toads, and for that reason we owe them every protection.

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On Artificial Parthenogenesis of the Sea-urchin Egg

From July 1 until now I have been studying artificial parthenogenesis in *Arbacia punctulata*. I succeeded in rearing the larvæ made parthenogenetic by treatment with carbonated sea-water (5 minutes) followed by hypertonic sea-water (about 30 minutes) for several weeks in Roscoff filter-aquaria. At the end of a month there were so few alive that I did not consider further attention to them worth while, owing to the possibility (though improbable) of contamination with foreign plutei when renewing the water (that was dipped up daily at the end of the wharf at high tide). I found no constant difference between parthenogenetic and fertilized larvæ.

It thus being doubtful that I could produce sexually mature adults parthenogenetically, I confined my further studies to early phases. J. Loeb considers the essential event in artificial parthenogenesis to be the production of a free-swimming embryo or larva—but why larva rather than any other stage. In natural parthenogenetic development the end result may be a maturation or segmentation stage or a larva or adult. Though only the reproductive adults are of significance to the species, all are of significance to science. It might also be remembered that Loeb's parthenogenetic *Chætopterus* "larvæ" were unicellular structures, resembling trochophores only in the possession of cilia and by an irregular redistribution of cytoplasm, and were incapable of further development.

In *Arbacia punctulata* maturation takes place in the ovary, but no segmentation occurs without fertilization or an artificial stimulus. The ovarian egg is surrounded by a thick coat of a jelly-like proteid that swells slightly and gradually dissolves in sea-water. It is practically invisible, but can be located by adding to the medium, Chinese ink, the particles of which stick to its surface. The inner surface of the jelly fits tightly against the egg. The jelly is stained by neutral red or methylene blue, which causes it to contract and pull away from the egg. Acids cause it to contract and become more dense and sticky. Tannin coagulates it into a coarsely granular yellowish mass. Alkalies cause it to dissolve more rapidly, as does also agitation. When the egg is fertilized or put in "membrane-forming" solutions a fluid is extruded which pushes the jelly out from the surface of the egg. The inner surface of the jelly is then sharply defined and is probably bounded by a thin membrane (the "fertilization-membrane") as spermatozoa wriggle freely through the jelly but can not pass its inner surface.

As membrane formation does not occur in all parthenogenetic *Arbacia* eggs it was considered of secondary importance. The next change seen in developing *Arbacia* eggs is the migration of the red pigment plastids to the surface. I first thought this due to the formation of asters, but on sectioning could find none. In the living egg these plastids take up neutral red or methylene blue before other parts of the egg, and in fixed material stain with Delafield's hæmatoxylin stronger than other parts of the cytoplasm. Parthenogenetic reagents when used in sufficient concentration cause the pigment to diffuse out of these plastids into the surrounding cytoplasm and from it into the sea-water, showing that both plastid membrane and cell plasma membrane are permeable at this time.

Loeb showed a similarity between hæmolysis and artificial membrane formation. It has long been supposed that hæmolysis is due to an increased permeability of the plasma membrane as hæmoglobin diffuses out. Ralph Lillie supposes artificial parthenogenesis and stimulation to be due to an increased permeability of the plasma membrane. This assumption is supported by my observation of the diffusing out of the pigment in the *Arbacia* egg.

Repeating the experiments of others and making new ones, I tried various types of agents that cause hæmolysis or stimulation to see whether they caused parthenogenetic development in *Arbacia*. I succeeded in causing segmentation by isotonic NaCl and by the

following chemicals and conditions in sea-water: acids, alkalis, hypertonicity, hypotonicity, ether, greatly diminished oxygen, potassium-cyanide, heat, cold, induction shocks and mechanical agitation. In many cases the eggs segmented while they remained in the artificial solution. They would not segment in sea-water charged with carbon dioxide unless most of the gas were allowed to leave the sea-water. They segmented in weak alkalis, hypertonic sea-water, diminished oxygen, KCN and cold.

From the above we may conclude that the various parthenogenetic agents could not have a similar chemical action and must have some common physico-chemical action, most probably changing the permeability of the plasma membrane, thus allowing the escape of carbon dioxide (as suggested by Lillie). The fact that eggs will not segment in concentrated carbon-dioxide demonstrates the last point. Lyon showed that the escape of carbon-dioxide from sea-urchin eggs varied rhythmically during cleavage, which suggests that a period of increased permeability is necessary for each cleavage. The stimulus to parthenogenetic development need only be applied once and the egg becomes automatic like any other cell.

The question arises whether these agents have any additional effect besides changing the permeability of the membrane. When the membrane becomes permeable some of the reagents must enter the cell. Probably this is the reason that some reagents start development that continues indefinitely, whereas after others development soon ceases (the eggs being injured by the reagent). Some chemicals may cause an irreversible permeability that does not initiate segmentation but causes death, but these will not be considered. It seemed to me that if the reagents caused a simple physical change, one could be made to act as quickly as another by finding the proper concentration, and this I tried to do. Fifteen seconds' exposure was sufficient with acetic acid while about seventeen hours was necessary with potassium cyanide. It is evident that the actions of the two are different. Probably the KCN slowly enters the egg while the membrane is relatively impermeable and by retarding certain enzyme actions brings about increased permeability of the membrane. Or the KCN may make the membrane permeable immediately and then enter the egg, retarding the production of carbon-dioxide and thus necessitating a longer period of permeability.

Since the egg becomes automatic after one of a number of stimuli the question arises why it did not remain automatic like every other cell in growing regions of the mother. In studying the cell lineage of parasitic Copepods I found that the germ cells could first be distinguished from the soma cells by their slow rate of division. In the thirty-two cell stage, one cell is the primary germ cell and it does not divide as soon as the other cells do, but grows larger than they do. Probably its failure to become sufficiently permeable to divide as soon as the others allows it to grow larger and become the germ cell. This may be true of all its progeny and in the final generation, the primary oocytes, enormous growth takes place and division is impossible without a special stimulus. The plasma membrane may not be sufficiently permeable for cell division and yet allow the passage of nourishment. Perhaps fats and lecithin may enter the cell by dissolving in the lipoids of the membrane.

To sum up, we may conclude that all agents initiating parthenogenetic development in the egg of *Arbacia* cause increased permeability of the plasma membrane, but some agents act differently from others, either by having an indirect action or by producing additional effects.

J. F. MCCLENDON

WOODS HOLE, MASS.,
Aug. 31, 1909

SOCIETIES AND ACADEMIES

AMERICAN MATHEMATICAL SOCIETY

THE sixteenth summer meeting and sixth colloquium of the society were held at Princeton University during the week September 13 to 18, 1909. The four sessions of the summer meeting proper occupied the first two days. Thirty-nine members were in attendance. At the opening session Professor Fine presided, being relieved at the later sessions by Professor Morley and Vice-presidents Kasner and Van Vleck. The following new