GAMETOGENESIS DURING THE ANNUAL REPRODUCTIVE CYCLE IN A CIDAROID SEA URCHIN (STYLOCIDARIS AFFINIS) [1]

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Within the Echinoidea, the cellular events of gametogenesis have been described in detail for several species: *Echinocardium cordatum* (Caullery, 1925), *Mespilia globulus* (Tennent and Ito, 1941), *Diadema setosum* (Yoshida, 1952), *Strongylocentrotus nudus* and *Strongylocentrotus intermedius* (Fuji, 1960), *Strongylocentrotus purpuratus* (Holland and Giese, 1965) and *Sterechinus neumayeri* (Pearse and Giese, 1966). All of these species, in the presently accepted echinoid classification, belong to the subclass Euechinoidea, and to date there has been no detailed investigation of gametogenesis in any species of the echinoid subclass Perischoechinoidea. The purpose of the present investigation is to describe the cellular events of gametogenesis in *Stylocidaris affinis* (Philippi), a member of the order Cidaroida of the subclass Perischoechinoidea. In addition to occupying an interesting taxonomic and phylogenetic position, the cidaroid sea urchins investigated were living in an environment in which several physical factors had been investigated locally (Hapgood, 1960); this permitted a discussion of possible exogenous control over the annual course of gametogenesis, a topic recently reviewed for echinoderms in general by Boolootian (1966).

Materials and Methods

Urchins were collected by dredge from a population living in about 70 meters of water in Bocca Piccola, the strait between the Isle of Capri and the tip of the Sorrentine Peninsula. Twelve to 17 adult urchins, each weighing between 10 and 40 g., were taken for each sample. In the ten samples taken, the distribution of male and female urchins was as follows: 9 March 1965 (10 M + 6 F); 27 August 1965 (8 M + 5 F); 5 October 1965 (5 M + 7 F); 9 December 1965 (8 M + 5 F); 31 January 1966 (9 M + 3 F); 9 March 1966 (6 M + 8 F); 20 April 1966 (11 M + 6 F); 23 May 1966 (8 M + 7 F); 1 July 1966 (9 M + 6 F); and 2 August 1966 (6 M + 7 F). From each urchin one of the five gonads was removed on the day of collection and fixed overnight in sea water-Bouin's fluid. Fixed gonads were dehydrated in ethanol, cleared in xylene, imbedded in paraffin and sectioned at 7 microns. The gonad sections were stained with haematoxylin and eosin, alcian blue, azure A, periodic acid-Schiff (PAS) or mercuric bromphenol blue by the methods given on page 284 of Holland and Nimitz (1964).

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Oogenesis in female specimens of *S. affinis* was studied by the frequency polygon method of Pearse (1965, page 53). The frequency of different sizes of primary oocytes in the sectioned ovaries stained with haematoxylin and eosin was estimated by measuring at random the diameters of 50 primary oocytes per animal. To be counted as a primary oocyte, a germinal cell had to be sectioned through its nucleolus and had to be at least 11 microns in diameter. Since most such cells were somewhat elliptical in outline, the diameter was always calculated by adding the long and short axes of the ellipse and dividing by two. The germinal cells less than 11 microns in diameter apparently comprised the oogonia and the smallest primary oocytes; such cells could not be counted reliably, since some of them lacked well defined nucleoli and the two cell types were difficult to tell apart. Although the transition of oogonia into primary oocytes is an important part of oogenesis, the present investigation was unable to determine when during the annual reproductive cycle new primary oocytes were produced from oogonia. In the one animal with ripe eggs present in the ovary, the germinal cells were quantified by measuring at random the diameters of a total of 50 primary oocytes (larger than 11 microns and sectioned through the nucleolus) and eggs (sectioned through the nucleus). For each animal, the range of germinal cell diameters from 11 to 110 microns was divided into nine groups at 11-micron intervals and the percentage of cells in each group was calculated. The frequency of occurrence of all size groups in each female was then plotted as a frequency polygon. Also, average frequency polygons were constructed by averaging data from all females in a sample.

Spermatogenesis in the male specimens of *S. affinis* was studied by measuring the thickness of the mass of germinal cells along the wall of testicular lobes that had been cut in approximate cross-section. The type of germinal cell being measured was recorded as spermatocyte, spermatid or spermatozoan. When no spermatids or spermatozoa were present in the germinal cell layer measured, the inner edge of the spermatocyte layer was always sharply demarcated from the central region of the testicular lobe, which was usually filled with non-germinal cells. However, in those testicular lobes containing spermatids or spermatozoa, the entire central region of the lobe was filled with the germinal cells. In such lobes, where the germinal cells did not form an actual layer, the thickness of the germinal cell layer was arbitrarily taken as half of the total diameter of the testicular lobe. In the testes of most male urchins, measurement of a single testicular lobe sufficed to describe the spermatogenic stage in all the lobes. However, in the testes of a few male urchins, some lobes were in one stage of spermatogenesis while other lobes of the same testes were in a second stage; for such testes, it was necessary to measure lobes of each sort and to record the relative abundance of the two types of lobe.

Results

In *S. affinis*, each of the five gonads is essentially an axial gonoduct from which spring elongate, more or less ramified lobes. A histological section through a gonadal lobe reveals an outer visceral peritoneum, a middle connective tissue-muscle layer and an inner layer of germinal as well as non-germinal cells. Within each of the nine samples collected between August, 1965 and August, 1966, the cytometric data from the ovaries were averaged and expressed as nine average fre-

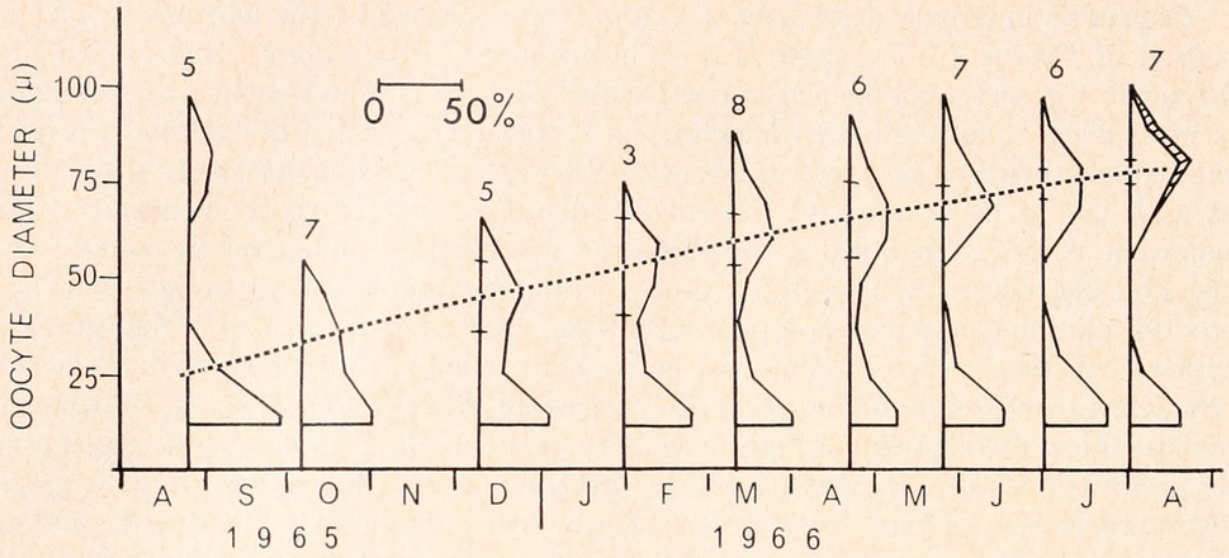

FIGURE 1. Polygons showing the frequencies (see scale) of primary oocyte diameters in the ovaries of *S. affinis* averaged from samples collected from August, 1965 to August, 1966. Each of the nine average frequency polygons was constructed from cytometric data from the number of animals indicated above the polygon. The mean diameter (plus or minus one standard deviation) of the group of growing primary oocytes has been included for most of the polygons. The hatched area of the polygon at the far right indicates ripe eggs. The dotted line drawn through these means describes the progressive increase in the diameter of the group of growing primary oocytes.

quency polygons. By plotting these average polygons, the yearly pattern of oogenesis could be visualized (Fig. 1). As the figure demonstrates, many small primary oocytes began to grow during the first part of September and at no other time during the annual reproductive cycle. In subsequent months, while the growing primary oocytes increased in diameter, numerous small primary oocytes, apparently not growing, continued to be present. Thus, two distinct size classes of primary oocytes could be detected in the ovaries throughout most of the annual reproductive cycle. The curve in Figure 1, drawn through the estimated average diameter of each sample's group of growing primary oocytes, demonstrates that their increase in diameter was not linear with time. It can be calculated, however, that the average volume increase between early December and early August was approximately linear. During this period, the average growing primary oocyte increased in volume at a steady rate of about 200 cubic microns per day.

From Figure 1, it may be seen that the growing primary oocytes attained maximum size and disappeared from the ovaries during the period from early August to late September. The details of this disappearance of the grown primary oocytes from the ovaries may best be visualized by presenting the frequency polygons for each individual female urchin collected in the samples of August, 1965 and August, 1966 (Fig. 2). Figure 2 shows that the ovaries from six of the seven female urchins collected on 2 August 1966 each contained a group of small primary oocytes and a group of grown primary oocytes. The grown primary oocytes had an average diameter of about 80 microns and each cell had a nucleus 35 to 40 microns in diameter containing a nucleolus about 8 microns in diameter. The ovary taken from the seventh female contained a group of small primary oocytes and a group of large germinal cells, more than 90% of which were ripe

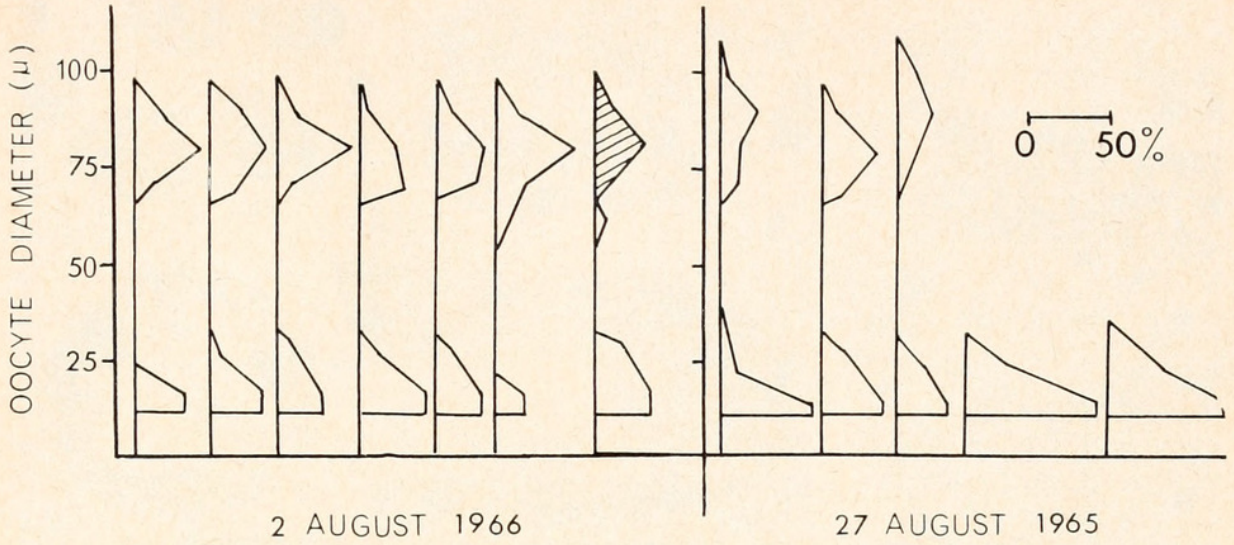

FIGURE 2. Polygons showing the frequencies (see scale) of germinal cell diameters in the ovaries of all female specimens of *S. affinis* collected in August of 1965 and 1966. Unhatched areas indicate primary oocytes; the hatched area indicates ripe eggs. In the sample of 2 August 1966, one of seven females had ripe eggs instead of grown primary oocytes. In the sample of 27 August 1965, three of five females had grown oocytes, while the other two females were devoid of all large germinal cells, presumably because spawning had taken place before collection.

eggs; the few primary oocytes belonging to this group of large cells were its smallest members. On the average, a ripe egg was about 80 microns in diameter and contained an anucleolate nucleus about 14 microns in diameter. In three out of five female urchins collected on 27 August 1965, the ovaries contained a group of small primary oocytes and a group of grown primary oocytes. In the other two females of the sample, the ovaries contained only small primary oocytes and were completely devoid of large primary oocytes or ripe eggs. The most reasonable interpretation of these findings is that the grown primary oocytes matured in the ovary, probably *en masse,* becoming secondary oocytes and then ripe eggs in rapid succession. The ripe eggs were then spawned after being retained in the ovary for a relatively short time (perhaps only a few hours). In the present investigation, the number of samples was insufficient to give precise information about the time elapsing between maturation and spawning. A photographic summary of oogenesis during the annual reproductive cycle is given in Figure 3 A–C.

In sections of ovaries stained with haematoxylin and eosin, all primary oocytes with a diameter of from 11 to 20 microns, as well as the germinal cells smaller than 11 microns in diameter (the smallest primary oocytes and oogonia), had a weakly basophilic cytoplasm, often organized into a loose fibrous meshwork. All primary oocytes larger than 24 microns in diameter had a granular eosinophilic cytoplasm. The primary oocytes ranging in diameter from 20 to 24 microns were in transition between fibrous basophilic cytoplasm and granular eosinophilic cytoplasm. Throughout the annual reproductive cycle, the largest cells in the group of small, non-growing primary oocytes contained cytoplasmic granules. The granular eosinophilic cytoplasm of the primary oocytes was crowded with minute granules of yolk material a fraction of a micron in diameter. These granules stained intensely with PAS after diastase digestion and gave a positive reaction with mercuric bromphenol blue. However, they did not stain with alcian blue

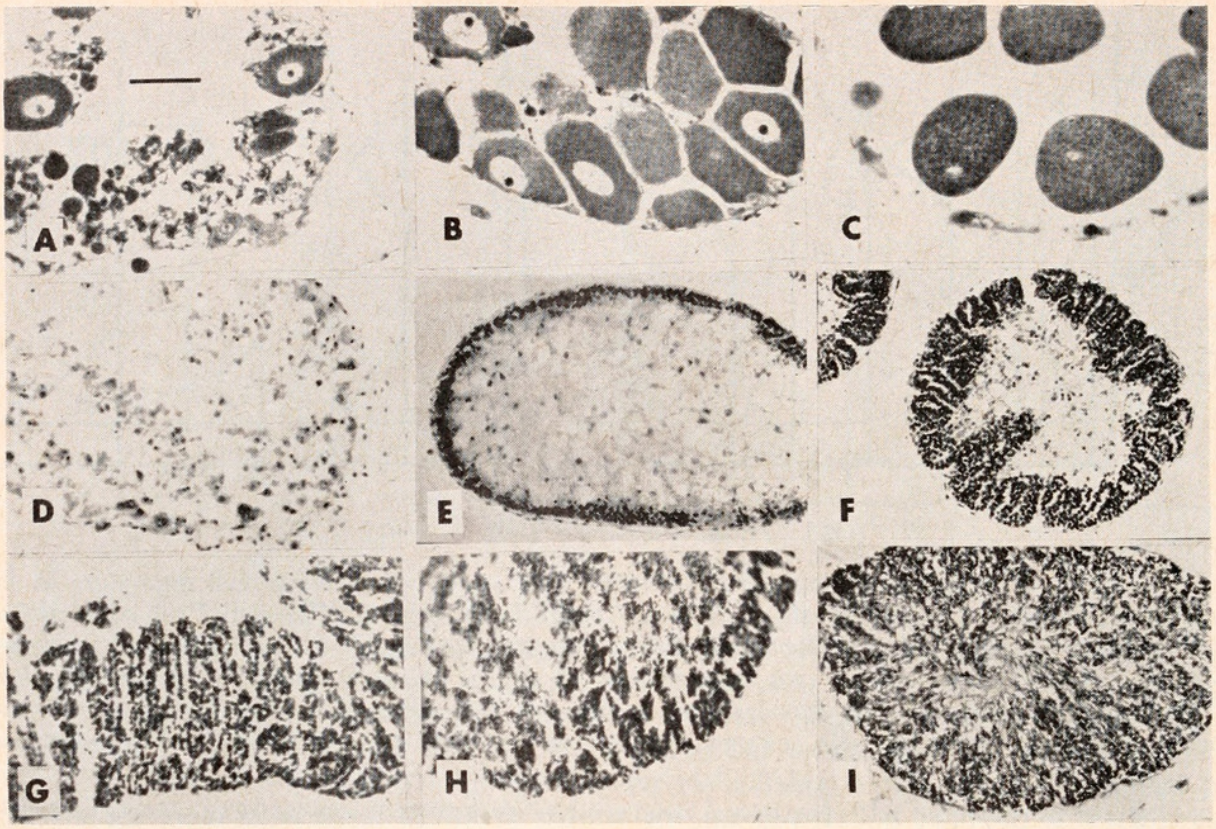

FIGURE 3. Photographs of histologic sections of gonadal lobes of *S. affinis*. Each photograph shows a cross-section of a gonadal lobe or a portion of a lobe with its center toward the top. The ovaries (A–C) were from urchins collected on 9 December 1965 (A), 23 May 1966 (B) and 2 August 1966 (C). In addition to a few small primary oocytes, A and B contain growing primary oocytes. The ripe eggs shown in C are in an ovarian lobe of the only egg-containing specimen of *S. affinis* collected during this investigation. The testes (D–I) were from urchins collected on 5 October 1965 (D), 31 January 1966 (E), 23 May 1966 (F) and 27 August 1966 (G, H and I). In D, there are a few scattered spermatogonia near the edge of the lobe. The next three photographs (E, F and G) show the progressive increase in the spermatocyte layer at the periphery of the lobe. In H (August lobe of type II), the spermatocytes nearer the center of the lobe have given rise to spermatids. In I (August lobe of type IV), the lobe is filled with spermatozoa. All the photographs are shown at the same magnification; the scale line in A is 50 microns long.

and failed to stain metachromatically with azure A. These findings suggest that protein and neutral mucopolysaccharide were conspicuous components of the yolk granules; no acid mucopolysaccharides were histochemically demonstrable. In ovaries sampled in August, some of the grown primary oocytes contained a few cytoplasmic flecks of alcian blue-positive material; this may have been an acid mucopolysaccharide precursor of the cortical material of the ripe egg. In the one ovary that contained ripe eggs, their cortical regions were stained with alcian blue and showed beta-metachromasia when stained with azure A. Thus, the egg cortex in *S. affinis* contained acid mucopolysaccharide components which may have been sulfated. The elaboration of these cortical components apparently occurred suddenly when the primary oocytes were approaching their maximum size. The histochemical reactions of the cytoplasmic yolk granules of the ripe egg resembled those of the primary oocytes, although the PAS reaction was somewhat less intense in the egg.

In sections of testes from all urchins sampled, regardless of the time of year, spermatogonia could be found scattered singly or in small groups just within the basement membrane delimiting the connective tissue-muscle layer from the inner layer of the testis. A typical spermatogonium, although irregular in outline, had an average diameter of about nine microns, and its cytoplasm remained almost unstained by haematoxylin and eosin. The nucleus typically had a diameter of about six microns and was relatively vesicular, containing a large amount of nuclear sap and a loosely organized meshwork of threadlike chromatin; a single, small nucleolus was present in most of the spermatogonia. Since they were always scattered, never forming a continuous layer of cells in the testicular lobes, the spermatogonia could not be quantified by the method used for the other types of male germinal cells. However, the constant presence of spermatogonia in all samples of testes is indicated by open circles interpolated into the base line of the bar graph describing the average course of spermatogenesis during the annual reproductive cycle (Fig. 4).

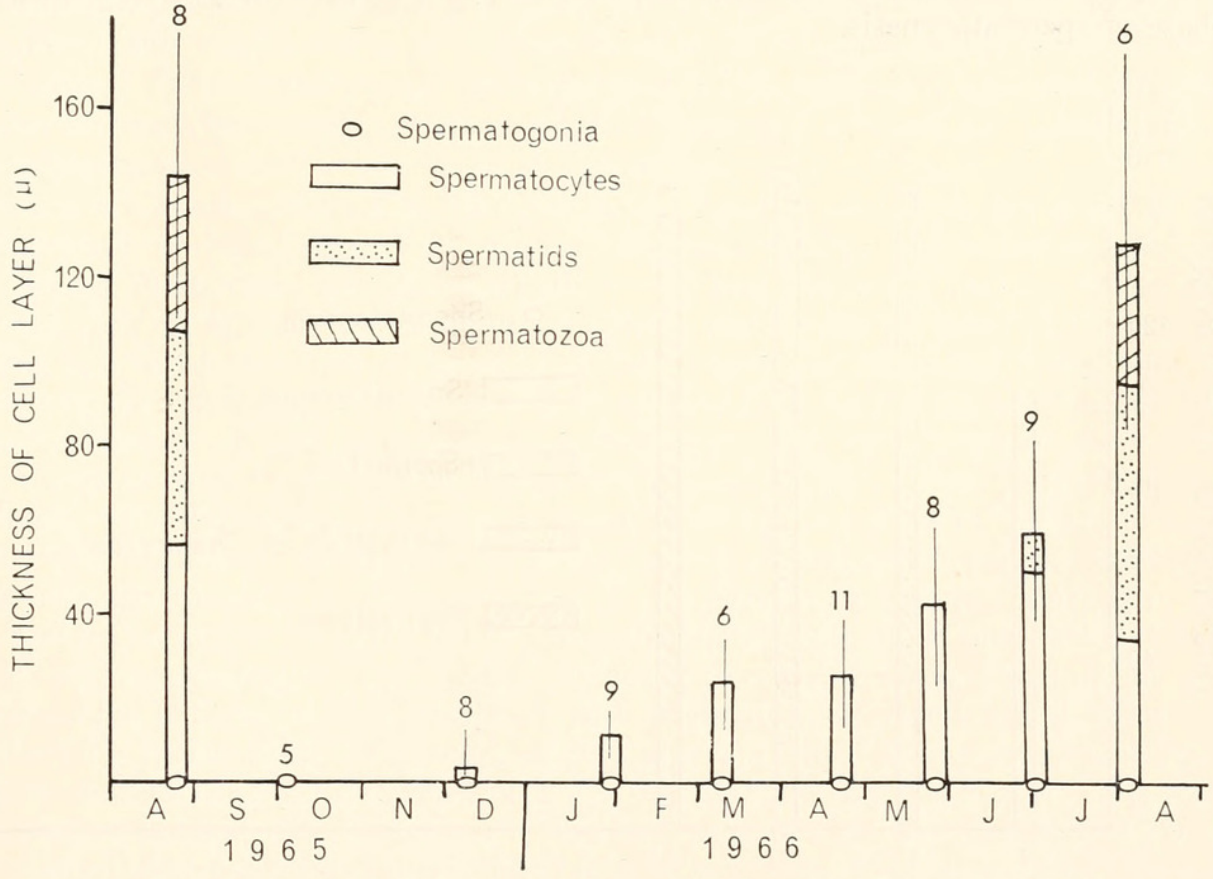

FIGURE 4. A bar graph showing the average thickness and composition of the peripheral layer of germinal cells in the testes of *S. affinis* sampled from August, 1965 to August, 1966. For each of the nine samples, the mean thickness of the germinal cell layer is shown plus or minus one standard deviation. The number of male urchins in each sample is given above each bar; for most samples, only one testicular lobe per animal was measured. However, in some animals sampled in August, two testicular lobes were measured. The open circles interpolated into the base line of the graph indicate the constant presence of spermatogonia. The clear area of each bar indicates spermatocytes, the stippled area spermatids and the hatched area spermatozoa.

The spermatogonia presumably gave rise to more spermatogonia by dividing and also differentiated into spermatocytes. Figure 4 shows that production of spermatocytes began during December and continued until the following August. The basophilic spermatocyte nucleus measured about 4 microns in diameter and was filled with a close-packed tangle of chromatin threads; around the spermatocyte nucleus was a thin shell of cytoplasm, often obscured through crowding by other cells. In the testes of *Stylocidaris affinis* there was no structural intergradation between the spermatogonia and the spermatocytes, as has been reported in the testes of *Strongylocentrotus purpuratus* (Holland and Giese, 1965). In *S. affinis,* the primary spermatocytes apparently could not be distinguished from the secondary spermatocytes. However, it was likely that most of the spermatocytes seen were primary, since the secondary spermatocytes of most animals constitute a very transitory cell type. Throughout the winter, spring and summer the spermatocytes accumulated as an ever-thickening layer of cells. During this extended period of accumulation, the spermatocytes did not differentiate into more advanced germinal cell types. In the sample of 1 July 1966, one out of nine male urchins had spermatids in its testes, heralding the end of the spermatocyte-accumulation phase of spermatogenesis.

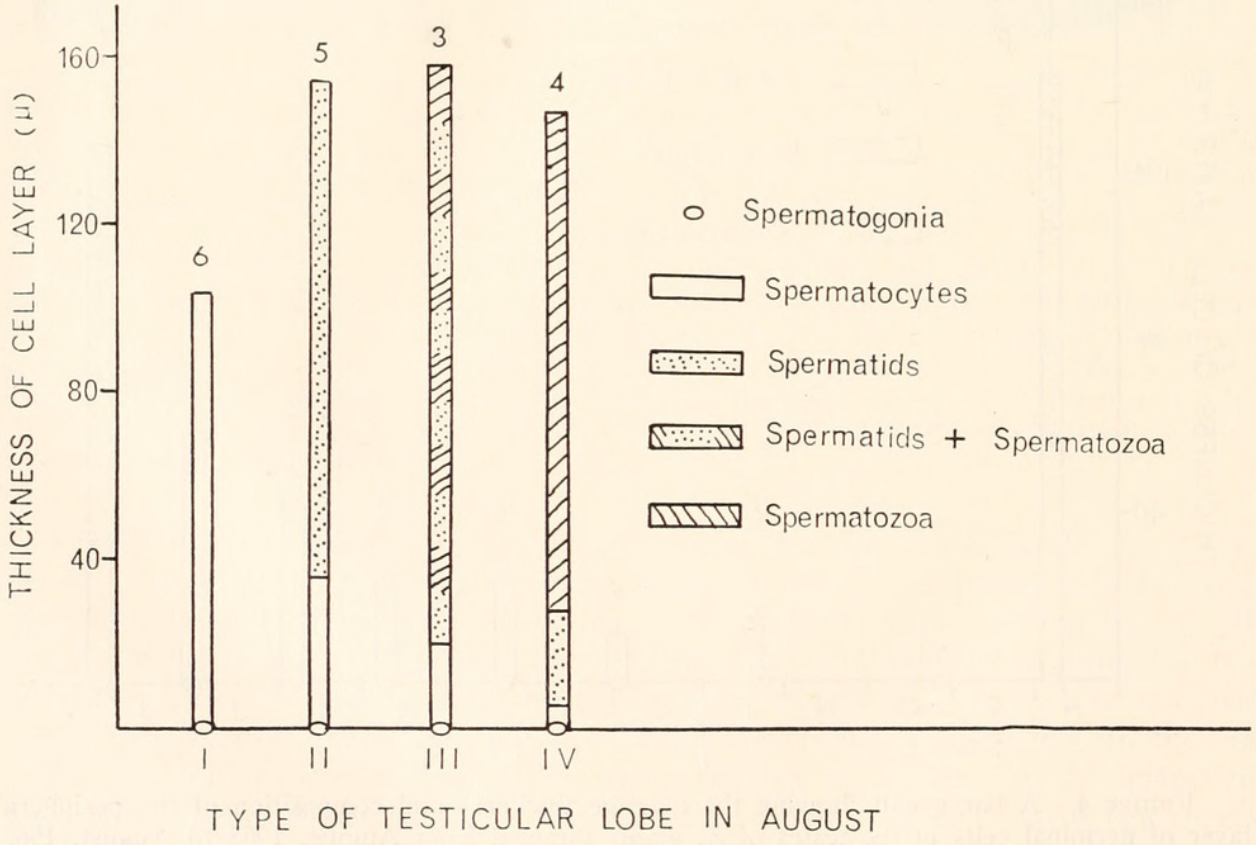

FIGURE 5. A bar graph showing the thickness and composition of the peripheral layer of germinal cells in each of four different types of testicular lobes in the testes of all male urchins sampled in August. The graph is based on data from eighteen testicular lobes from fourteen male urchins collected on 27 August 1965 and 2 August 1966. The respective bars represent an average of six lobes of type I, five lobes of type II, three lobes of type III and four lobes of type IV (the number of lobes averaged is given above each bar). The symbols are the same as in Figure 4.

The testicular lobes of most male urchins sampled in August of 1965 and 1966 contained not only spermatogonia and spermatocytes, but also spermatids and spermatozoa (Fig. 4). Each spermatid had a basophilic nucleus about 2.5 microns in diameter around which no cytoplasm could be demonstrated, while each spermatozoan had a basophilic, pointed conical head, 3.0 microns long and 1.4 microns in maximum diameter; the middle piece and tail were scarcely stained by haematoxylin and eosin. Each primary spermatocyte could give rise to four spermatids and subsequently each spermatid differentiated into a spermatozoan. In general, the spermatids and spermatozoa were less closely packed than the spermatocytes. These factors helped to account for the marked increase in the average thickness of the germinal cell layer of the testes in the August samples. In Figure 4, the bar graphs for the two August samples are averages of four different types of testicular lobes. In the first type of lobe, the layer of germinal cells measured was composed only of spermatocytes. The second type of lobe contained a peripheral layer of spermatocytes and an inner region of spermatids. The third type of lobe contained a peripheral layer of spermatocytes and an inner region of spermatids mixed with spermatozoa (for the calculation of the average bar graphs in Figure 4, this region was arbitrarily assumed to be half spermatids and half spermatozoa). The fourth type of lobe contained a thin peripheral layer of spermatocytes and an inner region of spermatozoa; interposed between the spermatocytes and spermatozoa was a thin layer of spermatids.

For the construction of the bar graphs in Figure 5, data from the testes of the eight male urchins collected 27 August 1965 and the six male urchins collected 2 August 1966 were considered together. Of these fourteen urchins, three had testes with all lobes of the first type, two had testes with all lobes of the second type, two had testes with all lobes of the third type and three had testes with all lobes of the fourth type. The other four urchins had more than one type of lobe in their testes. Of these urchins, three had testes with an average of about 60% of the lobes of the first type and 40% of the second type. The other urchin had a testis with about 80% of the lobes of the third type and 20% of the fourth type. For each of the four lobe types (from six, five, three, and four lobes, respectively), the average thickness and composition of the peripheral germinal cell layer are presented in Figure 5. The most reasonable interpretation of this figure is that the long spermatocyte accumulation stage of spermatogenesis came to an end during the latter part of the summer with a massive differentiation of spermatocytes into spermatids. This spermatocyte differentiation did not necessarily take place simultaneously in all lobes of a given testis, and, on the average, the spermatocytes farthest from the center of the lobe were the last to differentiate. Once produced, the spermatids differentiated into spermatozoa which were probably shed from the testes during the last part of August and the first part of September. The testes of all five male urchins sampled on 5 October 1965 contained no spermatocytes or spermatids and virtually no spermatozoa, with the exception of a few scattered spermatozoa which had been engulfed for digestion by the non-germinal cells (nutritive phagocytes) of the testis. A photographic summary of spermatogenesis throughout the annual reproductive cycle is given in Figure 3 D–I.

In the foregoing descriptions, oogenesis and spermatogenesis have been treated as if the annual reproductive cycle of August, 1965 through August, 1966 follows

essentially the same course year after year. This assumption was strengthened by the data from a collection made on 9 March 1965. From the ovaries of six females of this sample, the average frequency polygon of primary oocyte size classes was calculated and found to be virtually congruent with the average frequency polygon of primary oocyte size classes of urchins collected exactly one year later on 9 March 1966. From the testes of the ten males collected on 9 March 1965, the average thickness of the spermatocyte layer was calculated to be 20 microns, and did not differ significantly from the average thickness of 24 microns found exactly one year later on 9 March 1966.

The inner layer of the gonad of *S. affinis* contained, in addition to germinal cells, a population of non-germinal cells (the nutritive phagocytes). In the ovaries and testes, the nutritive phagocytes were similar and may be discussed together. The nutritive phagocytes, each of which had an irregularly shaped nucleus about 5 microns in average diameter, alternated between two morphological phases. They were globulated throughout the fall, winter and spring and they became deglobulated during the summer. In the globulated phase, each cell was typically elongate, measuring about 20×12 microns and often containing a voluminous central vacuole surrounded by a shell of cytoplasm. In the cytoplasm there were globules which ranged in diameter from less than 1 to about 10 microns and stained intensely with PAS. The cidaroid's globulated nutritive phagocytes were much like those of *Strongylocentrotus purpuratus;* one such cell is shown in Figure 2 on page 244 of Holland and Giese (1965). In some ovaries there were larger globules, up to 20 microns in diameter, which were probably the remains of broken-down oocytes and eggs. At no time during the annual reproductive cycle did the nutritive phagocytes of *Stylocidaris affinis* become as full of cytoplasmic globules as did those of *Strongylocentrotus purpuratus.* In the male urchins collected on 5 October 1965, the cytoplasm of the nutritive phagocytes contained a few engulfed spermatozoa in addition to globules; these spermatozoa had been left behind in the testis after the majority of the spermatozoa had been shed during August–September. In the testicular lobes with a peripheral layer of spermatocytes, the globular nutritive phagocytes were found internal to the spermatocyte layer. In the ovaries, during much of the reproductive cycle, nutritive phagocytes could be found in the spaces between adjacent primary oocytes as well as in the central part of each ovarian lobe. By the end of summer, in ovaries as well as testes, most nutritive phagocytes had lost their globules and vacuole, and their cytoplasm was extended into long pseudopodial strands. Many deglobulated nutritive phagocytes were mixed with spermatids and spermatozoa in the testes and were found in the spaces between the grown primary oocytes and ripe eggs in the ovaries. It seems likely that some of these non-germinal cells were expelled from the gonads with the ripe gametes at spawning. Presumably, however, enough nutritive phagocytes remained after spawning to repopulate the gonads during the next reproductive cycle.

Discussion

In the last decade, Mortensen's classification of the class Echinoidea (as summarized in Hyman, 1955) has been profoundly revised. Most contemporary students of echinoderm evolution, excepting Philip (1965), have agreed that the

former taxa Regularia and Irregularia are to be abandoned and replaced with the subclasses Euechinoidea and Perischoechinoidea. The subclass Euechinoidea contains some fifteen extant orders comprising all irregular echinoids and most regular echinoids, while the subclass Perischoechinoidea contains only one extant order, the order Cidaroida, as can be seen in the diagram on page 369 of Durham (1966). It is generally held that certain members of the order Cidaroida, during the Carboniferous period, gave rise to the ancestral stock of the present-day subclass Euechinoidea, while other members of the order continued in the evolutionary line leading to the present-day order Cidaroida. The cidaroid sea urchins have outlasted all other perischoechinoid orders, which became extinct by the end of the Paleozoic era. The new classification makes living cidaroids phylogenetically more remote from all other living echinoids than previously suspected, and it is of interest to compare selected aspects of gametogenesis in *S. affinis,* in euechinoids and in other echinoderms.

A striking difference between oogenesis in *S. affinis* and the euechinoids which have been studied is the absence in the cidaroid of the extended period of egg accumulation and storage characteristic of the euechinoids. The euechinoid phenomenon appears to be caused by the relatively asynchronous initiation and culmination of growth by the primary oocytes as well as by the prompt maturation of the oocytes on reaching their maximum size. In *S. affinis* as well as in the non-echinoid echinoderms which have been studied, the ovaries never contain ripe eggs for an extended portion of the annual reproductive cycle. Instead, the primary oocytes undergo maturation divisions only a short time before spawning as in *S. affinis* and in the crinoid *Comanthus japonica* (Dan, 1952); or while spawning is in progress as in holothurians (Ohshima, 1925); or a short time after spawning as in asteroids and at least some ophiuroids (Costello *et al.,* 1957). This egg production only at the approximate time of spawning is a widespread, and presumably primitive, characteristic of the phylum Echinodermata, being found in non-echinoid echinoderms as well as in *S. affinis,* where it may represent the retention of an ancient echinoderm trait. By contrast, the extended period of egg accumulation and storage characteristic of euechinoids is an alteration of a presumably primitive echinoderm trait and probably had its origins in the ancestral stock of the subclass Euechinoidea.

Spermatogenesis in *S. affinis* has an extended spermatocyte accumulation phase stretching through much of the annual reproductive cycle; this phase ends abruptly with the production of spermatozoa not long before they are to be shed. In other echinoderms studied, non-echinoids as well as euechinoids, there is no extended phase of spermatocyte accumulation. Instead, since developing germ cells experience no extended arrest as spermatocytes, they continue to differentiate into spermatozoa, and there is an extended stage of accumulation of spermatozoa. Thus, the accumulation of spermatozoa is a widespread, and presumably primitive feature of echinoderm spermatogenesis. The extended phase of spermatocyte accumulation is peculiar to *S. affinis* and is an alteration of a presumably primitive echinoderm trait. In this discussion, I have avoided extrapolation of the findings in *S. affinis* to the order Cidaroida. Clearly, there is a need for detailed studies of more species of cidaroid sea urchins before it is possible to speak of a cidaroid type of gametogenesis.

The population of *S. affinis* sampled was living in Bocca Piccola, one of the straits connecting the Gulf of Naples with the Tyrrhenian Sea. Useful maps of the region of the Gulf of Naples as well as a summary of its geology, meteorology and oceanography can be found in Puri *et al.* (1964) and in Düing (1965). In this region, from January, 1957 to January, 1958, Hapgood (1960) collected oceanographic data once a month at a number of stations, including one in Bocca Piccola (Station D, 75 meters) very close to the sampled urchin population. While Hapgood's data were collected seven years before the present investigation, they are probably a good approximation of the conditions for 1965–1966. The temperature at 75 meters in Bocca Piccola was almost constant throughout the year, averaging 14.3° C.; the lowest reading (13.7° C.) was in early spring and the highest reading (14.8° C.) was in early winter. Oxygen concentrations throughout the year averaged 7.9 mg./liter, fluctuating only slightly from the lowest concentration of 7.4 mg./liter to the highest of 8.4 mg./liter. The average yearly salinity was 37.82‰, and the lowest and highest salinities of the year were 37.58‰ and 38.06‰, respectively. The lack of seasonal fluctuations in temperature, oxygen concentration and salinity indicated that these exogenous factors do not control and synchronize the gametogenesis and spawning of *S. affinis*. On the other hand, the marked annual fluctuations in photoperiod could possibly influence reproductive events of the urchins in Bocca Piccola. The water of Bocca Piccola is clear, having an average annual Secchi disc reading of about 21 meters, and there is sufficient submarine light at 70 meters to support growth of macro-algae. However, the long periods of oocyte growth and spermatocyte accumulation showed no close relation to photoperiod; nor did the short periods of spawning, initiation of oocyte growth, and initiation of spermatocyte accumulation. However, even if light does not have a direct influence on reproduction in the urchins (comparable to photoperiodic induction in some plants), the photoperiod might possibly be used as a periodic reference point to synchronize an endogenous reproductive rhythm, if such exists in the urchin. It is also quite likely that photoperiod could have an important indirect influence on reproduction by influencing the quantity and quality of the algal food available to the urchins. To sum up, the factors (exogenous or endogenous) controlling the clear-cut annual reproductive cycle in this species remain enigmatic. Tortonese (1965) reported that *S. affinis* has a wide geographical distribution (Mediterranean, northwest coast of Africa, Cape Verde Islands, Bermuda, Caribbean and Gulf of Mexico) and a bathymetric range of 30 to 1000 meters. Perhaps a study of gametogenesis in several populations of *S. affinis* living at different depths and in different parts of its geographic range would help shed light on the control of gametogenesis in this species.

The apparent failure of the small primary oocytes to grow in the presence of the growing primary oocytes suggested that the growing oocytes might produce a growth-inhibiting factor to which they themselves were immune but which acted to prevent growth of the small oocytes. On the assumption that lack of growth would be correlated with biosynthetic inactivity, pieces of ovaries of female urchins collected on 23 May 1966 were labeled *in vitro* in H^3-glycine or H^3-uridine for one hour and then prepared for autoradiography. Surprisingly, the autoradiography revealed that there was synthesis of proteins (nuclear and cytoplasmic) and RNAs (nuclear) in both the large and the small primary oocytes. There was

certainly no striking suppression of protein or RNA synthesis in the small oocytes; indeed, on the whole, the rate of RNA synthesis in the small primary oocytes appeared higher than in the large primary oocytes. These observations suggest that the small primary oocytes may be growing after all. Perhaps oogonia are giving rise to primary oocytes throughout the year; these primary oocytes subsequently grow to a diameter of 25 to 30 microns and then, if large oocytes are present in the same ovary, break down. This suggestion is supported by the presence in most ovaries of a few small primary oocytes that are apparently deteriorating. If this suggested scheme is indeed the case, the rates of production and destruction of the small primary oocytes would have to be nearly equal to maintain the cell population at the relatively constant size indicated by Figure 1.

Summary

1. Periodic sampling of a Neapolitan population of the cidaroid sea urchin, *Stylocidaris affinis,* revealed an annual reproductive cycle.

2. In female urchins, primary oocyte growth begins only in September and continues for almost a year until maximum size is attained the following August.

3. In the oocytes of this species, conspicuous components of the yolk granules are protein and neutral mucopolysaccharide. Acid mucopolysaccharides, probably destined to be cortical components of the ripe egg, are synthesized only as the primary oocytes are nearing their maximum size.

4. After reaching their maximum size in August, the primary oocytes undergo maturation divisions (probably *en masse*) to become ripe eggs. The ripe eggs are apparently shed soon after being produced in August or September.

5. In male urchins, spermatogonia give rise to spermatocytes, which accumulate in an ever-thickening layer in the testes during the winter, spring and summer. The spermatocytes seem blocked from differentiating into more advanced germinal cell types until late summer, when they abruptly differentiate into spermatids and subsequently spermatozoa. The spermatozoa are apparently shed in late August or early September.

6. The lack of an extended period of egg accumulation in the female urchins, as well as the presence of an extended period of spermatocyte accumulation in the male urchins, sets gametogenesis in this cidaroid (subclass: Perischoechinoidea) apart from gametogenesis in other echinoids that have been studied (subclass: Euechinoidea).

7. The possible control of the annual reproductive cycle by exogenous environmental factors is discussed.

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