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Stages and Stage Distribution in Early Oogenesis in the Annelid, *Platynereis dumerilii**

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Summary. 1. The coelomic stages of oogenesis up to early vitellogenesis are described in *Platynereis dumerilii.*

2. Early stages up to 26—27 μm in diameter are attached to each other in variable numbers thus forming clusters of variable size. Sheath cells cover the oocyte clusters initially and in later stages wrap up the clustered oocytes individually.

3. Thereafter, the oocytes fall apart and loose their sheath cells. Instead, the oocytes may make contact to eleocytes.

4. A series of four succeeding stages with specific nuclear phases, cell shape and cell size could be established for the clustered oocytes. Primary oocytes in interphase ("polygonal oocytes") are the earliest coelomic stages found (Stage 1). In Stage 2, meiotic synapsis is observed ("synaptic oocytes"). During Stage 3 the chromosomal structures become dispersed ("late prophase oocytes"). Stage 4 starts with the onset of vitellogenesis ("vitellogenic oocytes"). Asynchrony among the oocytes of a cluster is not observed prior to Stage 3.

5. During early oogenesis oocytes of different size and stage are encountered simultaneously in individual females, whereas mature females are known to contain only oocytes of one size class. Mechanisms are discussed which might serve to make an initially heterogeneous oocyte population synchronous during later oogenesis and which are in harmony with current knowledge on the endocrine control of oogenesis in nereids.

Key words: Oogenesis — Meiosis — Vitellogenesis — Control of oogenesis — *Platynereis dumerilii* (Annelida).

Zusammenfassung. 1. Die im Coelom von *Platynereis dumerilii* auftretenden Stadien der Oogenese werden bis zur frühen Vitellogenesephase beschrieben.

2. Oocyten bis zu 26—27 μm Durchmesser hängen in unterschiedlicher Anzahl in Ballen unterschiedlicher Größe zusammen. Hüllzellen überziehen die Ballen mit den jüngsten Stadien; in späteren Stadien umkleiden die Hüllzellen jede Oocyte eines Ballens einzeln.

3. Die Oocytenballen zerfallen danach in einzelne, von Hüllzellen freie Oocyten, die nun oft mit Elaeocyten besetzt sind.

4. Bei den zu Ballen vereinten Oocyten können aufgrund ihrer spezifischen Kernstruktur, Gestalt und Größe 4 Stadien unterschieden werden. Die jüngsten im Coelom auffindbaren Stadien sind Oocyten I. Ordnung in Interphase ("polygonale Oocyten"): Stadium 1. In Stadium 2 beobachtet man Chromosomenpaarung („synaptische Oocyten"). In Stadium 3 verschwinden die chromosomalen Strukturen („Oocyten in später Prophase"). Stadium 4 setzt mit der Dotterbildung ein. — Erst ab Stadium 3 kommt Asynchronie zwischen den Oocyten desselben Ballens vor.

5. Während geschlechtsreife Weibchen dieser Spezies bekanntlich ausschließlich Oocyten von einheitlicher Größe enthalten, beherbergen Weibchen in frühen Stadien der Oogenese gleichzeitig Oocyten unterschiedlicher Größe und unterschiedlichen Stadiums. Unter Bezug

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auf die bekannte endokrine Steuerung der Oogenese bei Nereiden wird diskutiert, auf welche Weise solch eine anfänglich heterogene Population von Oocyten schließlich synchronisiert wird.

Introduction

Many papers have appeared in recent years concerning oocytes in nereid polychaetes (for reviews, see Clark and Olive, 1973; Durchon, 1970, and Hauenschild, 1974). There are several reasons for choosing the oocytes of nereids for research work: Oogenesis is "diffuse" in the sense that the oocytes grow everywhere in the coelomic cavity seemingly without permanent contact with other cells. Oocytes grow synchronously, at least during later oogenesis, and are therefore a valuable test system to evaluate the degree of sexual maturation in the worms. Since both oocytes and somatic tissues respond to a common morphogenetic hormone, evaluation of oocyte size relates to the hormonal status as well as to the degree of somatic maturation.

Nevertheless, information on early phases of oogenesis in nereids is scanty. No report of the staging of early nereid oocytes is known to the author. Early oogenesis can be inferred to be fairly asynchronous in several nereid species from data available (see Discussion) but exact figures are lacking for this early phase. The aim of the present paper is to give an account of the structural phases of early oogenesis as well as data on the variability of oogenetic stages among the oocytes of young nereid females.

Material and Methods

All observations were made on living laboratory-bred material unless otherwise stated. Worms were anaesthetized in a mixture of one part of sea water to one part of an aqueous solution of 7% by weight of $MgCl_2$. A worm was placed on a slide and one parapodium in the 12th setigerous segment was cut off by aid of a piece of razor blade. With a pair of fine needles coelomic contents were squeezed out from the coelomic cavity. A sample of oocyte clusters and oocytes could be obtained thereby of such a quantity that the entire size range of oogenetic stages in a female was represented. The representativeness of the sample was tested in the following manner: Twenty females tested for their range of oocyte size in the manner described above were dissected 1 or 2 days later along their body length. Hundreds of oocytes obtained by this method from a single worm all fell within the same range of size as found in the simpler test. Thus, careful examination of coelomic corpuscles obtained by removing just one parapodium is a sufficient test for oocyte size range in a single animal.

Oocytes of *Platynereis dumerilii* after a short exposure to natural sea water mixed up with a solution of $MgCl_2$ do well not only morphologically but also physiologically as shown by transplantation of oocytes (Fischer, 1974). Living oocytes on slides were examined and photographed by Nomarski optics (Zeiss). One micrograph is included (Fig. 4) from a 1 μm section of material fixed with glutaraldehyde — osmic acid and embedded in Araldite M.

Results

Staging of Early Oocytes

All stages described here were found in the coelomic cavity, either freely floating or loosely attached to groups of coelomic cells (eleocytes).

External sex differences are lacking in atokous specimens of *Platynereis dumerilii*. Worms in the atokous phase can only be sexed if gametocytes proper are present. Cells of the earliest coelomic stages of oogenesis might be confused with spermatogonia. However, different stages of oogenesis are found simulta-

neously in young females (see p. 41). Therefore, the sequence of oogenetic stages can be traced even in early oocytes which resemble spermatogonia because of the simultaneous presence of oocytes in later stages characterized as oocytes by their yolk inclusions. The observation of a series of structural transitions leads to the following description of successive stages in oogenesis.

Stage 1. The earliest stages of oogenesis detected in the coelomic fluid are cells which are packed very tightly together to form clusters (Fig. 1). The surface of the cluster is smooth. The nuclei of the germ cells appear to be in interphase with one nucleolus visible. Cytoplasm is scarce and may contain an intensely refracting body. Each cluster is enclosed by a cellular sheath. The sheath cell nuclei are flattened and appear spindle-shaped or sickle-shaped in profile. Individual germ cells of a cluster are squeezed against each other so that a polyhedral interface results between a cell and its neighbor cell. A polygonal outline is therefore observed in the microscope (*"polygonal stage"*). The number of germ cells per cluster varies. Several clusters, each one measuring 20–50 μm in length, may be packed together to a larger unit, again enclosed by a cellular sheath. Transitions were observed between this stage and Stage 2.

Stage 2. At Stage 2 the gametocytes are still packed together to clusters (Figs. 2–4). The nuclei, irregular in contour before, are now almost spherical. Since the cytoplasm is still scarce, the spherical nucleus makes the cell roughly isodiametric rather than elongate. The gametocytes are 9–10 μm in diameter. The surface of the germ cell cluster is bulged out slightly by what appear to be individual gametocytes. The leading feature of the female germ cells at this stage is the structure of the chromosomes. Chromosomes are now visible as short thick strands which at high magnification (Fig. 3) turn out to be bivalents. Such chromosomes must be in synapsis, and therefore in prophase of the first meiotic division (so-called "premeiotic oocytes"). One nucleolus is found usually but a second one occasionally may occur. Chromomeres show up in stained sections (Fig. 4). The cytoplasm contains 1 or 2 strongly refracting bodies. The term *"synaptic oocytes"* is suggested for this stage. All the oocytes of a cluster are in phase.

In addition to the spherical nuclei of the gametocytes a differently shaped nucleus is now found inside the cluster (Fig. 3b): its outline conforms to the angular space left by the surrounding oocytes. No chromosomes are visible in this nucleus and cell outlines are not visible in the light microscope. This is the sheath cell nucleus which now has been displaced from the cluster surface into the interior.

Stage 3. In Stage 3 the oocytes have become roughly spherical so that the cluster attains an aciniform aspect (Figs. 2, 5). The oocytes, still 10 μm in diameter, are now separated by clefts which contain lamellate structures connected to the sheath surrounding the cluster. The oocyte nuclei are still spherical. Individual chromosomes are no longer discernible but a reticulum of chromosomal strands can be seen, which represent various stages of dispersal of the visible chromosome structures (*"late prophase oocytes"*). The oocyte nuclei of a cluster are not always in phase. The nucleolus is larger than that found in Stage 2. The cytoplasm, still scarce, resembles that of Stage 2. Transitory stages between Stages 2, 3 and 4 are found.

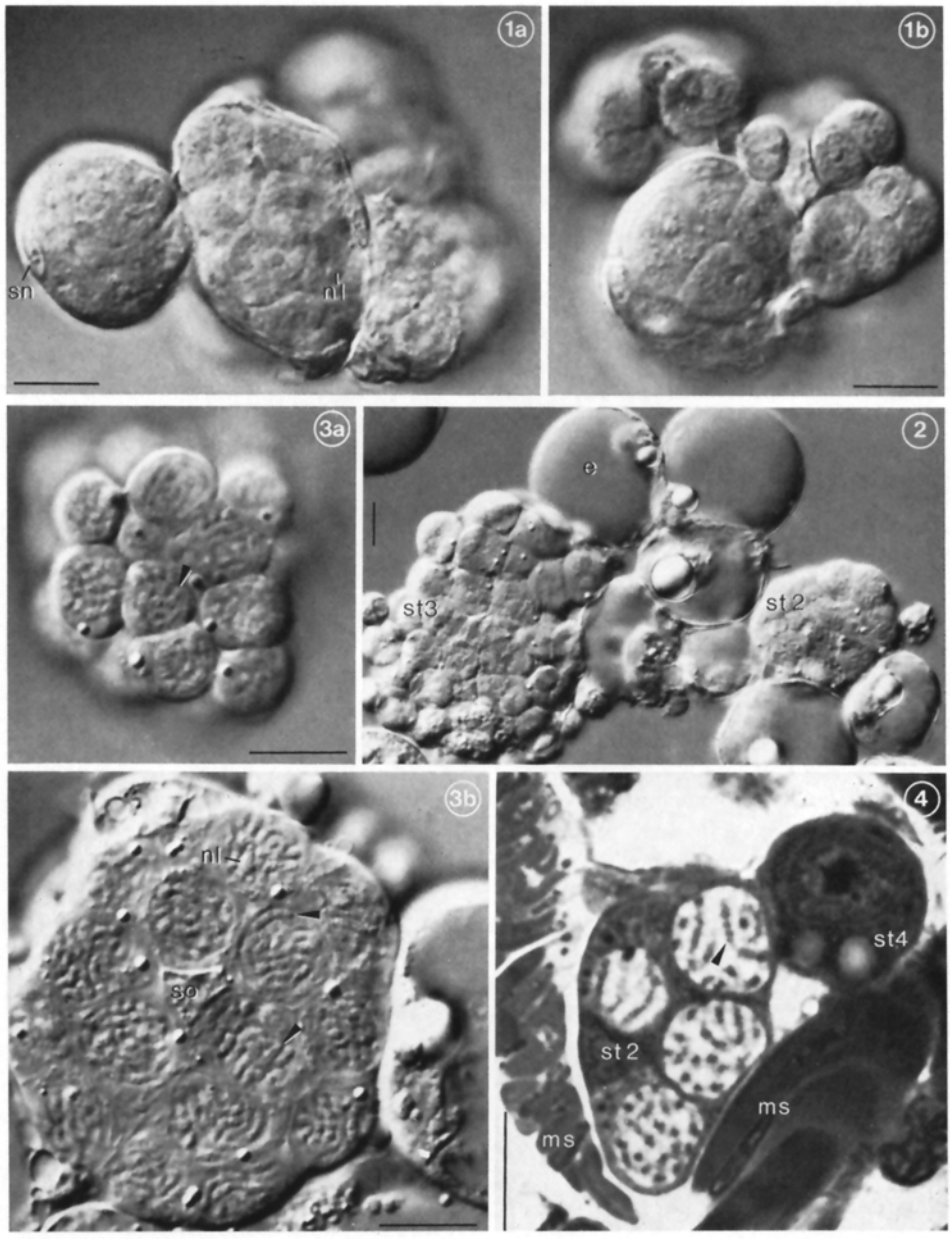

Fig. 1. (a and b) *Platynereis dumerilii*. Germ cells in the polygonal stage are packed in clusters with a peripheral cellular sheath (*sn*, sheath cell nucleus). The oocytes have a globular nucleolus of a uniform size (*nl*). In this and in all the following micrographs the bar equals 10 μm

Fig. 2. *Platynereis dumerilii*. This micrograph shows a cluster of oocytes in early meiotic prophase (*st 2:* Stage 2) and a cluster of oocytes in Stage 3 (*st 3*) with chromosomal structure already dispersed to form a loose meshwork. *e* eleocyte

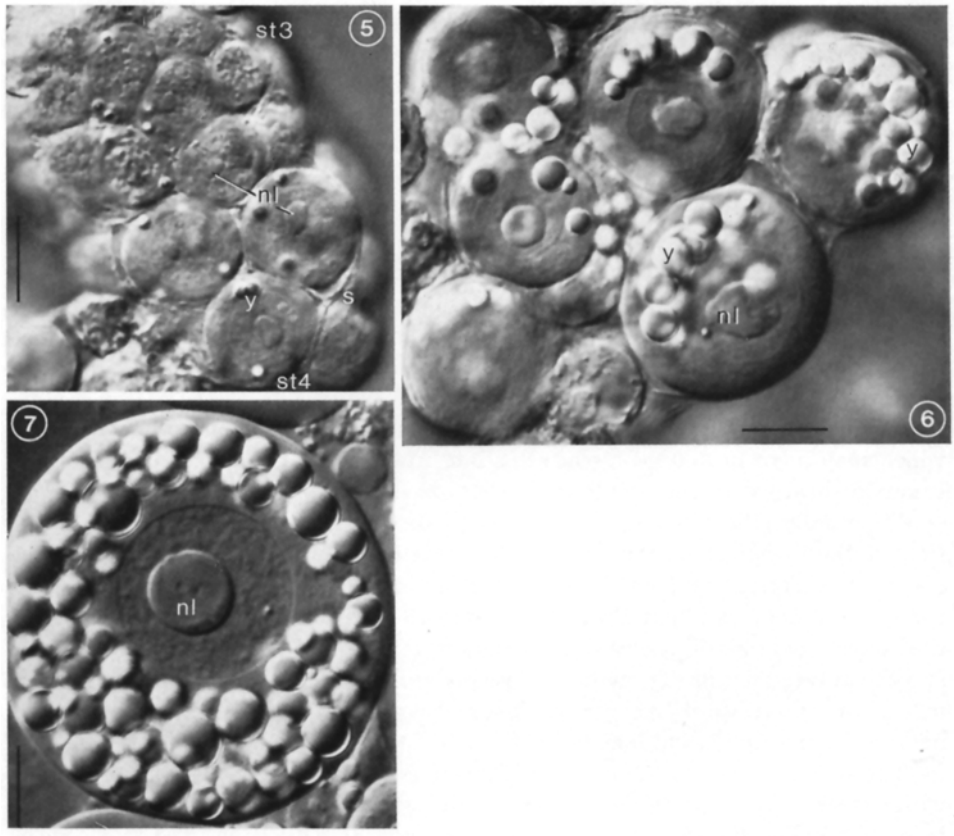

Fig. 3. (a and b) *Platynereis dumerilii*. Oocytes in early meiotic prophase at higher magnification. The nucleus is roughly spherical. Chromosomes are found as bivalents (*arrows*). A nucleolus (*nl*) is maintained during this stage. The nucleus of a somatic cell (*so*) is seen in the middle of the oocyte cluster in Fig. 3b

Fig. 4. *Platynereis dumerilii*. A cluster of Stage 2 (*st 2*) oocytes with chromomeres visible (*arrow*) *in situ* adjacent to muscle cells (*ms*), and a Stage 4 oocyte (*st 4*). Section stained with methylene blue — azur B

Fig. 5. *Platynereis dumerilii*. The oocytes of this cluster are of two different stages: Stage 3 (*st 3*) in the upper left with dispersing chromosomal structures and Stage 4 (*st 4*) oocytes with yolk droplets (*y*) beginning to appear. Note the enlargement of the nucleolus (*nl*). The lamellar sheath (*s*) which encloses the Stage 4 oocytes is now easily seen

Fig. 6. *Platynereis dumerilii*. As the oocytes proceed in vitellogenesis, the nuclear shape may become irregular. The nucleoli (*nl*) are now especially large. In this phase, shortly before dissociation of the clusters into single oocytes, the yolk droplets (*y*) are still distributed asymmetrically

Fig. 7. *Platynereis dumerilii*. This Stage 4 oocyte was 38 μm in diameter (slightly deformed for microphotography). Note yolk droplets and the large nucleolus (*nl*)

Stage 4. Stage 4 oocytes are characterized by the onset of vitellogenesis (*"vitellogenic oocytes"*). Highly refractive yolk droplets of variable size appear in the cytoplasm (Figs. 6, 7). Yolk droplets are first observed in oocytes 12 μm in diameter. Oocytes 15 μm in diameter contain about 4, oocytes 20 μm in diameter about 10 granules; the number of yolk granules then increases rapidly with increase in oocyte diameter. The relative volume of the nucleus decreases and its outline may become irregular. The nucleolus becomes especially large in early vitellogenesis. Chromosomal structures are no longer visible.

During the initial phases of vitellogenesis the oocytes are still in clusters. The sheath covers the oocytes less tightly than before and becomes easily visible by interference contrast optics as a web (Fig. 6). The oocytes of one cluster may be, but need not be, in phase. If they are not in phase their size as well as their yolk content then may increase gradually from one pole of the cluster to the other. Occasionally, oocytes of a cluster are in synchrony esxcept one or a few cells which may have proceeded already far into vitellogenesis though still sharing a common sheath with the oocytes in earlier stages.

Eventually, the vitellogenic oocytes break free from the clusters and float free in the coelomic cavity. Among 31 females with both clustered and free oocytes the largest oocytes still found together in clusters were 36 μm in diameter, the smallest oocytes found already free were 18 μm in diameter. On the average, the largest oocytes still enclosed in clusters were 27 μm in diameter in this sample and the average size of the smallest single oocytes found along with oocyte clusters was 26 μm. Overlap of free versus clustered oocyte sizes does not exceed a few microns in any individual female.

Throughout early oogenesis specific internal structure in oocytes is correlated with specific cell shape and well-defined cell size or size range. This is found also in early vitellogenic oocytes, whether they have already come free from their sheath or they are still enclosed in clusters. Therefore, cell size, cell shape and internal structure of *Platynereis* oocytes seem correlated.

Eleocytes in Early Oogenesis

Coelomic cells of nereids, called eleocytes, are known to be phagocytic to muscle debris during epitokous metamorphosis. Eleocytes are also found adhering to oocytes in *Platynereis dumerilii* as well as in other species. In coelomic samples of *Platynereis dumerilii*, single oocytes were, to a large part, covered by numerous eleocytes, the latter making broad contact to the oocytes by adhesion. Most of the oocyte clusters, however, were not associated with eleocytes. Statements on the existence of adhesive contact between oocytes and/or sheath cells and eleocytes do not, to this author, seem to be reliable when only supported by histological observation. In sections, oocyte clusters often appear to be embedded in eleocyte masses but in coelomic samples adhesion between sheath cells and eleocytes seems weak and mostly casual.

Distribution of Stages of Oogenesis in Individual Females

The oocytes of *Platynereis dumerilii* reach full size synchronously. Synchronous oocyte growth is thought to be dependent upon a response to a decrease

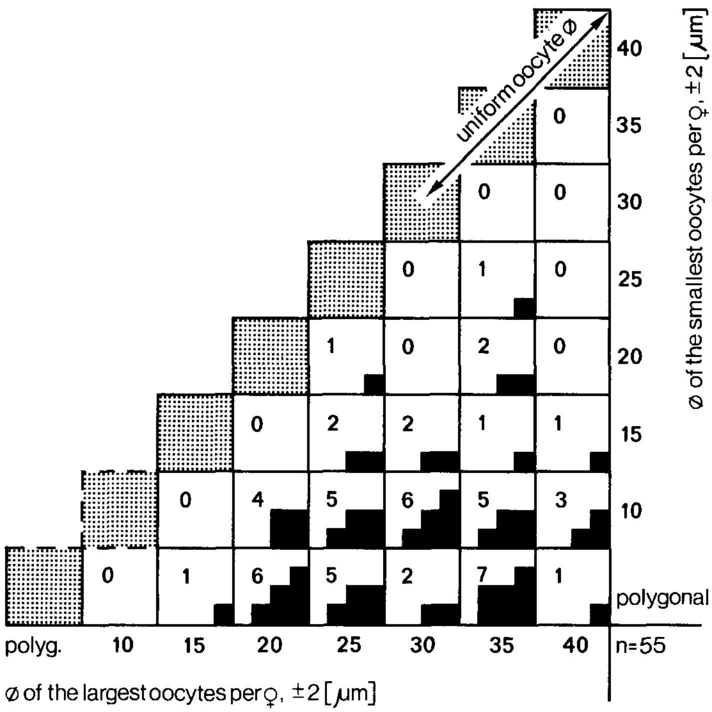

Fig. 8. Variability of oocyte size ranges in 55 females of *Platynereis dumerilii* with oocytes not larger than 42 μm in diameter. Each large square represents a size range of smallest versus largest oocytes. Small black squares and the figure in each large square represent the number of females with this particular oocyte size range. Large squares which would represent uniform oocyte sizes (± 2 μm) are stippled

in brain hormone activity (see Discussion). One would expect therefore synchronous oocyte growth in a *Platynereis* female throughout oogenesis. This is not the case. When females of this species are examined during early oogenesis, the oocyte population in any one specimen is found to cover a wide range of oocyte diameters or even different stages of oogenesis. Fifty-five females which were in the early stages of oogenesis (oocytes not exceeding 42 μm in diameter) were measured individually for their smallest and largest oocytes. The distribution of oocyte diameters is represented in Fig. 8. Three parameters are simultaneously represented such that each large square represents a range of oocyte size, smallest to largest, and the frequency of females having that particular range is superimposed thereupon. Were synchrony the rule, the distribution would be in or adjacent to the stippled squares. Among these females only one worm was found with oocytes differing less than 10 μm in diameter. In the remaining 54 worms the oocyte sizes differed to a larger extent. In addition, two other females were found with oocyte size ranges of 9–60 μm and 9–65 μm.

It follows, that oocytes in *Platynereis dumerilii* do not become uniform in size and stage until they have completed large part of vitellogenesis.

Discussion and Conclusions

The Course of Early Oogenesis

The previtellogenic stages of nereid oogenesis are mentioned only occasionally in the literature despite the fact that many papers deal with nereid oogenesis (reviews: Clark and Olive, 1973; Durchon, 1970; Hauenschild, 1974). Klesch (1970) gives an account of early oogenesis in *Laeonereis culveri*. Most details are at variance with the observations made in *Platynereis dumerilii* except that in both species the earliest stages of oocytes form clusters which fall apart in early vitellogenesis. Dhainaut (1972) presents a picture of sectioned oocytes from *Nereis pelagica* in meiotic prophase. Here too the previtellogenic oocytes are found gathered in clusters. Schroeder (1966) mentions "clumps of tiny oogonia" in the coelom of *Nereis grubei*. He found females in this species which contained simultaneously such clumps and oocytes of up to 120 μm in diameter. In *Neanthes lightii* early gametocytes are not identified as oocytes until germinal vesicles show up, since this species is hermaphroditic (Smith, 1950).

In this study on the oocytes of *Platynereis dumerilii*, certain internal structural features of the female germ cells were always found combined with a specific cell shape and cell size. The nuclear events are particularly evident in *Platynereis* oocytes during Stage 2: the existence of bivalents makes it clear that the nuclei are in early first meiotic prophase. Following this synaptic stage, the chromosomal structures disappear (Stages 3, 4), to give an apparent "diffuse diplotene" stage for the rest of oogenesis in agreement with a pattern distributed widely among metazoan oocytes. The first meiotic division is not completed and polar bodies are not formed until the oocytes are spawned and fertilized.

Since no nuclear divisions were observed between Stages 1 and 2, Stage 1 cells are inferred to be primary oocytes in interphase prior to meiotic prophase. Stage 1 cells are more frequent among the coelomic cells as compared with Stage 2 cells. Thus, the interphase of primary oocytes in this species seems to surpass Stage 2 by far in duration. Even though no mitoses have been observed among coelomic germ cells till now, oogonia and mitotic oogonial divisions perhaps do occur occasionally among the coelomic germ cell clusters. A germ cell type differing from the 4 stages described has not been observed, however.

A nucleolus was observed in each stage of early oogenesis. The nucleolus is particularly large at the onset of vitellogenesis, when its diameter may be half that of the nucleus. The structural development of the nucleolus in nereid oocytes is treated in detail by Dhainaut (1972) in *Nereis pelagica* and by Bertout (1973) in *Nereis diversicolor*.

Eventually during early oogenesis the oocytes of a cluster fall apart. This is not just due to the loss of the sheath cell. In fact, in an electron microscopical investigation (Fischer and Weigelt, 1974; Fischer, in press) the "oocytes" of a cluster have always been found to be connected by cytoplasmic bridges. Oocyte nuclei which stem from a common oogonial nucleus thus remain connected, up to early oogenesis, in a syncytium.

Oocytes which have come free from sheath cells may make adhesive contact to eleocytes, a well defined type of coelomocyte. Eleocyte attachment to oocytes has not yet been found to be correlated with a specific developmental stage of

the oocytes concerned. Adhesive contact between eleocytes and the sheath cells of oocyte clusters is found only occasionally. Nothing is known about the function of adhesion between eleocytes and oocytes in nereids with exception of fine structural evidence reported for polysaccharide transmission from eleocytes adhering to oocytes in *Nereis pelagica* (Dhainaut, 1966).

Asynchrony of Early Oogenesis

Since nereid females at epitoky only contain oocytes of equal size, one would expect that the oocytes in these animals should grow synchronously throughout oogenesis. Such a notion draws support from the many reports on nereid oocytes as target cells for an inhibiting brain hormone (Hauenschild, 1956, for *Platynereis dumerilii*; Choquet, 1962; Clark and Ruston, 1963; Porchet, 1970; Schroeder, 1971). The hormone, by definition, should be expected to act on all oocytes in the same manner, inhibiting growth in early oogenesis while, in later oogenesis, through a decrease in activity, giving way to oocyte growth. However, all data available including the present study show that the early oocytes in individual females vary considerably in size (Schroeder, 1966, 1971, for *Nereis grubei*; Brafield and Chapman, 1967, for *Nereis virens*; Bertout and Dhainaut, 1971, for *Nereis diversicolor*). Oocytes become uniform in size but only gradually as they approach their final size. The present paper shows that early oocytes in *Platynereis dumerilii* do not only vary in volume (as mentioned briefly by Hauenschild, 1966), but that a young female in this species may temporarily contain presynaptic, synaptic and vitellogenic oocytes at the same time. Comparable variability has been recorded by Schroeder (1966) in *Nereis grubei*, as mentioned above. The question therefore arises: by what mechanism do the oocytes in an individual worm become synchronized during later oogenesis?

Epitokous species among nereids are known to be monotelic in the sense that they reach sexual maturity only once in their life (Clark and Olive, 1973). Consequently, successful oogenesis in these worms finally must produce a batch of full-sized synchronized oocytes. This is in fact observed. It is known that in epitokous nereids oogenesis proceeds, to a large extent, at the expense of maternal materials. Many body tissues are histolyzed as epitoky approaches so that after spawning the female is left no longer viable. Durchon (1952) determined the weight ratio of somatic tissues versus coelomic corpuscles (for the most part oocytes) in a sample of mature females of *Perinereis cultrifera* as 62% to 38%. It is not known how much this figure varies under natural conditions but from the strongly determinate mode of epitokous metamorphosis it can be concluded that only as much somatic tissue is left unconverted as is necessary for the worm to perform the spawning activities. Thus, the number of oocytes spawned by an epitokous nereid may be assumed to make up a fairly constant proportion of the worm's mass. In fact, the oocyte numbers spawned by female *Platynereis dumerilii* vary to a large extent with the size of the worms.

Two different mechanisms might alternatively serve to adapt the final number of oocytes in a female nereid to its individual trophic resources:

1. A female might proliferate just as many oocytes into its coelom as are likely to find nourishment throughout oogenesis. All oocytes except perhaps a few abnormal ones would reach full size. Since later oogenesis in *Platynereis*

dumerilii is known to depend upon a brain hormone (see Hauenschild, 1974) endocrine activity might well directly control the extent of proliferation of oocytes by oogonia. Alternatively, a feedback mechanism, as found for spermatocyte proliferation in *Arenicola* (Howie and McClenaghan, 1965) and in *Cirratulus* (Olive, 1972), might account for a repression of oocyte proliferation, developing oocytes themselves inhibiting further proliferation. Initial size diversity among oocytes in a female would reflect an extended period of proliferation and would vanish gradually as the smaller oocytes catch up with the larger ones. As the activity of the hormone, which inhibits oocyte enlargement, decreases, the upper limits for oocyte growth would be pushed towards full size. The largest oocytes in a female, then, indicate the actual activity of the hormone inhibiting oocyte growth.

2. Oocytes might be proliferated in numbers far exceeding the final oocyte number. Because of limited maternal resources only part of the oocytes would be able to keep up with the developmental potential prescribed by the actual level of the inhibiting hormone and indicated by the largest oocytes in a female. Competition for organic matter among oocytes would have to come into play. Oocytes would degenerate to a large extent during oogenesis. It would seem most likely that the largest oocytes in a female reach full size whereas the smaller ones degenerate. Such a pattern is inferred from data on *Nereis grubei* (Schroeder, 1971) by Clark and Olive (1973).

Both patterns are in agreement with our present knowledge on the inhibiting effect of the nereid brain hormone on the enlargement of oocytes. The patterns differ, however, in that, in the first, the smaller oocytes would grow faster and catch up with the larger ones while in the second the smaller oocytes would slow down in growth and finally degenerate. Which of these two patterns might be descriptive of oogenesis in *Platynereis dumerilii* cannot be concluded merely from the observation of simultaneous stage diversity among the oocytes in young females. No other facts are known to the author which substantiate one or the other pattern of oocyte number control among nereids. Degeneration of early oocytes has been observed only under experimental conditions in nereids (Schroeder, 1971; Porchet, 1970). Since the oocytes are widespread throughout the body cavity in these animals a census of early oocyte numbers compared with final oocyte numbers would be difficult.

Different kinds of observations and experiments are thus needed to explain how the initial diversity of oogenetic stages in individual nereids is lost during later oogenesis. A new approach does, however, seem worthwhile since monotelic animals such as nereids might be particularly well suited to answer the question of how oocyte numbers are controlled.

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