AMER. ZOOL., 14:447-456 (1974).

Gametogenesis in the Genus Hydra

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SYNOPSIS. This paper comments on the induction of gametogenesis, on microscopical and electronmicroscopical aspects of spermatogenesis and oogenesis and on fertilization in the genus *Hydra*. Spermiogenesis does not present any peculiarities. The ripe sperm contains no detectable acrosome. Egg-formation involves phagocytosis of entire oogonia by growing oocytes. Several oocytes merge to a single oocyte, in which one nucleus becomes the germinal vesicle. The egg shell is formed only when the egg is fertilized. Various factors such as the synchronization of gametogenesis, the length of sexual periods, continuous release of sperm and the long life span of sperm are considered to guarantee the fertilization of the eggs.

INTRODUCTION

Our knowledge about sexual activities in the *Hydridae* is based mostly on laboratory observations. Nothing is known as to when and under what circumstances free living hydras start producing gametes. We also lack information about the extent and the significance of sexuality in free living populations of the various species as compared with asexual reproduction. A project aiming at the solution of some of these problems is actually under way in the lake of Zurich.

This paper will give a brief survey of the data available on the subject emphasizing the problems which need to be investigated in more detail.

MATERIAL AND METHODS

The species with which our laboratory is mainly concerned are: *Hydra attenuata* Pallas 1766, *Hydra fusca* L. (Syn.: *Pelmatohydra oligactis* Schulze 1917), *Hydra circumcincta* Schulze 1914 (Syn.: *Hydra stellata* Schulze 1914, *Hydra ovata* Boecker 1920) and *Hydra viridis* L. (Syn.: *Chlorohydra viridissima* Pallas 1766). These species are readily available in the lake, the ponds, and rivers around Zürich.

In the laboratory they are kept in Loomis and Lenhoff (1956) artificial culture medium and fed with zooplankton (*Cladocera, Copepoda*) of the lake or with larvae of *Artemia salina*. Under routine conditions they are kept at 18 ± 1 C and exposed to a 12 hr light-12 hr dark rhythm.

For histological investigations (Zihler, 1972) individual polyps were fixed with Carnoy's fixative and stained with Mayer's hemalum. For electronmicroscopic studies the specimens were fixed for 1 to 2 hr in glutaraldehyde (3% in phosphate buffer, *p*H 7.2), rinsed with phosphate buffer, and fixed once more for 1 hr with osmium tetroxide (2% in phosphate buffer), and imbedded in Durcopan (Fluka AG.). The sections were treated according to Reynolds (1963) and examined with a Hitachi electronmicroscope (HU 8).

INITIATION AND INTENSITY OF SEXUAL ACTIVITIES

According to what is known so far, the factors initiating gametogenesis and the periodicity and intensity of sexual activities are distinctly species specific.

Hydra fusca L. is a clear-cut gonochoristic species (Schulze, 1917; Brien, 1961; etc.). The determination of the sexual status can within certain limits be altered by bringing fragments of both sexes into parabiosis. Under the influence of the male partner the female also becomes male (Brien, 1961, 1963; etc.). Under laboratory

This work was supported by the Swiss National Science Foundation (Grant 3.205.69).

conditions gametogenesis can be induced by exposing the animals to low temperatures (Brien, 1966; Brien and Reniers-Decoen, 1949; etc.). Particularly in males gamete production is so intense that we can speak in terms of a "gametic crisis" ("crise gamétique," Brien, 1966) leading to a complete exhaustion and death of the animals.

This species is very abundant in our lakes and ponds, where the populations reach a density which is never matched by the other species mentioned here. In the lake of Zurich this density reaches its maximum in fall just before the population enters its gametogenic period lasting for 4 to 6 weeks. As a consequence of gametogenesis the previously dense populations collapse, leaving a small number of individuals which reconstitute the population by means of asexual reproduction (Tardent, unpublished). From the few observations available we must conclude that in this species gametogenesis takes place once a year and that in the natural environment factors other than lowering the temperature are inducing gametogenesis.

Hydra attenuata Pallas is what could be called an unstable gonochorist (Tardent, 1966). In this species individuals will for months and even years and for many subsequent sexual periods produce either eggs or sperm. For still unknown reasons the relatively stable state of sexual determination can spontaneously switch to the other sex (Tardent, 1966). Inversion of the sexual status from female to male can be experimentally obtained by bringing fragments of both sexes into parabiosis (Tardent, 1968). Furthermore, a high percentage of males treated with low doses of X-rays (Tardent, 1968) or exposed to nitrogen-mustard (Figi, 1969) will become females. In this species we still do not know which are the factors that induce gametogenesis. According to laboratory experiments in which environmental conditions were kept as constant as possible, gametogenesis was initiated more or less synchronously even when the animals were kept in separate jars or aquaria.

In this species, gametogenesis is not restricted to particular seasons of the year (Tardent, 1966) and it is neither in the laboratory nor in the natural environment as intense and fatal as in *Hydra fusca*. The natural populations of *H. attenuata* are always much less dense than those of *H. fusca*.

Hydra viridis L. (Brien, 1950*a*; etc.) and *Hydra circumcincta* Schulze 1914 (Tardent et al., 1968). Both of these species are hermaphrodites, although we may encounter sexually active specimens which, at a given time, produce only testes or only eggs. In both species there is no evidence for a distinct annual periodicity of gametogenesis and nothing is known about the mechanisms releasing gametogenesis. We still lack information about how the populations of these two species behave in their natural environment.

THE CELLULAR ASPECTS OF GAMETOGENESIS

The microscopical aspects of gametogenesis in the genus *Hydra* have been quite extensively studied by a number of authors (Korotneff, 1878; Downing, 1905, 1909; McConnell, 1933; Brien, 1950*a,b*, 1966; Brien and Reniers-Decoen, 1949, 1951; Tokin, 1955). In *Hydra* gametogenesis takes its issue from the interstitial cells of the ectoderm. There is no reason to assume that amongst this population of interstitial cells there are particular lineages of gametocytes and somatocytes. When at the end of a sexual period of *H. attenuata*, e.g., we examine the ectoderm of the distal body region where spermatogenesis had taken place, there are practically no interstitial cells left. If there were gametocytes and somatocytes amongst interstitial cells we should at least find a population of somatically determined I-cells which were not engaged in gametogenesis.

SPERMATOGENESIS

In *Hydra attenuata* L. spermatogenesis is initiated by local accumulations of interstitial cells within the intercellular spaces of epithelio-muscular cells (Brien and Reniers-Decoen, 1951). These clusters of what can now be called spermatogonia are

most probably formed by both centripetal cell migration and local cell proliferation. As the clusters grow larger they lift up the stratum of epithelio-muscular cells, which then forms the protruding outer wall of the testis. Spermatogenesis and spermiogenesis take place in the conspicuous spaces formed between the outer wall of the testis and the mesoglea. Within these chambers the various cell layers, starting from the mesoglea outwards, represent the succession of the various stages of spermatogenesis (Brien, 1966).

This process does not present any peculiarities except that the meiotic and following mitotic divisions do not always lead to a separation of the spermatidia. As shown by Zihler (1972), in some cases following the meiotic and also the subsequent mitotic divisions of the spermatocytes the heads of the spermatids will remain within a common membrane (Fig. 1). Thus, spermiogenesis of two or four sperms will take place within a common membrane. The three types of spermatids shown in Figure 1 can be found within the same testis.

From an ultrastructural point of view spermatogenesis and spermiogenesis do not offer anything out of the ordinary (Schin-

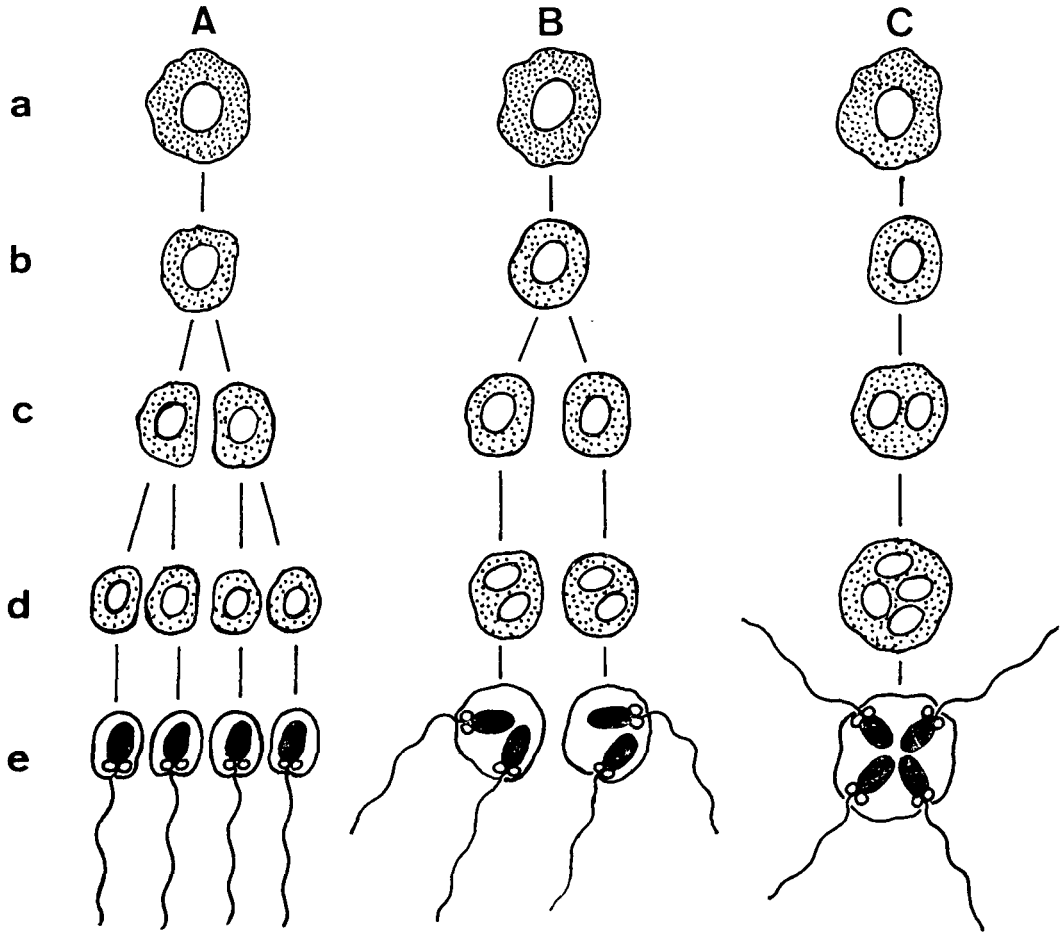


FIG. 1. Three pathways of spermatogenesis observed in *Hydra attenuata*. *A*, Meiosis and mitosis lead to four separated spermatids. *B*, Spermiogenesis of two spermatids occurs within a common membrane. *C*, Meiosis, mitosis, and spermiogenesis occur within a common membrane. *a*, Spermatogonia; *b*, spermatocytes I; *c*, spermatocytes II; *d*, spermatids; *e*, spermiogenesis. (Redrawn from Zihler, 1972.)

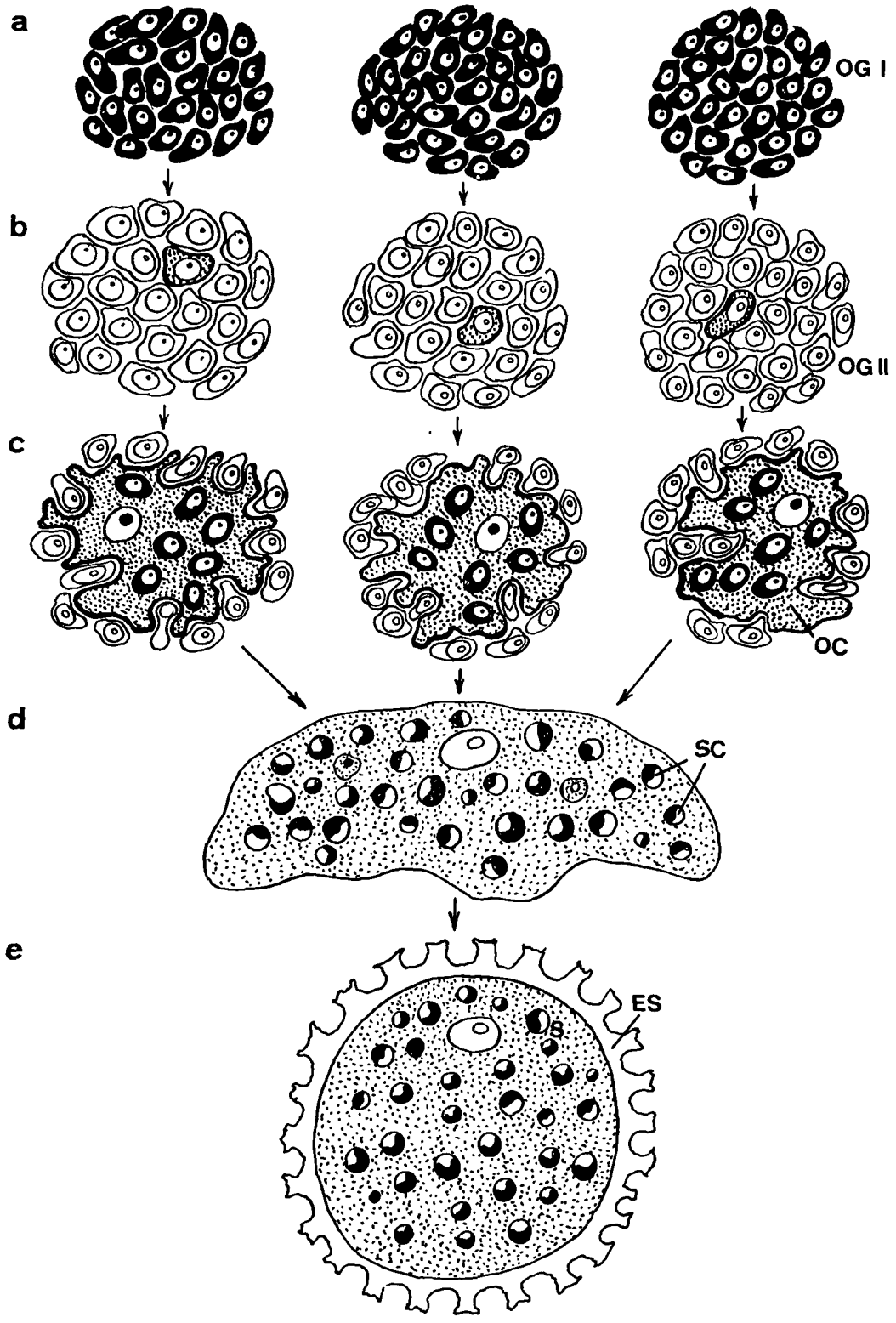


FIG. 2. Oogenesis in *Hydra circumcincta:* schematic drawing of the process. *a,* Clustering of oogonia I; *b,* clusters of oogonia II, in which one cell becomes the oocyte I; *c,* the oocyte engulfs by phagocytosis the remaining oogonia II and part to one oocyte containing "shrinking cells" (SC) and the nuclei of the other oocytes; *e,* fertilized egg with egg-shell. (ES, egg-shell; OG I, oogonia I; OG II, oogonia II; OC, oocyte.) (Redrawn from

cariol et al., 1967; Weissmann et al., 1969; Stagni and Lucchi, 1971; Zihler, 1972). So far electronmicroscopic studies have failed to reveal an acrosome or acrosome-like structure, although the spermatid cell contains a conspicuous Golgi-body. In *Hydra attenuata* the middle piece encloses four mitochondria and the centriole (Zihler, 1972, Figs. 20–28).

The ripe sperms of *Hydra attenuata* and *H. circumcincta* have a total length ranging between 35 and 38 μm while that of *Hydra fusca* is 10 μm longer. In all these species the head and middle piece claim $\frac{1}{8}$ to $\frac{1}{9}$ of the total length of the sperm (Zihler, 1972).

The ripe spermatozoa, which already move within the testis, are released into the surrounding medium in small portions of 10 to 100 at a time. In a single testis 3 to 5 such subsequent ejaculations are normally followed by a period of rest lasting several hours (Zihler, 1972). By this means a sexually active polyp of *H. attenuata* bearing 5 to 10 testes can guarantee a continuous and lasting supply of spermatozoa to the surrounding medium.

OOGENESIS IN HYDRA CIRCUMCINCTA

Like spermatogenesis, oogenesis is initiated by an accumulation of a great number of interstitial cells within the egg-forming area of the body's ectoderm (Fig. 2*a*). At first a variable number of cell clusters of these oogonia I (see Fig. 8) are separated from each other by epitheliomuscular cells. At this stage the oogonia I contained in these clusters start synthesizing yolk (see Fig. 9), leading to a considerable increase of their cytoplasmic volume. One cell of each cluster is in some unknown way determined to become a primary oocyte (Fig. 2*b*). This cell now gradually engulfs all other oogonia of the cluster by phagocytosis (Fig. 2*c*). The developing oocyte is, therefore, not a plasmodial plurinucleated system, but it consists of one cell which has phagocytosed all its neighboring oogonia II. The engulfed cells, while they are within the cytoplasm of the oocyte (Fig. 5), retain their membranes and their organelles (nucleus, endoplasmic reticulum, mitochondria) (Figs. 10–11), but they are gradually shrinking. For this reason, we called these cells "shrinking cells."

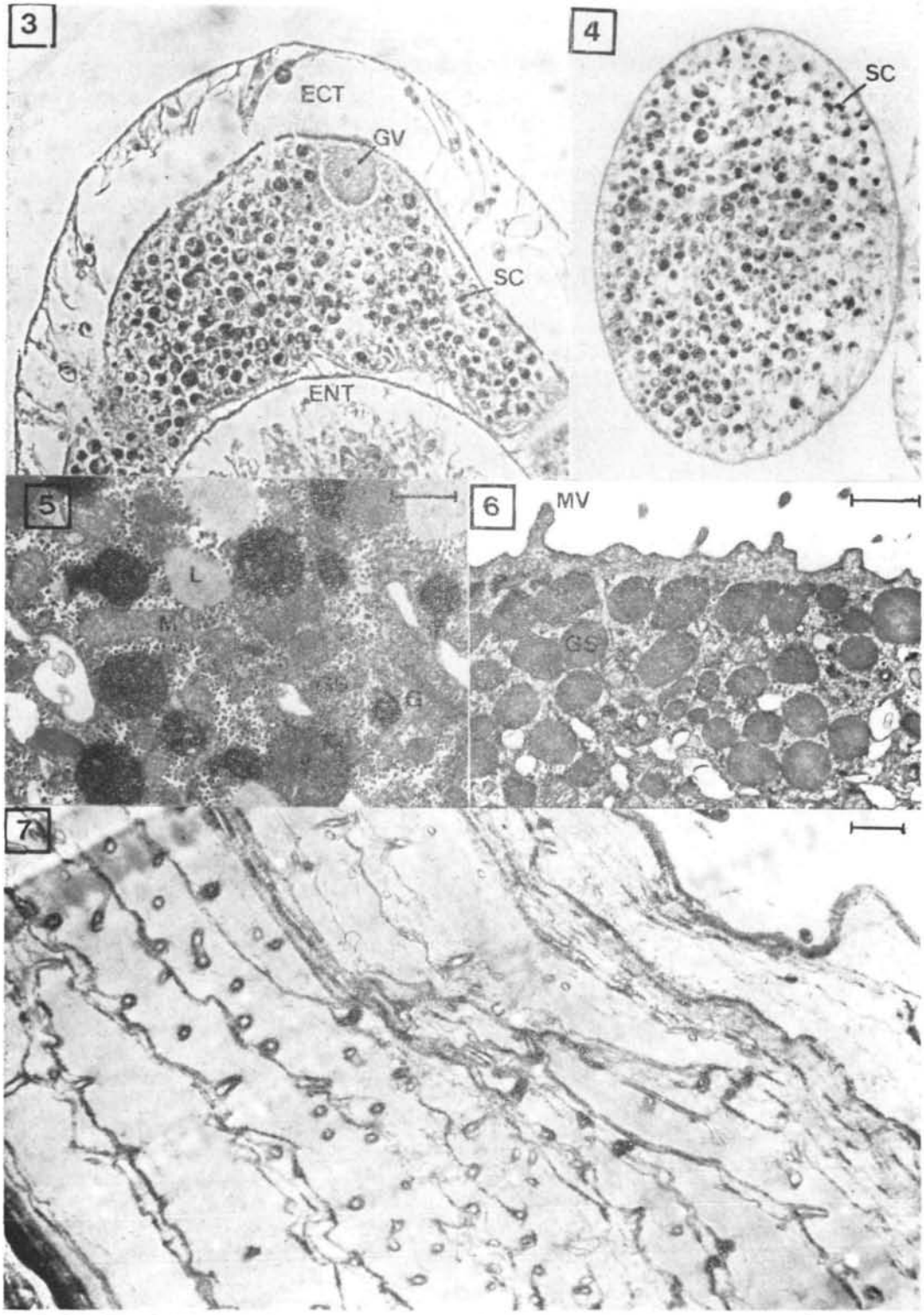
Several of these quickly growing oocytes now merge to form an initially plurinucleated oocyte (Fig. 2*d*), in which one nucleus persists as the germinal vesicle (Fig. 3) while all others are inactivated and share the fate of the nuclei of phagocytosed "shrinking cells." It would be interesting to know when and how this decision-making takes place. By dividing the oocyte into two portions at this stage of merging, Zihler (1972) found that each fragment, when fertilized, will initiate development. This means that any one of these oocyte nuclei can become a functional egg nucleus.

As shown, no follicle-like cells are involved in egg maturation. The numerous oogonia are first phagocytosed by a varying number of primary oocytes, which in their turn become an integrated part of the secondary oocyte (Fig. 2*d*).

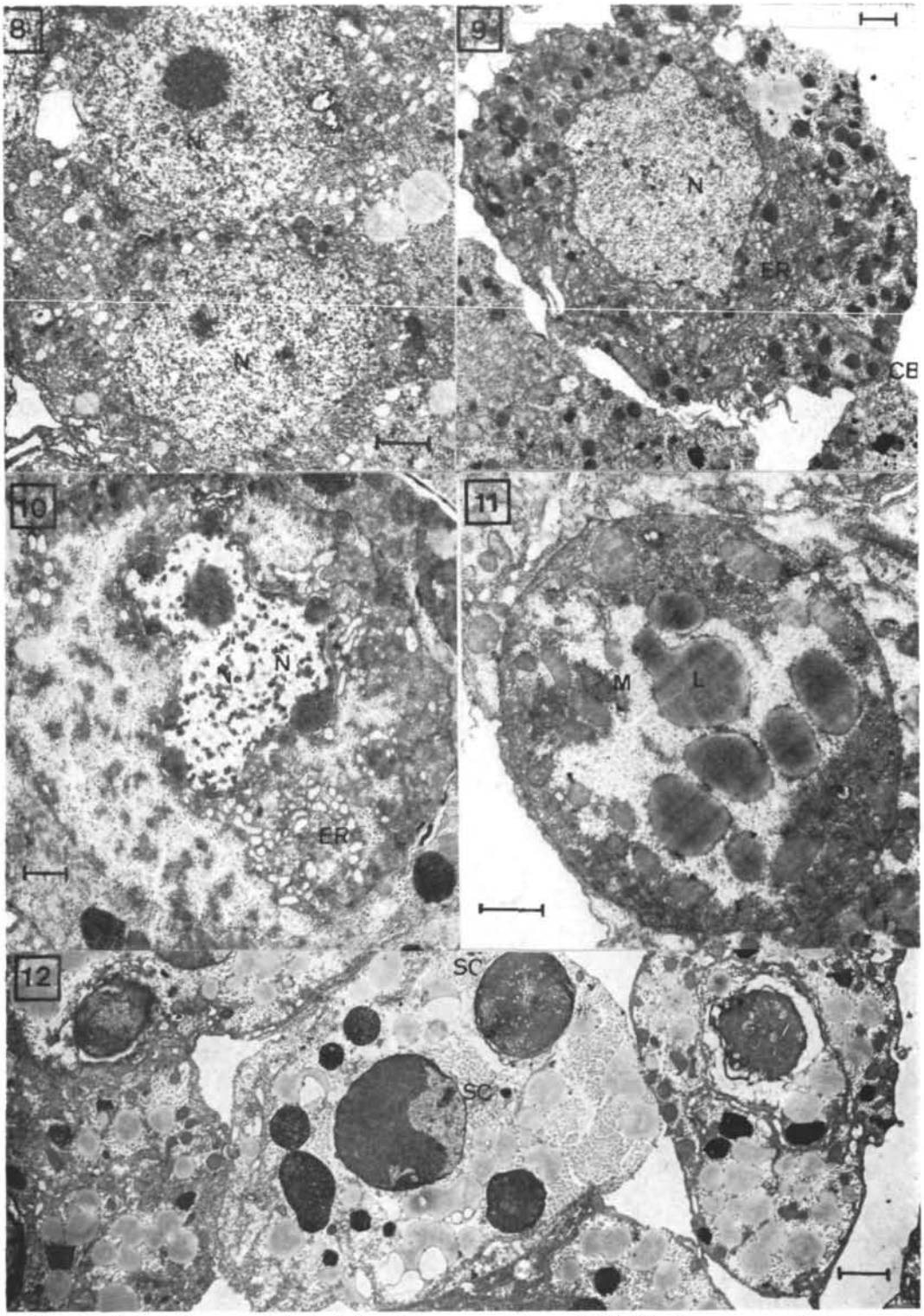
The secondary oocyte is gradually assuming a spherical shape (Fig. 4) and bursts through the sheet of epitheliomuscular cells. At this stage the egg is fertilized (Zihler, 1972). Immediately after fertilization has taken place, cleavage begins (Fig. 12) and the outer surfaces of the blastomeres start secreting the egg shell which consists of three layers (Fig. 7). These layers are perforated by small tubules which are formed presumably by protruding microvilli of the blastomeres' outer surfaces (Fig. 6). When the egg is not fertilized and cleavage does not occur, no shell is formed and the egg disintegrates within 12 hr (Zihler, 1972).

FERTILIZATION

In most laboratory populations of the species mentioned, the rate of fertilization is near to 100%. We have so far been unable to detect any chemotactic guidance of the sperm towards the egg surface as shown by Miller (1966) in *Campanularia*.



FIGS. 3–7. FIG. 3. Histological section of an oocyte still covered by an ectodermal (ECT) sheet. The oocyte nucleus and the "shrinking cells" (SC) are clearly visible. FIG. 4. Histological section through an unfertilized egg still attached to the ectoderm of the polyp. FIG. 5. Electronmicrograph of the central portion of a ripe unfertilized egg in between "shrinking cells." FIG. 6. Cortical plasma of an unfertilized egg. FIG. 7. Fine structure of the egg case perforated by microvilli. Left, inner surface; right, outer surface. (ECT, ectoderm; ENT, entoderm; GS, secretory products of the Golgi; G, Golgi-body; GV, germinal vesicle; L, lipids; M, mitochondria; SC, "shrinking cells"; Y, yolk.) Magnifications: Fig. 3, 60×; Fig. 4, 40×; Figs. 5–7, width of bars = 1 micron.



FIGS. 8–12. FIG. 8. Oogonia I derived from interstitial cells. FIG. 9. Oogonia II. Note the cytoplasmic bridge (CB) between neighboring oogonia. FIG. 10. Oogonium II transforming to a "shrinking cell." FIG. 11. "Shrinking cell" within the egg cytoplasm. FIG. 12. Blastomeres of the morula stage containing "shrinking cells" (dark bodies). (CB, cytoplasmatic bridge; ER, endoplasmatic reticulum; N, nucleus; SC, "shrinking cells.") Width of bars = 1 micron.

We think that even if such a mechanism is absent there is a fair chance that in a natural population of *Hydra* the sperm will reach the egg surface. We think that the following factors, when acting in combination, are sufficient enough to guarantee casual collisions of sperm with the egg.

1) Within a given population gametogenesis of both sexes is synchronized (Tardent, 1966), although it is still unknown how this synchronization is brought about.

2) The average duration of the gametogenic period is longer in males than in females (Tardent, 1966; Zihler, 1972), thus insuring an uninterrupted supply of free swimming sperm while oogenesis in females takes place.

3) A male polyp bearing several testes is releasing continuously small quantities of sperm into the surrounding water (Zihler, 1972).

4) The life span of free moving spermatozoa is relatively long. Under optimal conditions (10 C and *p*H 7.1) it reaches 200 min (Zihler, 1972).

5) The oocyte of *Hydra* is not enclosed in a gonotheca. Its surface is freely exposed to the surrounding water.

These factors seem to be sufficient to guarantee a successful fertilization even in the absence of a far reaching chemical interaction between the gametes. Nothing is known yet about how egg and sperm interact once the latter has collided with the egg surface, nor how the sperm enters the egg cytoplasm. We do not think that the spherical bodies found in the cortical layer of the egg (Fig. 6) are true cortical granules involved in a cortical reaction. They probably contain the materials for the formation of the egg shell.

DISCUSSION

In the genus *Hydra* oogonia and spermatogonia derive from the ubiquitous interstitial cells, which can also differentiate into somatic cell-types such as nematocytes, nerve cells, and others. It would be of great interest to know how the decision-making takes place, when an interstitial cell leaves its "undifferentiated" state to become a somatic element or a male or female gamete. This is one amongst several possible alternatives. If we knew the environmental factor or factors which release gametogenesis or, in other words, which induce a considerable portion of the interstitial cell population to become gametocytes, we could possibly track the sequence of events that at the level of the genome determine the fate of the cell. Much work needs to be done before we can recognize these factors by which gametogenesis can be released and therefore experimentally controlled.

The same holds true for another alternative in connection with interstitial-cell determination. It includes the events which decide, in a hermaphroditic species, whether the cell that was determined to become a gametocyte will differentiate to a spermatocyte or an oocyte. Burnett (1966), in proposing his model of cell differentiation in *Hydra,* has offered an interpretation of phenotypic sex determination within the interstitial cell population, but here again we lack sufficient experimental information to support his or any other explanation.

Spermatogenesis as such, as emphasized in this paper, does not present any peculiarities. Our electronmicroscopic studies (Zihler, 1972) have failed to demonstrate the presence of an acrosome or acrosome-like structure at the tip or near the tip of the sperm head. Hinsch (1974) has shown, in the ripe spermatozoa of other Cnidaria, the presence of particular formations which could be interpreted as being acrosome-like structures. They are not, however, situated at the tip of the sperm, but alongside the sperm nucleus. It would be of interest to extend these comparative studies to investigate more closely the behavior of the sperm when it collides with and enters the egg surface.

Oogenesis, as it was described for *Hydra circumcincta* (Zihler, 1972), is in our view one of the most primitive ways of building an egg. No nourishing follicle-like cells seem to be involved. Instead, a large number of potential oogonia gather and a few of them phagocytose the remaining oogonia. The bodies found within the cyto-

plasm of the oogonia are therefore not just nuclei of cells which have fused to a plasmodium, but actually entire cells. Although we have no experimental proof as yet, the synthesis of egg cytoplasm and its compounds seems to occur at the expense of the "shrinking cells," which in a certain sense simply represent nourishing cells engulfed by the growing oocyte. We do not know whether, in addition to this way of introducing raw material into the ovocyte's cytoplasm, there is also transfer of materials from the neighboring ectodermal and entodermal cells to the ovocyte. We plan to study the metabolic aspects using radioactive precursors.

The type of oogenesis observed in *Hydra* is to our knowledge unique in the animal kingdom. It raises a number of questions related to the selection of those oogonia which later will become oocytes. The same holds for the later stage when the primary oocytes fuse and one nucleus, by suppressing the others, becomes the germinal vesicle.

This system offers many possibilities for studying the building-up of a hierarchical situation amongst cells which before seemed to be identical and equivalent and, furthermore, the establishment of dominance and the resulting interactions amongst nuclei situated within a common cytoplasm.

Very little is known so far about the physiology of sperm and egg interaction. Whether there is a classical cortical reaction to sperm entry or not still remains to be verified electronmicroscopically. We do know that, after entry of the sperm, the egg surface and the microvilli protruding from it start secreting the egg shell. This egg-shell consists of three layers and is not formed if no fertilization occurs after egg maturation. Although this shell formation is induced by sperm entry, it is not certain whether it has anything to do in its initial stage with preventing polyspermy.

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