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Initiation of Development in the Egg of Arbacia. I. Effect of Hypertonic Sea-water in Producing Membrane Separation, Cleavage, and Top-Swimming Plutei

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INITIATION OF DEVELOPMENT IN THE EGG OF *ARBACIA*.

I. EFFECT OF HYPERTONIC SEA-WATER IN PRODUCING MEMBRANE SEPARATION, CLEAVAGE, AND TOP-SWIMMING PLUTEI.

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It is well known from Morgan's work that unfertilized eggs of *Arbacia* may be induced to develop through exposure to hypertonic sea-water. Morgan, however, did not investigate the action of hypertonic sea-water on the unfertilized egg much beyond its effect in producing cleavage. Loeb extended these results of Morgan: he was able by the use of hypertonic sea-water to produce plutei from the unfertilized eggs of *Arbacia*. Two outstanding features of Loeb's work strike the reader: first, he was not able with the use of hypertonic sea-water to call forth "membrane formation"; nor was he able to obtain plutei of great viability, since these failed to swim at the surface as do plutei from normally fertilized eggs.

With his now classic method of exposing urchins' eggs to butyric acid in sea-water before or after exposure to hypertonic sea-water, Loeb was able to correct both these defects. On the basis of these findings Loeb founded his famous lysin theory of fertilization. He reasoned that butyric acid, as all hæmolytic agents, brings about a "superficial cytolysis" of the egg and thus the formation of the "fertilization membrane." This "superficial cytolysis," however, tends to be lethal and hence the egg must have a corrective treatment to offset the initiation of death changes. The hypertonic sea-water acts as this corrective factor. According to Loeb, it is of no moment whether he uses the corrective agent first and follows with the cytolytic agent or *vice versa*. In other words, the "uncorrected" egg may be first corrected, then superficially cytolyzed; or the egg may be first superficially cytolyzed and saved from death by the corrective factor. In any event, it is clear not only from Loeb's work, but that of others,

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that this double treatment of the eggs of sea-urchins produces top-swimming larvæ.

Loeb's work with agents of superficial cytolysis and the corrective factor led him to solve the fertilization problem in this wise: The sperm carries a lysin which initiates a superficial cytolysis of the egg; thus the first effect of the sperm is comparable to the action of butyric acid. But the sperm, reasons Loeb, also carries a corrective factor which checks the action of the lysin that otherwise would kill the egg. This reasoning is aided by the fact that in many ova the internal changes of fertilization leading to cell division are preceded by demonstrable cortical changes.

I have attempted to point out that this theory of Loeb fails to explain fertilization, and this for several reasons. Waiving not only the fact that Loeb has produced cell division and swimming plutei from uninseminated urchin eggs with the use of hypertonic sea-water before or after the treatment with butyric acid, whereas in the fertilization of these eggs the cortical changes always precede the internal—cell division—phenomena, but waiving also the fact that hypertonic sea-water alone will give cleavage and plutei, we must discard the superficial cytolysis-corrective factor theory of fertilization for two reasons: First, this theory emphasizes too much purely hypothetical substances in the sperm for which we have not a single bit of evidence; and, secondly, it wholly ignores the fact that the egg is a highly irritable system, thus in no wise different from other living substance; that there are naturally parthenogenetic eggs would indicate this. Moreover, the high degree of susceptibility to shaking of such eggs as those of *Asterias, Amphitrite, Nereis,* and the effect of sea-water in starting up maturation in eggs of *Podarke, Chætopterus,* etc., show how labile are some uninseminated marine ova. This work on the experimental production of cell division and larvæ is of importance in showing that ova are independent, activable systems; they are inherently irritable—not a difficult physiological conception. But as a means of elucidating the problem of fertilization, this work on "artificial parthenogenesis," so called, has failed; it has actually obscured the fertilization problem.

For these two reasons, then, the superficial cytolysis-corrective factor theory of experimental parthenogenesis has no logical status as an explanation of fertilization. Fertilization can be explained only by observation and experiment on ova and sperm during fertilization. It can not be explained by mere analogy of the processes in experimental parthenogenesis.

But the superficial cytolysis-corrective factor theory as an explanation of experimental parthenogenesis itself is open to grave suspicion. First, the corrective factor may operate alone and give results. In the second place, the corrective factor, says Loeb, may act first when, according to the theory, there is nothing to correct, and the cytolytic agent may follow presumably to vitiate the action of the corrective factor. Again, as I have previously pointed out, the theory is largely built on the assumption that the proper exposure to butyric acid for inducing membrane formation is cytolytic because an over-exposure is lethal. This does not follow. One might just as well argue that since stimulation of the cardiac components of the vagus causes cessation of the heart beat the normal function of these fibers is to kill the animal.

Nevertheless some may hold, despite these criticisms, that the superficial cytolysis-corrective factor hypothesis is still a valid explanation of experimental parthenogenesis; that while it is true that most marine ova need but a single agent to induce development, eggs of sea-urchins need two. If, now, we can show for the egg of *Arbacia* that a *single* agent acting alone can induce both membrane formation and cleavage, then again is the famous theory put to question. And if, more than this, we can show that this single agent is the corrective factor—*anti-cytolytic,* if you please—then the superficial cytolysis-corrective factor theory must be rejected, for the egg of *Arbacia* at least, as an explanation not only of fertilization, but also of experimental parthenogenesis as well.

The present communication aims to present data, accumulated during the season of 1921 at the Marine Biological Laboratory, Woods Hole, Mass., to show that hypertonic sea-water alone acting on the uninseminated eggs of *Arbacia* will give membranes, cleavage, and viable surface-swimming plutei scarcely to be distinguished from those resulting from normally fertilized eggs.

I.

If the uninseminated eggs of *Arbacia* be exposed to sea-water made hypertonic by the addition of NaCl or KCl in the proportions 50 parts sea-water plus 8 parts 2½ *M* NaCl or KCl, on return to normal sea-water they are induced to cleave and develop plutei. The per cent. of eggs that develop depends upon the length of exposure which will vary somewhat with different lots of eggs. Too brief an exposure will call forth merely the monaster condition and few, if any, of the eggs cleave; too long an exposure will produce cytasters, the resulting cleavage being abnormal. These eggs do not form membranes.

If 15, 16, and 17 parts 2½ *M* NaCl or KCl plus 85, 84, and 83 parts sea-water, respectively, are employed, the results are similar to those obtained with the hypertonic sea-water mentioned above (in the proportion 8 parts 2½ *M* NaCl or KCl plus 50 parts sea-water). With hypertonic sea-water made up with 20, 22, and 24 parts 2½ *M* NaCl or KCl plus 80, 78, and 76 parts sea-water, respectively, however, the results are quite different. In these and stronger hypertonic solutions of sea-water *the eggs lift off membranes while in the solutions.* The time from the instant that one treats eggs with a solution to that at which the eggs form membranes will vary with the strength of the solution. Thus in full strength 2½ *M* NaCl or KCl eggs lift off membranes in 15 seconds. In the solution 24 parts 2½ *M* NaCl or KCl plus 76 parts sea-water the eggs lift off membranes in five to ten minutes. Solutions between these two strengths call forth membranes at rates proportional to the degree of hypertonicity. The rate at which eggs lift membranes while in the solutions depends thus upon the strength of the solution.

The solution 18 parts 2½ *M* NaCl or KCl plus 82 parts sea-water gives about 3 per cent. membranes. It is thus the minimum concentration for the production of membranes. Hypertonic sea-water below this concentration does not yield membranes.

On the whole, the optimum concentration is that which gives the highest per cent. of membranes and which likewise allows an exposure longer than that to produce membranes without any deleterious effect on the eggs as revealed by their subsequent fate

on restoral to normal sea-water. Such an optimum lies around 22 parts 2½ *M* NaCl or KCl plus 78 parts sea-water. The solutions 20, 22, and 24 parts 2½ *M* NaCl or KCl plus 80, 78, and 76 parts sea-water, respectively, were the ones used most extensively. In general, portions of eggs from one female were exposed to each of these concentrations to cover any variation of the eggs with respect to their response to treatment with hypertonic sea-water, since these concentrations are around the optimum. The following table summarizes results of a part of the forty experiments on this point. It is scarcely necessary to say that in all this work extreme precautions were taken against accidental insemination. In none of the experiments did the control, uninseminated eggs in sea-water, show a single membrane.

TABLE I.

EFFECT OF HYPERTONIC SEA-WATER ON EGGS OF ARBACIA AS SHOWN BY THE PER CENT. OF EGGS THAT SEPARATE MEMBRANES WHILE IN THE HYPERTONIC SEA-WATER.

Date of Experiment.	Per Cent. of 2½ *M* NaCL[1] in Sea-water to which Eggs Exposed.	Per Cent. of Eggs that Separate Membranes.
June 26	14	0
"	16	−1
"	18	3
"	20	35
"	22	60
"	24	78
June 27	14	0
"	16	−1
"	18	3
"	20	20
"	22	46
"	24	56
June 30	20	70
"	22	93
"	24	84
July 1	20	94
"	22	78
	24	63
July 25	20	48
"	22	69
"	24	94
July 30	20	83
"	22	87
"	24	80

[1] 2½ *M* KCL gives closely similar results.

This table shows, I think, that hypertonic sea-water alone will induce membrane separation. In the most successful experiments

every single mature egg showed a membrane. Since, moreover, experiments were made throughout the season, the results can not be interpreted as mere incidental findings based on insufficient data.

These membranes induced by hypertonic sea-water separate more slowly than membranes lifted from the eggs following normal insemination. These membranes are, nevertheless, as clear and as distinct and possess as wide a perivitelline space as normally fertilized eggs. In the hypertonic sea-water the egg shrinks, its periphery retaining a smooth contour. One gains the impression that the perivitelline space arises in part as the result of this shrinkage. That this is not wholly correct seems to be indicated by those eggs that undergo an equal amount of shrinkage without forming membranes. Moreover, on return to sea-water the egg, though it increases in size, does not obliterate the perivitelline space.

If the intensity of the membrane separation process be too great, the membrane formed is eccentric; the perivitelline space is not of the same width in all zones of the egg. In such cases the egg, as seen in optical section, is flattened in that zone above which the membrane is at its greatest distance from the egg. On return of the egg to normal sea-water this eccentricity of the membrane persists. The cortex of that zone, in these cases, from which the membrane has separated least, is apt to be swollen. This seems to indicate that the reaction underlying membrane must be of a certain order to insure best results.

The membrane does not always arise in the manner described. In some cases the egg presents a crenated surface beneath the membrane. This crenation may quickly disappear, leaving the egg cortex below the membrane perfectly smooth. If the crenation persist, on return to normal sea-water the perivitelline space is very narrow; indeed, it may be absent, in which case the membrane is closely stuck to the swollen cortex.

Finally, in some cases the membrane may be extremely thin, though otherwise the egg and perivitelline space are about as found in the normally fertilized egg.

These observations on the effect of hypertonic sea-water in bringing about membrane separation, fortunately, do not stand alone. I find that Loeb almost twenty years ago made a similar

observation on the egg of *Strongylocentrotus*. Using concentrated solutions (2½ and 1½ *n* NaCl and 2½ *n* and 2 *n* cane sugar), Loeb[1] found that the unfertilized eggs of *S. purpuratus* form membranes in the same way as in fertilization. The details of his description differ very little from those I have given above for the egg of *Arbacia*.

Moore, working with *Arbacia*, was able by the use of hypertonic sea-water alone (16 c.c. 2½ *M* NaCl plus 50 c.c. sea-water) to obtain "quite a considerable number of membranes." According to Moore, however, these membranes are not like the fertilization membranes produced in normal fertilization.

In those lots of eggs that show a high per cent. of immature eggs, some mature eggs may fail to show membrane separation in any concentration of hypertonic sea-water. Stale eggs often fail to respond to hypertonic solution with membrane separation. Blood inhibits membrane separation and enhances the cortical changes that give the thick swollen cortex. Eggs that fail to form membranes in the hypertonic sea-water are invariably from lots that yield a low per cent. of membranes following normal insemination. We may consider these points in detail.

On June 29, July 8, July 18, for example, uninseminated eggs were mixed with blood. In each experiment the eggs were divided into three lots—*A, B,* and *C*. *A* was untreated (control), *B* inseminated, and *C* exposed to hypertonic sea-water. Not a single egg in any of the lots *B* formed membranes. The lots (*C*) treated with hypertonic sea-water (20, 22, and 24 parts 2½ *M* NaCl plus 80, 78, and 76 parts sea-water, respectively) gave a low per cent. of very poor membranes; instead, in the majority the egg cortex became badly swollen. Nothing was more clearly brought out in the work than this sharp inhibition by blood both in fertilization and in experimental parthenogenesis.

Several experiments, for example, those of August 1, 2, and 3, were made on washed eggs. These established that eggs lose their capacity for artificial activation more quickly than their capacity for fertilization. In one case eggs washed but four times in an hour were highly fertilizable, as shown by the presence of 96 per cent. membranes and subsequent normal development.

[1] *Pflüger's Archiv*, '04, 103.

Uninseminated eggs from this same lot exposed to hypertonic sea-water gave only 17 per cent. membranes. Eggs a day old that have been repeatedly washed never gave membranes with hypertonic sea-water, though they were capable of responding to insemination with complete membrane separation.

Immature eggs give no response to treatment with hypertonic sea-water, as experiments early in June revealed.

The best criterion, we may conclude, for the capacity of the eggs to respond to treatment with hypertonic sea-water is their response to insemination. Eggs from the same lots as those which, when inseminated, rapidly lift off fine membranes everywhere equidistant from the eggs with wide perivitelline spaces are the best for hypertonic sea-water treatment. Eggs in the presence of blood, stale eggs, and immature eggs, lift few or no fertilization membranes. Such eggs yield poor or no results with hypertonic sea-water.

I pass now to the consideration of the type of cleavage and plutei resulting from *Arbacia* eggs exposed to hypertonic sea-water (20, 22, and 24 parts 2½ *M* NaCl or KCl plus 80, 78, and 76 parts sea-water, respectively, and sea-water of greater hypertonicity). And I may say at the outset that the quality and per cent. of membranes separated in hypertonic sea-water are indices of cleavage and the production of plutei. The production of cleavage and of surface-swimming plutei are of the best quality and most numerous from those lots of eggs with best membranes, provided, always, that the exposure is optimum. Data on this point are summarized in Table II.

TABLE II.

PER CENT. OF CLEAVAGE AND OF PLUTEI FROM EGGS OF ARBACIA FOLLOWING EXPOSURE TO HYPERTONIC SEA-WATER IN WHICH THE EGGS SEPARATE MEMBRANES.

Date of Experiment.	Per Cent. of Membranes Formed in the Hypertonic Sea-water.	Per Cent. of Cleavage in Eggs on Return to Sea-water.	Per Cent. of Top Swimming Plutei (Estimated).
July 17	96	93	85
" 19	34	37	25
" 20	41	32	25
" 29	88	79	70
" 30	92	89	85
Aug. 1	84	77	65
" 2	0	7	0

The experiments here cited, a fraction of the total, show that the best cleavage and plutei, both as to quality and per cent., are invariably found in those eggs that produce the best membranes. At times the results are perfectly wonderful. Thus on August 9 eggs exposed the day before to KCl hypertonic sea-water gave gastrulæ (and later plutei) that were scarcely to be distinguished from those arising from normally fertilized eggs. The seven dishes were simply alive with surface-swimming forms. These eggs had lifted off very fine membranes. On the other hand, on August 6 the eggs treated with KCl hypertonic sea-water lifted off very poor membranes. They produced inferior cleavages and larvæ. The cleavage and larvæ resulting from exposure to sea-water of such concentration that membrane separation does not take place in no wise compare to those from eggs in which membrane separation takes place in hypertonic sea-water.

In my experience insemination of eggs on return to normal sea-water following an exposure to hypertonic sea-water that calls forth membrane separation is not possible. If the cortical reaction is complete and full membranes separate, insemination does not increase the per cent. of cleavage and of plutei. In eggs induced to form membranes by hypertonic sea-water the cortical reaction is therefore complete and irreversible.

The results here reported are in every way equal to those obtained with the butyric acid-hypertonic sea-water method. Indeed, in my experience the results with the use of the strong hypertonic solutions have proved superior to the butyric acid-hypertonic sea-water method. And certainly the use of hypertonic sea-water alone is far more simple. With butyric acid one must get just the right exposure for membrane separation. In any lot of eggs, a mixed population, all eggs do not have precisely the same optimum point of exposure to butyric acid for perfect membranes. Moreover, even with the very highest per cent. of membranes following butyric-acid treatment, the worker must again at intervals give the eggs exposure to hypertonic sea-water of various lengths. Three optima must the worker, therefore, obtain for best results: optimum exposure to butyric acid, optimum length of time in sea-water following butyric-acid treatment before exposure to hypertonic sea-water, and optimum exposure to hypertonic sea-

water. With the hypertonic solutions used in the experiments here presented, the case is quite otherwise: one simply notes the time in hypertonic sea-water to membrane separation and allows roughly twice this length of time before removal to normal sea-water.

But the main point in these experiments, it seems to me, is not the inferiority or the superiority of this method of a single exposure to hypertonic to the butyric acid-hypertonic sea-water method. If the experiments here reported simply revealed that the single hypertonic sea-water treatment only calls forth membrane lifting, they would be, it seems to me, worthy of report. And for this reason: If hypertonic sea-water be capable of inducing membrane separation, then we must discard the superficial cytolysis-corrective factor hypothesis for experimental parthenogenesis, as we have already discarded this hypothesis as explaining fertilization. I propose, therefore, to discuss these results, since they involve to a far-reaching degree current conceptions of the mechanism of experimental parthenogenesis.

II.

The evidence submitted above shows (1) that sea-water, if made sufficiently hypertonic, is alone capable of inducing membrane separation in the eggs of *Arbacia;* (2) that such eggs give good cleavage and practically normal gastrulæ and plutei; and (3) that the highest per cent. and normality of cleavage and of plutei result when the membrane separation most closely simulates the separation of the vitelline membrane as a cortical response to insemination. If this be true, several important considerations follow with regard to the nature of the processes underlying membrane separation and to the interpretation of these processes in the physiology of the developing egg cell. These considerations follow:

1. In the first place, membrane separation certainly can not be due to any mere surface tension change. According to Traube,[1] substances are effective in calling forth membrane separation the more they lower surface tension. From this it follows that hypotonic sea-water should be capable of inducing membrane separa-

[1] "Ueber Parthenogenese," J. Traube, *Biochem. Zeitschr.*, Bd. 16, 1909, pages 182–186. Cf. also, McClendon, *Am. Jour. Phys.*, 10, 27, 240.

tion. This is true, as Schücking found. Toluene, etc., should likewise be effective, and they are (cf. Herbst). But surely one could scarcely insist upon this same explanation for the effect of the hypertonic sea-water employed in the experiments here reported.

Moreover, in the eggs both of *Arbacia* and of *Echinarachnius* any competent observer can see that membrane separation following insemination is no mere surface tension effect, but an active progressive dissolution of cortical material. In the egg of *Echinarachnius* one can actually observe the cortex going into solution; in the egg of *Arbacia* pigment escapes at this time. If, therefore, we experiment with agents that induce membrane separation, in order to solve the problem of the cortical changes in normal fertilization; despite the fact that such agents do lower the surface tension of the sea-water, we are not justified in the light of the observed phenomena in normal fertilization to postulate any theory at variance with these observed phenomena. Such postulates must cease to have any scientific value.

To be sure, it may well be that the membranes induced by these agents are not at all comparable to those induced by sperm. Nor, indeed, does it follow that membranes induced by hypertonic sea-water are like those induced by sperm. The main point, however, is something more than this. Hypertonic sea-water, which certainly is not of lower surface tension than normal sea-water, does call forth membranes while the eggs are in the solution. If we must adhere to the surface tension hypothesis, then we must conclude that the effect of hypertonic sea-water is an exception—as is the effect of the sperm in calling forth membrane separation by a cortical breakdown which follows in a wave beginning at the entrance point of the sperm.

2. Again, the experiments here reported are at variance with the notion that the separation of the membrane is due to a superficial cytolysis.

As I understand it, the term cytolysis connotes a cellular disintegration. One certainly can not use the term in its strict etymological sense particularly since that misnomer "superficial cytolysis" has now become widely current. Unfortunately many zoölogists use the terms cytolysis and plasmolysis interchangeably.

If we define cytolysis as a breaking up of the cell within the membrane or actual liberation of the cell contents, we may define plasmolysis as a shrinkage of the cell contents. Now, certainly hypertonic sea-water as employed in these experiments never caused any liberation of the cell contents. We can not, therefore, regard the action of hypertonic sea-water as cytolytic.

There is another way of reaching a conclusion in this matter. Prolonged exposure to butyric acid in sea-water will cause the uninseminated egg of *Arbacia* on return to normal sea-water to form a fine gelatinous film instead of a membrane. Such eggs, as Loeb noted, soon cytolyze. We may accept this specific instance as a definition. Now, such eggs go to pieces by droplet formation; thus they disintegrate. Or, if eggs with membranes induced by exposure to butyric acid are allowed to lie in normal sea-water, they eventually disintegrate by the formation of globules in the cortex. The disintegration eventually involves the whole egg.

In hypotonic sea-water both *Arbacia* and *Echinarachnius* eggs take up water, lose pigment, and assume a granular appearance. The contents then slowly disappear as if washed away. Rarely do the contents of the eggs burst through the membrane before total disintegration.

Now, the effect of hypertonic sea-water on these eggs is unlike that of butyric acid or hypotonic sea-water. Rather the effect of hypertonic sea-water is plasmolytic. In it the egg shrinks, becomes darker. On return to normal sea-water such an egg, if it fail to develop, remains intact for hours.

Unless, therefore, we change the meaning of the term cytolysis, the hypertonic sea-water employed in these experiments is not cytolytic. Instead of disintegrating, the eggs on return to normal sea-water cleave, gastrulate, and reach the pluteus stage.

3. If we admit that hypertonic sea-water does not call forth membranes by superficial cytolysis, then we must conclude that the hypothesis of a superficial cytolysis as part of the mechanism of experimental parthenogenesis is as unnecessary for a theory of experimental parthenogenesis as it is superficial and inadequate for a theory of fertilization. This must follow for several reasons.

First, we well know from older work that hypertonic sea-water

alone is sufficient for the production of plutei. So-called agents of superficial cytolysis do, of course, improve results, but are not absolutely essential. Moreover, for many eggs hypertonic sea-water alone will initiate development; the majority of ova that respond at all to agents that initiate development need but a single agent. The egg of *Arbacia* is no exception. It is entirely unnecessary to use an agent of superficial cytolysis as either a primary or secondary factor for the production of a high per cent. of plutei of great degree of normality.

Secondly, according to Loeb, the agent of superficial cytolysis may be used either before or after the hypertonic sea-water. If butyric acid is as effective after hypertonic sea-water treatment as before, on what logical grounds can we speak of hypertonic sea-water as a corrective factor for the superficial cytolysis yet to take place?

Finally, the hypothesis of a superficial cytolysis as part of the theory of experimental parthenogenesis is untenable because it assigns a rôle to hypertonic sea-water which is the opposite of that of any agent of superficial cytolysis. Since, as shown above, the hypertonic sea-water alone, if of sufficient strength, does just what the butyric acid will do—call forth membranes—the case falls. In order to save the theory, it would be necessary to assume that the hypertonic sea-water of the strength used by the writer to induce membranes has two effects.[1] First, it acts as butyric acid by superficially cytolyzing the egg; and, second, it acts as a corrective factor to correct its first effect. This interpretation in turn entails assumptions which together make it worthy of no serious consideration.

If, for example, we insist that the first effect of hypertonic sea-water is cytolytic, then we must change the connotation of the word cytolysis. Further, the hypertonic sea-water employed by the writer brings about membrane separation while the eggs are *in the solution*. This fact, now, entails an interesting assumption: since following butyric-acid treatment as employed by Loeb the egg of *Arbacia* cytolyzes on return to sea-water, therefore indi-

[1] Loeb does make just this assumption. I must confess, though, that I fail to follow his reasoning. See Loeb ("Artificial Parthenogenesis and Fertilization," The University of Chicago Press, 1913, page 159.)

cating that the butyric acid renders the egg more susceptible to sea-water cytolysis (that is, the acid acts as a catalyst to the ordinary cytolytic action of sea-water on the uninseminated egg), the hypertonic sea-water of the concentrations used by the writer possesses three distinct actions: (1) It prepares the egg for cytolysis as does butyric acid; (2) it cytolyzes as does the normal sea-water following butyric-acid treatment; and (3) it corrects this cytolysis as does the hypertonic sea-water as used by Loeb. Of course, this may well be. It does seem, however, a rather cumbersome suggestion.

It would thus appear that the hypertonic sea-water being alone sufficient, butyric acid is not necessary. Since, moreover, as I have elsewhere pointed out (Just, '20), there are cogent reasons for the position that butyric acid *does not* cause membrane separation through a superficial cytolysis, the superficial cytolysis-corrective factor hypothesis becomes untenable. Rather it is far more simple to explain the action of butyric acid and of the hypertonic sea-water as used by Loeb as *additive:* together they accomplish what the hypertonic sea-water alone in my experiments accomplishes. The butyric acid-hypertonic sea-water method, beautiful though it is and technically brilliant, confuses the picture because of the superficial cytolysis-corrective factor theory to which it gave rise.

In any field the pioneer work is usually qualitative. The work is none the less important therefor. And yet one can not but feel that it is a pity that Loeb did not make exact observations with various concentrations of salt—particularly so since the method involved is such a simple quantitative one.

If, now, we reject the superficial cytolysis-corrective factor hypothesis as an explanation of experimental parthenogenesis, what explanation do we offer? While it seems to me, in the present state of our knowledge of this subject, far more profitable to collect data than to theorize, it is nevertheless true that the data presented above permit at least a provisional hypothesis. Certainly, we may draw conclusions from the work if these be consistent with the data.

To begin with, it is difficult to conceive of the initiation of development being fundamentally different for different ova. The

differences encountered are doubtless merely incidental. Any explanation of experimental parthenogenesis ought, therefore, be congruous—it ought to be applicable to all eggs capable of experimental initiation of development.

But there are serious difficulties in the way of reducing all work on experimental parthenogenesis to a common basis. Leaving out work which is manifestly erroneous, we still have a large body of data purporting to deal with "artificial" parthenogenesis which as a matter of fact merely details results in producing membranes, or some slight cortical change, in initiating maturation, etc. In much of this work indubitable death changes are mistaken for cleavage. And we are told that all these are important for science. And even where experimental parthenogenesis is specifically defined as the production of cell division many substances are named as agents of experimental parthenogenesis, whereas such agents if allowed to act but an extremely short time either call forth membrane separation merely and initiate coagulative death changes. Such results have far less significance for the problem of experimental parthenogenesis than death stiffening for the theory of muscle contraction. We are thus forced to discard much of this work also.

On the other hand, it would be unscientific to reach conclusions for all ova from the results obtained on one. Fortunately, however, we possess many investigations in the field of experimental parthenogenesis of undoubted value. Such, for example, is the work of R. S. Lillie on the egg of *Asterias,* of Miss Allyn's on the egg of *Chætopterus,* in addition, of course, to Loeb's work. Now, in all this work the only common factor is the use of a single agent, heat, butyric acid, or hypertonic sea-water. If we add the eggs of *Nereis* and of the frog to those just mentioned, we have, with respect to the stage in maturation at which fertilization takes place or experimental parthenogenesis is possible, all types of eggs represented. It may be generally true, therefore, wherever experimental parthenogenesis is possible, that a single agent suffices.

In both fertilization and experimental parthenogenesis one fundamental reaction takes place, namely, the cortical reaction. This is no mere arbitrary assumption. Eggs pass through a period of

fertilizability. This period is likewise the optimum for experimental parthenogenesis. In some cases this fertilizability we know is due to the presence in or at the cortex of a substance, fertilizin. Complete fertilization-reaction depends upon the combination of fertilizin and sperm. The cortical explosions leading to membrane separation are the sign and sequela of this complete fertilization-reaction. There is evidence that in experimental parthenogenesis also the same phenomena obtain (Lillie, '14, '20[a], '20[b]; Moore, '16, '17; Just, '15, '19[a], '19[b]).

Now, in fertilization the primary object is the incorporation of the sperm nucleus to the end that chromosomes of each parent are alike present in the ensuing division. This object is attained by the reaction between sperm and fertilizin by which the sperm head is made to swell and to form an aster out of aster-forming substance present in the egg. The sperm thus carries the aster to the egg nucleus and cell division ensues.

There is evidence that indicates that the aster-forming substance and fertilizin are not identical, though they may be spatially related. The work of Delage, Wilson Yatsu, and R. S. Lillie shows that in the eggs studied the capacity for merogeny, fertilization, and experimental parthenogenesis depends upon the presence in the cytoplasm of material from the germinal vesicle. We might interpret this to mean that the fertilizability depends upon the presence of fertilizin alone, and that fertilizin and the aster-forming substance are identical. But on this basis how shall we account for fertilization in *Nereis* and *Platynereis?* In these eggs the fertilization-reaction takes place while the egg is in the germinal vesicle stage. At this stage fertilizin is already at the cortex. The sperm aster, on the other hand, never forms until the sperm is in the endoplasm into which germinal vesicle sap has diffused. The sperm aster arises similarly in the eggs of *Chætopterus* and of *Allolobophora.* Where, as in the eggs of *Arbacia* and of *Echinarachnius,* the fertilization-reaction normally takes place in the mature egg, the germinal vesicle material by diffusion has previously reached the ectoplasm; the sperm aster forms, therefore, shortly after the sperm passes the cortex.

In experimental parthenogenesis as in fertilization cell division depends upon the localization of aster-forming substance around

the egg nucleus. Optimum localization is enhanced by complete cortical reaction and by exposure to the agent beyond that sufficient for cortical change. (See R. S. Lillie, effect of butyric acid or heat on starfish eggs; Miss Allyn, effect of heat on *Chætopterus* eggs; hypertonic sea-water on urchin eggs as in this paper.)

Instead of the superficial cytolysis-corrective factor theory of experimental parthenogenesis I suggest that the activating agent binds fertilizin, thus leading to complete cortical change. This complete cortical change makes it possible that the additional action of the agent brings about an optimum concentration of aster-forming substance around the egg nucleus. The nucleus swells, a bipolar spindle forms, and development begins.

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