

the transfer of fluid is in itself the essential or "inogenetic" part of the process. It is of course obvious that any theory which (*e. g.*) regards contraction as due to a swelling of the isotropic segments by fluid absorbed from the anisotropic—as discs of gelatin or fibrin swell in acidulated water—must require that the interchange of fluid should be rapid and promptly reversible; hence that part of the present interpretation which regards the structure of striated muscle as essentially a means for facilitating transfer of fluid within the cell is equally consistent with this latter theory. Nevertheless the point of view that regards absorption of water by an acidulated sheet of gelatin as the analogue of what occurs in muscular contraction is radically different from that set forth in this paper, according to which the energy of contraction is the transformed surface-energy of the ultimate structural elements or colloidal particles (sub-microns) composing the fibrils. There is undoubtedly a movement of fluid between the muscle-segments during contraction; but this fact in itself is consistent with either of the two theories just contrasted. The decision between the two must be made on the basis of other evidence.

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FERTILIZATION OF THE EGGS OF VARIOUS INVERTEBRATES BY OX-SERUM

I

THE chemical method of artificial parthenogenesis has thus far been worked out with any degree of completeness, only for the Californian sea urchin, *Strongylocentrotus purpuratus*. In this form it was shown by Loeb that the process of fertilization is composed of two entirely different phases. The one is an alteration or destruction of the surface layer of the egg. This alteration of the cortical layer may or may not result in the formation of a fertilization membrane. The alteration of the surface can be brought about by a great many different means, all of which have

a cytolytic effect. The superficial cytolysis starts the development of the egg but leaves the latter with a tendency to perish during the further development. The sickly condition is remedied by a second treatment of the egg, which may consist in putting the eggs for about from 30 to 50 minutes into hypertonic sea water of a certain concentration. If taken out of this solution, the egg develops practically normally.

Experiments on heterogeneous hybridization which Loeb carried out, furnished the evidence that the spermatozoon also causes the development of the egg by carrying two agencies into it, one of which is a cytolytic substance, a lysin, which causes the membrane formation.

Lysins are contained not only in the spermatozoon but in all the cells and in the blood of any animal. Loeb found five years ago that the blood of a worm, *Dendrostoma*, calls forth membrane formation in the unfertilizing egg of the sea-urchin. This blood retained its fertilizing power when diluted as much as several hundred times with sea water.

The same author found subsequently that the blood and tissue extract of many animals had the same effect, *e. g.*, the blood of cattle. The fact that the blood of each female does not cause the parthenogenetic development of its own eggs, Loeb explained by the theory, that while the lysins contained in the blood of foreign species can diffuse with comparative ease into the egg and the cells of an animal, the lysins contained in its own blood are prevented from such a diffusion.

It was found impossible to cause the development of the eggs of all female sea-urchins by means of foreign blood. This difficulty was overcome by treating the eggs with strontium chloride before they were exposed to the foreign serum. If the sea-urchin eggs were put for a short time into a $\frac{3}{8}$ or $\frac{1}{4}$ M solution of strontium chloride, a subsequent treatment with ox blood caused them all to form fertilization membranes. When subsequently treated for a short time with hypertonic sea water, most of the eggs developed into normal plutei.

II

While in this way the mechanism of fertilization was cleared up to a large extent for the sea-urchin egg, very little had been accomplished with the eggs of other invertebrates. The eggs of a great many forms had been caused to develop by artificial means but the development was often very abnormal.

Artificial parthenogenesis was caused in the eggs of molluscs by Kostanecki as well as by Loeb, but the development was abnormal in as much as it resulted in the production of larvæ without previous segmentation. In the egg of *Cumingia*, another mollusc, efforts to produce artificial parthenogenesis had failed entirely. In annelids the results were not very satisfactory either. In *Chaetopterus*, e. g., Loeb produced parthenogenetic larvæ, but they developed without segmentation as he first observed and as was later ascertained beyond doubt by F. Lillie.

If the lysin theory of fertilization was correct, it was necessary to find out whether artificial parthenogenesis with an approximately normal type of development can be caused in the eggs of all animals by foreign blood. Experiments which we have carried on this year seem to indicate that this can be done to a large extent.

III

We first ascertained that the eggs of *Arbacia* behave essentially like those of *Strongylocentrotus purpuratus*. The eggs of *Arbacia* are sensitized by putting them for a short time into a $\frac{3}{4}$ M solution of strontium chloride. They were then exposed for about 10 minutes to ox-serum which had been rendered isotonic with sea water through the addition of sodium chloride. The eggs were then transferred for 20 minutes into hypertonic sea water. Such eggs developed normally into plutei. The only difference between the behavior of the eggs of *Arbacia* and *Strongylocentrotus* is that the eggs of *Arbacia* do not form a very distinct membrane. It is needless to say that the necessary controls were made and that we made sure that the treatment of the eggs with strontium choride or with strontium chloride

and subsequently with the hypertonic solution, did not lead to the formation of embryos, although occasionally a few segmentations could be brought about in this way.

We next worked with the eggs of *Cumingia* which had been found to be refractory to the other methods of artificial parthenogenesis. We obtained an apparently perfectly normal segmentation of the eggs and the formation of larvæ, by treating them in the following way: The eggs were sensitized to the effects of serum by placing them for from 2 to 4 minutes into a $\frac{3}{4}$ M solution of strontium chloride. They were then placed for five minutes into ox-serum rendered isotonic with sea water and diluted with an equal part of a $M/2$ solution of $\text{NaCl} + \text{CaCl}_2 + \text{KCl}$. After having been freed from all traces of serum by repeated washing in a Ringer solution they were transferred for 60 minutes into hypertonic sea water (50 c.c. sea water + 8 c.c. $2\frac{1}{2}$ M NaCl). Control experiments showed that the treatment with serum is the essential factor in this process.

We induced segmentation in the eggs of *Chaetopterus* by putting them for from $1\frac{1}{2}$ to $2\frac{1}{2}$ minutes into a mixture of 25 c.c. $\frac{3}{4}$ M strontium chloride + 25 c.c. $M/2$ $\text{NaCl} + \text{CaCl}_2 + \text{KCl}$, then for ten minutes into ox-serum diluted with its own volume of the above mentioned solution and then by putting them for thirty minutes into hypertonic sea water. From fixed and stained preparations which Dr. Bancroft made for us, we made sure that the nuclear and cell division was real and not merely apparent.

While the method needs to be perfected in some details, the experiments show that it is possible to induce, with the aid of foreign blood serum, parthenogenetic segmentation and development into larvæ, in eggs which had been found refractory to the other methods of artificial parthenogenesis. The lysin theory of fertilization is therefore more generally applicable.

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