamella that separates the spadix from the germ cell mass. The secondary mesolamella is about 0.2 µm thick and is penetrated by pores 0.22-0.28 µm in diameter. Small vesicles of homogeneous cytoplasm 0.25-0.50 µm in diameter are dispersed among the germ cells and may form a layer between them and the secondary mesolamella (Fig. 27). These vesicles appear to pass through the pores in the secondary mesolamella (Fig. 26).

During the development of the oocyte-nurse cell complexes, four categories of germ cells are distinguishable morphologically using transmission electron microscopy. These are the pre-vitellogenic germ cells, vitellogenic nurse cells with small vesicles of yolk, vitellogenic oocytes with large vesicles of yolk, and the engulfed nurse cells or shrunken cells. Observations of pre-vitellogenic germ cells apply to the oocytes and to the nurse cells. These cells are indistinguishable until vitellogenesis begins and the difference in the size of their vesicles becomes apparent (Figs. 31, 32, 35).

The pre-vitellogenic germ cells of Tubularia are identical to hydrozoan I-cells. They are 8.0-8.5 µm in diameter and have nuclei 3.5-4.0 µm in diameter with 1.5-1.7 µm nucleoli. There is usually no Golgi apparatus and they have only a few mitochondria, and many ribosomes. As the pre-vitellogenic germ cells or oogonia of complex proliferate by mitosis, they do not complete cytokinesis but remain interconnected by cytoplasmic bridges (Figs. 28, 29) with a diameter of 0.55-0.70 µm. Longitudinal sections of intercellular bridges often reveal the presence of septate desmosomes connecting the cytoplasmic membranes of the two cells. The septate desmosomes between germ cells were only found in close proximity to an intercellular bridge. While some sections of bridges lacking associated desmosomes were encountered, most bridges between the pre-vitellogenic germ cells had at least one septate desmosome; three bridges were observed to have a desmosome on each side. In one thin section, a septate desmosome was encountered between a very early vitellogenic germ cell and one of the cells of the inner ectoderm. Numerous bridges were detected between spermatids in male gonophores, but no desmosomes were visible. No cross sections of intercellular bridges were encountered using TEM and the number and arrangement of the desmosomes associated with each intercellular bridge is not known. No desmosomes were detected between larger, vitellogenic germ cells (Fig. 30).

Yolk was not seen in germ cells smaller than 9.5 µm in diameter.

These cells have nuclei 4.2-4.5 µm and nucleoli 2.0-2.2 µm in diameter. It was not possible to distinguish between nurse cells and oocytes at this stage. In young nurse cells the vesicles of yolk are 0.24-0.40 µm in diameter. As the nurse cells enlarge they eventually produce vesicles that are up to 1.4 µm in diameter. In the nurse cells there are no indications of fusion of these vesicles. As vitellogenesis commences in an oocyte-nurse cell complex there is an increase in the number of Golgi apparati and mitochondria in the nurse cells. At this time, the nuclei of the nurse cells are often located near the periphery of the cell on the side facing away from the oocytes. Mitochondria are commonly found in one or two clusters and the numerous Golgi apparati are clustered in groups of five or less. Each Golgi apparatus is 0.7-1.0 µm long, consists of 5-7 lamellae about 0.2 µm thick, and produces vesicles 0.05-0.06 µm in diameter. The intercellular bridges between the nurse cells normally increase in diameter to a maximum of about 1.8-2.1 µm, although one intercellular bridge was 3.4 µm.

Using TEM, the primary oocytes and nurse cells are distinguishable when the vesicles of yolk commence to fuse in the oocytes. There are two size classes of vesicles in the oocytes once fusion starts. Smaller vesicles are 0.5-1.6 µm in diameter (Fig. 35) and are most abundant at the periphery of the oocytes near the nurse cells. In a large primary oocyte, most of the larger vesicles are 3.5-8.0 µm in diameter with a few approximately 10 µm. In the oocytes, vesicles that are intermediate between the two size classes are surrounded by a cluster of small vesicles (Fig. 36). As vitellogenesis progresses, the oocytes become densely packed with large vesicles of yolk which are eventually separated by only a very thin layer of cytoplasm. In one micrometer thick plastic sections of the gonophores of T. larynx there is a distinct difference in the staining reactions of the two size classes of yolk vesicles. The smaller vesicles in both the nurse cells and oocytes stain strongly with toluidine blue, while the larger vesicles in the oocytes of the same plastic section stained lightly or not at all (Fig. 31). This differential staining was not nearly as pronounced in T. indivisa in which the smaller vesicles did not stain very strongly.

Engulfed nurse cells or shrunken cells (Fig. 33) were found in the large, fusing oocytes and larvae, and were enclosed by a membrane. Most of the shrunken cells viewed by TEM had been engulfed singly, but up to three were found within a single vesicle. Serial polyester sections revealed up to 12 shrunken cells in a single vesicle although most vesicles contain 1-4. The shrunken cells contained a large amount of heterochromatin in their nuclei and a small amount of cytoplasm with a few vesicles of yolk 0.5-0.7 µm in diameter.

The microvillar layer found on the surface of the embryo appears to begin to develop before the oocytes complete fusion and the single, fused oocyte ruptures through the manubrial layer of the inner ectoderm into the subumbrellar space. The microvillar layer on the surface of the embryo is 0.8-1.2 µm thick and is morphologically identical to the layer of periderm on the outside of the gonophore. The two layers of inner ectoderm have a similar microvillar layer that is 0.5-0.70 µm thick and is sometimes visible before the opening of the subumbrellar space. Both T. larynx and T. indivisa contain striated muscle fibers not found in the manubrial layer of the inner ectoderm covering the germ cells. The septal cells, which attach the manubrial layer of the inner ectoderm to the mesolamella of the spadix, are commonly seen among the germ cells in sections of the germ cell mass. The spetal cells are much less electron dense than the germ cells (Fig. 28).

Figure 25. *T. larynx*. Electron micrograph showing engulfment of nutrients (N) by the cells of the spadix and the secodary mesolamella (ML) separating the germ cells (GC) and spadix (SP). (X 1800)

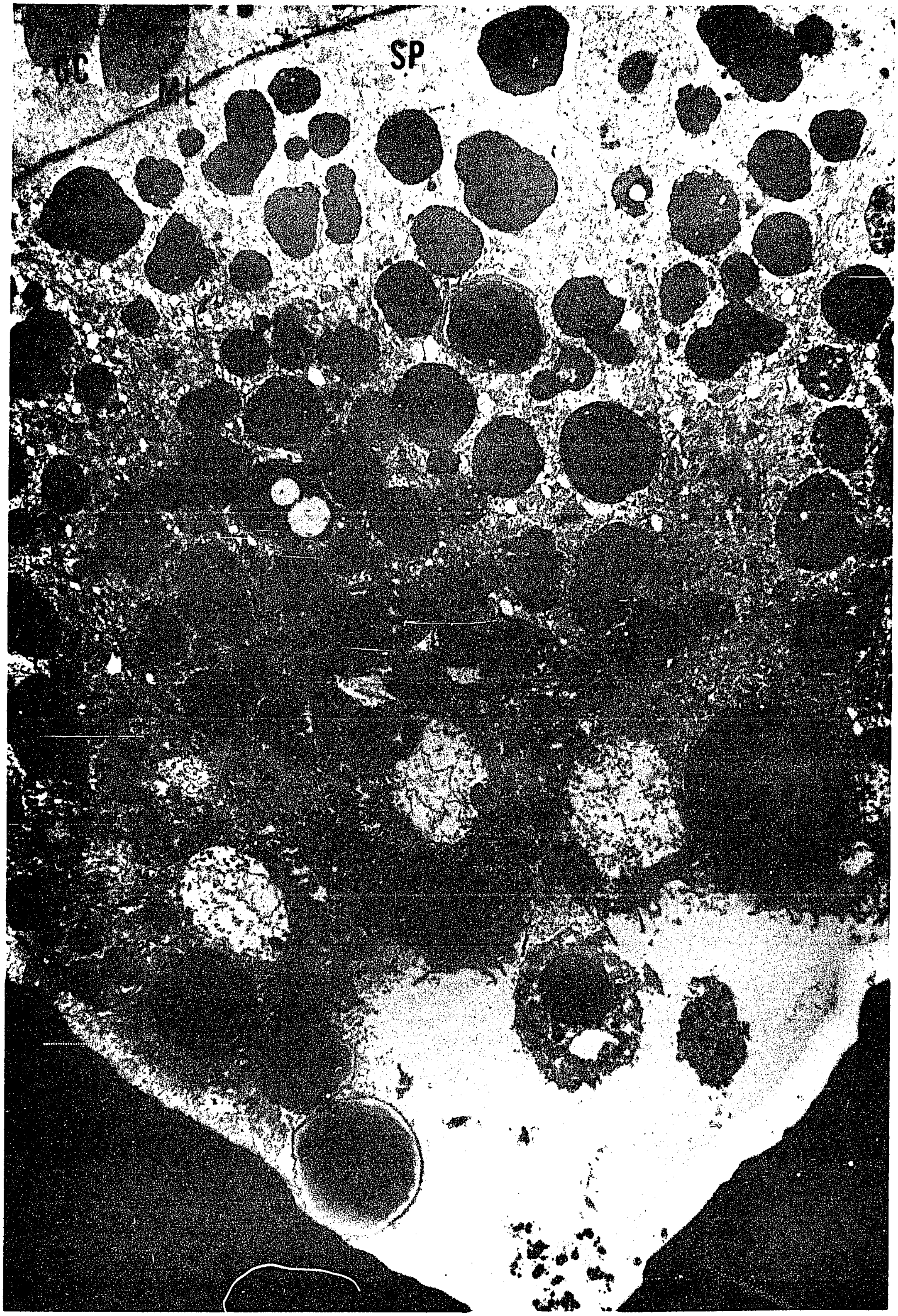

GC
ML
SP

Figure 26. *T. larynx*. Micrograph showing material (arrow) passing from the spadix to the germ cell mass (GCM). (X 2900)

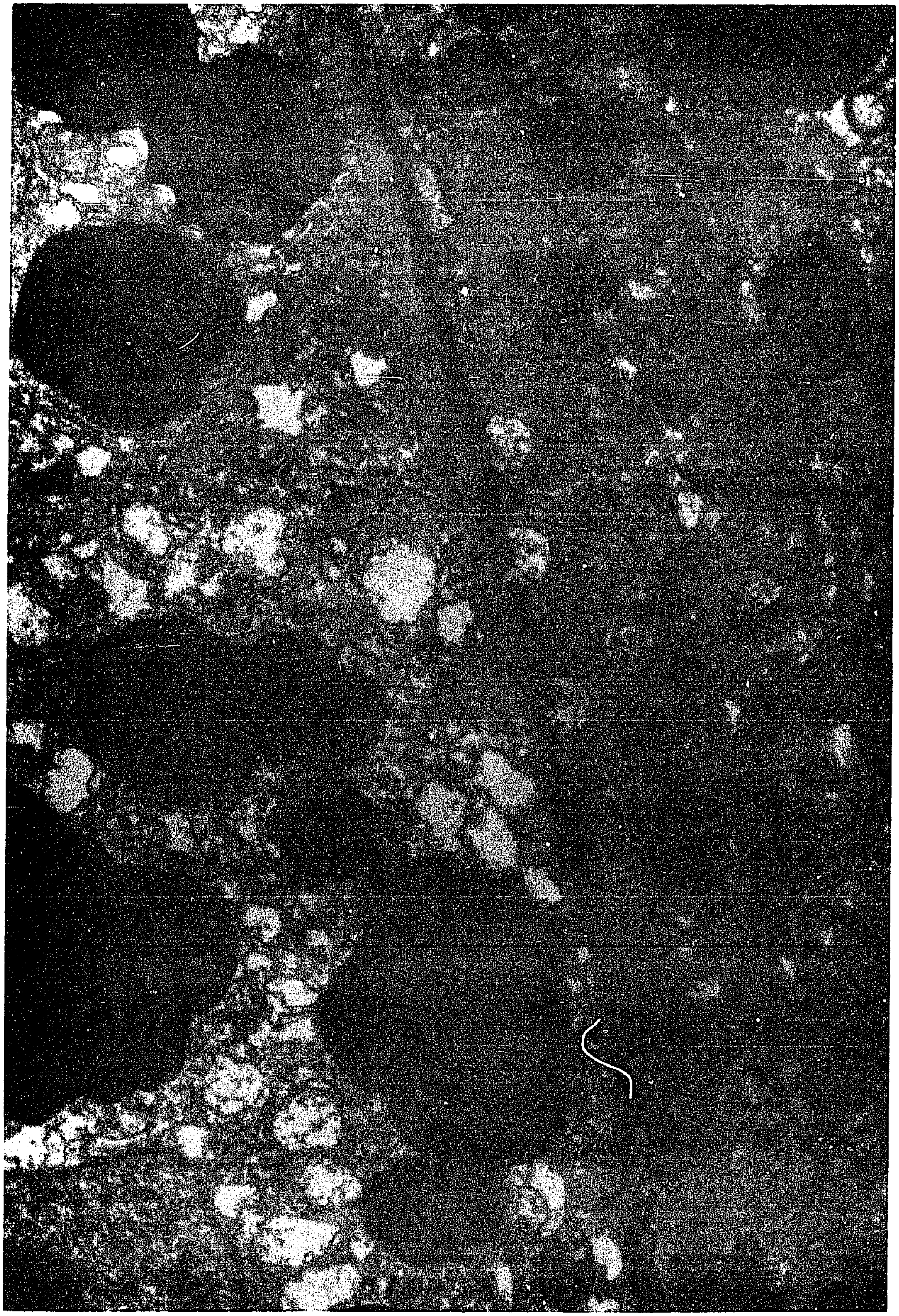


Figure 27. *T. larynx*. Micrograph of nutrient vesicles (NV) next to the secondary mesolamella.

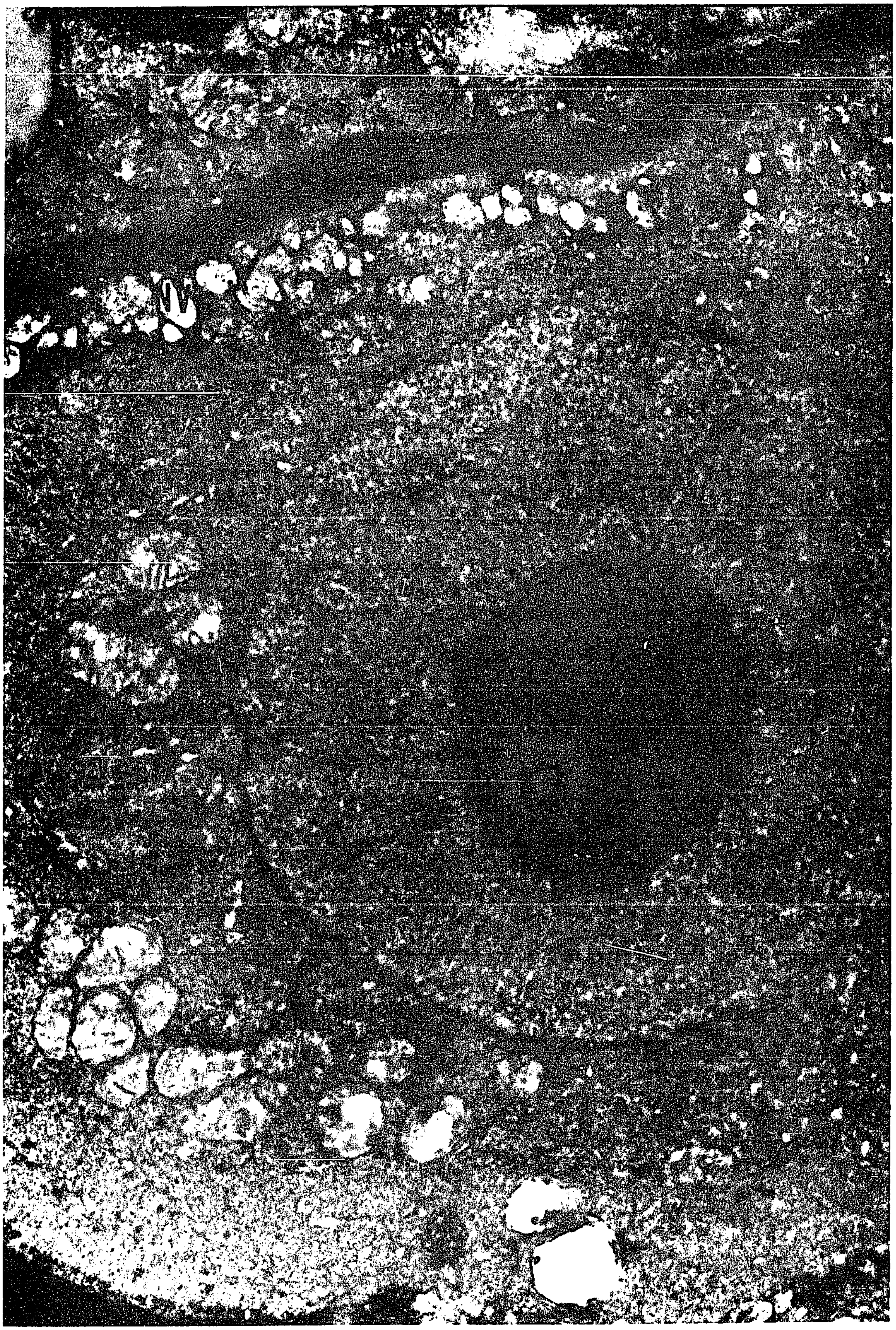


Figure 28. *T. larynx*. Micrograph of intercellular bridges (arrows) between previtellogenic germ cells and a cross section of a septal cell (SC). (X 5400)

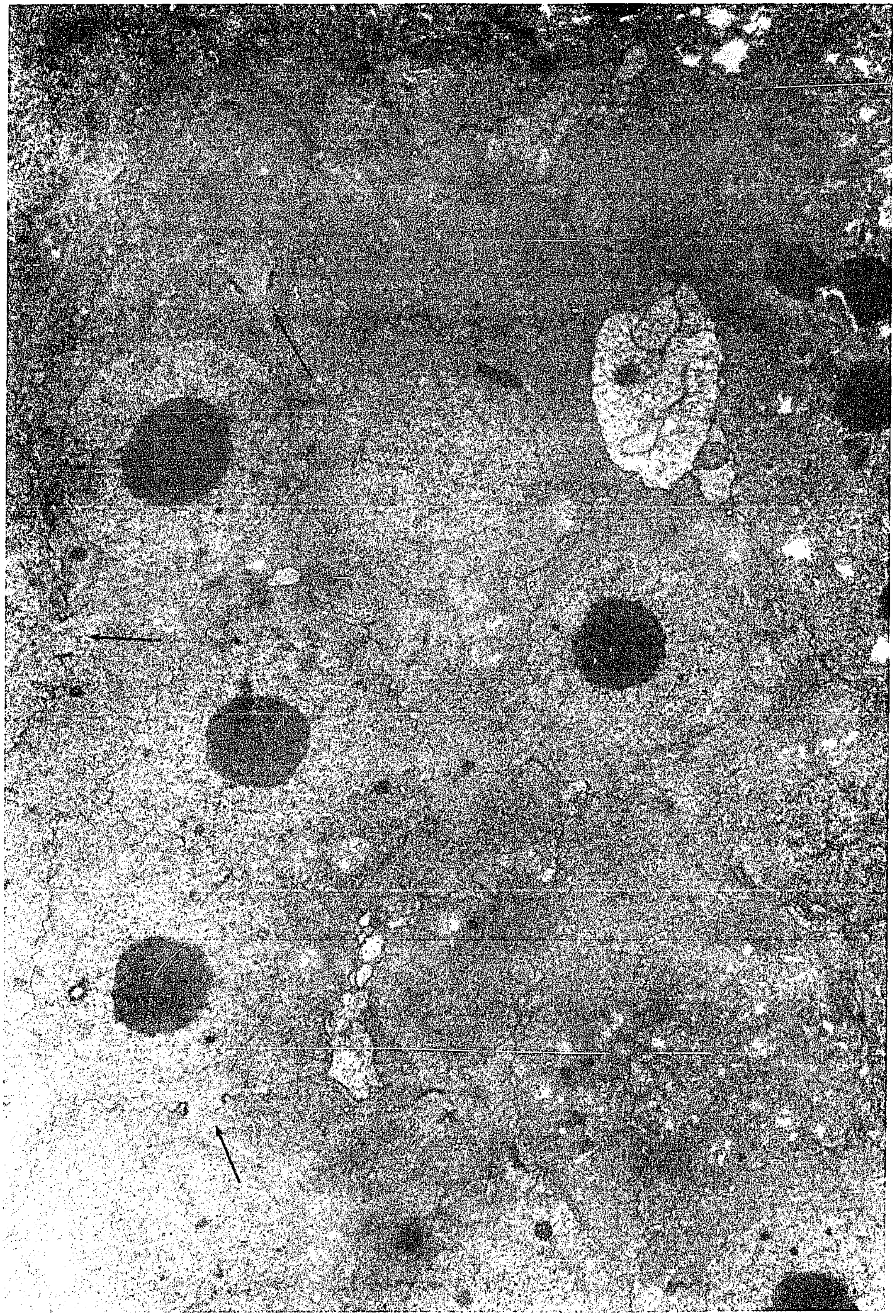


Figure 29. *T. larynx*. Micrograph of an intercellular bridge with a septate desmosome (arrow). (X 10,800)

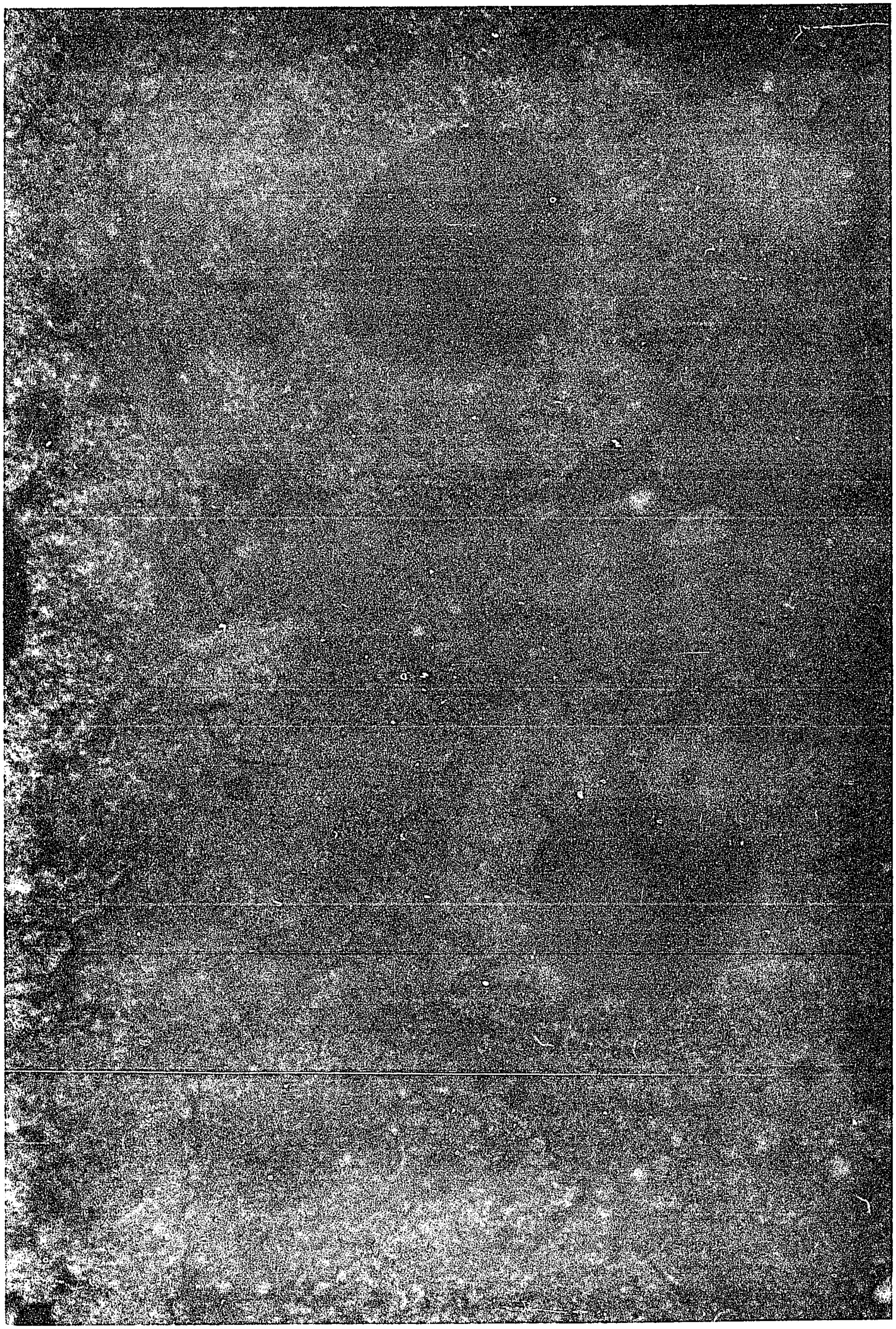


Figure 30. *T. larynx*. Micrograph of an intercellular bridge between young vitellogenic germ cells. (X 6700)

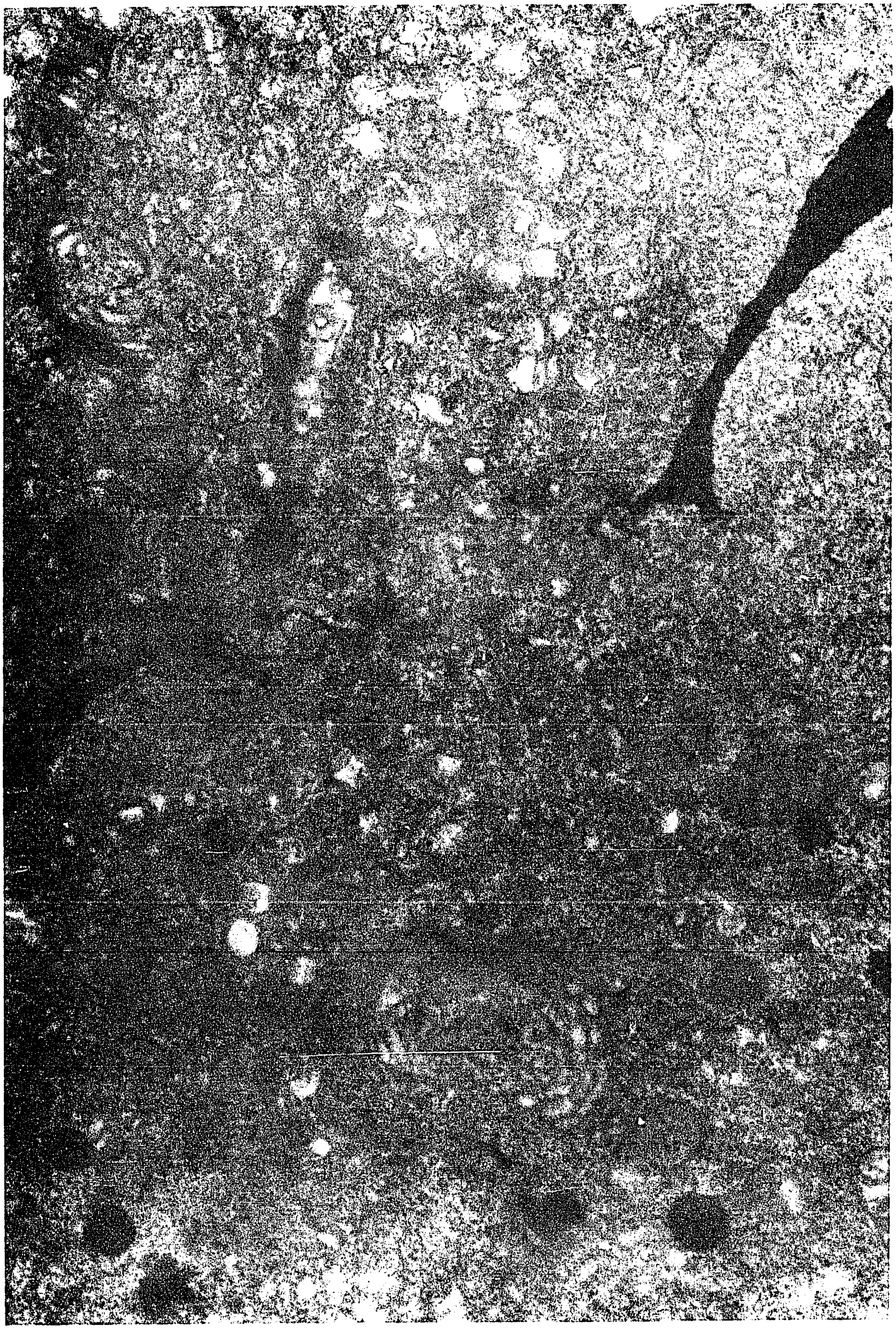


Figure 31. *T. indivisa*. One micrometer thick plastic section of nurse cells (NC) with small vesicles of yolk and an oocyte (O) with large and small vesicles. (X 830)

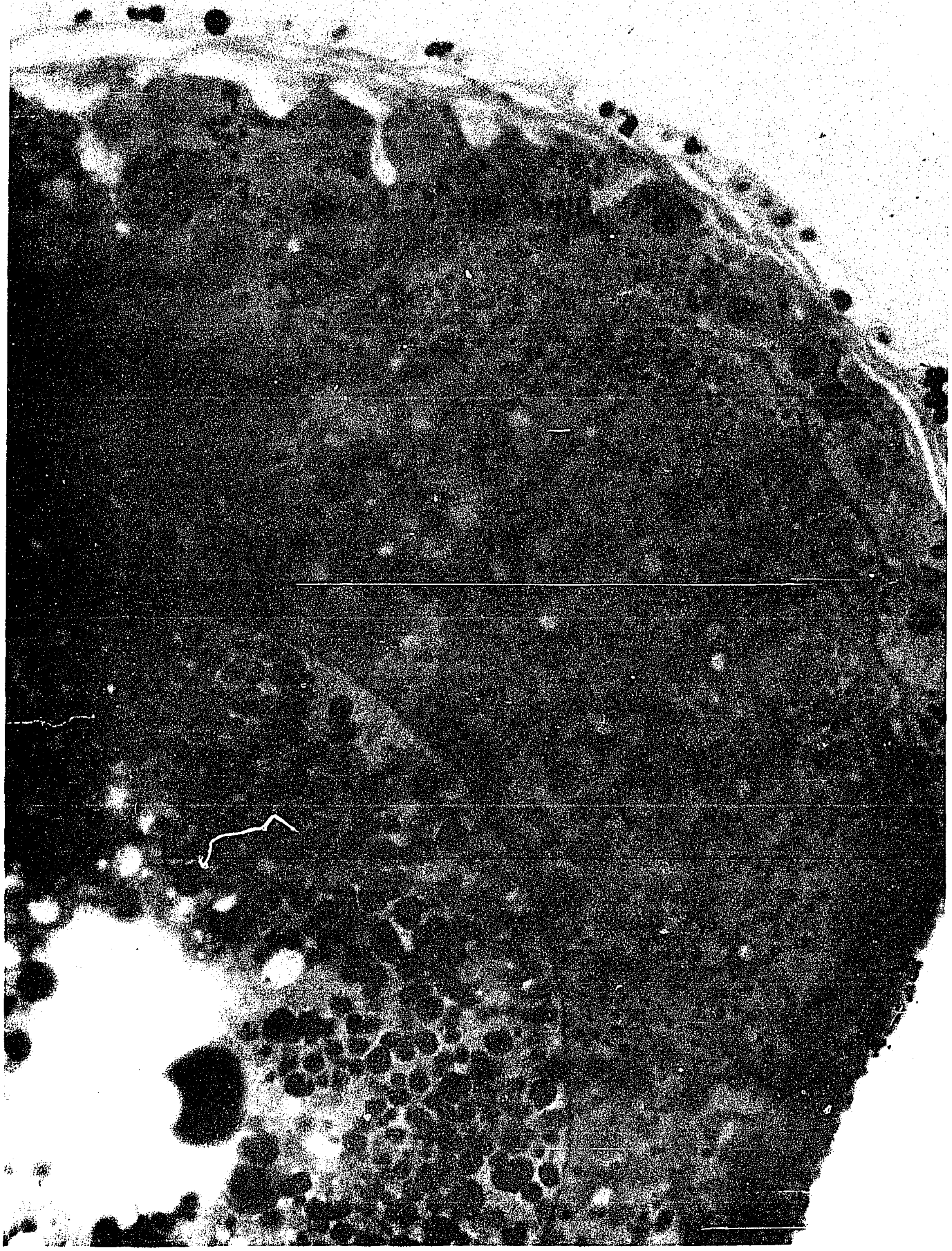


Figure 32. *T. larynx*. Micrograph of a nurse cell (NC) with small vesicles of yolk and oocytes (O) with both large and small vesicles. (X 3600)

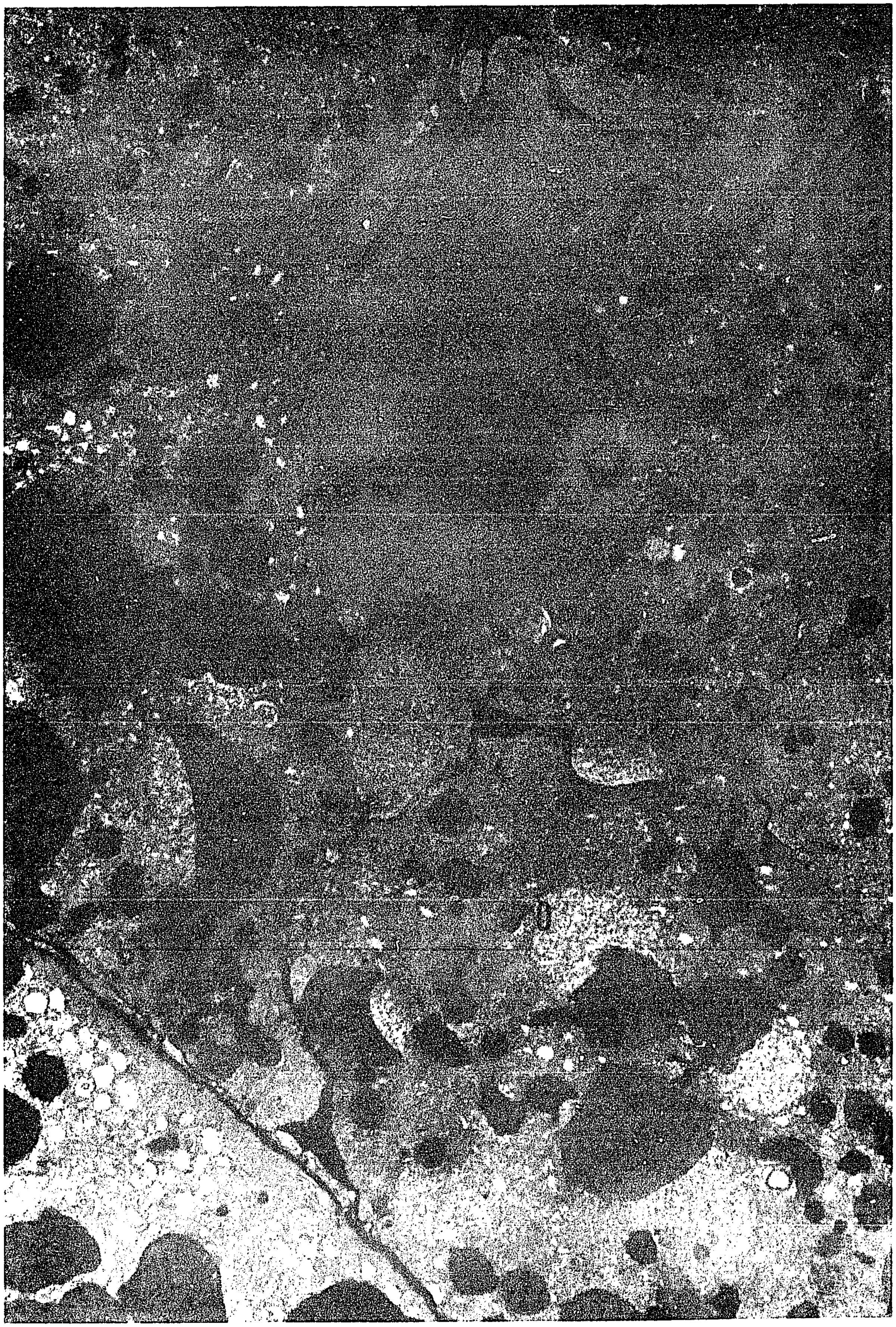


Figure 33. _T. larynx_. Micrograph of a shrunken cell with a nucleus containing heterochromatin. (X 7000)

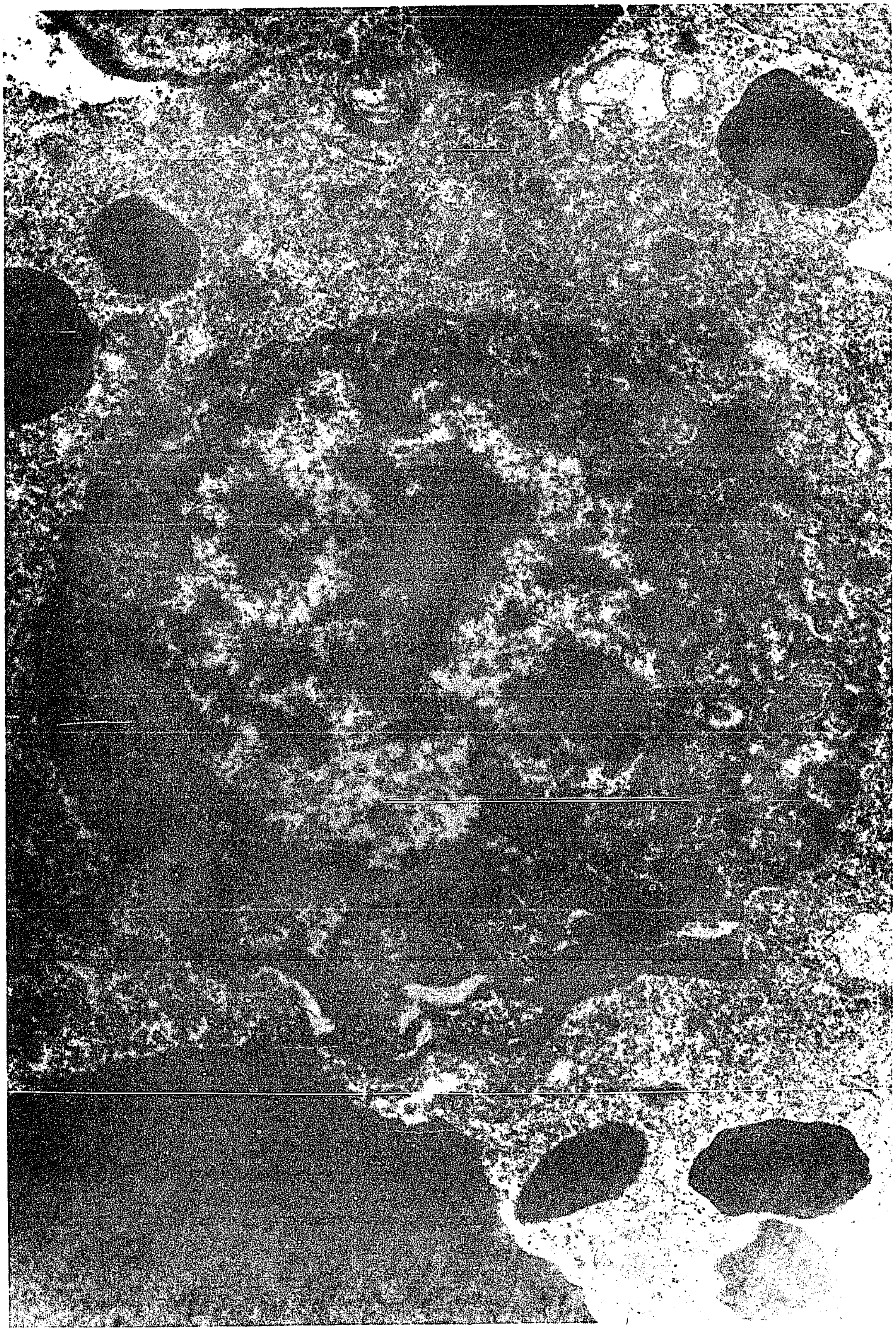


Figure 34. *T. larynx*. Micrograph of the spadix with microvilli and discoidal coated vesicles (CV). (X 16,500)

Figure 35. *T. larynx*. Micrograph of early vitellogenic germ cells.
(X 4300)

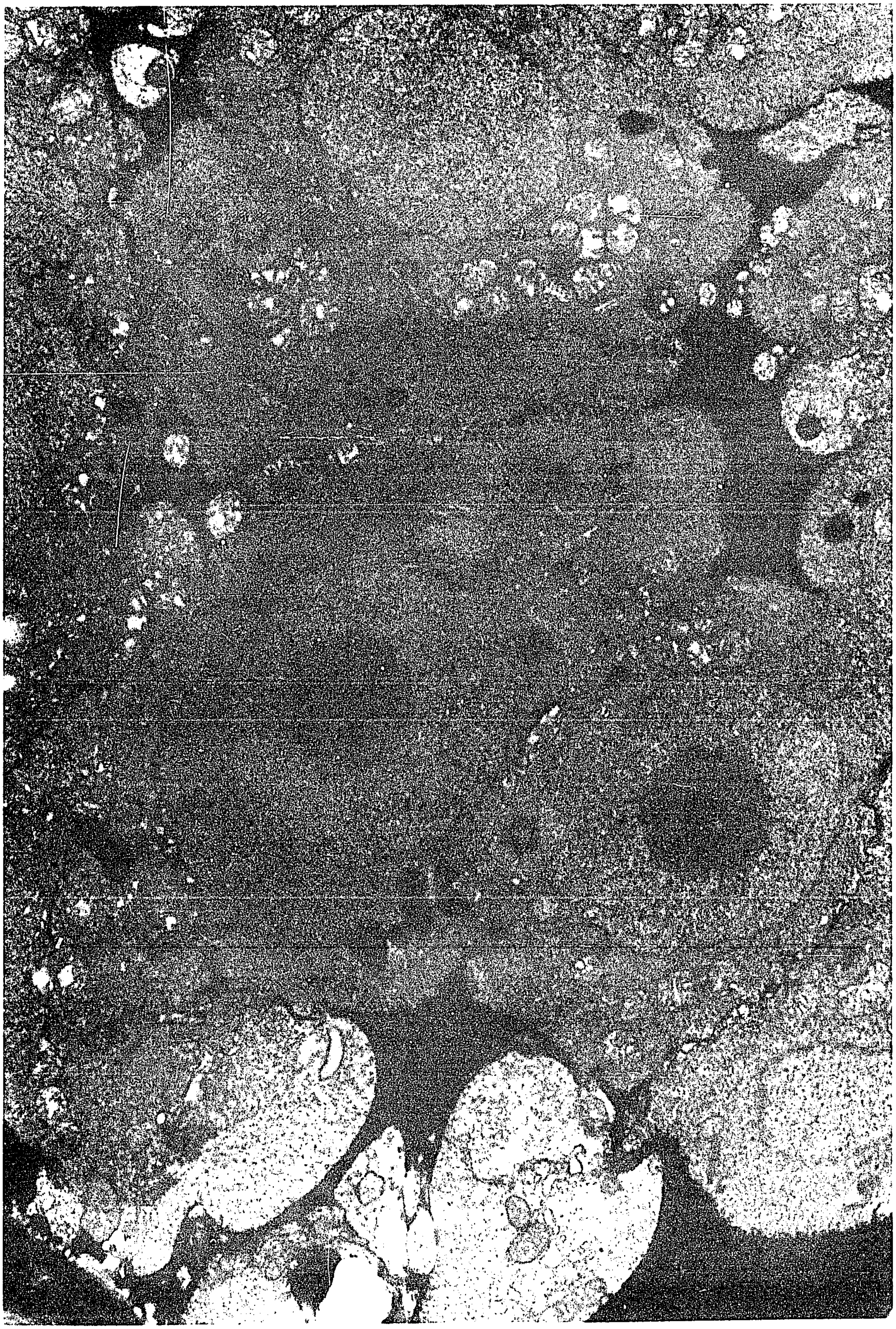
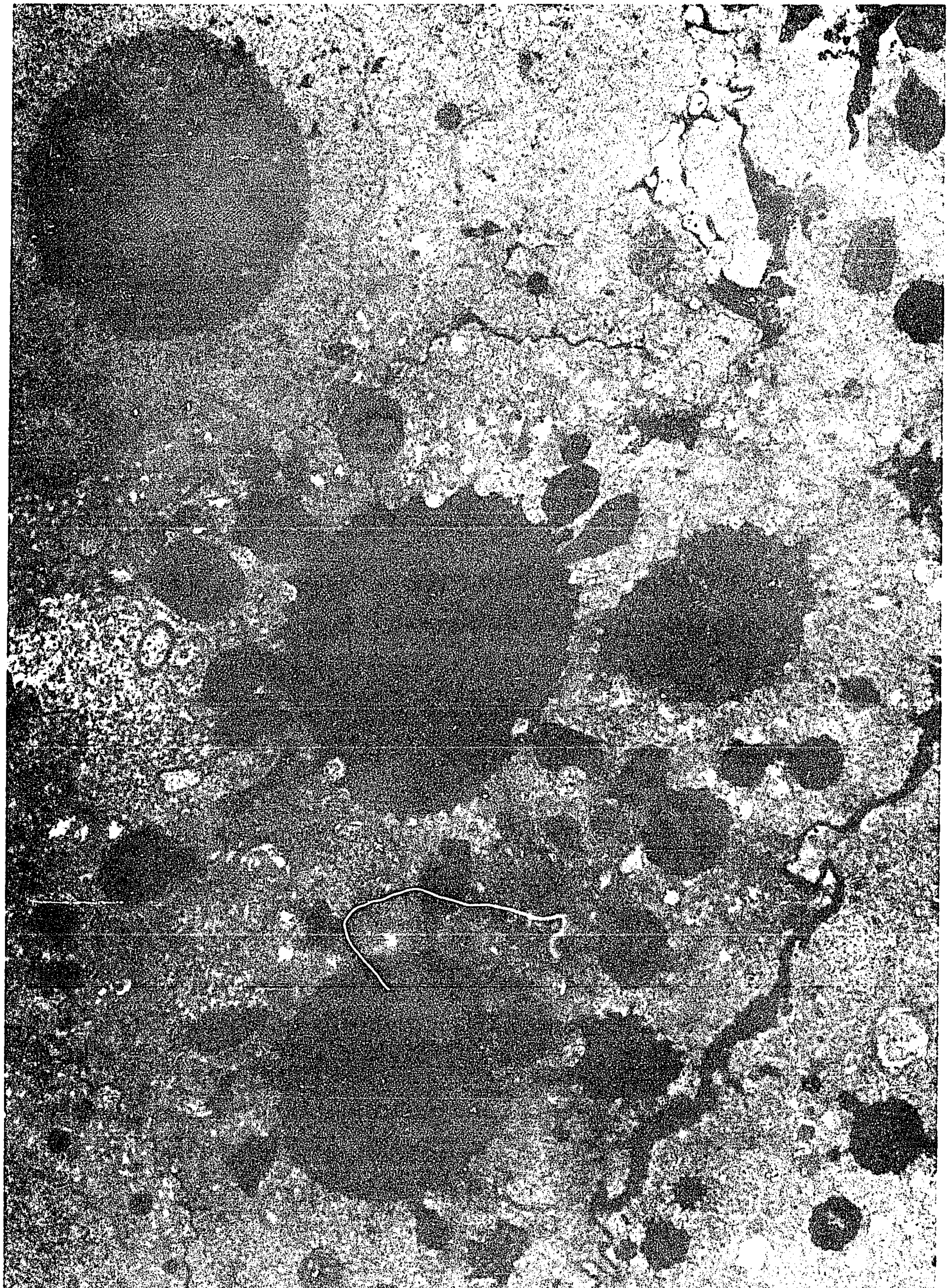


Figure 36. *T. larynx*. Micrograph of an oocyte in which the small yolk vesicles appear to fuse to form the large yolk vesicles found only in the oocytes. (X 6100)



DISCUSSION

After Weismanns work (1883) it seemed definitely established that in the Hydrozoa the germ cells have an ectodermal origin and migrate to the gonophores, although the germ cells have endodermal origin in the Scyphozoa and Anthozoa according to Campbell (1974), Szmant-Froelich *et al.* (1980), and Larkman (1983). Other work on various hydroids, including species of *Tubularia* by Koch (1876), Hargitt (1909), Liu and Berrill (1948), and Berrill (1961), seemed to indicate that, in at least some hydroids, germ cells have an endodermal origin. Goette (1907) concluded that the spermatogonia in *Clava multicornis* had an ectodermal origin while the oogonia were derived endodermally.

My observations on the female gonophores of *T. larynx* and *T. indivisa* indicate that the oogonia are derived from undifferentiated cells (I-cells) that proliferate in the ectoderm of the stalk of the gonophore and which migrate in the endoderm to an apical position in the gonophore bud. This contrasts sharply with the work of Liu and Berrill (1948) on *T. crocea* and Benoit (1925) on *T. mesembryanthemum* who reported the direct conversion of endodermal cells into germ cells without migration. My observations, for the most part, agree with those of Lowe (1926) who reported the proliferation of I-cells in the ectoderm of the gonophore stalk and subsequent migration to the gonophore. However, she reported that the migration of I-cells occurred in the ectoderm while I found an endodermal migration. It is interesting to consider that the migration of the primordial germ cells from the endoderm at the base of the allantois and yolk sac to the germinal ridge in vertebrates is well established, while in the "primitive" and structurally simple hydrozoans the problem is basically unresolved.

Weismann's (1883) discovery of germ cell migration in hydroids helped to shift the emphasis from the cellular layer in which recognizable germ cells first appear, to also include the cellular type from which they originate. The origin of hydrozoan germ cells has been a controversial topic and the subject of numerous studies by many investigators. The solution to this problem has been made especially difficult since the studies have involved interpretations of a dynamic process based largely on the information based on fixed and sectioned material. Even where modern investigators have agreed that the cells that will form a gonophore bud or medusa bud are derived from I-cells, there is then the question of the origin of the I-cells. Bierbach and Hofmann (1973) argued that the localized accumulations of I-cells that produce meudsa buds are derived form I-cells present in the ectoderm of the stolons. Bouillon and Werner (1965), on the other hand, considered that local somatic cells dedifferentiated to form the I-cells for medusa development.

In the Cnidaria, according to Campbell (1974, p. 152), "Germ cells and their accessory cells arise from interstitial cells." Adult hydrozoans maintain a proliferating population of I-cells in the ectoderm (Hyman, 1940; Bouillon, 1968), however, these I-cells originate from the larval endoderm during early cleavage in at least some hydroids. According to Nagao (1965) and Summers and Haynes (1969), the smaller cells produced from unequal divisions of the large endodermal cells become the I-cells and migrate to the ectoderm of the larva. While it is clear from recent work on Hydra that the ectodermal I-cells, regardless of their larval orgin, produce the germ cells under normal conditions (Burnett, Davis and Ruffing, 1966; Tardent, 1974; Honegger, 1981), this does not hold in the laboratory. Normandin (1960), Haynes and Burnett (1963), Davis _et al_. (1966), and Burnett _et al_. (1966) have demonstrated the differentiation of I-cells from endodermal cells under laboratory conditions. Burnett _et al_. (1964), after their work with _Hydra viridis_, concluded that there can be no separate lines of interstitial cells to form the cnidoblasts, neurons, or germ cells since endodermal gland cells can dedifferentiate and produce a normal, sexually mature polyp.

In _Tubularia_, enlargement of the oocytes that have entered the prophase of meiosis I does not begin until after the migrating I-cells reach the apex of the gonophore. This makes it difficult to separate the I-cells which develop into germ cells from the I-cells which will differentiate into nematoblasts, especially when both processes occur in the same region of the polyp. Lowe (1926) also noted this problem in _T. larynx_ and in some of my sections of both _T. larynx_ and _T. indivisa_ nematocytes were found among the germ cells in young gonophores. A potential solution to this problem may be to study hydroids in which the oocytes differentiate and begin the growth phase of meiosis I during or even before migration. In _Clava leptostyla_, ooctyes with a distinct germinal vesicle are first distinguishable in the endoderm of the polyp, before they reach the gonophores where they mature and are fertilized (Hargitt, 1906; Hargitt, 1916; personal observations). Wasserthal (1973) found _Eudendrium armatum_ an even better species to study since the ooctyes begin to enlarge before migration commences. He found the young oocytes growing in the ectoderm of the hydrocaulus (stalk or stem of the polyp) surrounded by nematocytes in various stages of nematocyst development. Later stages of oocyte growth were found in the ectoderm of the gonophore stalk, but at some location they penetrate the mesolamella of the gonophore and complete oogenesis in the endoderm of the gonophore.

David and Challoner (1974) used the thiolacetic acid-lead nitrate staining technique of Lentz and Burnett (1961) to locate the developing nematocytes in intact hydra. It might be possible to develop a similar histochemical technique to differentiate the nematoblasts and nematocytes from the early germ cells. This is especially feasible in a hydroid such as Eudendrium armatum where a morphological distinction between the two types develops quickly. This technique could then be applied to hydroids such as Tubularia in which there seem to be no morphological characters or simple staining techniques to differentiate young germ cells and nematoblasts. It might then be possible to determine young germ cells and nematoblasts. It might then be possible to determine the location of germ cell origin in living Tubularia. Campbell (1974, p. 152) considered the problem of the germinal interstial cells and concluded that, "it is generally impossible to assess the orgin of a cell type which cannot be distinguished from other interstitial cells, at least by classical histological techniques employed."

In addition to the question of the location of origin of the germ cells, a number of other problems concerning hydrozoan reproduction have not been resolved, in spite of numerous studies. These questions include the cellular type from which germ cells originate and whether or not the germ cells actually migrate to the gonophores and are not simply carried by tissue movements. Other questions concern the formation of the entocodon. In many species it is not known if the entocodon develops in the ectoderm or endoderm and whether the germ cells develop from the entocodon or have a separate origin. While these topics are no longer as controversial as they were in the late 1800's and early 1900's, the wide variety of results, sometimes with the same species, is troublesome. Resolution of the inconsistencies in the interpretations have been made difficult since all of the investigators have used fixed and sectioned material to interpret dynamic processes.

The present results on germ cell movement and origin are, unfortunately, also based on circumstantial evidence and interpretations of fixed and sectioned material. Examination of my specimens of T. larynx and T. indivisa has indicated that the major site of I-cell proliferation is the ectoderm at the base of the gonophores. Between the site of I-cell proliferation and the apex of the gonophore bubs, single and groups of 2-4 I-cells are abundant in the endoderm. These endodermal I-cells appear to be in the process of migrating to the apex of the gonophore bud after having been produced in the ectoderm at the base of the gonophore. Endodermal I-cells were rare in other areas of the polyps including the distal portions of the racemes which contain only older gonophores. At the apex of the gonophore buds the I-cells accumulate and proliferate in the endoderm on the inside of the mesolamella that separates the endoderm and ectoderm. The entocodon and germ cell mass which form these apical I-cells are thus both endodermal structures. Brauer (1981), working with T. mesembryanthemum, and Lowe (1926), working with T. larynx, also reported that the germ cells originate in the ectoderm at the base of the gonophores. However, both of these investigators also agreed that the entocodon and the germ cells developed in the ectoderm at the apex of the gonophores. While Brauer reported that the germ cells migrated in the endoderm, Lowe found an ectodermal migration in _T. larynx_, one of the species in which I am reporting an endodermal migration. Obviously, better techniques and more than circumstantial evidence are required to resolve this problem.

Tubularia larynx and _T. indivisa_ produce large, yolky eggs (about 410 µm and 700 µm respectively) and brood the embryo within the gonophore to an actinula before the larva is released. The results of Hargitt (1919) and Rees (1957) indicate that the species of hydroids with large polyps produce relatively large eggs, usually greater than 200 µm in diameter. These species include colonial hydroids such as _Pennaria_, _Hybocodon_ and _Tubularia_ which Rees considers to be essentially solitary, and the solitary hydroids including _Corymorpha_ and _Hydra_. On the other hand, most of the colonial species, including _Obelia_, _Campanularia_, _Clava_, _Gonothyrea_, and _Hydractinia_, have small polyps and produce relatively small eggs that are less than 170 µm in diameter. Hargitt (1919) also noted that during oogenesis in the species that produce large eggs, numerous excess oocytes, which are a type of accessory cell, are produced and consumed by the relatively few growing oocytes in each gonophore. However, the species that produced small eggs are apparently nourished directly from the enteron without any accessory cells.

A variety of accessory cells have been described and the participation of accessory cells of somatic origin, or follicle cells, especially during vitellogenesis, is known in many animals, including vertebrates. Balinsky (1975) reported follicle cells and oocytes with interdigitatiing processes in all vertebrates that have been studied, with the oocyte surface typically possessing numerous microvillar projections (Anderson, 1974). These projections increase the surface area of contact between the plasma membranes of the oocyte and its follicle cells which provide an exogenous source of yolk. All of the material utilized by the oocytes are not produced by the follicle cells which may act as a barrier between the oocytes and other exogenous sources of nutrients. Bellairs (1965, p. 215) reported, "In the adult hen each oocyte is surrounded by a capsule of follicle cells and all the raw materials that enter the oocytes must pass through this capsule."

Not all of the somatic accessory cells develop interdigitations with the oocytes for the passage of nutrients. At least some lizards develop another type of follicle cell, the pyriform cell (Naeves, 1971; Taddei, 1972). Pyriform cells are unusual in that, while they are somatic cells, they develop intercellular bridges with the oocytes. These bridges were not found in small follicles and are thought to form secondarily, as the oocytes grow, and not as a result of incomplete cytokinesis. Neaves reported a system of about 10,000 pyriform cells surrounding each oocyte and suggested that the pyriform cells function in the maintenance of dormant oocytes since they disappear before the onset of yolk accumulation.

While accessory cells of germ line origin, usually termed nurse cells, have not been reported in vertebrates, numerous investigators have reported intercellular bridges between the oocytes in mammalian ovaries. Such intercellular associations have been reported in humans (Stegner, 1967; Gondos, 1973), mice (Ruby _et al_., 1969), rats (Franchi and Mandl, 1962), hamsters (Weakly, 1966, 1967), and rabbits (Zamboni and Gondos (1968). Zamboni and Gondos suggest that the intercellular bridges may account for the synchronoous differentiation, maturation, and degeneration of groups of germ cells and that syncytia of germ cells may form in all mammals. They also speculate that the oocytes that survive may be the ones that complete cytokinesis. Baker (1963) reported that in humans less than one oocytes in 20 survives to the age of seven years. McReynolds _et al_. (1984) reported the presence of mitochondria and ribosomes in the cytoplasm of the bridges and suggested that they may allow the exchange of organelles and nutrients. It is interesing to speculate that perhaps one oocyte in each cluster survives, while the remaining oocytes function as nurse cells before degenerating.

A wide variety of types of accessory cells of germ line and somatic origin are known from different groups of invertebrates, including ascidians which have been studied by Tucker (1942), Kessel and Kemp (1962), and Berrill (1975). Ascidians develop both an outer follicular epithelium and a layer of test cells during oogenesis. The primary follicle cells are derived from the germinal epithelium, from which the oocytes also differentiate. The test cells differentiate from the cells of the primary follicle and penetrate into the superficial cytoplasm of the oocyte, increasing the surface area of contact. Both the test cells, which are eventually extruded from the oocyte, and the follicle cells pass nutritive material to the oocyte. Since the unspecialized germinal epithelium produces the oocytes and the follicle cells, Berrill (1961) considered the follicle cells to be descendents of abortive oocytes. If this reasoning were applied to hydrozoans, the cnidoblasts could be considered to be descended from abortive oocytes since they also differentiate from I-cells. However, Berrill (1975) reported that totipotent cell types exist in tunicates and no distinct germ line had yet been demonstrated.

Projections of follicular cells penetrate into the oocytes of the copepod, Labidocera aestiva, according to Blades-Eckelbarger and Youngbluth (1984), but a much more extensive penetration is found in the decapod cephalopods. Arnold and Williams-Arnold (1977) reported that the cells of the follicular epithelium proliferate mitotically and that folds of this tissue, consisting of numerous follicle cells, penetrate deeply into the oocytes. The interdigitations tremendously increase the surface area of contact between the follicular epithelium and the plasma membrane of the oocyte. In addition, the follicle cells develop multiple nucleoli and a larger nucleus to cytoplasm ratio and eventually form a syncytium surrounding the oocyte. Autoradiography with ^{3}H-leucine was used by Selman and Wallace (1972) to demonstrate that the follicle cells are the major site of protein synthesis in the follicle cell-oocyte complexes in Loligo pealei.

In some species of turbellarians and in a few narcomedusae, the follicular epithelium has been reduced to a single acessory cell which surrounds the oocyte. Newton (1970), working with a freshwater turbellarian, Hydrolimax grisea, found that one of the accessory parenchymal cells completely envelops a growing oocyte and that both nutrition by diffusion and fertilization take place through the accessory cell. A similar development was noticed in the marine turbellarian, Alaurina composita (personal observation). Berrill (1950) described work by a number of investigators including Bigelow (1909) on Pegantha smarygdina and Metschnikoff (1886) on Cunina proboscidea, both of which are narcomedusae. In both species a specialized "nurse cell", the phorocyte, whose origin is unknown, surrounds the oocyte and early embryonic stages. The nucleus of the phorocyte of Cunina was later incorporated into the embryo. The phorocytes of Pegantha become multinucleate, but their fate was not described. Davidson (1968) mentioned that certain annelids develop two "nurse cells" with polytene nuclei along with each oocyte, but did not mention the species.

The accessory cells described above have not been demonstrated to come from the same cell line as the oocytes with which they are associated and, although they are sometimes referred to as nurse cells, are more accurrately considered follicle cells (Berrill and Kapp, 1976). However, numerous examples also exist of accessory cells of germ line origin that participate in the nutrition of the oocyte or embryo.

Thorson (1950) reported examples of embryos with non-pelagic development that feed on nurse eggs in crinoids, ophiuroids, polychaets, nemerteans, and prosobranchs. In this method of embryonic nutrition, a large number of eggs are deposited together, but only a few develop into embryos. The nurse eggs are devoured by the embryos before they hatch. The extreme example was the prosobranch mollusc, Volutopsis norvegica, in which he reported that 50,000-100,000 nurse eggs are consumed by a single embryo.

In other invertebrates, the oocytes that do not complete development are used as nourishment for the developing oocytes. Pollock (1975) mentions this process in tardigrads and describes the abortive oocytes functioning as "nurse cells" while Fell (1969) reports that the oocytes in the sponge, Haliclona ecbasis, engulf whole nurse cells. However, in both tardigrads and sponges the engulfed cells were not directly connected to the oocytes by intercellular bridges. If we define the term nurse cell to include only those cells of germ line origin and connected by intercellular, cytoplasmic bridges to the sucessful oocyte (Anderson, 1974; Berrill and Kemp, 1976; Browder, 1980), then we are left needing a term for the accessory cells of germ line origin which lack intercellular bridges to the oocytes and have not completed meiosis. Pollock (1975) used the term abortive oocytes while Raven (1961) called them nurse cells or nutrimentary eggs and considered them to be abortive germ cells. Hargitt (1919) also mentioned the problem of accessory cells which are called nurse cells, but which are "consumed instead of preparing food", but did not suggest an alternative. The term, nutritive oocytes, which has already been used (Spracklin, 1978), still appears appropriate since it implies both the function and the stage of meiosis.

The most highly organized development of accessory cells is found in the species in which true nurse cells, possessing intercellular bridges to the oocyte, are produced during oogenesis and provide the oocytes with nutrients. This type of germ cell nutrition is best known from the insects which have evolved two different types of meroistic ovaries, polytropic and telotropic, as well as the panoistic ovaries which do not contain nurse cells (Davidson, 1968). In the telotropic ovaries the nurse cells are connected to the oocytes by a long nutrient cord. Woodruff and Anderson (1984) have used microinjected dyes to demonstrate the physiologic continuity between the oocytes and nurse cells that is provided by the cords, movement of dye along the cord, and the coupling of the oocytes and follicle cells, presumably by gap junctions. In the polytropic ovaries, of which Drosophila melanogaster is a typical example, a series of cell divisions, with incomplete cytokinesis, produces a complex of an oocyte and a species specific number of nurse cells. In Drosophila, a series of four divisions produces a complex consisting of a central oocyte and 15 interconnected nurse cells (Brown and King, 1964; King and Aggarwal, 1965; Koch and King, 1966). Growth of the oocyte in Drosophila is supported by the nurse cells and the cytoplasmic volume of the oocyte can increase by 90,000 times in only 3 days. After the four cycles of mitosis with incomplete cytokinesis, each of the two central cells of the oocyte-nurse cell complex have 4 intercellular bridges, a common one and 3 each to nurse cells. The other 14 cells have a maximum of 3 bridges each. The two central cells, which Koch et al. (1967) refer to as pro-oocytes, begin synapsis of meiosis I. Synapsis is later arrested in one of the two pro-oocytes which develops into another nurse cell, while the remaining pro-oocyte becomes the successful oocyte. They found that no oocytes develop in abnormal complexes in which no cell has 4 intercellular bridges, but were left with the question of what factor determines which of the pro-oocytes becomes the oocyte.

Davidson (1968) reported that chromosome diminution occurs during nurse cell formation in some beetles that also produce an oocyte-nurse cell complex of 15 nurse cells and 1 oocyte. Only the oocytes retain the normal chromosomal complement in these species. In *Drosophila*, the nurse cells actually become highly polyploid as their DNA content reaches a maximum of 1024C and their nuclei increase in volume up to 6000-fold (Koch and King, 1966). This may actually represent amplification of only a small portion of the genome, similar to what has been reported in the oocytes of *Xenopus laevis* by Perkkowska *et al*. (1968). In *Xenopus* oocytes the number of copies of the ribosomal DNA is increased by 2500 times and 1400 to 1600 nucleoli are produced in the germinal vesicle.

As vitellogenesis progresses in *Drosophila*, the germinal vesicles enlarge and may increase to 900 times their original volume; however, the nurse cell nuclei are much larger. Raven (1961) reported that the nuclei of oocytes of animals with nurse cells are generally smaller than the oocyte nuclei of similar species that do not form nurse cells. Pianka (1974) has reported an animal in which the ooctye nuclei do not enlarge at all during oogenesis. In the ctenophore, *Bolinopsis microptera*, the oocyte is part of a complex of about 100 cells, including 99 nurse cells and 1 oocyte. In contrast to the 4 intercellular bridges of each *Drosophila* oocyte, each oocyte and the inner nurse cells of *Bolinopsis* have only 3 intercellular bridges. However, in each of the 3 clusters of nurse cells attached to each oocyte, the 7 nurse cells closest to the oocyte became highly polyploid. According to Pianka, "all major synthetic functions of oogenesis in *Bolinopsis* are apparently performed by the nurse cells, and not by the oocyte."

Hydroids display a wide range in the structural complexity of the gonophores and in the involvement of accessory cells during oogenesis.

The structurally simplest are the styloid gonophores, whose walls are only continuations of the ectoderm and endoderm of the polyp (Kuhn, 1913; Teissier, 1926; Hyman, 1940). The gonophores of Eudendrium armatum are typically styloid. Each gonophore contains only a single oocyte and no accessory cells of any kind, although the spadix is well developed and wraps about two thirds of the way around the oocyte (Wasserthal, 1973). The other types of hydrozoan gonophores, hetermedusoid, cryptomedusoid, and eumedusoid, are increasingly complex and develop additional cellular layers, an endoderm lamella and inner ectoderm, but there has been no suggestion that any of these cells function as follicle cells. However, in many hydroids, cells of germ line origin are used as food or to produce nutrients for the developing oocytes. Pennaria tiarella has been investigated by Smallwood (1899), Hargitt (1900), Hargitt (1909, 1919), Berrill (1952, 1961), and Cowden (1964). In the gonophores of this species, several thousand oocytes differentiate in each gonophore, but only 6-10 of the oocytes complete meiosis. The remainder of the oocytes are absorbed or engulfed and quickly digested by the growing or definitive ooctyes. No trace of the engulfed oocytes remains at the completion of meiosis. Similar examples of this form of oocyte nutrition, which Cowden (1964) referred to as the "most primitive form of nutrimentary oogenesis", have been reported in other species of hydroids. Large numbers of nutritive oocytes have been reported in the gonophores of Acaulis primaris by Berrill (1952), Rhizogeton fusiformis by Spracklin (1975), and in Millepora by Mangan (1909). In all of these species, the excess or nutritive oocytes are engulfed and quickly digested and no trace of the engulfed oocytes remains in the eggs. Intercellular bridges have not been reported among the oocytes in any of these species.

In other hydroids in which large numbers of nutritive oocytes are consumed by a few successful oocytes, the remains of the engulfed cells persist through embryonic development and are known as pseudocells (Brauer, 1891) or shrunken cells (Zihler, 1972). May (1903) reported that single nutritive oocytes and clusters of up to 7-8 are engulfed and enclosed in vacuoles in the eggs of *T. crocea* while, during this research, as many as 12 shrunken cells were found in a vacuole in early cleavage stages of *T. indivisa*. The shrunken cells have been reported in all of the species of *Tubularia* and *Hydra* that have been investigated, most recently by Lowe (1926), Nagao (1965), and Boelsterli (1975) in *Tubularia*, and by Zihler (1972) and Tardent (1974) in *Hydra*. Zihler demonstrated conclusively that the shrunken cells were the remains of engulfed whole cells, and not simply the remains of nuclei.

During oogenesis in both *Tubularia* and *Hydra*, a single egg is produced in each reproductive structure from the originally large number of germ cells that differentiate. There are initially over 1200 germ cells in each gonophore of *Tubularia* and, according to Zihler's figures, at least 100 in *Hydra*. Based on the work on *Hydra circumcincta* by Zihler and Tardent, oogenesis in each *Hydra* ovary begins with the accumulation of a variable number (at least 3) of clusters of I-cells which differentiate into the initial germ cells, called OOgonia I. The Oogonia I develop into Oogonia II, which grow and synthesize yolk. One of the Oogonia II in each cluster becomes a primary oocyte and engulfs the remaining Oogonia II, transforming them into shrunken cells. All of the oocytes then fuse to form a single large ooctye which completes meiosis. Only one of the oocyte nuclei survives. Tardent found intercellular bridges between the Oogonia II in a cluster, but made no mention of the number or arrangement of bridges. Burnett _et al_. (1966) reported the mitotic proliferation of I-cells to form syncytial nests of up to 32 cells, which differentiate into cnidoblasts or spermatogonia. The intercellular bridges in the clusters of Oogonia II suggest that each cluster is descended from a single I-cell. However, neither investigator found intercellular bridges between the primary oocytes.

There have been some previous indications of incomplete cytoplasmic divisions during oogenesis in two species of _Tubularia_. Lowe (1926), working with _T. larynx_, suspected that the cytoplasmic divisions that produced the germ cells were incomplete since the shrunken cells were engulfed in groups, but did not see the bridges. Boelsterli (1975), reporting on _T. crocea_, included one electron micrograph of an intercellular bridge between oogonia and in Figure 15 included drawings of clusters of young oocytes interconnected by cytoplasmic bridges. Boelsterli believed that most of the oocytes disintegrated and were incorporated, through some unknown process, by the approximately 15 growing oocytes which fused to form one large oocyte. He also briefly reported on egg fragmentation experiments with _Tubularia_ and _Hydra_. His account is not extremely specific, but he confirmed that the germinal vesicles of the oocytes which fuse to form the single _Hydra_ oocyte persist until just before the continuation of meiosis. However, he reported that similar experiments failed with _Tubularia_. These results seem to indicate that the oocytes which fuse in _Hydra_ to produce the egg are essentially identical cells, while the cells which fuse to produce an egg in Tubularia are not identical.

The current research on T. larynx and T. indivisa has revealed an extensive network of intercellular bridges among the germ cells in the female gonophores of both species. The cytoplasm of 1200 to 200 germ cells is incorproated into a single oocyte which completes meiosis, and it appears that all of these cells are interconnected into a single complex by intercellular bridges. It can be hypothesized that each complex, consisting of at least 1200 cells, is formed by the proliferation of one initial cell, since, except for the pyriform cells of lizards, intercellular bridges normally form only as a result of incomplete cytoplasmic division. The lizard pyriform cells also represent a much simpler system, with each pyriform cell directly connected to the oocyte. It is hard to imagine a mechanism by which secondary cell contact could result in the repeated formation of essentially identical complexes of cells, interconnected by cytoplasmic bridges in a highly organized pattern. Since they are all descended from a single cell and connected to the oocyte by intercellular bridges, the 1200 to 2000 cells in each complex that are engulfed and transformed into shrunken cells meet even the highly restricted definition of nurse cells used here.

While the majority of the cells in the germ cell complexes of Tubularia are nurse cells, the 15-18 centermost cells present a problem, although at least one of them has to be considered a primary oocyte. All previous investigators have considered the central cells to be oocytes; however, the existence of the intercellular bridges was previously unknown. Hargitt (1909) noted that here were two different types of oocyte nuclei in the gonophores of T. crocea and believed that only the oocytes with a particular type of nucleus (the cells starting meiosis) enlarged and formed the egg. In both T. larynx and T. indivisa, the nuclei of the central cells show changes typical of meiosis I, and later all develop germinal vesicles which are identical except for slight differences in size. These germinal vesicles are distinctly different from the nuclei of the nurse cells. In addition, the oocytes produce large vesicles of yolk and fuse together at the completion of vitellogenesis, while the nurse cells contain only the smaller type of yolk vesicles and are engulfed and transformed into shrunken cells. Koch et al. (1967) reported that one of the two pro-oocytes that formed synaptinemal complexes reverted back and became a nurse cell in Drosophila. This does not appear to happen in Tubularia in which all 15-18 central cells are morphologically indistinguishable. Therefore, while only one of the germinal vesicles will persist in the fused primary oocyte, there are grounds for continuing to refer to all of the central cells as primary oocytes. An alternative would be to separate each complex into a single oocyte, about 15 large nurse cells, and about 1200 small nurse cells. Separating the cells of the complexes of Tubularia into three categories seems cumbersome and it is not entirely clear which oocyte will contribute the surviving germinal vesicle.

The oocyte-nurse cell complexes found in T. larynx and T. indivisa are similar to those reported by Pianka (1974) in the ctenophore, Bolinopsis, although there are some major differences. The complexes in Tubularia are over 10 times as large, each initially has more than 1 oocyte, and the oocytes are larger and have larger nuclei than the nurse cells. Attempts to resolve and map the interconnections in the complexes of Tubularia have produced some confusing results. Most of the complexes contained oocytes with a maximum of 3 intercellular bridges on each cell and the largest germinal vesicle was in a cell at the center of the complex. However, some complexes were found to contain ooctyes with 4 bridges and 2 germinal vesicles. These oocytes were probably produced by early fusion of the oocytes, which normally does not occur until the end of vitellogenesis when all of the oocytes fuse together. If two cells with 3 bridges apiece, including 1 common bridge, fuse together, the resulting cell will have 4 bridges and 2 nuclei. Unfortunately, some oocytes were seen that had 4 bridges and only a single nucleus, and the largest germinal vesicle was not always in a central cell of the complex. The largest complex that was mapped consisted of 43 cells and contained 3 oocytes with 4 bridges, but only 2 of these oocytes had 2 nuclei. The largest germinal vesicle in this complex was in one of the peripheral oocytes with 2 nuclei, although it was not the largest oocyte of the complex. The factors which determine the relative growth of the oocytes and their germinal vesicles and may also select the surviving germinal vesicle are not known. It is possible that all of the germinal vesicles are initially equivalent and that the one which will survive may be gradually determined during vitellogenesis.

The cooperative effort of the large number of cells of the oocyte-nurse cell complexes of Tubularia results in the production of large eggs which develop into actinulae. The actinula larva of the Tubulariidae is also found in other families of hydroids, including the Margelopsidae, Corymorphidae and Myriothelidae, as well as in other hydrozoans including the Trachymedusae, Narcomedusae, and Chondrophora

(Totton, 1954). Garstang (1946, p. 154) considers the actinula "merely a planula with precocious adult characteristics." Rees (1957) observed that an actinula is present in the more primitive families of hydroids and many authors, including Swedmark and Teissier (1966) and Rees (1966) have proposed that the ancestral hydroid was an actinula. While it is interesting to speculate about the evolutionary implications of nutritive oocytes, shrunken cells and actinulae and to make comparisons, many pieces of the puzzle are still missing. The reproductive biology of many hydroids is poorly known and few authors include sufficiently detailed information on both oogenesis and larval development.

Shrunken cells are well known in *Hydra* (Zihler, 1972) and have also been found in *Acaulis primaris* (Berrill, 1952). Hyman (1940) reported that the encysted larvae of *Hydra* hatch in a relatively advanced condition, with indications of tentacles, and Brinkman-Voss (1970) reported a similar process in *Acaulis ilionae*; however, these species do not develop an actinula. The larvae of *Corymorpha palma* (Torrey, 1907) and *C. nutans* (Rees, 1937) are also encysted and develop directly into polyps, without a free-swimming planula stage, but in these species there are no indications of shrunken cells. While all of the species whose eggs contain shrunken cells do not develop actinulae, the converse does appear to hold. According to Van De Vyver (1968a,b) the families of hydroids that develop an actinula larva have large eggs, which digest neighboring oocytes, and which accumulate shrunken cells. There are no reported exceptions to this generalization.

Hargitt (1919) found that most of the species that utilize large numbers of accessory cells are solitary species or colonial species with large polyps, and the eggs produced are at least 200 um in diameter. Eudendrium ramosum oocytes engulf large numbers of somatic accessory cells, while all of the other hydroids utilize germ line accessory cells (Hargitt, 1919). Rees (1957) reported a similar phenomenon and found that solitary species produced large eggs, while colonial species produced relatively small eggs. Other hydroids with large eggs and numerous germ line accessory cells, in addition to the species reported by Hargitt and Rees, include Acaulis ilionae which has 190 µm eggs (Brinkman-Voss, 1970), Millepora with 210-275 µm eggs (Mangan, 1909), Ectopleura dumortieri with 350 µm eggs (Aurich and Werner, 1954), and Acaulis primaris with eggs 200-225 µm in diameter (Berrill, 1952). While the eggs of Ectopleura dumortieri are relatively large when compared to most hydroids, they are the smallest eggs of any hydroid reported to produce an actinula.

There are two major advantages to oogenesis in which many small nutritive oocytes or nurse cells support the growth of a relatively few definitive oocytes. The first is the relatively large surface area to volume ratio of many cells which increases the amount of surface area available for food absorption as compared to a single large cell. There is also the tremendous increase in the amount of DNA available to code for ribosomal RNA. Each accessory cell has at least the normal 2C DNA content and if they are germ line accessory cells that have started meiosis their DNA content will be at least 4C. The nutritive oocytes and nurse cells effectively increase the rate of synthesis of ribosomes for the definitive oocytes since each cell has at least one nucleolus where ribosomal RNA production occurs. The net result is the accelerated development of large eggs reported by Van de Vyver (1968a) in the families of hydroids with actinulae.

Campbell (1974) reported that gametogenesis in the Cnidaria usually does not involve accessory cells. However, regardless of the type of oocyte nutrition, the eggs of hydroids with sessile gonophores are usually fertilized internally and develop in or on the gonophores. The species of hydroids with small eggs produce many of them in each gonophore and brood them to a planula before release, while the species that produce relatively few, large eggs in each gonophore brood them to more advanced stages. According to Pearse (1979) the brooding species of chitons are often relatively small compared to the non-brooders. Chia (1974) attributes brooding by small species of invertebrates to energy limitiations. In general, brooding requires a greater investment in stored nutrients for each embryo than for planktotrophic species in which the egg is fertilized after being released by the parent and development is external. However, brooding increases the larval survival rate by decreasing the time a planktonic larva is exposed to filter feeders. Brooding species therefore have to release many fewer larvae than planktotrophic species to produce the same number of larvae that survive to settle and metamorphose. This may reduce the total yolk requirement during a reproductive period. The actinulae of _Tubularia_ may have a very short planktonic existence and often settle close to the colony that released them (Pyefinch and Downing, 1949).

An alternative view of brooding is provided by Menge (1975) who suggests that internal space limitations prevent small species from producing and broadcasting the necessarily large numbers of planktotropic larvae and that they therefore concentrate on producing a relatively small number of lecithotrophic larvae with a large yolk supply. Hydroids appear to do the opposite. All of the large solitary species produce relatively large eggs and many brood them to an actinula larva before release. The colonial species with small polyps produce relatively small eggs while _Pennaria tiarella_, a colonial species with large polyps, broods large eggs which are produced by the utilization of many nutritive ooctyes. In general, among hydroids, the fewest eggs per gonophore and the largest eggs are produced by the largest species. Rees (1957, p. 481) may have provided the answer in his suggestion that the large eggs of the solitary hydroids "may only reflect the minimum size at which such a polyp can become self supporting and at the same time be a miniature of the adult." This type of argument may also be applied to the eggs that develop into actinulae. The eggs of _Ectopleura dumortieri_, which are 360 µm in diameter, may represent the minimum size at which an egg contains enough material to form an actinula. The eggs of _Hydra_ are only slightly smaller, 340 µm according to Hargitt (1919), and produce an advanced, encysted larva, but do not form an actinula.

Whatever the advantages that are involved in oogenesis when hundreds or thousands of germ line accessory cells support the production of a large egg, it is apparently a method utilized by many species of athecate hydroids. While most of these species are capitate hdyroids, at least one species, _Rhizogeton fusiformis_, is in the suborder filifera. These species may be separated into two general groups depending on the presence or absence of shrunken cells in the eggs. During this investigation, intercellular bridges and nurse cells were discovered in _T. larynx_, _T. indivisa_, and _Acaulis primaris_, all species which produce shrunken cells. The reports of bridges in *T. crocea* by Boelsterli (1975) and in *Hydra* by Tardent (1974), both species that produce shrunken cells, makes it likely that the accessory cells in these species are also nurse cells. On the basis of this information, it is also possible to make two other, more general, predictions. The first of these is that the shrunken cells of other species, including *Ectopleura* and *Hybocodon*, will also prove to be nurse cells and that intercellular bridges will be found among the germ cells of these species. The second prediction, which is simply the converse of the first, is that no bridges will be found among the germ cells of species that lack shrunken cells and that the accessory cells of these species are nutritive ooctyes.

LIST OF REFERENCES

Agassiz, L. 1860. *Contributions to the Natural History of the United States of America*. III. 301 p. Little, Brown & Co., Boston.

————. 1862. *Contributions to the Natural History of the United States of America*. IV. 380 p. Little, Brown & Co., Boston.

Allen, C.M. 1900. A contribution to the development of *Parypha crocea*. Biol. Bull. 1:291-315.

Allman, G.J. 1871. *A Monograph of the Gymnoblastic or Tubularian Hydroids*. 450 p. Roy. Society, London.

Anderson, E. 1974. Comparative aspects of the ultrastructure of the female gamete. Intl. Rev. Cytol. (Suppl.) 4:1-70.

Arnold, J.M. and L.D. Williams-Arnold. 1977. Cephalopoda:Decapoda. pp. 243-290. In *Reproduction of Marine Invertebrates IV* (A.C. Giese and J.S. Pearse, eds.). Academic Press, New York.

Aurich, V.H. and B. Werner. 1954-56. Uber die entwicklung des polypen von *Ectopleura dumortieri* van Beneden, und die verbreitung der planktischen stadien in der sudlicher Nordsee (Athecatae-Anthomedusae). Helgol. wiss. Meersunter. 5:234-250.

Baker, T.G. 1963. A quantitative and cytological study of germ cells in human ovaries. Proc. Roy. Soc. Lond., Series B. 158:417-433.

Balinsky, B.I. 1975. *An Introduction to Embryology*. 648 p. Saunders, Philadelphia.

Barnes, H. 1948. Studies on anti-fouling compositions. J. Iron and Steel Inst. 1948:175-185.

Barth, L.G. 1940. The process of regeneration in hydroids. Biol. Rev. 15:405-420.

Bellairs, R. 1965. The relationship between oocyte and follicle in the hen's ovary as shown by electron microscopy. J. Embryol. exp. Morphol. 13:215-233.

Berrill, N.J. 1948. Temperature and size in the reorganization of *Tubularia*. J. Exp. Zool. 107:455-464.

————. 1950. Development and medusa-bud formation in the hydromedusae. Quart. Rev. Biol. 25:292-316.

————. 1952. Growth and form in gymnoblastic hydroids. V. Growth cycle in *Tubularia*. J. Morphol. 90:583-601.

————. 1961. *Growth Development and Pattern*. 555 p. W.H. Freeman, San Francisco.

__________. 1975. Chordata:Tunicata. pp. 241-282. In Reproduction of Marine Invertebrates (A.C. Giese and J.S. Pearse, eds.). Academic Press, New York.

Berrill, N.J. and G. Karp. 1976. Development. 566 p. McGraw-Hill, New York.

Bier, K. 1963. Autoradiographic untersuchungen uber die Leistungen des Follikelipithls und der nahrzellen bei der dotterbildung und eiweissynthese im fliegenovar. Wilhelm. Roux's Arch. 154:522-575.

_______. 1975. Zur funktion der nahrzellen im meroistischen insektenovar unter besonder berucksichtigung der oogenese adephager Coleopteren. Zool. Jahrb. Physiol. 71:371.

Bierbach, M. and D.K. Hofmann. 1973. Experimentelle untersuchungen zur stochwachsturn und zur medusenbildung bei den marine hydrozoon Eirene viridula. Helg. wiss. Meesunter. 25:63-84.

Bigelow, H.B. 1909. The medusae. Mem. Mus. Comp. Zool. 37:9-245.

Blades-Eckelbarger, P.I. and M.J. Youngbluth. 1984. The ultrastructure of oogenesis and yolk formation in Labidocera aestiva (Copepoda:Calanoida). J. Morphol. 179:33-46.

Boelsterli, V. 1975. Notes on oogenesis in Tubularia crocea Agassiz (Athecata,Hydrozoa). Pubbl. Staz. Zool. Napoli (Suppl.) 39:53-66.

____________. 1977. An electron microscopic study of early developmental stages, myogenesis, oogenesis, and cnidogenesis in the anthomedusa, Podocoryne carnea M. Sars. J. Morphol. 154:259-290.

Bouillon, J. and B. Werner. 1965. Production of medusa buds by the polyps of Rathkea octopunta (M. Sars.) (Hydroida,Athecata). Helgol. wiss Meeresunters 12:137-148.

Brauer, A. 1891. Uber die entstehung der geschlechtsproducten und die entwicklung von Tubularia mesembryanthemum. Zeit. f. wiss. Zool. 52:551-579.

Brien, P. 1950a. Etude d'Hydra viridis. Ann. Soc. Roy. Zool. Belg. 81:33-110.

________. 1950b. La vesicle germinative de l'oocyte des Hydres. Bull. Cl. Sci. Acad. Roy. Belg. 36:561-573.

________. 1961. Induction sexuelle et intersexualite chez une Hydre gonochoric (Hydra fusca) par la method des greffes. C.R. Acad. Sci. Paris 253:1997-1999.

_______. 1963. Contribution a l'etude de la biologie sexualle chez les hydres d'eau douce. Bull. Biol. Fr. Belg. 97:213-283.

Brien, P. and M. Reniers-Decoen. 1949. La croissance, la blastogenese, l'ovogenese chez Hydra fusca (Pallas). Bull. Biol. Fr. Belg. 23:293-386.

________________________. 1951. La gametogenese et l'intersexualite chez Hydra attenuata. Ann. Roy. Socl. Zool. Belg. 82:285-327.

Brinkman-Voss, A. 1970. Anthomedusae/Athecatae (Hydrozoa,Cnidaria) of the Mediterranean. Capitata. Fauna E. Flora del Golfo di Napoli 39.

Broch, H. 1915. Hydroiduntersuchungen IV. K. Norske. Vidensk. Selsk. Skr. 1914(2):1-17.

Browder, L.W. 1980. Developmental Biology. 602 p. Saunders College, Philadelphia.

Brown, E.H. and R.C. King. 1964. Studies on the events resulting in the formation of an egg chamber in Drosophila melanogaster. Growth 28:41-81.

Burnett, A.D., L. Davis, and F. Ruffing. 1966. A histological and ultrastructural study of germinal differentiation of interstitial cells arising from gland cells in Hydra viridis. J. Morphol. 120:1-8.

Burnett, A.L., R. Baird, F. Diehl. 1962. Method of introducing tritiated thymidine into the tissues of Hydra. Science 138:825-826.

Calder, D.R. 1975. Biotic census of Cape Cod Bay: hydroids. Biol. Bull. 149:287-315.

Callahan, W.P. and J.A. Horner. 1964. The use of vanadium as a stain for electron microscopy. J. Cell Biol. 20:350.

Campbell, R. 1965. Cell proliferation in Hydra: an autoradiographic approach. Science 148:1231-1232.

Campbell, R. 1974. Cnidaria. pp. 133-199. In Reproduction of Marine Invertebrates (A.C. Giese and J.S. Pearse, eds.). Academic Press, New York.

Chia, F.-S. 1974. Classification and adaptive significance of developmental patterns in marine invertebrates. Thalassia Jugol. 10:121-130.

Clarkson, S.G. 1969. Nucleic acid and protein synthesis and pattern regulation in Hydra I. J. Embryol. Exp. Morphol. 21:33-54.

Cloney, R.A. and E. Florey. 1968. Ultrastructure of cephalopod chromatophore organs. Z. Zellforsch. Mikroskop. Anat. 89:250-280.

Congdon, E.D. 1906. Notes on the morphology and development of two species of Eudendrium. Biol. Bull. 11:27-46.

Cowden, R.R. 1964. A cytochemical study of gonophore and oocyte development in Pennaria tiarella. Acta Embryol. et Morph. Exp. 7:167-179.

David, C.N. 1973. A quantitative method for maceration of Hydra tissue. Wilhelm Roux's Arch. 171:259-268.

David, C.N. and D. Challoner. 1974. Distribution of I-cells and differentiating nematocytes in nests in Hydra viridis. Amer. Zool. 14:537-542.

Davidson, E.H. 1968. Gene Activity in Early Development. 375 p. Academic Press, New York.

Davidson, M.E. and N.J. Berrill. 1948. Regeneration of primordia and developing hydranths of Tubularia. J. Exp. Zool. 107:465-477.

Davis, L.A., A.L. Burnett, J. Haynes, and V. Mumaw. 1966. A histological and ultrastructural study of dedifferentiation of digestive and gland cells in Hydra viridis. Develop. Biol. 14:307-329.

Eisenstadt, E. 1971. Transfer of photosynthetic products from symbiotic algae to animal tissue in Chlorohydra viridissima. pp. 202-208. In Experimental Coelenterate Biology (H.M. Lenhoff, L. Muscatine, L.V. Davis, eds.). Univ. of Hawaii Press, Honolulu.

England, H.M. 1926. Development of the gonophores of the Stylasteridae. Proc. Zool. Soc. 1926:265-283.

Fell, P.E. 1969. The involvement of nurse cells in oogenesis and embryonic development in the marine sponge Haliclona ecbasis. J. Morphol. 127:133-150.

_________. 1974. Porifera. pp. 51-132. In Reproduction of Marine Invertebrates (A.C. Giese and J.S. Pearse, eds.). 546 p. Academic Press, New York.

Fenchel, A. 1905. Uber Tubularia larynx Ellis. Rev. Suisse Zool. 13:507-580.

Fulton, C. 1959. Re-examination of an inhibitor of regeneration in Tubularia. Biol. Bull. 116:232-238.

Franchi, L.L. and A.M. Mandl. 1962. The ultrastructure of oogonia and oocytes in the fetal and neonatal rat. Proc. Roy. Soc. Ser. B 157:99-114.

Fraser, C.M. 1944. Hydroids of the Atlantic Coast of North America. 451 p. Univ. of Toronto Press, Toronto.

Hargitt, C.W. 1900. A contribution to the natural history and development of *Pennaria tiarella* McCr. Am. Nat. 34:387-414.

____________. 1904. Notes on some hydromedusae from the Bay of Naples. Mittheil. Zool. Stat. Neapel 16:553-586.

____________. 1906. The organization and early development of the egg of *Clava leptostyla* Ag. Biol. Bull. 10:207-232.

Hargitt, G.T. 1909. Maturation, fertilization, and segmentation of *Pennaria tiarella* and of *Tubularia crocea*. Bull. Mus. Comp. Zool., Harvard 53:161-212.

____________. 1916. Germ cells of coelenterates. II. *Clava leptostyla*. J. Morphol. 27:85-97.

____________. 1917. Germ cells of coelenterates. III. *Aglantha digitalis*; IV. *Hybocodon prolifer*. J. Morphol. 28:593-641.

____________. 1918. Germ cells of coelenterates. V. *Eudendrium ramosum*. J. Morphol. 33:1-59.

____________. 1919. Germ cells of coelenterates. VI. General considerations, discussion, conclusions. J. Morphol. 33:1-59.

Haynes, J.F., R.G. Summers, and M.I. Kessler. 1974. The application of autoradiography to developmental studies in the hydrozoa. Amer. Zool. 14:783-795.

Haynes, J.F. and A.L. Burnett. 1963. Dedifferentiation and redifferentiation of cells in *Hydra viridis*. Science 142:1481-1483.

Hincks, T. 1868. *A History of British Hydroid Zoophytes*. 338 p. John Van Voorst. London.

Hisaw, F.L. 1963. *Physiology of Reproduction*. Oregon State Univ. Press, Oregon.

Honegger, T.G. 1981. Light and scanning electron microscopic investigations of sexual reproduction in *Hydra carnea*. Intl. J. Invert. Rep. 3:245-255.

Humason, G.L. 1972. *Animal Tissue Techniques*. 641 p. W.H. Freeman & Co., San Francisco.

Garstang, W. 1946. The morphology and relations of the siphonophora. Quart. J. Micr. Sci. 87:103-193.

Goette, A. 1907. Vergleichende Entwickslungsgeschichte des geschleschtsindividuen der hydropolypen. Zeitsch. f. wiss. Zool. 87:1-335.

Gondos, B. 1973. Germ cell degeneration and intercellular bridges in the human fetal ovary. Z. Zellforsch 138:23-30.

Gutzeit, H.O. 1980. Yolk synthesis in ovarian follicles of Drosophila. Wilhelm Roux's Archives 189:221-224.

Hyman, L.H. 1940. The Invertebrates, Vol. I. 726 p. McGraw Hill, New York.

Jorgensen, M. 1913. Zellen-Studien, I-III. Arch. f. Zellforsch 10.

Josephson, R.K. 1965. The coordination of potential pacemakers in the hydroid Tubularia. Amer. Zool. 5:483-490.

King, R.C. and S.K. Aggarwal. 1965. Oogenesis in Hyalophora cecropia. Growth 29:17-83.

Kingsley, J.S. 1910. Hydroids of New England. Tufts College Studies Sci. Ser. 1910, 3:13-38.

Koch, E.A. and R.C. King. 1966. The origin and early differentiation of the egg chamber of Drosophila melanogaster. J. Morphol. 119:283-303.

Koch, E.A., P.A. Smith, and R.C. King. 1967. The division and differentiation of Drosophila cystocytes. J. Morphol. 121:55-70.

Koch, G. von. 1876. Mittheilungen uber coelenteraten. Morph. Jahrb. 2:83-86.

Kessel, R.G. and N.E. Kemp. 1962. An electron microscopic study of the oocyte, test cells, and follicular envelope on the tunicate, Molgula manhattensis. J. Ultrastruct. Res. 6:57-76.

Korotneff, A. 1878. Sur le development de l'oeuf de la Hydra. C.R. Acad. Sci. Paris 87:412-414.

Kuhn, A. 1910. Die entwicklung des geschlechtsindividuen der hydromedusen. Zool. Jahrb. Abt. Anat. 30:43-174.

_______. 1913. Die entwicklunggeschte und vermandtschaftsbeziehungen der hydrozoen. I. Tiele: die hydroiden. Ergeb. Forschr. Zool. 4:1-284.

Larkman, A.V. 1983. An ultrastructural study of oocyte growth within the endoderm and entry into the mesoglea in Actinia fragacea (Cnidaria,Anthozoa). J. Morphol. 178:155-177.

Lenhoff, H.M. 1968. Feeding response, digestion and nutrition. pp. 157-221. In Chemical Zoology, Vol. II (M. Florkin, B.T. Scheer, eds.). 639 p. Academic Press, New York.

Lentz, T.L. and R.J. Barnett. 1961. Enzyme histochemistry of Hydra. J. Exp. Zool. 147:125-149.

Liu, C.K. and N.J. Berrill. 1948. Gonophore formation and germ cell origin in Tubularia. J. Morphol. 83:39-55.

Lowe, E. 1926. The embryology of Tubularia larynx (Allman). Quart. Jour. Micr. Sci. 70:599-627.

Mackie, G.O. 1966. Growth of the hydroid Tubularia in culture. pp. 397-410. In The Cnidaria and Their Evolution. 449 p. Symp. Zool. Soc. London 16. Academic Press, New York.

Mangan, J. 1909. The entry of zooxanthellae into the ovum of Millepora and some particulars concerning the medusae. Quart. J. Microsc. Sci. 53:697-709.

May, A.J. 1903. A contribution to the morphology and development of Corymorpha pendula Ag. Am. Nat. 37:579-599.

McConnell, C. 1933. The growth of the nucleus in the developing egg of Chlorohydra viridissima. Biol. Bull. 64:103.

McReynolds, H.D., L.J. Blanchet, D.C. Bowman, A.A. Hakin, and M.L. Kiely. 1984. Intercellular bridges in the ovaries of the newborn gerbil. J. Morphol. 180:29-35.

Menge, B.A. 1975. Brood or broadcast? The adaptive significance of different reproductive strategies in two intertidal sea stars, Leptasterias hexactis and Pisaster ochraceus. Mar. Biol. 31:87-100.

Mergner, H. 1971. Cnidaria. pp. 1-84. In Experimental Embryology of Marine and Fresh-Water Invertebrates (G. Reverberi, ed.). North-Holland Publ. Co., Amsterdam.

Metschnikoff, E. 1886. Embryologische Studien an Medusen. 159 p. Alfred Holder, Vienna.

Mollenhauer, H.H. 1963. Plastic embedding mixtures for use in electron microscopy. Stain Technol. 39:111-114.

Morse, M. 1909. The autotomy of the hydranth of Tubularia. Biol. Bull. 16:172-182.

Muscatine, L. and H.M. Lenhoff. 1963. Symbiosis: on the role of algae symbiotic with hydra. Science 142:956-958.

Nagao, Z. 1965. Studies on the development of Tubularia radiata and Tubularia venusta (Hydrozoa). Publ. Akkeshi mar. biol. Stat. 15:9-35.

Neaves, W.B. 1971. Intercellular bridges between follicle cells and oocyte in lizard, Anolis carolinensis. Anat. Rec. 170:285-302.

Newton, W.D. 1970. Oogenesis of the freshwater turbellarian Hydrolimax grisea (Platyhelminthes:Plagiostomidae) with special reference to the history of the supernumerary asters and central bodies. J. Morphol. 132:27-46.

Normandin, D. 1960. Regeneration of Hydra from the endoderm. Science 132:678.

Pearse, J.S. 1979. Polyplacophora. pp. 27-85. In Reproduction of Marine Invertebrates. V. (A.C. Giese and J.S. Pearse, eds.). 369 p. Academic Press, New York.

Perkowska, E., H.C. Macgregor, and M.L. Birnstiel. 1968. Gene amplification in the oocyte nucleus of mutant and wild-type Xenopus laevis. Nature 217:649-650.

Pianka, H.D. 1974. Ctenophora. pp. 201-265. In Reproduction of Marine Invertebrates, Vol. I. (A.C. Giese and J.S. Pearse, eds.). Academic Press, New York.

Pollack, L.W. 1975. Tardigrada. pp. 43-54. In Reproduction of Marine Invertebrates, Vol. II. (A.C. Giese and J.S. Pearse, eds.). Academic Press, New York.

Postlethwait, J.H. and P.D. Shirk. 1981. Genetic and endocrine regulation of vitellogenesis in Drosophila. Amer. Zool. 21:687-700.

Pyefinch, K.A. and F.S. Downing. 1949. Notes on the general biology of Tubularia larynx Ellis and Solander. Jour. Mar. Biol. Assn. 28:21-43.

Raven, C.P. 1961. Oogenesis. Pergammon Press, New York.

Rees, W.J. 1957. Evolutionary trends in the classification of capitate hydroids and medusae. Bull. Brit. Mus. (Nat. Hist.) Zoology 4:454-534.

__________. 1966. The evolution of the hydrozoa. pp. 199-222. In The Cnidaria and Their Evolution (W.J. Rees, ed.). 449 p. Academic Press, New York.

Reynolds, E.S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17:208.

Richardson, K.C., L. Jarett, and E.H. Finke. 1960. Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain Tech. 35:313-323.

Riser, N.W. 1950. Notes on toto-mount techniques. Proc. Helm. Soc. Wash. 17:132-133.

Rose, S.M. 1974. Bioelectric control of regeneration in *Tubularia*. Amer. Zool. 14:797-804.

Ruby, J.R., R.F. Dyer, and R.G. Skalko. 1969. The occurrence of intercellular bridges during oogenesis in the mouse. J. Morphol. 127:307-340.

Russell, F.S. 1953. *The Medusae of the British Isles*. 530 p. Cambridge.

Schmid, V., R. Stidwel, A. Bally, B. Marcum, and P. Tardent. 1981. Heat Dissociation and Maceration of marine cnidaria. Wilhelm Roux's Arch. 190:143-149.

Selman, K. and R.A. Wallace. 1972. A role for the follicle cells during vitellogenesis in the squid *Loligo pealei*. Biol. Bull. 143:477.

Smallwood, M. 1899. A contribution to the morphology of *Pennaria tiarella* McCrady. Am. Nat. 33:861-870.

Spracklin, B.W. 1975. Aspects of the biology of *Rhizogeton fusiformis* Agassiz, 1892 (Hydrozoa:Clavidae). M.S. Thesis, Northeastern Universtiy (unpublished).

______________. 1978. Correlated light and electron microscopic study of oogenesis in *Tubularia larynx* (Hydrozoa,Athecata). Amer. Zool. 18:646.

______________. 1980. Autoradiography and microscopy of oogenesis in *Tubularia*. Amer. Zool. 20(4).

Spurr, A.R. 1969. A low-viscosity epoxy-resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26:31-43.

Stegner, H.-E. 1967. Die elektronenmikroskopische strukture der eizelle. Ergeb. Anat. Entwicklung 39:1-112.

Summers, R.G. and J.F. Haynes. 1969. The ontogeny of interstitial cells in *Pennaria tiarella*. J. Morphol. 129:81-87.

Swedmark, B. and G. Teisser. 1966. The actinulida and their evolutionary significance. In *The Cnidaria and Their Evolution* (W.J. Rees, ed.). 449 p. Academic Press, London.

Szmant, A.M. 1971. Patterns of $^{14}CO_2$ uptake by *Chlorhydra viridissima*. In *Experimental Coelenterate Biolgoy* (H.M. Lenhoff, L. Muscatine, L.V. Davis, eds.). Univ. of Hawaii Press, Honolulu.

Szmant-Froelich, A., P. Yevich, and M.E.Q. Pilson. 1980. Gametogenesis and early development of the temperate coral *Astrangia danae* (Anthozoa:Scleractinia). Biol. Bull. 158:257-269.

Taddei, C. 1972. Significance of pyriform cells in ovarian follicles of *Lacerta sicula*. Exptl. Cell. Res. 72:562-566.

Tannreuther, G.W. 1908. The development of *Hydra*. Biol. Bull. 14:261-280.

________________. 1909. Budding in *Hydra*. Biol. Bull. 16:210-214.

Tardent, P. 1963. Regeneration in the hydrozoa. Biol. Rev. 38:293-332.

__________. 1974. Gametogenesis in the genus *Hydra*. Amer. Zool. 14:447-456.

Teissier, G. 1926. Notes critiques sur la morhpologie des gonophores chez les hydroids. Arch. Zool. Exp. et Gen. 65:75-86.

Thorson, G. 1950. Reproductive and larval ecology of marine bottom invertebrates. Biol. Rev. 25:1-45.

Totton, A.K. 1965. *A Synopsis of the Siphonophora*. 230 pp. British Mus. Natural Hist., London.

Tucker, G.H. 1942. The histology of the gonads and development of the egg envelopes of an ascidian (*Styela plicata* Lesueur). J. Morphol. 70:81-113.

Tucker, S.G. and C.R. Wyttenbach. 1974. Differentiation of the female reproductive body in the colonial hydroid *Campanularia flexuosa*. Wilhelm Roux's Arch. 177:163-181.

Tweedell, K.S. 1974. DNA synthesis and cell movement during regeneration in *Tubularia*. Amer. Zool. 14:805-820.

Van de Vyver, G. 1964. Etude histologique du development embryonnaire d'*Hydractinia echinata*. Cah. De Biol. mar. 5:295-310.

_______________. 1967. Etude du developpment embryonnaire des hydraires athecates (gymnoblastiques) a gonophores. I. Formes a planula. Arch. Biol. (Liege) 78:451-518.

_______________. 1968a. Etude du developpment embryonnaire des hydraires athecates (gymnoblastiques) a gonophores. II. Formes a actinulas. Arch. Biol. (Liege) 79:327-363.

_______________. 1968b. Etude du developpment embryonnaire des hydraires athecates (gymnoblastiques) a gonophores. III. Discussion et conclusions generales. Arch. Biol. (Liege) 79:365-379.

Wager, R.E. 1909. The oogenesis and early development of *Hydra*. Biol. Bull 18:1-38.

Wasserthal, W. 1973. Zur Ei-und embryonalentwicklung des hydroidpolypen Eudendrium armatum. Helg. wiss. Meers. 25:93-125.

Weakley, B.S. 1966. Electron microscopy of the oocyte and granulosa cells in developing ovarian follicles of the golden hamster (Mesocricetus auratus). J. Anat. 100:503-534.

___________. 1967. Light and electron microscopy of developing germ cells and follicle cells in the ovary of the golden hamster. J. Anat. 101:435-459.

Weismann, A. 1880. Uber den ursprung des geschlechtzellen bei den hydroiden. Zool. Anz. 3:367-370.

West, D.L. and R.W. Renshaw. 1970. The life cycle of Clytia attenuata (Calyptoblastea:Campanulariidae). Mar. Biol. 7:332-339.

Woodruff, R.I. and K.L. Anderson. 1984. Nutritive cord connection and dye-coupling of the follicular epithelium to the growing oocytes in the telotrophic ovarioles in Oncopeltus fasciatus, the milkweed bug. Wilhelm Roux'a Arch. 193:158-163.

Zamboni, L. and B. Gondos. 1968. Intercellular bridges and synchronization of germ cell differentiation during oogenesis in the rabbit. J. Cell Biol. 36:276-282.

Zihler, J. 1971. Zur gametogenese und befructungsbiologie von Hydra. Wilhelm Roux's Arch. 169:239-267.