THE PRESENCE OF CENTRIOLES IN ARTIFICIALLY ACTIVATED SEA URCHIN EGGS

E. ROTER DIRKSEN. From the Department of Zoology, University of California, Berkeley

The origin of the cleavage center or centriole of fertilized eggs has been of a controversial nature for many years. There is much evidence which shows that in cases where meiosis has been completed and the egg is in the pronucleus stage when fertilization takes place (sea urchins, tunicates, some nemertines and amphibians) the centriole of the fertilized egg is derived from the sperm middle piece, the mature egg seeming to have no active center of its own. The sperm-derived centriole then acts as an initiator or organizer for the asters and spindle of the mitotic figure and continues to duplicate itself in subsequent divisions. An excellent example of this type of behavior was observed by Boveri (1888) in eggs of the sea urchin, *Echinus*. He was able to show that when the sperm center outstrips the nucleus in its migration in the fertilized egg, it forms an amphiaster with the chromosomes of the female pronucleus, leaving the male pronucleus behind.

There are, however, conditions under which the egg, without mediation from the spermatozoön, may be made to produce all the structures required for division. For example, in artificial parthenogenesis the egg can produce asters and, under optimal conditions, a complete division figure (see reviews by Wilson, 1924, and Tyler, 1941). Clearly, these figures cannot derive from the sperm. Since (in eggs which have completed meiosis before fertilization takes place) the fate of the formerly existing egg centriole is unknown, there has been much speculation (Wilson, 1924; Briggs and King, 1959) as to whether the centers of artificially produced asters derive from this centriole or whether they arise *de novo*. Studies by E. B. Harvey (1936) on artificial activation of enucleated halves and quarters of sea urchin eggs strongly suggest that the asters formed under these conditions have been produced by centrioles with *de novo* origin.

On the other hand, there is convincing evidence that normally (*i.e.*, in cells undergoing mitosis) the centriole is a truly self-duplicating structure (Pollister, 1933; Cleveland, 1957). It would seem unlikely that such a structure would also have the ability to arise *de novo*. For this reason, it has been suggested (Brachet, 1957) that artificially produced cytasters do not arise from true centrioles at all, but may arise from any cytoplasmic granule.

Recent electron microscopic studies which show a highly organized structure of the centriole, both in vertebrate material (de Harven and Bernhard, 1956) and in marine eggs (Harris, 1961; Rebhun, 1960), stimulated the present preliminary investigation, for they offered the opportunity to determine whether the asters produced by artificial parthenogenesis might have at their center similar structures.

METHODS

Eggs of the California coast sea urchin, *Strongylocentrotus purpuratus*, were artificially activated by a modification of the "double method" of Loeb (1913). At all times suitable precautions were maintained against contamination with sperm. To every 25 cc of egg suspension, 1 cc of 0.11 N butyric acid was added for a period of 2 minutes. After this treatment

FIGURE 1

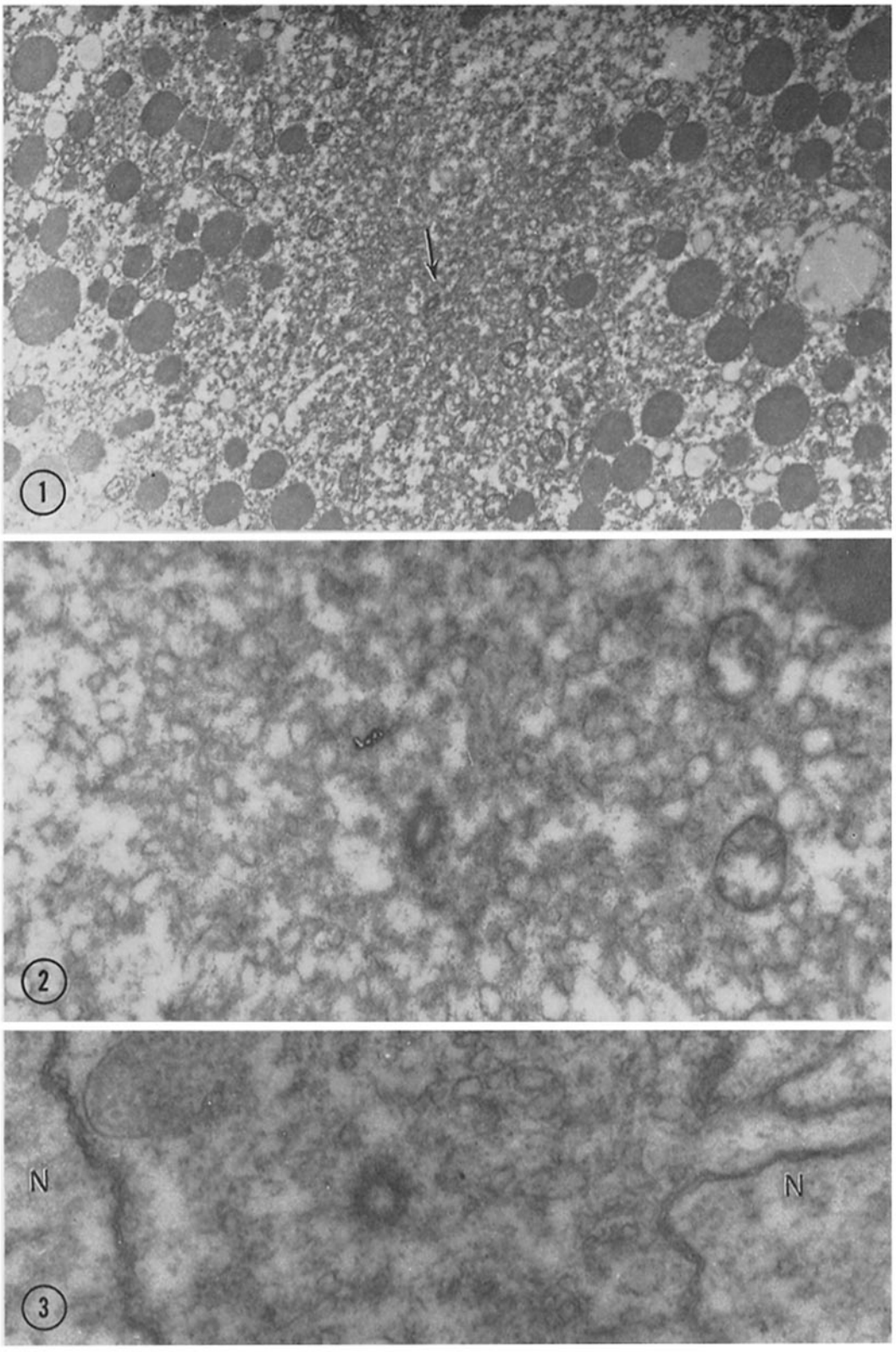
A low-power electron micrograph through an aster. The arrow points to the centriole within the astral region. × 8,480.

FIGURE 2

A higher magnification of the astral center shown in Fig. 1. Notice the small body close to the centriole. × 35,380.

FIGURE 3

A centriole between two reconstituted nuclei (*N*). × 45,140.



the eggs were immediately centrifuged and transferred to a beaker, and the volume adjusted to 100 cc. To this was added a solution of versene (EDTA), Ca-free sea water, and mercaptoethanolgluconamide (MEGA) designed to soften the fertilization membranes produced by the butyric acid activation. This method of fertilization membrane removal has been developed by Mazia and others (1961) and was used to insure proper impregnation of the fixative.

The suspension, having been allowed to settle by gravity, and the supernatant decanted, was ready for the second part of the activation treatment. To every 50 cc of eggs and sea water was added 8 cc of 2.5 M NaCl, the eggs being kept in suspension in this hypertonic solution by a glass paddle turned by an electric motor. After 45 minutes of this treatment the eggs were allowed to settle, the hypertonic solution was decanted, and sea water was added. Stirring was continued throughout the development of the eggs to insure proper aeration and a homogeneous environment.

Approximately 45 minutes after removal from the hypertonic solution asters begin to appear in the cytoplasm; at this point, and at subsequent periods of aster formation, small samples of eggs were placed in the fixative. Fixation was done in a 1 per cent osmium tetroxide solution made up in filtered sea water and buffered to pH 7.5. Due to the long procedure required for artificial activation, it was difficult to carry out all steps in one day; hence the eggs were fixed overnight at 0°C. The osmium-fixed eggs were dehydrated in a graded series of ethyl alcohols and embedded in Araldite following the procedure of Glauert and Glauert (1958). Sections were cut on a Porter-Blum microtome using glass knives, and examined with an RCA EMU-3E or 3F electron microscope.

OBSERVATIONS AND DISCUSSION

Fig. 1 is a low-power electron micrograph of a section showing a fairly typical astral region of the activated egg. Notice that the area lacks yolk particles, and that the centrosomal region, inside which the centriole can be seen (arrow), is denser and somewhat more organized. This centrosomal area is delineated by several mitochondria. The centriole is seen to be a small, oblong, electron-dense body in the very center of the aster.

Fig. 2 is a higher magnification of the central area of the aster seen in Fig. 1. This area is clear and free of large particles, an organization similar to that obtained in the fertilized sea urchin egg by Harris (1961). The clear area of the whole aster would then correspond to the transparent area in the living egg as seen with the light microscope. In the center of the aster one can observe the electron-dense structure which has come to be associated with the centriole as observed in mammalian leucocytes (de Harven and Bernhard, 1956) or chick spleen (Bernhard and de Harven, 1958). In the eggs of the surf clam *Spisula*, fixed 11 minutes after fertilization, Rebhun (1960) also shows a similar structure in the center of the aster.

It is of further interest to note that associated with the centriole is what appears to be a small body of similar electron density. Bernhard and de Harven (1958) have called these structures "satellites" or "pericentriolar" bodies. The duplication of the centrioles may be associated, according to them, with these structures, which seem to be related to certain phases of centriolar activity.

Fig. 3 shows a centriole between two reconstituted nuclei, the development of the activated egg having proceeded through several phases of nuclear breakdown, chromosome condensation, and nuclear reconstitution. (Occasionally, when two asters are formed at a considerable distance from each other, the cell does not cleave but instead becomes binucleate). There is good resolution of internal structure in this example of a centriole in an activated egg.

There is no question, then, that the structures observed in the center of the asters are true centrioles and not random particles around which fibers can orient themselves to form astral rays (Brachet, 1957; Pollister and Pollister, 1943).

The important question which remains to be answered is the question of the origin of the centriole. Their presence in the asters of activated eggs does not entirely solve the problem which has interested cytologists and embryologists for many years, namely, whether an ordinarily self-duplicating body may, under certain conditions, seem to be created *de novo*. It does solve the problem as to the nature of the astral centers.

Although there is much work which on the surface might argue for the *de novo* formation of centrioles (Yatsu, 1905; Harvey, 1936; Lorch, 1952), this evidence is based upon the ability of the eggs to produce asters, the formation of centrioles being inferred. The fact that prior to activation there is no evidence of a centriole in the egg may only mean that it was not present at a microscopically visible level.

If the centriole can generate the previously

mentioned "pericentriolar bodies," it is possible that the egg contains the material which composes these "bodies" in a disperse form, and that activation induces their aggregation with subsequent formation of centrioles. If this material is in a disperse form, it may be an evolutionary necessity which would act as a control of cell division. If the egg maintained a fully functioning centriole, then perhaps nothing would prevent it from dividing spontanously. The production of large numbers of centrioles by artificial activation can be attributed to the fact that usually these chemical means are hardly gentle or localized compared to the activation caused by the sperm. But the fact that the activated egg can produce more than one aster, and often an odd number of them, (see Wilson, pp. 684–690), may mean that the cytoplasm contains material capable of producing many centrioles, and that at the center of each aster, whether connected with the nucleus or purely cytoplasmic in origin, there is a centriole.

SUMMARY

Using a modification of the classical methods of parthenogenetic activation, asters were produced in eggs of the sea urchin, *S. purpuratus*. The activation procedure consisted of a very short exposure to butyric acid followed by incubation of the eggs in sea water made hypertonic by the addition of NaCl. After 45 minutes in hypertonic sea water the eggs were transferred to filtered sea water and allowed to develop. At the appropriate times small samples were removed and fixed in buffered osmium tetroxide. The eggs were subsequently embedded in Araldite and sectioned. Electron microscopic studies revealed the presence of centrioles in the center of the asters. These centrioles are similar in structure and electron density to those observed in both vertebrate tissues and fertilized eggs.

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