IMMUNO-ELECTRON MICROSCOPE ANALYSIS OF THE SURFACE LAYERS OF THE UNFERTILISED SEA URCHIN EGG

I. Effects of the Antisera on the Cell Ultrastructure

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ABSTRACT

The response of unfertilised *Paracentrotus lividus* eggs to γ-globulin fractions of antisera against isolated homologous jelly coat substance or homologous homogenates of jellyless eggs has been studied at the ultrastructural level. The antijelly γ-globulin caused precipitation of the jelly layer, the density of precipitation varying between different eggs and being proportional to the γ-globulin concentration. Agglutination of the jelly substance of adjacent eggs, which is species specific, occurred frequently with higher γ-globulin concentrations. Antiegg γ-globulins (from antiserum against total homogenates of jelly-free eggs or the heat-stable fraction thereof) did not produce these effects. Instead, these γ-globulins caused various structural alterations mostly representing stages in parthenogenetic activation. This species-specific activation was induced by the reaction of antibodies with some heat-stable egg antigens different from those involved in jelly precipitation. Surface alterations included the formation of small papillae, membrane blisters, hyaline layer, and activation membrane, the release of material from the cell surface, and the breakdown of cortical granules. These alterations were dependent on both γ-globulin concentration and the variable reactivity among different females. Aster formation, found intracellularly, verified that the surface responses represented real parthenogenetic activation and were not the result of immune lysis. No such alterations appeared in the controls.

INTRODUCTION

An understanding of the surface layers of the unfertilised egg is essential when studying fertilisation, since the egg surface obviously plays an important role during the initial stages, particularly in determining the specificity of sperm-egg interactions. It therefore seems logical to extend previous immunological analyses of the surface of the sea urchin egg (1–4) to the electron microscopical level, firstly by observation of the effects exerted on the ultrastructure of the living egg by the homologous antisera, and secondly by submicroscopic localisation of the various surface antigens (see following paper).

It has already been shown microscopically

that unfertilised sea urchin eggs exposed to antiserum against the isolated jelly coat around the egg or to antiserum against the unfertilised egg itself undergo precipitation of the jelly layer or parthenogenetic activation of the egg respectively (2, 5). The action of these antisera is both species specific and specific for the various surface layers of the egg. The biological effects produced reflect the presence of distinct antigens located in the jelly substance and on the egg surface (1–4). Variation in the response of eggs, particularly those from different females, indicates some inconsistency either in the number of available antigenic sites or in their reactivity (1, 2, 5).

The jelly coat of the unfertilised egg, apart from acting as a mechanically protective layer, may act as a sieve in selecting the spermatozoa capable of attachment to the actual egg surface (6). The jelly substance is the first surface layer with which the sperm comes in contact and thus presumably provides the initial step, although not the only one, in fertilisation specificity. It contains the so called fertilizin, known to react specifically with an antifertilizin in the sperm (7, 8). The jelly may also be important in releasing the sperm acrosomal reaction (9–11). The significance of the various jelly activities for the fertilisation process is unknown.

The study of parthenogenetic activation by the antiegg serum throws light on the mechanisms involved in fertilisation and allows the separation of egg activation and sperm penetration. Observation of antiserum-treated eggs is particularly helpful since the surface changes are slower and often less complete so that it is possible to build up a picture of a succession of events which is completed very rapidly in a normally fertilised egg (12). These experiments help in elucidating the degree of heterogeneity of the cell surface (*cf.* reference 13). They also show the relationship existing between the plasma membrane and the vitelline membrane and the role which these membranes have in fertilisation and its specificity.

MATERIALS AND METHODS

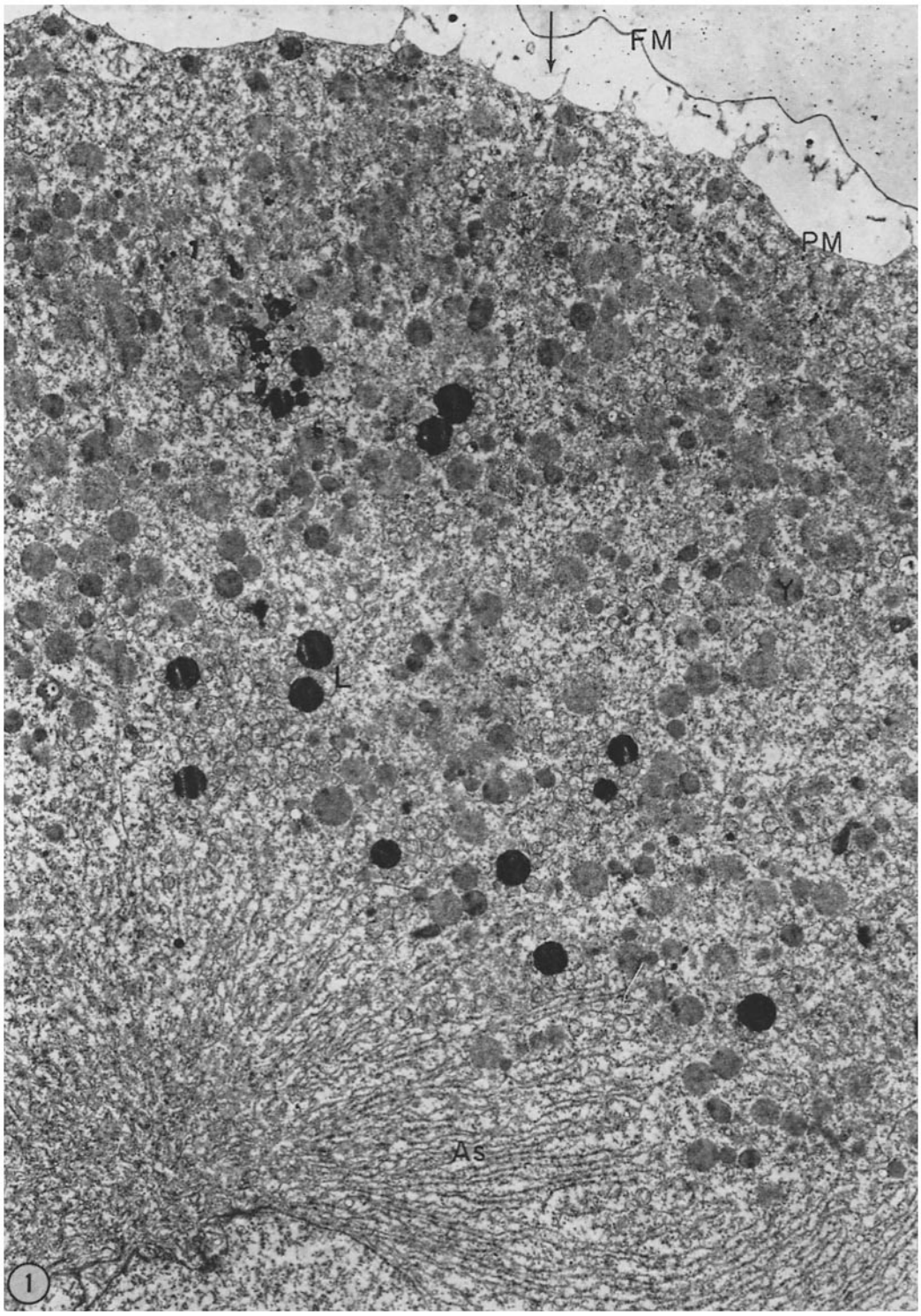
Antisera against two of each of the following preparations of *Paracentrotus lividus* gametes were prepared in rabbits as described previously (2); lyophilised homogenate of total jellyless unfertilised eggs; lyophilised homogenate of jellyless unfertilized eggs heated to 100°C for 2 hours; isolated jelly substance (2); lyophilised spermatozoa. Normal serum was also taken from unimmunised rabbits as a control.

Sera from 3 to 5 immunised or untreated rabbits were pooled and only the γ-globulin fractions were used in all experiments. These γ-globulin fractions were isolated from the whole sera by chromatography on diethylaminoethyl (DEAE) cellulose, using elution with 0.0175 M phosphate buffer at pH 6.3 (14). The eluates were concentrated to 30 to 40 mg protein/ml by dialysis against polyethylene glycol or polyvinyl pyrrolidone (15), and before use were dialysed against sea water.

For the absorption experiments, 0.5 ml of γ-globulin was mixed with 5 mg antigen, left at room temperature for a few hours, at 4°C for 2 days, and centrifuged to obtain a clear absorbed solution (3, 4). This gave complete absorption, judging by the removal of all light microscopically detectable effects (3) and by the absence of labelling when a ferritin–anti-γ-globulin conjugate was subsequently added to these absorbed preparations (16). The lyophilised preparations of unfertilised *P. lividus* eggs used for absorption were untreated or heated (100°C, 2 hours) jellyless whole eggs and isolated jelly substance. Control absorptions were also performed with *P. lividus* sperm and with eggs from the sea urchins *Arbacia lixula*, *Sphaerechinus granularis*, and *Psammechinus microtuberculatus*.

The absorbed and unabsorbed antijelly and antiegg γ-globulins were tested by the reactions they produced on living eggs; *i.e.*, jelly precipitation and activation respectively (1, 2). Fresh unfertilised eggs with intact jelly coats were taken from *P. lividus* (17) and were washed in sea water. Small numbers of eggs (500 eggs in 0.2 ml sea water) were mixed with the γ-globulin. In the jelly precipitation experiments eggs were treated for 2 hours at room temperature and in the egg activation experiments they were kept with the γ-globulin for 1 to 2 hours in the cold and then for 2 hours at room temperature. The percentage of reactive eggs in a dilution series of 80 per cent to

FIGURE 1 Electron micrograph of a control fertilised egg showing astral fibres (*As*) radiating from a region outside the nucleus. At the egg periphery is the fertilisation membrane (*FM*) and beneath is the cell membrane (*PM*) comprising the original plasma membrane and the membrane of opened cortical granules. The hyaline layer (arrow) can be seen at places. In the cytoplasm there are a number of lipid droplets (*L*) and many yolk granules (*Y*). × 5,000.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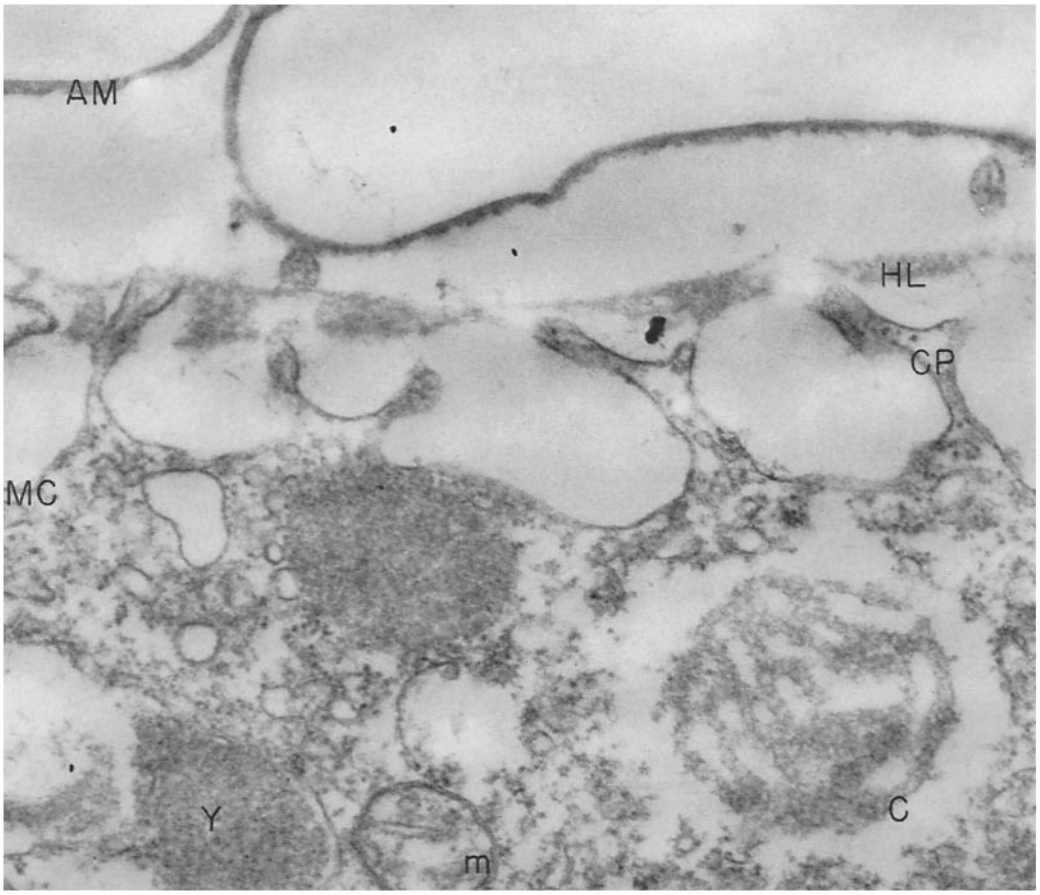


FIGURE 2 An egg 29 minutes after artificial activation by butyric acid. A distinct activation membrane (*AM*) has been formed and most of the cortical granules have opened up and their membranes (*MC*) have been incorporated into the new cell surface. The contents of one cortical granule (*C*) remain more or less intact although its membrane appears to have broken down. Cytoplasmic projections (*CP*) extend into the hyaline layer (*HL*). Yolk granule (*Y*), mitochondrion (*M*). × 48,000.

2.5 per cent γ-globulin (80 per cent = 0.8 ml of original γ-globulin solution/milliliter reaction mixture, etc.) was estimated from light microscopic observations after 2 to 4 hours of treatment as described earlier (2).

In experiments carried out for electron microscope examination (18), the eggs were treated before fixation with γ-globulin in varying concentrations as described above (unless stated, experiments with 80 per cent solutions are described here). After γ-globulin treatment the eggs were washed thoroughly in sea water, fixed with 2 per cent osmium tetroxide dissolved in sea water, dehydrated, and embedded in Epon 812 (19). Sections were cut on an LKB Ultrotome microtome. Some sections were treated either with a saturated solution of uranyl acetate (20) or with lead hydroxide (21) in order to give greater contrast. The sections were examined with a Siemens Elmiskop I at 60 kv and with a 20 or 30 μ objective aperture, except for the low magnification micrographs when a 50 μ aperture was used.

As a control of the antiserum-induced activation, chemical parthenogenetic activation experiments with butyric acid (22) were also made for electron microscopical examination.

RESULTS

The Untreated Egg

A brief description of the normal egg will be given in order to clarify the subsequent results (see also references 23–25). The unfertilised egg is surrounded by a wide jelly layer which under

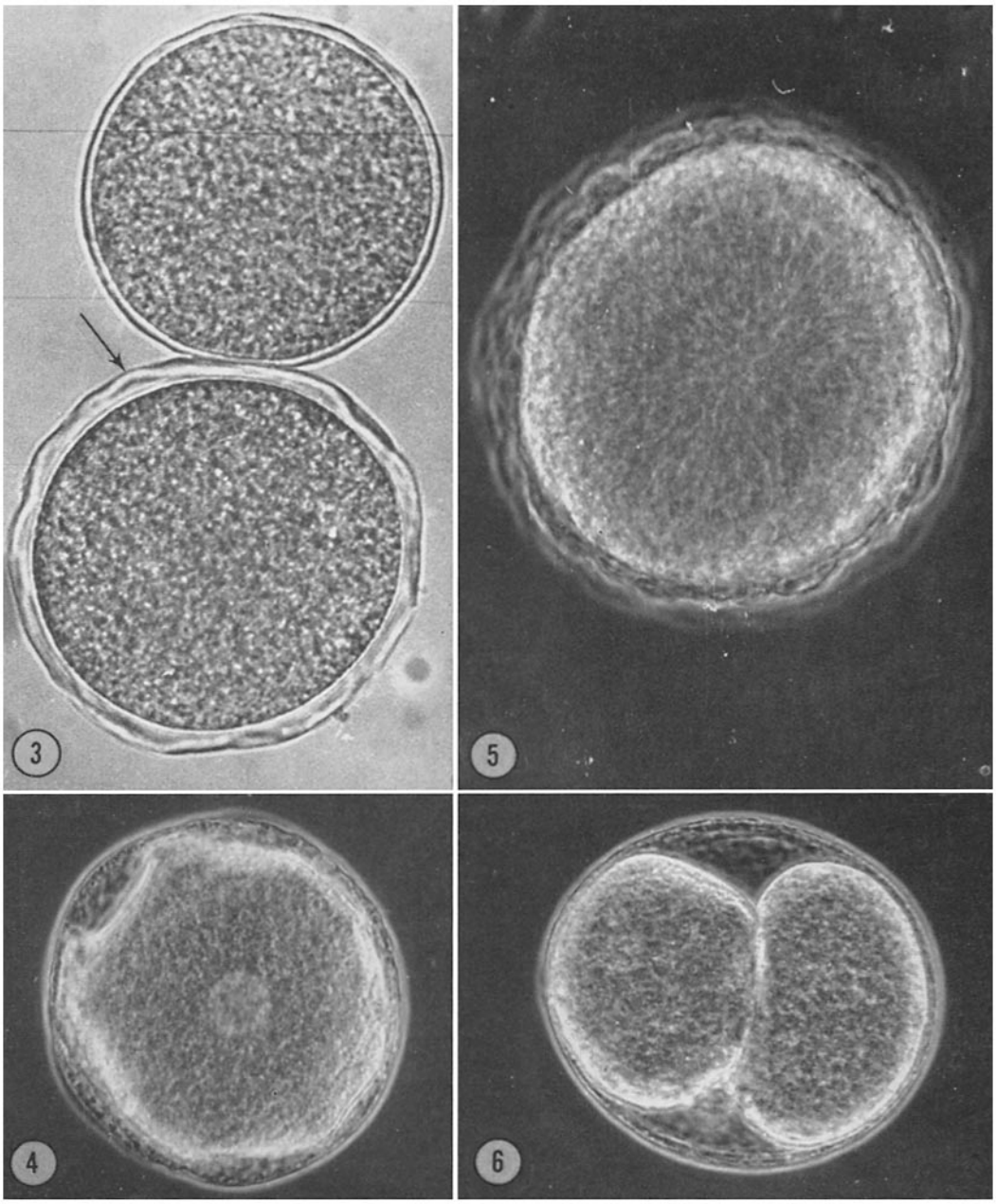


FIGURE 3 Light micrograph of unfertilised eggs after treatment with antijelly γ-globulin prior to fixation for electron microscopy. The jelly layer surrounding each egg is densely precipitated (arrow) and the eggs have become agglutinated by their adjacent jelly substances. × 600.

FIGURES 4 TO 6 Light micrograph (phase contrast) of eggs treated with γ-globulin from antisera against homogenate of jelly-free eggs. The eggs have reacted to the antiserum by becoming parthenogenetically activated, and show surface changes and nuclear migration (Fig. 4), aster formation (Fig. 5), and division (Fig. 6). × 600.

normal conditions is not sufficiently electron-opaque to be detected with the electron microscope. Its presence can be inferred, however, from the distance between the eggs lying in the embedding medium since normal eggs, in contrast with jellyless eggs, do not lie close together. The egg cell itself is enclosed by a plasma membrane of 60 to 65 A. Immediately outside this membrane, contain electron-opaque lamellae with a very distinctive periodic structure in *P. lividus* (18), electron-opaque hemispheres, and are bounded by a definite membrane about 60 A thick. Yolk granules are occasionally seen among the cortical granules at the egg periphery, but they are generally below the cortex, distributed throughout the cell cytoplasm.

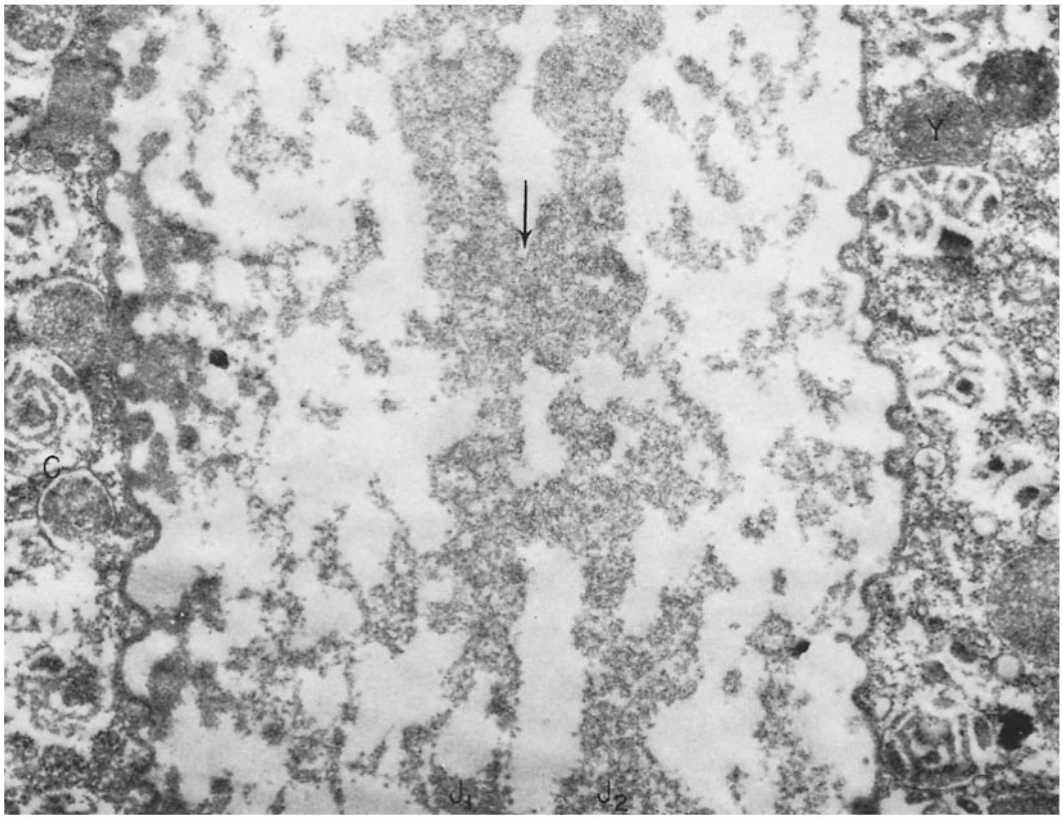


FIGURE 7 Unfertilised eggs treated for 3 hours with a concentrated solution of an antijelly γ-globulin. The jelly layer appears precipitated, particularly at the periphery (J_1, J_2). The jelly substance of the two eggs has become agglutinated in several places (arrow) thus uniting the eggs. Cortical granules (*C*) and yolk granules (*Y*) are within the egg. Compare with Fig. 3. × 25,000.

and often indistinguishable from it, lies the vitelline membrane, an extracellular layer present in the sea urchin egg (26). When separated from the plasma membrane, this layer can be seen to be about 30 A wide. The egg surface is not spherical but has very small protrusions or papillae. Cortical granules are situated very close to, and possibly in contact with, the plasma membrane in the periphery of the egg cytoplasm. These granules

In the fertilised egg, (Fig. 1), the vitelline membrane becomes lifted off over, at first part of and then all of, the egg surface, and the cortical granules open up releasing their contents which combine with the vitelline membrane to form the thick fertilisation membrane. The plasma membrane and the membrane bounding the cortical granules unite to form the intricate cell membrane with many cytoplasmic projections which reflect

the outline of the original cortical granules. Outside this cell membrane is the perivitelline space containing the diffuse hyaline layer which is partially formed from cortical granule material, from the electron-opaque hemispheres. Cortical globules (23, 27, 28) and cortical rods (23, 28, 29) may also be present in the perivitelline space. Intracellularly there is subsequently diaster formation prior to nuclear division and cell cleavage within the fertilisation membrane.

the jelly coats (1, 3, 12). Under light microscopic observation the jelly layer around the egg clearly became narrower and denser with a less uniform outline and frequently it seemed to fuse with or adhere to the precipitated jelly layer of an adjacent egg. In general, when a small number (500) of reactive eggs were mixed with a concentrated solution of the antijelly γ-globulin, all eggs reacted as described above. Decreasing the γ-globulin concentration led to a gradual disappearance

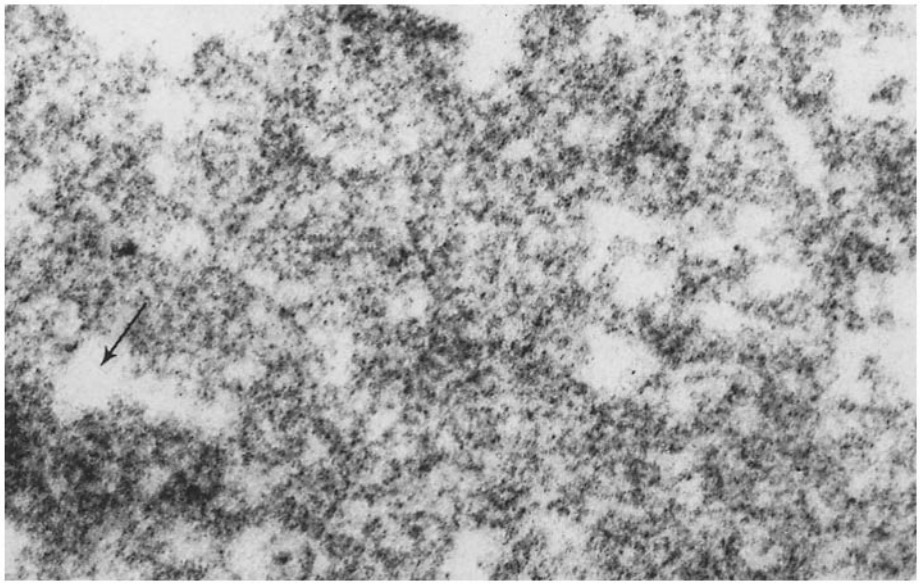


FIGURE 8 Higher magnification showing the region arrowed in Fig. 7, where the jelly layers of the two eggs are agglutinated. The jelly, which appears to have a granular structure here, is unevenly precipitated and there are areas without any apparent jelly substance (arrow). × 90,000.

The Butyric Acid-Activated Egg

In the early stages (Fig. 2) the surface of the eggs artificially activated by butyric acid appeared very similar to fertilised eggs except that the activation membrane formed was thicker and more uneven than the fertilisation membrane. There also seemed to be more cytoplasmic projections, and a number of cortical granules remained unopened at the surface although they appeared to be breaking down within the cytoplasm.

The Action of Antijelly-γ-Globulin (Figs 3, 7, and 8)

The chief effects which the antijelly γ-globulin exerts on the living egg are precipitation of the jelly substance and egg-to-egg agglutination by

of both jelly precipitation, contraction and agglutination. At 2.5–5 per cent γ-globulin concentration (see Materials and Methods) most eggs appeared normal in the light microscope. With the antiegg globulins used in these studies no reactions visible under the light microscope appeared in the jelly layer. Absorption of the antijelly globulin with 50 μg isolated jelly substance/ml completely removed all visible effects. In contrast, large amounts of jellyless eggs did not abolish the effects of the antijelly globulin. (For details and discussion see the references given above.)

Electron microscopically, a constant pattern of jelly precipitation was apparent with high concentrations (80 per cent) of γ-globulin in these experiments as well as in those where a ferritin label was subsequently added (16). The egg

surface had more papillae than in untreated eggs or in those treated with low concentrations of antijelly γ-globulin, but there were no other structural changes similar to those found in anti-egg γ-globulin treated eggs. Outside the plasma membrane there was a layer of jelly substance about 0.05 μ wide. Next to this was an electron-translucent area containing clumps of thickly precipitated jelly material and, finally, around the periphery was a wide layer (0.5 to 1.5 μ) of precipitated jelly. The outer edge of the precipitated jelly coat was 1 to 3 μ from the actual egg surface as compared with approximately 30 μ in the untreated egg (26).

The width, density, and heterogeneity of the precipitated jelly coat varied considerably among different eggs and was also dependent on the concentration of γ-globulin. It appeared that the jelly layer was more strongly precipitated on the periphery, and even this peripheral layer often seemed to be more dense on the outer margin. The precipitated jelly substance had a slightly granular, rather than fibrous, structure.

Agglutination of eggs by their jelly layers was a frequent occurrence, the connection between the eggs being intimate so that it was often impossible to tell the exact transition point separating the two different jelly substances. In some sections the outer part of the jelly layer was pulled away from the egg surface for some distance, enabling the eggs to agglutinate more firmly since a greater area of jelly coat was in mutual contact.

The Action of Antiegg-γ-Globulin (Figs. 4 to 6, 9, and 10 to 17)

As already indicated, the γ-globulin fractions from the antisera against homogenates of total jelly-free *Paracentrotus* eggs or against the heat-stable part of them did not visibly affect the jelly layer. In the light microscope, the main effects of the antiegg globulin were wrinkling of the egg surface and parthenogenetic activation. High concentrations exhibited the strongest influence on the eggs although there was a marked variation in sensitivity among the eggs of one female and among these of different females (1, 2, 5). Fig. 9 illustrates the typical decrease of the percentage activated eggs (from a highly reactive female) upon dilution of the γ-globulin with sea water. This figure also demonstrates that the activating antibodies could be efficiently removed by absorbing the γ-globulin solution with egg homogenate of *Paracentrotus* eggs. As can be seen, the heat-stable part of the homogenate was as equally efficient an absorbant as the total untreated homogenate. In contrast, no activating antibodies could be removed by absorption with heterologous egg homogenates (*Sphaerechinus granularis* and *Arbacia lixula*). In addition, no absorption

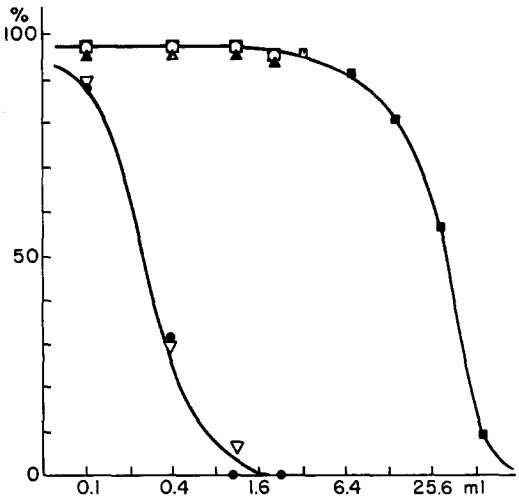


FIGURE 9 Inhibition of activation of *Paracentrotus* eggs after absorption of γ-globulin from antiserum against total jelly-free eggs. *Abscissa:* ml of sea water or antigen solutions added to 1.0 ml aliquots of γ-globulin solution. Each antigen solution contained 0.5 mg lyophilised antigen/ml. For testing, 0.8 ml of the diluted or absorbed serum was mixed with 0.2 ml of sea water containing 500 reactive eggs. *Ordinate:* per cent activated eggs. Each symbol represents 200 counted eggs. Explanation of symbols: *squares,* sea water; *open triangles,* absorption with homogenate of jelly-free *P. lividus* eggs; *solid circles,* the same homogenate first heated to 100°C for 2 hours; *solid triangles,* homogenate of *Arbacia lixula* eggs; *open circles,* homogenate of *Sphaerechinus granularis* egg.

was achieved with lyophilized preparations of *Paracentrotus* sperm while the absorbing effect of jelly substances was variable, depending on the degree of purity of the material (*cf.* reference 3).

As mentioned above, not all eggs were activated even when treated with antiegg globulin in high concentration. However, ultrastructurally such eggs showed an increase in the number and size of surface papillae. Moreover, the presence of a wide dense layer, adjacent to the plasma membrane, and the formation of a diffuse substance in the jelly region was observed (Fig. 10). This diffuse substance probably originated from the

egg surface or interior (*cf.* reference 16) and, when released, was retained by the jelly layer. Among some eggs (treated with the γ-globulin against total jelly-free eggs), fibrous material (20 to 40 A fibres), clearly visible after uranyl acetate staining (Fig. 11), was present outside the egg surface and connected to it at places. When lower

semble those occasionally found in butyric acid–activated eggs in that the bounding membranes have broken down while the lamellar structure remained intact.

The subsequent stage showed a definite separation of the vitelline membrane from the plasma membrane. It appeared thickened and in places

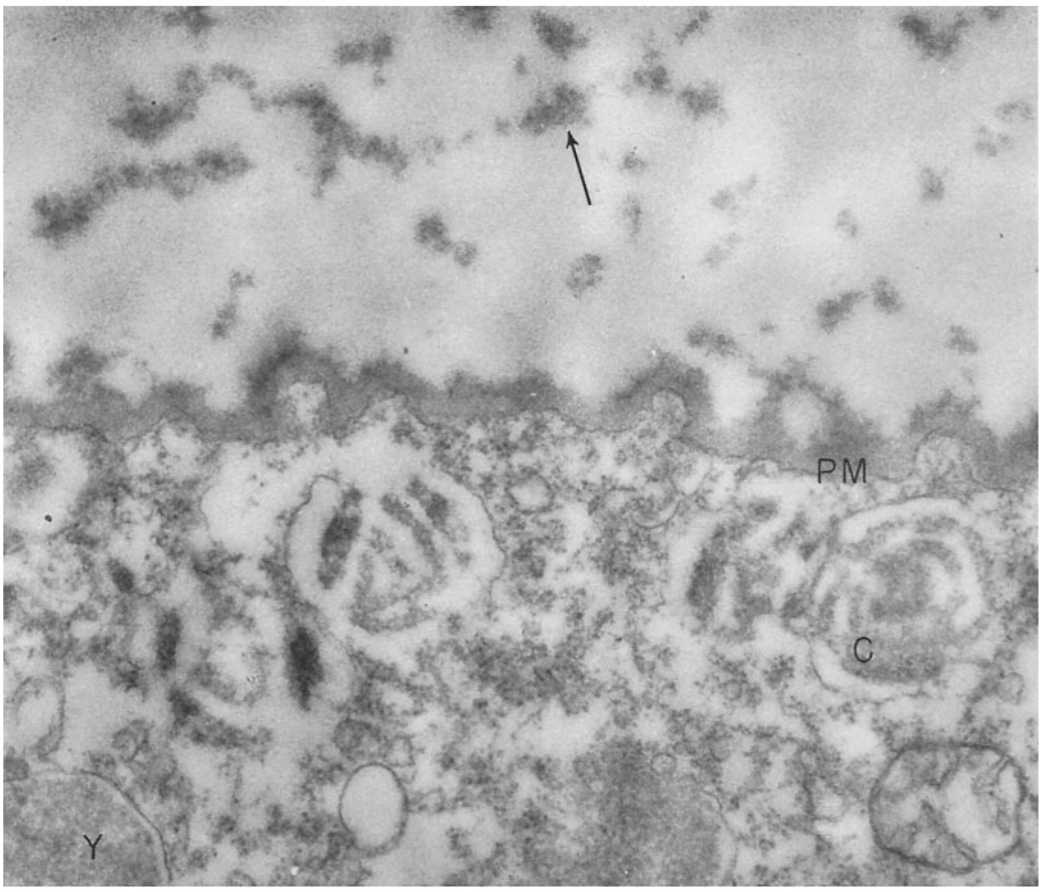


FIGURE 10 An unactivated egg which had been treated with γ-globulin from an antiserum against a homogenate of heated (100°C, 2 hours) jellyless eggs. Outside the plasma membrane (*PM*) is a diffuse layer at least 0.1 μ wide, and more of this material appears to have diffused into the jelly layer (arrow). The egg surface has more papillae than in untreated eggs. Cortical granule (*C*), yolk granule (*Y*). × 40,000.

γ-globulin concentrations (*e.g.* 20 per cent) were used, the surface changes were greatly reduced and the eggs appeared almost like the untreated controls.

The first evidence of parthenogenetic activation was seen in the cortical granules which broke down inside the cell without opening up at the egg surface (Fig. 11). These granules re-

was similar to the activation membrane. Concavities in the egg surface, formed by the plasma membrane, were covered by the vitelline or activation membrane (Figs. 12 and 14). In these eggs, as well as in fully activated eggs (Fig. 15), membrane-profiles (either membrane-bounded vesicles or cross-sectioned cytoplasmic projections) were present in the perivitelline space and at the

activation membrane. In the larger concavities, reaching a size of 15 × 22 μ, unaltered cortical granules could be seen lying below the plasma membrane as in the normal unfertilised eggs.

In the later stage of activation (Figs. 13, 15, 16) the cortical granules had opened up, although a few remained unaltered as was found in butyric acid–activated or occasionally in fertilised eggs. Many cytoplasmic projections were left along the new cell membrane which comprised the original plasma membrane and cortical granule bounding membrane. Within the perivitelline space were a few electron-opaque spheres or cortical globules and cortical rods (23, 24, 27–29) and other cortical material. The hyaline layer was generally not well formed although it could be distinguished from the activation membrane by its location rather than structure. A distinct, slightly elevated activation membrane was formed but it was less well defined and consistent than the fertilisation membrane or the membrane of the butyric acid–activated eggs. A wide layer of small aggregations of dense material was frequently found outside the activation membrane.

More evidence that these eggs were truly activated was seen intracellularly where aster formation occurred. Numerous astral rays in an orderly arrangement were visible near the nucleus, and many annulate lamellae (30–32) were found among the aster membranes (Fig. 17, *cf.* also Fig. 5). Similar results were obtained with γ-globulin from both antiegg sera (anti–total egg homogenate and anti–heat-stable part of egg homogenate).

Controls

Eggs which were treated with γ-globulin from normal rabbits in parallel with both the jelly precipitation and egg activation experiments showed no signs of reacting in either of these two ways under light and electron microscope examination. The surface appeared as in untreated eggs, although there often appeared to be a few more surface papillae.

As already described above, the addition of antijelly γ-globulin absorbed with isolated jelly substance produced no evidence of the jelly layer's being affected at all, while addition of the same antijelly γ-globulin absorbed with lyophilised jelly free-eggs resulted in jelly precipitation and agglutination. When eggs were treated with γ-globulin from antiegg serum (both anti–total and anti–heated egg homogenates) absorbed with total egg homogenate there was generally no apparent effect on the egg. Occasionally there was a small amount of slightly electron-opaque fuzzy material outside the egg surface. Figures of various control preparations will be given in the following paper (16).

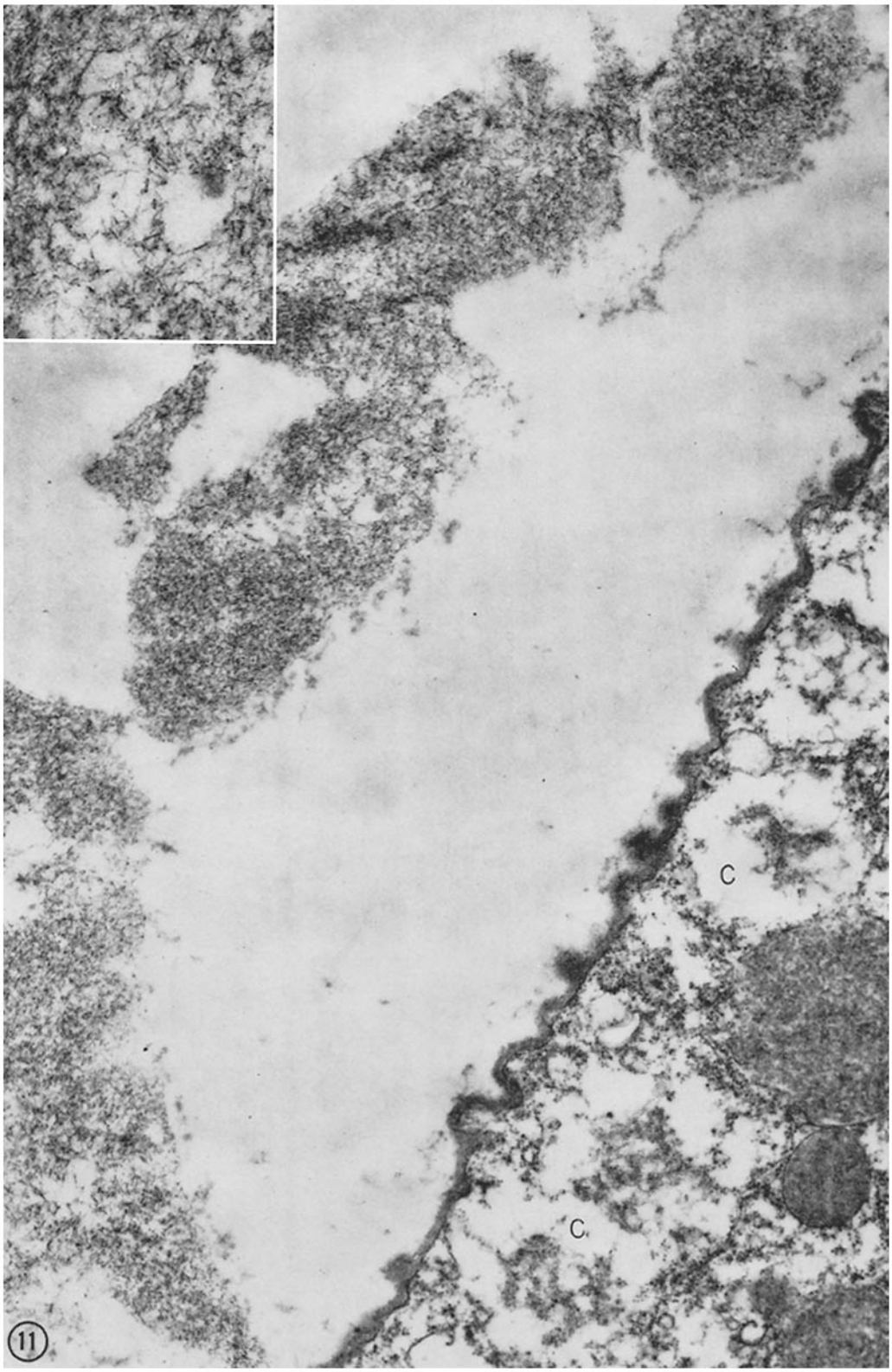
DISCUSSION

General Action of Antiserum

There are certain changes occurring in the surface of eggs treated with immune γ-globulins which can be considered as being general reactions of most living cells to antibody. The most striking effect is the formation of short papillae at intervals over the egg surface. This surface reaction is also evident, although less marked, with normal γ-globulin, but it seems to be initiated more readily by the immune γ-globulins. In antibody solution, the number and diameter of these papillae is approximately proportional to the concentration of the γ-globulin in which the eggs are incubated, those in low concentrations resembling untreated eggs.

Small projections and invaginations of the plasma membrane have also been observed in ascites tumour cells treated with antiserum (33, 34) where the complex surface folding resulted in interdigitation of adjacent cells. Although there may be some structural change in the cell surface, there are no signs of immune lysis either in the

FIGURE 11 The egg surface after treatment with γ-globulin from an antiserum against total jellyless eggs. This egg is not activated, but has reacted to the antiserum by the formation of many surface papillae and a thick layer outside the plasma membrane. The cortical granules (*C*) also appear to be breaking down. Outside the egg, and attached to it at places, is an aggregation of an electron-opaque fibrous material. Section stained with 2 per cent uranyl acetate. × 30,000. Inset: detail of the extracellular fibrous material. × 70,000.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egg cells used here or in other cells treated with γ-globulin alone (34, 35). Eggs treated with antiserum and complement together showed no signs of cell lysis, but the experimental conditions were unfavourable for complement action. It has been shown in other cells however that antibody in the presence of complement (33–38) or complement on antibody-sensitized cells (39), but not antibody with inactivated complement (33, 40), produces immune lysis allowing the escape of intracellular components through "functional holes" in an apparently intact membrane. The absence of cell lysis reported here is contradictory to the interpretation of results obtained with antiserum-treated eggs of other sea urchin species (41).

One specific reaction of the cells treated with γ-globulin from antiegg serum is the presence of relatively large amounts of extracellular material attached to the egg surface (Fig. 11). This material appears independent of either jelly precipitation or parthenogenetic activation and is not present after treatment of the eggs with antijelly globulin or antiegg globulin absorbed with egg homogenate. It has been shown to be both structurally and antigenically different from the jelly substance and is in fact immunologically an egg substance (16). The attachment of this fibrous material to the egg in certain regions also suggests that it may originate from the cell surface and is not miscellaneous cell debris present in the surrounding medium. The presence of electron-opaque extracellular debris has been reported after the incubation of ascites tumour cells with immune serum with inactivated complement (33), and has been tentatively attributed to the loss of structural elements from the cell membrane. The debris in the tumour cells did not appear to have the same distinctive fibrous structure that was seen in these egg cells however.

Jelly Precipitation

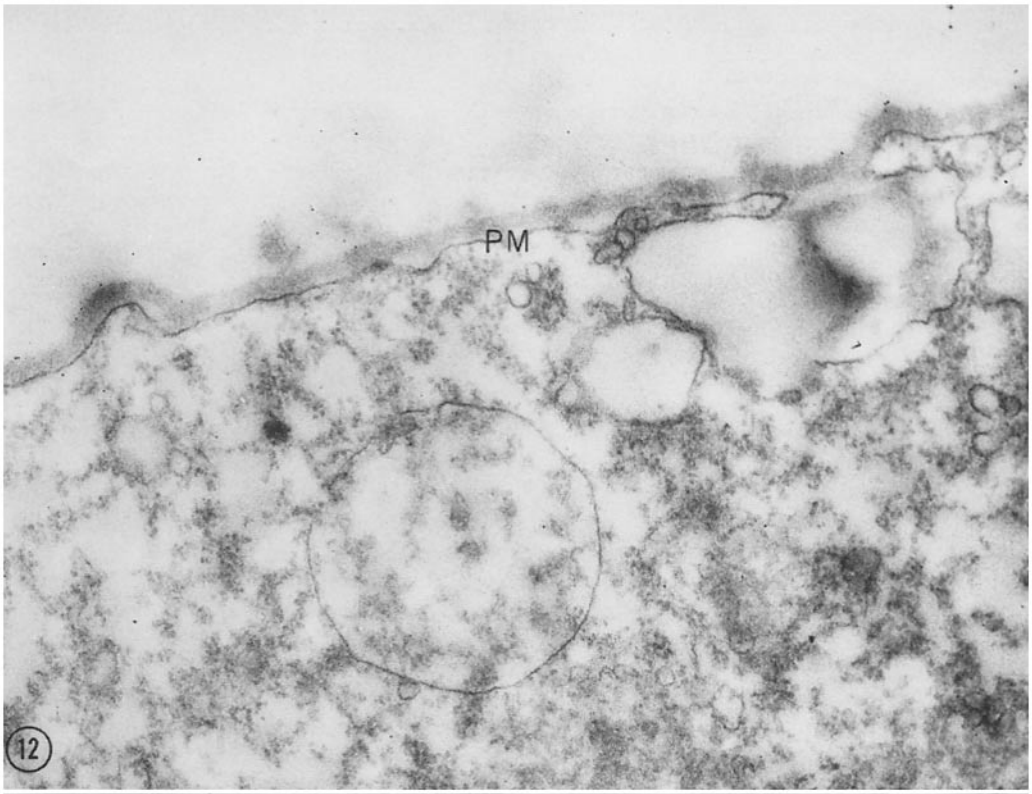
Evidence for jelly precipitation being brought about by species-specific antibodies present in the antijelly sera has been given above and in previous publications (1–3, 5). When seen in ultrathin sections, the precipitated jelly coat has a markedly heterogeneous appearance, the two densest regions being immediately adjacent to the egg surface and at the periphery of the jelly layer. Since light microscope examination also indicates a distinctive pattern of precipitation, it is unlikely that the arrangement of electron-opaque and -translucent regions observed were artefacts resulting from the preparative procedures (*e.g.* by unspecific chemical precipitation or coagulation of the jelly substance). The precipitated jelly substance has a relatively dense granular structure after antiserum treatment, but the large electron-translucent regions in the jelly layer contain no apparent jelly substance. Either a real lack of jelly substance exists in these regions or some jelly may be present which is incapable of interacting with the homologus antibody (see reference 16) and thus of being precipitated.

The binding of the precipitated jelly to the egg is presumably rather strong and stable since after antiserum treatment the jelly layer remains intact. In addition, eggs which show agglutination of adjacent jelly substances endure the washing and embedding procedures for electron microscopy and yet still remain united by a very small proportion of the surface area. In fact, the jelly-to-jelly binding is often more durable than the binding between the egg and jelly layer.

According to certain views, the jelly substance is said to be synonymous with fertilizin which is assumed to react with sperm antifertilizin in an antigen-antibody–like fashion (7, 8). Previous immunological work has shown that the jelly-

FIGURE 12 An egg after treatment with γ-globulin from an antiserum against a homogenate of total jellyless eggs. Immediately outside the plasma membrane (*PM*) is a layer approximately 600 A wide which is similar in appearance to the activation membrane. On the right side of the figure is a convolution of the plasma membrane. × 50,000.

FIGURE 13 Another egg (from the same experiment as in Fig. 12) in which parthenogenetic activation is more complete. The cortical granules have opened up and an activation membrane (*AM*) has been formed. As well as cytoplasmic projections (*CP*), there is apparently similar membrane-bounded material immediately below the activation membrane. In the perivitelline space several cortical rods (*CR*) and a partially formed hyaline layer (*HL*) are present. × 32,000.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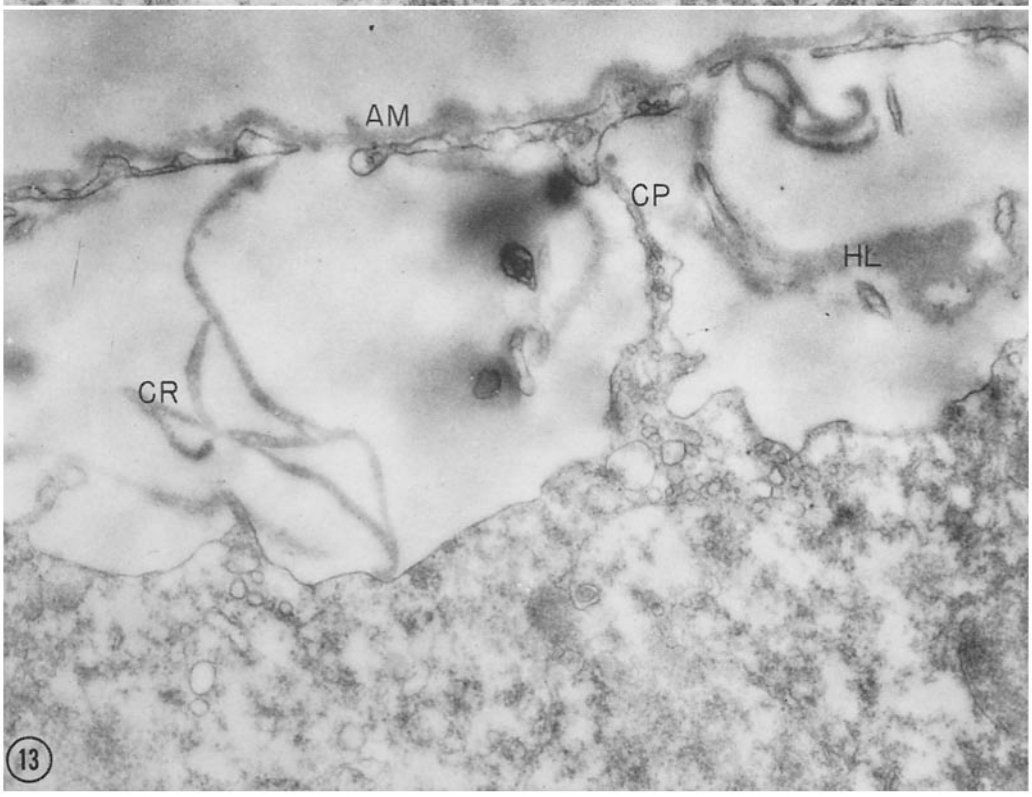
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precipitating antibodies, in spite of their reaction with the main polysaccharide constituting the jelly substance, do not seem to inhibit fertilisation (1–4), and the precise significance of the fertilizin-active principle for the specificity of sperm-egg interaction in fertilisation is at present unknown (*cf.* references 12, 41). The jelly-layer *in situ* lowers the incidence of polyspermy (42). Although this could be due to a sperm inactivation by "unsuccessful" fertilizin-antifertilizin reactions, it may also be brought about by the jelly's unspecifically diminishing the fertilising capacity of the sperm (42, 43). It is well known that removal of the jelly coat increases the fertilisability of the eggs by both homologous and heterologous sperm (44, 45). Regardless of the precise mechanism of this sperm-sieving action of the jelly layer (6), it is likely that the two main selection points are the dense peripheral and inner regions, respectively, that is, where the sperm first comes in contact with the egg at the outer surface of the jelly coat, and then, immediately prior to reaching the actual cell surface (vitelline membrane and plasma membrane), where the most important specificity selection presumably takes place (46).

Activation

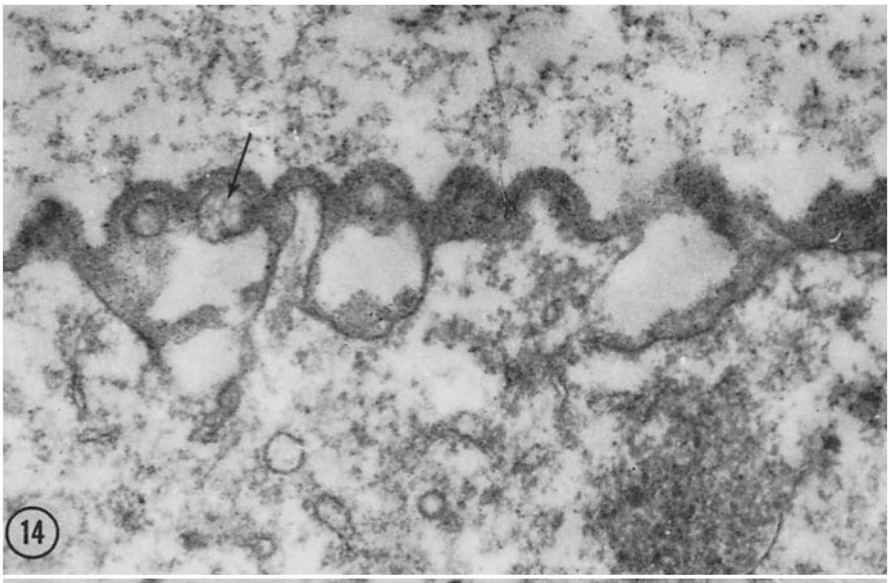
As shown above under Results and in earlier publications (1–3, 5), the surface changes which take place in the egg after treatment with γ-globulin from antiegg serum are induced by specific antibodies against antigens assumed to be part of the egg surface. The present electron microscope investigation allows the establishment of the following sequence of events involved in egg surface responses and in parthenogenetic activation. The first visible stage is an alteration of the vitelline membrane which appears considerably wider and denser. This increase could be due merely to the antigen-antibody reactions or also to additional physiological changes in the membrane, such as swelling or precipitation. These alterations may correspond to formation of an ectoplasmic layer which other workers have observed with the light microscope on the egg surface after antiserum treatment (47).

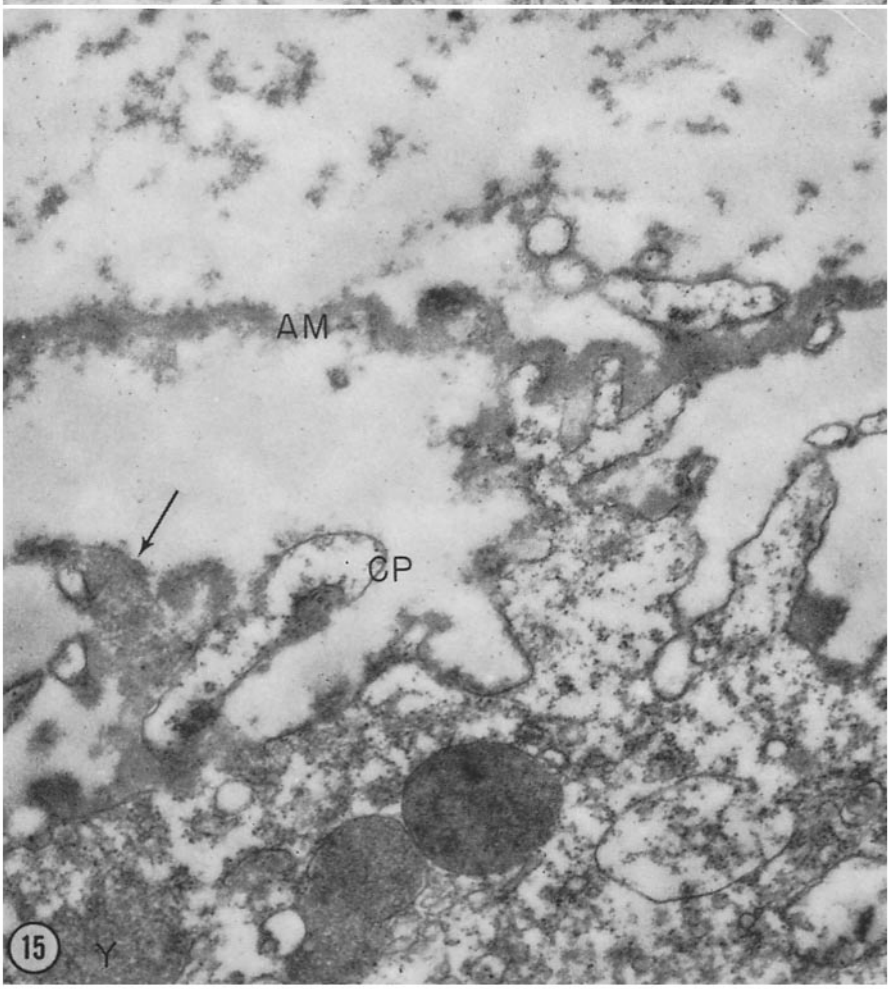
Subsequently there is the opening up of an occasional cortical granule (Fig. 12) which may occur initially only in those granules with a very low stimulation threshold for being broken down (48, 49). The plasma membrane is now covered by a distinct layer which structurally could be either the vitelline membrane or the activation membrane, since it closely resembles the membrane in fully activated eggs. This layer appears fairly well formed even without the contribution of the cortical granules. It often appears as if the membrane bounding the cortical granules is more sensitive than the actual contents of the granule, since in many partially activated eggs the membrane appears broken down while the internal structure of the granules is easily recognisable (Fig. 11), or even almost intact. In these cases the continuity of the plasma membrane itself is apparently unaffected although the outer layer adjacent to it is dense and thickened. Even if the cortical granule membrane is broken down the contents do not appear to undergo a structural change as normally occurs. This may be due to the membrane's disintegrating gradually all over, instead of breaking at one particular point, so that the sudden change in pressure necessary to eject the electron-opaque cortical granule material is lacking. The usual subsequent decrease in cortical rigidity (49) is presumably also absent.

In fully activated eggs there is a diffuse, but less

FIGURE 14 An unfertilised egg treated with γ-globulin from an antiserum against homogenate of total jellyless eggs. The egg shows an early stage in parthenogenetic activation. Small membrane blisters have been formed and are covered by a wide layer. Some membrane-bounded structures (arrow) are seen within this layer. Electron-opaque material has diffused into the region of the jelly layer. × 50,000.

FIGURE 15 The same experiment as in Fig. 10, showing an egg (from a different female). The egg has reacted to the antiegg γ-globulin by becoming parthenogenetically activated. The cortical granules have opened up leaving many cytoplasmic projections (*CP*). A distinct activation membrane (*AM*) has been formed. Outside it, material which has diffused into the jelly layer can be seen, as in the unactivated egg. No definite hyaline layer is present but some electron-opaque material (arrow) occurs in the perivitelline space. Yolk granule (*Y*). Section stained with uranyl acetate. × 30,000.


14


AM
CP
15
Y

distinct, elevated membrane which is thicker and less uniform than both the activation membrane after butyric acid–treatment and the normal fertilisation membrane. This is a layer rather than a true membrane, but is called activation membrane by analogy with the fertilisation membrane. The lack of uniformity in this activation membrane may well depend on the variable contribution of the cortical granules.

Apart from its localisation, the crudely formed activation membrane is often difficult to distinguish from other material below it (in the perivitelline space) which is presumably homologus with the hyaline layer of fertilised eggs. Where most of the cortical granules have reacted this layer is thick, whereas in partially activated eggs which do not have the full contribution from the cortical granules the hyaline layer is indistinct. This is in accordance with the observation of a quantitative (50) relationship between the breakdown of cortical granules and the establishment of the hyaline layer. Its completion is important in the fertilised, although not in the activated, egg as it may play some role in preventing polyspermy (49–51).

In the fully activated egg the cytoplasmic projections extending from the cell surface into the perivitelline space are abundant and somewhat disorganised. In contrast to fertilised or butyric acid–activated eggs, membrane-bounded material (possibly representing cytoplasmic projections) is found even in the activation membrane of serum-activated eggs.

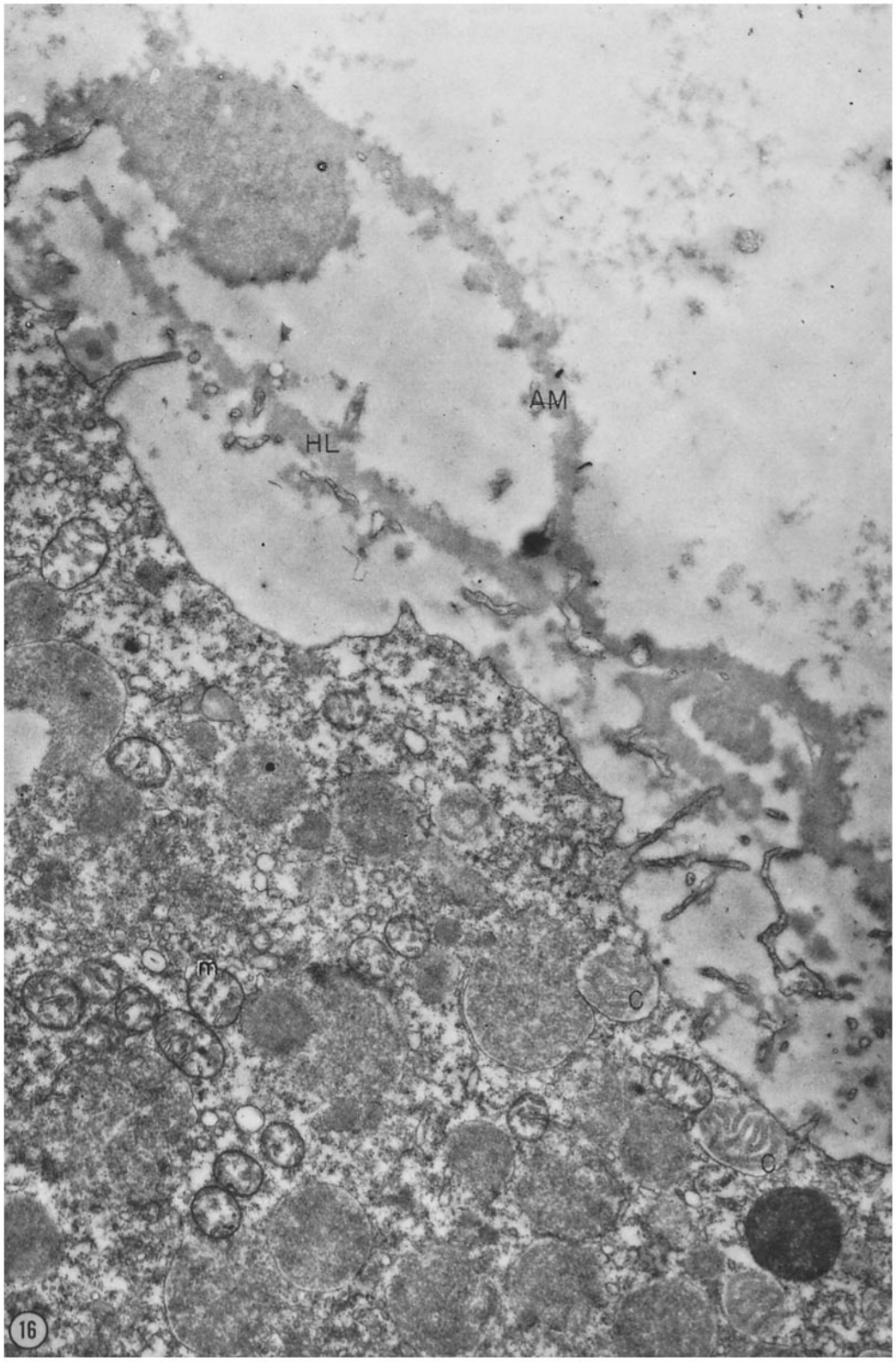
It seems probable, from the electron micrographs, that a propagating cortical wave (52, 53) is either absent or inhibited when the sea urchin eggs are activated by specific antibodies. In the earlier stages the localised effect of antibody can be recognized by the formation of small membrane blisters. Even in almost completely activated eggs the blister-like outline is still retained (Fig. 16).

At fertilisation the activation of the unfertilised egg by the sperm appears to involve sequential surface changes, such as cortical granule breakdown and membrane elevation, which are propagated in a wave or impulse over the entire egg surface (52). This propagatory mechanism is not apparent in serum-activated eggs however. The γ-globulin may therefore act without any propagatory mechanism, causing local cortical granule breakdown and other surface changes directly and only where the surface is exposed to antibody. Alternatively, the γ-globulin activation may initiate the stimulus causing the impulse as in fertilised eggs, but a secondary reaction would inhibit either its subsequent propagation or the expression of the propagated wave within the egg surface. In any event, since a propagating impulse does not carry the cortical changes rapidly over the whole egg surface, cortical granule breakdown and membrane elevation only occur locally. This is similar to the non-propagatory mechanism of activation induced by wasp venom or sodium choleinate (53). In contrast, butyric acid activation produces a propagation of the cortical reaction similar to that brought about by periodate (54) or by sperm in fertilised eggs.

In parthenogenetic activation, or in other cases where the contribution of the sperm to the egg cytoplasm is absent (55), a monaster is formed. Many of the antiserum-activated eggs showed aster formation similar to that seen in normally fertilised eggs (see references 56, 57), where the dense concentration of astral rays excluded most other cytoplasmic elements such as mitochondria, yolk granules and lipid droplets. No centrioles were observed in these experiments although it is probable that they were present but undetected, especially as centrioles have been found in chemically activated eggs (58).

The presence of the aster in electron micrographs and the occurrence of cell division observed with the light microscope (Fig. 6) give conclusive proof of the eggs being truly activated by the antiegg γ-globulin. This also indicates that most or all of the egg surface has reacted and that the surface alterations involved in activation are completed since unfertilised (and presumably

FIGURE 16 An unfertilised egg parthenogenetically activated by the γ-globulin from antiserum against a homogenate of total jellyless eggs. A wide activation membrane (*AM*) has been formed and at places appears to be diffusing into the jelly coat. The layer (*HL*) structurally similar to the activation membrane is homologous with the hyaline layer. Most of the cortical granules have opened up and released their contents, but a few remain more or less intact (*C*). Mitochondrion (*M*). Section stained with lead acetate. × 17,000.


AM
HL
M
C
C
16

unactivated) egg cytoplasm (59) or cortex (50) is believed to inhibit the subsequent nuclear processes. In the following paper (16), a study of the submicroscopic localisation of the various surface antigens will be presented.

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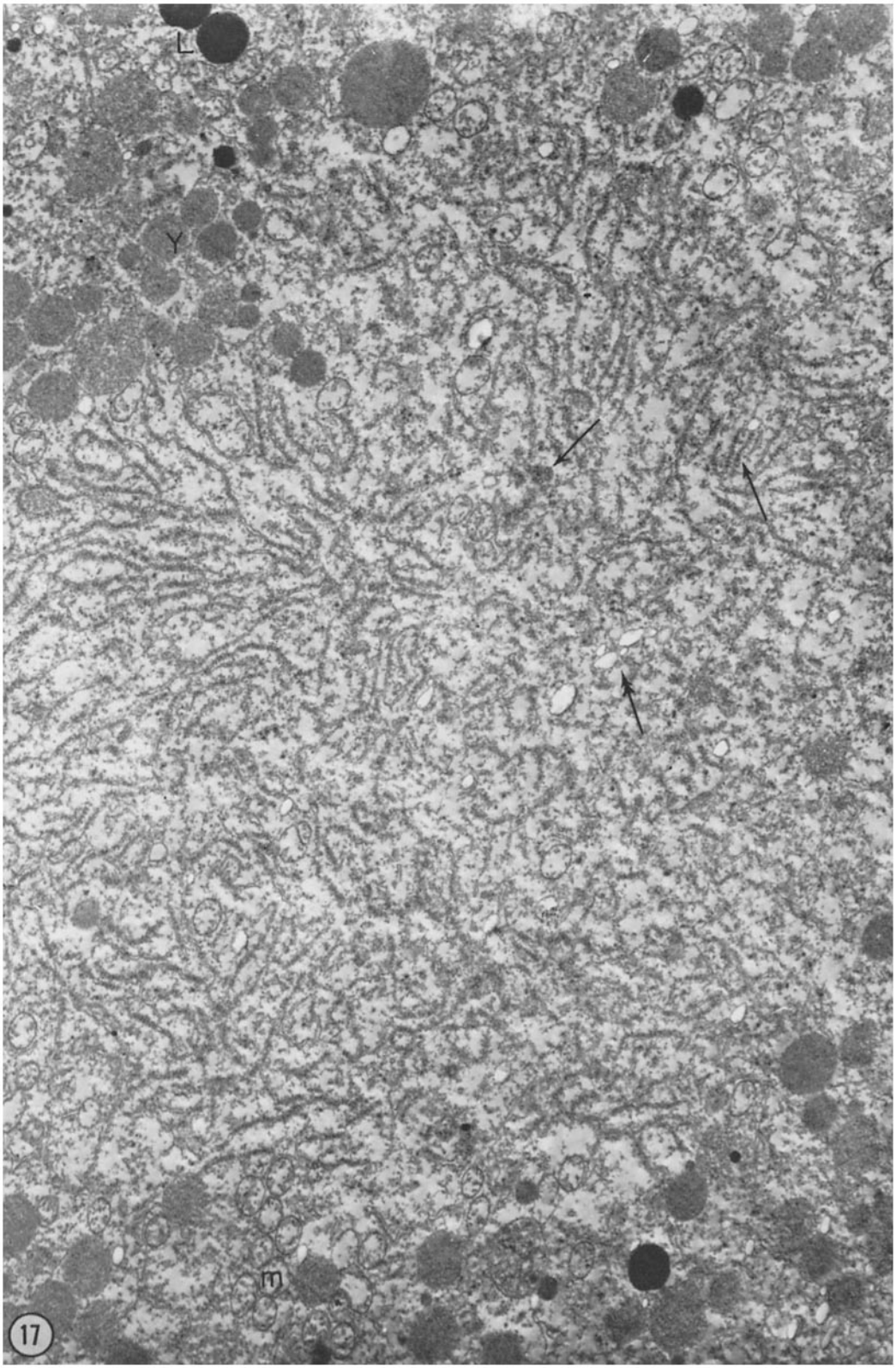
FIGURE 17 An egg from the same experiment as in Fig. 14. The antiegg γ-globulin has caused parthenogenetic activation, and aster formation is evident intracellularly. Here the aster region has a circular outline and the membranes are in an approximately radial arrangement. A large number of annulate lamellae (arrow) occur among the astral rays, and vesicles with contents even less electron-opaque than the embedding medium are also present (double-barbed arrow). Yolk granules (*Y*), lipid droplets (*L*), and most of the mitochondria (*M*) are excluded from the aster region. Compare with Fig. 5. × 11,000.


L
Y
m
17

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