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ON THE CHEMICAL CHARACTER OF THE PROCESS OF FERTILIZATION AND ITS BEARING UPON THE THEORY OF LIFE PHENOMENA[1]

I

THERE may be a difference of opinion as to whether or not it will ever be possible to produce living matter from inanimate; but I think we all agree that we can not well hope to succeed in making living matter artificially unless we have a clear conception of what living matter is. Living organisms have the peculiarity of developing and reproducing themselves autom atically, and it is this automatic character of reproduction and development which differentiates them, for the time being, from machines made of inanimate matter. Hence the answer to the question of what living matter is will have to be an answer to the question what determines the phenomena of automatic development and reproduction. Since all life phenomena are ultimately purely chemical, the answer must consist in pointing out one or more series of definite chemical reactions, for which it can be proved that they are identical with the phenomena of development and self-perpetuation. It always seemed to me that the natural starting point for a search after this definite chemical reaction or series of reactions was the analysis of that process which causes the resting egg to develop into an embryo, namely, the process of fertilization.

[1] Address delivered at the International Zoological Congress at Boston, August 22, 1907.

II

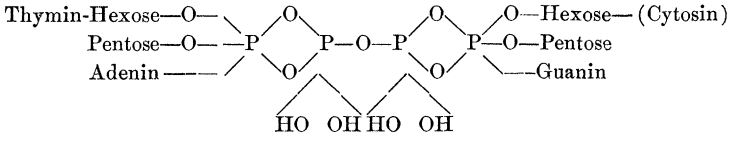
The spermatozoon produces two kinds of effects upon the egg. It causes the egg to develop and it transmits the paternal qualities to the offspring. We are here concerned only with the developmental effects of the spermatozoon. If the question be raised as to what is the most obvious chemical reaction which the spermatozoon causes in the egg, the answer must be, an enormous synthesis of chromatin or nuclear material from constituents of the cytoplasm. After the entrance of the spermatozoon the egg has one nucleus which during segmentation is successively divided into two, four, eight, sixteen, etc., nuclei. Boveri has shown that each new nucleus has the same size as the first nucleus after fertilization. It is therefore obvious that one, and, to all appearances, the foremost chemical effect of the spermatozoon upon the egg is an enormous synthesis of nuclear matter, and to this we must give our attention.

The main mass of the nucleus consists of a salt, the acid of which is nucleic acid, the base a protein substance of the type of protamins, the synthesis of which has been accomplished by A. E. Taylor, or histones. The skeleton of the nucleic acid molecule seems to be phosphoric acid, to which are coupled at least two chemical groups, namely, purin bases (adenin, guanin and possibly other representatives of the same group) and carbohydrates—a pentose and a hexose. The constitution of the nucleic acid may be represented by the following diagram by Burian, of which he states, however, that it can not be entirely correct since it has forty-one instead of forty atoms of carbon in the molecule.

From where does the material for this synthesis of nucleic acid after fertilization come? Our attention is first called to the phosphoric acid. In the case of eggs developing in sea-water, one might think of the possibility that these phosphates are taken from the sea-water. I have made experiments with artificial sea-water from chemically pure salts which contained no phosphates. Eggs of the sea-urchin accomplish their nuclein synthesis just as fast in solutions free from phosphates as in sea-water. Since the same is true for eggs caused to develop by chemical methods, it is obvious that the phosphates for the synthesis of nucleins are derived from the egg itself. The same is true for the other constitutents of the nucleus, since the segmentation of the egg of the sea-urchin can proceed to the blastula and gastrula stage in a solution containing only the chlorides of sodium, potassium, calcium and magnesium.

Miescher found that the amount of lecithin in the blood of the salmon is increased during the period of the formation of sex cells, and he concluded that lecithin is one of the substances from which nucleic acid is formed. The egg itself does not seem to contain any preformed nucleic acid, according to the investigation of Kossel, in the hen's egg, and of Tichomiroff in the eggs of insects. All eggs and possibly all embryonic cells possess comparatively large quantities of lecithin, a fact to which Hoppe Seyler has already called attention .

The lecithin consists of two different groups of bodies, one being distearyl (or oleyl), glycero-phosphoric acid, the other being cholin.



$$C_3H_5\begin{matrix}\diagup\!\!\diagup (C_nH_{2n-1}O_2)_2 \\ O.PO \quad OH.O\end{matrix}\diagdown\!\!\diagdown C_2H_4N(CH_3)_3OH.$$

The cholin can apparently not be utilized for the synthesis of nucleins, but the other constituent is able not only to furnish the phosphoric acid skeleton of the nucleic acid molecule, but also the carbohydrates. The fatty acid could be rendered available for this purpose by oxidation, and we shall see indeed that phenomena of oxidation are the prerequisite of the synthesis of nucleins. If the lecithin of the egg is utilized for this synthesis it is obvious that the lecithin molecule must first undergo a cleavage, whereby it is freed from the cholin group. The question as to whether or not lecithin is the source of the phosphates and possibly some other constituents of the nucleic acid group can not be decided until the synthesis of the nucleic acid has been accomplished.

Some further data concerning the synthesis of nucleic acid in the egg can be obtained. The fertilized egg can not develop or increase the number of its nuclei unless an ample supply of free oxygen is present. Twelve years ago I showed that if all the oxygen is withdrawn from the egg, no nuclear, and no cell division is possible, and no increase in its mass of nuclein occurs. The same effect is accomplished if we inhibit the oxidations in the egg through the addition of KCN. The latter, or possibly the HCN formed by hydrolytic dissociation, inhibit the oxidations in the egg. The addition of $\frac{1}{2}$ c.c. of a 1/20 per cent. solution of KCN to 50 c.c. of sea-water is sufficient to bring to a complete standstill the effect of the fertilization in the egg of the sea-urchin, without otherwise injuring the egg, provided the latter does not remain too long in the absence of oxygen or the presence of KCN. As soon as oxygen is again admitted to the egg which has been deprived of it, the synthesis of nuclein and the segmentation begin again; the same occurs when the eggs are brought back from the cyanide sea-water to normal sea-water, provided they are sufficiently aired. It is therefore obvious that the nuclein synthesis depends absolutely upon a process of oxidation.

By way of digression I may mention that this rôle of oxidation in cell division should induce teachers of physiology to discontinue the antiquated statement that the sole object of oxidation in living cells is the production of heat. Oxidations occur in plants and animals which do not need to keep up a constant temperature. But in all these animals a synthesis of nuclein and cell divisions occurs.

We can further show that aside from the process of oxidation, still other processes are originated or accelerated through the entrance of the spermatozoon into the egg and that these processes occur even in the absence of oxygen. This follows from the difference in the resistance of the fertilized and the unfertilized eggs towards lack of oxygen. Fertilized eggs suffer much more quickly from lack of oxygen or from KCN than unfertilized eggs. Eggs were fertilized and a few minutes later put into sea-water free from oxygen, in which they remained twenty-four hours at a temperature of about 15° C. No segmentation or increase in nuclei occurred in the eggs while they were without oxygen. At the end of the twenty-four hours, air was admitted and segmentation began; many eggs, however, segmented abnormally and none developed beyond the blastula stage. Simultaneously the same experiment was made with the unfertilized eggs of the same female. When after twenty-four hours air was admitted and sperm added, all the eggs developed into normal plutei. The same experiment can be made more easily and strikingly by adding KCN to the sea-water. These facts show that lack of

oxygen or the suppression of oxidations in the egg injures the fertilized egg much more quickly than the unfertilized egg. This becomes easily intelligible under the assumption that the spermatozoon causes or accelerates still other processes in the egg than oxidations, *e. g.*, hydrolyses, and that the products of these hydrolyses are utilized in the processes of oxidation. If the oxidations do not occur the products of the hydrolyses accumulate in the egg or give rise to further reactions not compatible with the life of the egg. It thus becomes comprehensible why the fertilized egg suffers much more readily than the unfertilized egg, which seems to be able to stand the influence of lack of oxygen for several days, and possibly a longer period of time. It is of course possible that among the hydrolyses occurring in the fertilized egg might be those of lecithin.

III

Our present knowledge of the chemical structure of the spermatozoon does not enable us to state why the entrance of the spermatozoon into the egg should cause its development. That part of the spermatozoon which prevails by its mass is its head, which seems to have essentially the same chemical composition as the egg nucleus or any other cell nucleus. The tail of the spermatozoon is cytoplasm which is at present not characterized by anything special except a relatively large amount of lecithin and fat. If we wish to gain any further insight into the nature of the process of fertilization we must turn to those experiments in which the action of the spermatozoon on the egg can be imitated more or less completely by well-known chemical agencies. The data on heterogeneous hybridization seem to indicate that the substances which cause the egg to develop must be identical or closely related in widely different forms, otherwise we could not understand why the sperm of various starfish, of the brittle star, the crinoids, and, according to Kupelwieser, even of mollusks, should be able to fertilize the egg of the sea-urchin. It almost looks as if the only limitation to heterogeneous hybridization were the fact that for some reason the spermatozoon is not able to enter into the egg of a widely different family. This may explain why it is often necessary to change the constitution of the sea-water, for example, to raise its alkalinity, in order to enable the spermatozoon to enter the foreign egg. It follows from this fact that we can draw conclusions upon the nature of the process of fertilization only from such methods of artificial parthenogenesis as are of a more general application.

We shall begin our discussion with a consideration of the methods of artificial parthenogenesis in the sea-urchin, since they have been most thoroughly investigated in this form. The first method by which larvæ were produced from the unfertilized egg of the sea-urchin consisted in treating the eggs with sea-water whose osmotic pressure had been raised about 50 per cent. The method consisted simply in putting the unfertilized eggs for about two hours at a temperature of about 20° C. into a mixture of 50 c.c. sea-water plus $7\frac{1}{2}$ c.c. $2\frac{1}{2}N$ NaCl and then transferring them to normal sea-water. This method, which gave comparatively constant and good results on *Arbacia* at Woods Hole, gave unreliable results in a form of sea-urchin common at Pacific Grove, *Strongylocentrotus purpuratus*. Neither were the results obtained with this method, on the shore of France and at Naples, very satisfactory, according to reports by Giard, Herbst and others; while E. B. Wilson obtained good results with this method on *Toxopneustes* at Beaufort, North Carolina.

Whenever we notice such an inconstancy of results by a definite method it is probable that some essential variable of the experiment has been overlooked. It seemed difficult to suggest what this variable might be. But there was another chance of overcoming this block. I had noticed in my first experiments that the unfertilized eggs which had been caused to develop by the treatment with hypertonic sea-water differed typically in the form of their development from the eggs fertilized by sperm. This fact was at first welcome since it disposed of the general objection that my results were due to an infection by sperm. The main differences were the following: the egg fertilized by sperm forms immediately after the entrance of the spermatozoon a fertilization membrane, while in the egg caused to develop by the osmotic treatment no membrane was formed. It was also found that a segmentation and development of the egg occurred more rapidly and more regularly in the fertilized egg than in the egg treated osmotically. These and other differences led to the idea that the treatment of the egg with hypertonic sea-water initiated only certain, but not all of the developmental effects of the spermatozoon. It therefore seemed to be necessary to find a second agency which in combination with the osmotic treatment would allow a more complete imitation of the effects of the spermatozoon. In the pursuit of this idea it was found that if the unfertilized eggs of the Californian sea-urchin, *Strongylocentrotus purpuratus,* are treated for some minutes with sea-water to which a small but definite amount of a monobasic fatty acid (or any acid with only one carboxyl group) was added, they will form a typical membrane of fertilization after being transferred to normal sea-water. If these eggs are subsequently treated for from 30 to 50 minutes with hypertonic sea-water (50 c.c. sea-water plus 8 c.c. $2\frac{1}{2}N$ NaCl) at a temperature of 15° C., practically all the eggs will develop into larvæ, provided the time of exposure to the hypertonic sea-water is correctly chosen. In a part of these eggs the segmentation occurred in a perfectly normal way and these eggs developed into normal plutei. If the eggs are treated with only one of the two agencies, the fatty acid or the hypertonic sea-water (for from 30 to 50 minutes) no egg develops. The calling forth of the membrane formation alone leads, at room temperature, to the formation of the first nuclear spindle and division, and then soon to a rapid disintegration of the egg. The experiment can also be made in the reverse order, namely, the eggs can first be treated with the hypertonic sea-water and then be submitted to the treatment with fatty acid. If this order is adopted the eggs must be exposed to the hypertonic solution much longer than in the other case, namely, for from $1\frac{1}{2}$ to 2 hours. This difference in the duration of exposure is due to the fact that the process of membrane formation leads to an acceleration of certain chemical reactions in the egg, whereby the hypertonic sea-water can accomplish its effects more quickly than if it is applied to an intact egg. The superiority of this new method of artificial parthenogenesis over the old one is very striking in the eggs of *Strongylocentrotus.* It happened often enough that the old, purely osmotic method of artificial parthenogenesis led to the formation of none or a small percentage of larvæ, while the new method of combining the fatty acid treatment with the osmotic treatment led to the development of the majority or practically all the eggs of the same female.

It could be shown that in this method we are not dealing with the direct effect of the fatty acid upon the egg, but with the effect of the membrane formation caused

by the fatty acid. To cause the membrane formation it was necessary to put the unfertilized eggs at 15° C. for from 1½ to 2½ minutes into a mixture of 50 c.c. sea-water plus 2.8 c.c. *N*/10 butyric acid (or any other monobasic fatty acid). If the eggs were taken a little too early from the solution, after about eighty seconds, only part of the eggs formed membranes after being transferred to normal sea-water. If these eggs were afterwards treated for from 30 to 50 minutes with hypertonic sea-water, only those eggs developed into larvæ which had formed the membrane. A further proof lies in the following data. In 1887 O. and R. Hertwig published the fact that if chloroform is dissolved in sea-water the sea-urchin eggs may form membranes in such sea-water, and Herbst showed in 1893 that benzol, toluol, xylol, act in the same way. I suspected that all fat solvents have the same effect, and an experiment with amylen confirmed this expectation. If the membrane formation is called forth in the egg of *Strongylocentrotus purpuratus* with any of these fat solvents and the eggs are afterwards treated for from 30 to 50 minutes with hypertonic sea-water they will develop into larvæ. It is, however, important to realize that these fat solvents cause cytolysis of the eggs, unless the latter are transferred very rapidly to normal sea-water. On account of this cytolytic effect it is preferable for practical purposes to cause the membrane formation by a fatty acid.

It is therefore obvious that we are now in possession of a method which allows us to imitate more completely the effects of the spermatozoon than the previous purely osmotic method. My attention was again directed towards the fact that the purely osmotic method gave unreliable results with the eggs of the Californian *Strongylocentrotus,* while it gave better results with the eggs of the eastern *Arbacia.* Experiments on the effect of the alkalinity of the sea-water upon segmentation indicated that the sea-water in the laboratory at Woods Hole is considerably more alkaline than that used by me in Pacific Grove. It occurred to me whether this difference might have something to do with the difference in the result of the osmotic method in both places. This suggestion proved correct. It was found that a neutral hypertonic solution with a concentration of hydroxyl ions of 10^{-7} or 10^{-6} normal, as a rule does not cause the development of the unfertilized egg of *Strongylocentrotus,* no matter how high the osmotic pressure is; but that with a sufficiently high concentration of hydroxyl ions a comparatively small increase in the osmotic pressure of the sea-water is sufficient to cause the unfertilized eggs of *Strongylocentrotus* to develop into larvæ. It was, moreover, found that the minimal concentration of hydroxyl ions in the hypertonic solution necessary to call forth the development of unfertilized eggs differs considerably for the eggs of different females. For the eggs of some females this minimal concentration was as low as that found in the sea-water at Pacific Grove, namely, between 10^{-6} and 10^{-5} normal, but as a rule a higher concentration of hydroxyl ions was required. It is possible to obtain good and constant results with the purely osmotic method in *Strongylocentrotus* if only the concentration of the hydroxyl ions in the hypertonic solution is sufficiently raised through the addition of NaHO. In cases in which the eggs of *Strongylocentrotus* will not develop into larvæ when put for about two hours at 15° C. into a mixture of 50 c.c. sea-water plus 8 c.c. 2½*N* NaCl they will develop when about 1.5 c.c. *N*/10 NaHO is added to this solution.

The apparently purely osmotic method, therefore, turns out to be composed of two agencies, one being the increased osmotic

pressure of the solution, and the other the concentration of the hydroxyl ions. It could be shown that these two agencies can with good results be applied separately, and that therefore there exists a far-reaching analogy between the effects of the alkali in these experiments and the fatty acid in the experiments previously mentioned. If the unfertilized eggs of *Strongylocentrotus* are first put for two hours into a mixture of 50 c.c. of a neutral van't Hoff solution isotonic with sea-water plus ½ or 1 c.c. *N*/10 NaHO and then for from 30 to 50 minutes into hypertonic sea-water (50 c.c. sea-water plus 8 c.c. 2½*N* NaCl) many eggs or the majority will develop into larvæ. If the eggs are treated with the alkaline solution alone, without being subsequently treated with hypertonic sea-water, they will not develop. The treatment of the eggs for from two to three hours with NaOH acts, therefore, in a way similar to the treatment of the same eggs for about two minutes with a solution of the fatty acid of the same concentration. The analogy shows itself also in that with this method of combining the effects of alkali and hypertonic sea-water those eggs which develop into larvæ form often, if not always, a membrane. This membrane is not quite as distinct as the fatty-acid membrane for the reason that it surrounds the cytoplasm more closely. This membrane formation does not occur or does as a rule not become manifest until the eggs are returned from the hypertonic to the normal sea-water. If the order of events is reversed and the eggs are first put into the hypertonic sea-water and afterwards into the hyperalkaline solution, they must remain longer in the hypertonic sea-water, namely, for from 1½ to 2 hours; this also corresponds to the experience with the fatty-acid treatment.

We therefore possess two parallel methods by which we can imitate the fertilizing effects of the spermatozoon upon the egg of *Strongylocentrotus,* namely, we treat the unfertilized egg for from 1 to 2 minutes with a solution of fatty acid (50 c.c. *M*/2 van't Hoff solution plus 0.7 c.c. *N*/10 butyric acid) or for from 2 to 3 hours with an equivalent alkaline solution (50 c.c. *M*/2 van't Hoff solution plus 0.7 c.c. *N*/10 NaHO) and afterwards for from 30 to 50 minutes with hypertonic sea-water (temperature = 15° C.). The treatment of the eggs with fatty acid or alkali can be replaced by a treatment with a fat solvent. Fatty acids, alkalis and fat solvents all act in the same way, namely, by causing those changes in the egg which result in the process of a membrane formation.

IV

I have not yet had time to apply these results to the eggs of many other forms, but I believe from what I have seen that we are now in possession of at least some of the general methods and principles of artificial parthenogenesis. It seems that in general the treatment of the eggs with alkalis and acids, sometimes with, sometimes without subsequent treatment with hypertonic sea water, causes the development of unfertilized eggs.

The unfertilized eggs of *Polynoë,* a marine annelid, can develop into larvæ if they are permanently put into hyperalkaline sea-water, *e. g.,* 50 c.c. sea-water plus 1.5 c.c. *n*/10 NaHO. It is well to keep the eggs for this experiment in shallow watch-glasses which are loosely covered with glass plates; in this case the oxygen of the air diffuses more readily to the eggs, than if they are kept in dishes with a deep layer of liquid above them. These eggs of *Polynoë* are immature when taken from the ovary and do not become mature in ordinary sea-water unless they are fertilized by a spermatozoon. They become, however, mature without the aid of sperm,

when kept for some hours in hyperalkaline sea-water. They form a membrane and throw out the polar bodies. If we wait until this occurs and then transfer the eggs for from 2 to 3 hours at 15° C. into hypertonic sea-water (50 c.c. sea-water plus 8 c.c. $2\frac{1}{2}n$ NaCl) and after this time bring the eggs back into normal sea-water they develop more quickly and more eggs segment than if they remain permanently in the hyperalkaline sea-water without any treatment with hypertonic sea-water.

Three years ago I had found that a small number of the eggs of mollusks, *Lottia gigantea,* and various forms of *Acmæa,* can be caused to develop if put for 2 hours into hypertonic sea-water. I have convinced myself this year that no development occurs if they are treated with a neutral hypertonic solution; that, however, if the alkalinity of the hypertonic solution is raised sufficiently high by the addition of NaHO many, if not practically all, the eggs of *Lottia* can be caused to develop into larvæ. In these experiments it was also noticed that the concentration of the HO ions in the hypertonic solution necessary for the production of larvæ from the unfertilized eggs differed considerably for the eggs of different females.

I have convinced myself also that the unfertilized eggs of *Sipunculus* can be caused to develop into larvæ by putting them permanently into a solution with a comparatively high concentration of HO ions.

As far as the production of larvæ from unfertilized eggs with the aid of acids is concerned, we may mention the eggs of starfish, which can be caused to develop with the aid not only of the acids containing one carboxyl group, but apparently with the aid of all acids. They differ in this respect from the eggs of the sea-urchin. It is possible that the acids with one carboxyl group act also better in the case of the starfish egg than the other acids; and this might explain why Delage obtained better results with CO_2 than with other acids, although according to my own experience the results with the other acids are much more satisfactory than those of Delage.

For the eggs of the starfish the acid treatment suffices, and no further treatment with hypertonic sea-water is required. In the egg of one form of starfish, namely, *Asterina,* the spermatozoon causes a membrane formation which is just as distinct as in the sea-urchin egg. The membrane formation can be induced in *Asterina* by exactly the same methods as in the sea-urchin egg, namely, a treatment with a fat solvent (benzol, or amylen) or a fatty acid. In these eggs the production of the membrane is sufficient to cause the development into normal larvæ at least of a number of eggs and an after-treatment with hypertonic sea-water is not required. The starfish eggs differ also from the sea-urchin eggs in the former having a tendency to develop spontaneously if left in sea-water, although the number of eggs developing in this way is, as a rule, very small. This development may be due to the action of the HO ions in the sea-water, or the action of an acid, *e. g.,* CO_2, formed in the egg itself. In *Asterina* it can also be noticed that if eggs remain in sea-water occasionally some of them form a membrane spontaneously, possibly also through the influence of the HO ions of the sea-water or an acid formed in the egg.

In the eggs of *Thalassema mellita,* a marine worm, Lefevre has produced membrane formation and normal segmentation by treating them with acid. The eggs of this form are immature when removed from the ovary, and the entrance of the spermatozoon causes them to form a membrane, to throw out their polar bodies, and to segment and develop. Lefevre found

that by treating the unfertilized eggs with any acid, HCl, NHO_3, oxalic, acetic acid, CO_2, he could cause the membrane formation, maturation, normal segmentation, and the formation of normal larvæ in a large percentage of the eggs. In order to produce these results he put the eggs for about five minutes into a mixture of 85 c.c. sea-water plus 15 c.c. $N/10$ acetic acid.

V

These and similar facts may serve us as a basis for the further analysis of the nature of the process of fertilization.

If we call forth the membrane formation in the unfertilized egg of *Strongylocentrotus purpuratus,* either by treating it with benzol or with a fatty acid or with alkali, the same processes take place at first, as in the case of the entrance of a spermatozoon; after some hours a normal nuclear spindle is formed and the nucleus divides regularly into two nuclei. This shows that the synthesis of nuclein salts is started by the membrane formation. If the temperature is very low (from 2° to 5° C.) the segmentation continues slowly but regularly and a few normal blastulæ may be obtained. At a temperature of 15° or more the development does not go beyond the formation of the first nuclear spindle or nuclear division; soon after this the egg begins to disintegrate in a characteristic way. If, however, the egg is put after the membrane formation for from 30 to 50 minutes (at 15° C.) into hypertonic sea-water, all the eggs remain alive and develop, provided the time of exposure is chosen correctly; and in a number of these eggs segmentation and development occur in a normal way. *It is therefore obvious that the calling forth of the membrane formation in the egg starts the nuclein synthesis and the other processes of development, but that the chemical processes do not occur properly.* Through the subsequent treatment of such eggs with hypertonic sea-water these processes are carried back into the proper channels. In some forms, *e. g., Thalassema* and *Asterina,* the calling forth of the process of membrane formation obviously suffices to start the chemical processes in the egg in the right channels and no after-treatment with hypertonic sea-water is required. Our understanding of the developmental effects of the spermatozoon therefore depends upon the answer to the three following questions: (1) What is the physico-chemical character of the process of membrane formation whereby this process is able to start the development of the egg? (2) Why does it start this development in some forms, *e. g., Strongylocentrotus purpuratus,* in the wrong channels? (3) In which way does the treatment of such eggs with hypertonic solution carry the development back into the proper channels? We shall try to answer these three questions in turn.

As far as the physico-chemical character of the process of membrane formation in *Strongylocentrotus* is concerned, we have seen that it can be produced by very different means in the sea-urchin; first, by fat solvents, *e. g.,* benzol, toluol, amylen, etc. Since I had formerly expressed the suggestion that the process of membrane formation might be due to a coagulation and since it might be argued that the above-mentioned agencies might also have a slight coagulating effect, it was of importance to make certain whether they really act only through their fat-dissolving power. Benzol has a high fat-dissolving power and an extremely slight coagulating effect on proteins. Phenol, on the contrary, has a much smaller fat-dissolving power but a very great coagulating effect. If the process of membrane formation were due to a coagulating effect of these agencies, phenol should act much more powerfully in the membrane production than benzol;

if, however, these media act through their fat dissolving power, the reverse should hold. Benzol is practically insoluble in sea-water. For the purpose of the membrane formation about 2 drops of benzol were put into 50 c.c. of sea-water and the mixture shaken. In order to increase the solubility of the benzol in the sea-water the latter was heated slightly. The shaking caused an emulsion, but only a trace of the few drops of benzol went into solution; yet this caused the membrane formation instantly in the eggs of the sea-urchin. On the other hand, phenol is very soluble in sea-water. It was necessary to add 6 c.c. *m*/2 phenol (Kahlbaum) to 50 c.c. sea-water to produce the membrane formation. Moreover, although toluol has been used extensively in experiments on protein solutions, no author has ever noticed a coagulating effect. Yet it is just as effective as benzol for the production of the fertilization membrane. I think there can be no doubt that we are dealing with an action of benzol, amylen, toluol, on the solution of fatty compounds and not on coagulation.

The second agency for the membrane formation is a treatment of the eggs with alkali. The saponifying action of alkalis upon fat is too well known to require any further discussion.

The action of acids, however, is very peculiar and interesting. As already stated, only such acids as contain one (but not more) carboxyl group produce the membrane formation in *Strongylocentrotus purpuratus*. HCl, HNO_3, H_2SO_4, $NaH_2\ PO_4$, and dibasic or tribasic organic acids, *e. g.*, oxalic, succinic, citric acids, etc., were practically ineffective. This shows that the effect of the fatty acids can not be due to the hydrogen ion; the hydrogen ion inhibits the process of membrane formation, as can be shown by the fact that the membrane can not be formed as long as the egg is in the acidulated sea-water, but only after it has been transferred back to normal sea-water. Moreover, it can be shown that the ineffectiveness of such acids as HCl, HNO_3, etc., is not due to a secondary injurious effect upon the eggs, for an effective solution of butyric acid remains just as effective if we add to it the equivalent amount of hydrochloric acid. *We are obviously dealing here with a specific action of one group of acids, namely, of those which contain one carboxyl group.* Some of these acids, *e. g.*, acetic, are well-known fat solvents. Pflueger pointed out long ago the fat-dissolving action of oleic acid. All of these monobasic fatty acids are more soluble in fat than the other acids. It is therefore possible that these acids act as fat solvents and that it is due to this action that they cause the membrane formation.

But why should the membrane formation in the egg be connected with the process of fat solution? Several years ago I showed that the process of membrane formation in the egg is a transition stage in such cases of cytolysis of the egg, whereby the latter is transformed into a shadow. If we treat eggs with benzol or amylen they form a membrane and are a few seconds later transformed into shadows. The treatment of the unfertilized eggs with alkali also transforms them rapidly into shadows if the solution is free from Ca or Mg. In this process also a membrane is formed. The treatment of the eggs with a fatty acid does not cause cytolysis, but this is due to the inhibiting action of the H ions. Through the addition of acid to sea-water the cytolytic action of fat solvents like benzol is also inhibited. We can also produce cytolysis by treating the eggs with hypertonic sea-water of a very high osmotic pressure, *e. g.*, $1\frac{1}{2}$ to 2 m., or with very dilute sea-water; in both cases the process of membrane formation is a transitional stage in the cytolysis.

Experiments on cytolysis in red blood corpuscles seem to show that the mechanism of this process is the destruction of the membrane of the red corpuscles chiefly by lipolysis. Koeppe assumes (as is generally agreed) that the surface of the red blood corpuscle consists of a lipoid film which is liquefied, saponified, or otherwise destroyed, in cytolysis. I believe that the same is true for the cytolysis of the egg, with this difference only, that in the egg it is not the most superficial film which is liquefied, but the layer underneath it. The surface film is preserved in this process; it is at first quite thin and invisible, but very soon becomes visible, possibly through an imbibition with water which causes it to swell.

The process of membrane formation, according to these facts, seems to be due to a solution of the fatty layer underneath the surface film of the egg. This fatty layer forms together with the surface film a solid shell around the unfertilized egg. As soon as the fatty layer under the surface film is liquefied, water is squeezed out from the cytoplasm and forms a layer between this and the outside film which in the meanwhile has become toughened. But how could this process of fat solution and possibly lipolysis be connected with the synthesis of nucleins? We can not answer this question except by mentioning the possibility, that the lecithins may be involved in the liquefaction and hydrolysis of the surface layer of the egg.

The second question raised by us was: Why does the process of nuclein synthesis come to a standstill so soon after the membrane formation (unless the egg is treated with hypertonic sea-water) and why does the egg disintegrate so rapidly in this case? To this question we are able to give a pretty definite answer. We stated in the beginning of this paper that processes of oxidation are the *conditio sine qua non* of nuclein synthesis and development in the fertilized egg. The nuclein synthesis and the segmentation of the nucleus and the cytoplasm after the artificial membrane formation also depend upon oxidations and do not occur in the absence of O or the presence of KCN. It can be shown that the disintegration of the eggs does not occur if the eggs are put after the membrane formation into an atmosphere of pure hydrogen, or if the oxidations are suppressed in the egg by the addition of a trace of KCN. Eggs which after the membrane formation are thus treated remain intact and can be caused to develop if after a number of hours they are treated with hypertonic sea-water, while at this time the eggs of the same experiment which had remained in normal sea-water are already disintegrating. We must therefore conclude that the artificial membrane formation causes or allows the oxidations underlying the synthesis of the nucleins, but that these oxidations do not occur in the right direction; and that these faulty oxidations are the cause of the rapid disintegrations of such eggs. This disintegration occurs the sooner the higher the temperature.

This conception receives support through the experiments intended to give an answer to the third question, namely, how it happens that eggs which after the artificial membrane formation are treated for from 30 to 50 minutes with hypertonic sea-water develop normally. It was found in all experiments that a hypertonic solution acts in this way only if it contains free oxygen. If we substitute for the air pure hydrogen or if we add to the hypertonic solution a small amount of KCN this effect is not produced. If the eggs possessing membranes are brought back from the hypertonic solution free from oxygen or containing KCN into normal sea-water, they disintegrate in the same way as if

they had not been treated with the hypertonic solution; if the same eggs are put after the treatment with hypertonic sea-water free from oxygen for from 30 to 50 minutes into hypertonic sea-water containing oxygen they will develop normally when put back into normal sea-water. The dominant rôle of the oxygen in the action of hypertonic sea-water upon the unfertilized egg is still more manifest in experiments on eggs which possess no membranes. If we put the unfertilized eggs of *Strongylocentrotus* directly into hypertonic and hyperalkaline sea-water, *e. g.*, 50 c.c. sea-water plus 10 c.c. 2½NaCl plus 1 c.c. *N*/10 NaHO and leave them in such a solution at 15° C. for about two hours, many eggs will develop after they are transferred back to normal sea-water, while others will be injured and perish in a short time. Both effects, however, are only produced if the hypertonic solution contains oxygen. If it is carefully freed from oxygen or if the oxidations are inhibited by KCN the eggs are intact when taken out of the solution. They will neither develop nor disintegrate when put back into normal sea-water. If after a few hours sperm is added to such eggs they will develop. However one may vary the experiment, the result is always the same, namely that a hypertonic solution stimulates or modifies the development of the egg only in the presence of free oxygen. This seems to indicate that the effect of the hypertonic solution in artificial parthenogenesis consists in a modification of the phenomena of oxidation in the egg; the latter are led back into the right channel. This is the reason why the eggs do not disintegrate but develop if they are treated with hypertonic sea-water after the artificial membrane formation.

VI

If we summarize all the experiments on artificial parthenogenesis it seems that the essential feature of the process of fertilization consists first in a liquefaction or hydrolysis or both, of fatty compounds, and second, in the starting of processes of oxidation in the right direction. In some forms, *e. g.*, *Asterina*, the latter will take place naturally if only the former process is started. In the eggs of many forms the process of liquefaction or saponification of lipoids occurs under the phenomenon of membrane formation. These processes of the liquefaction of fats and hydrolysis and oxidation form apparently the basis of the synthesis of nucleins. It is possible, but far from proved, that among the fatty compounds involved in the process of hydrolysis are the lecithins.

These results are in harmony with the facts observed in the germination of oily seeds. The process of germination is an analogue to the starting of the development in the animal egg, inasmuch as resting cells are thrown into the process of cell division and this process is based upon the synthesis of nucleins. Experiments on the germination of the castor bean have shown, according to Hoyer, that as soon as the seeds are put into water a hydrolytic process is started which results in the formation of acid, chiefly carbonic, lactic, and to a smaller degree, acetic acid. Through these acids a lipolytic enzyme is activated by which the oil of the seed is rapidly hydrolyzed. The rest of the process of germination is primarily a nuclein synthesis. This synthesis depends, as in the case of the egg, upon the presence of free oxygen, since Moritz Traube has shown that seeds can not germinate except in the presence of free oxygen. I think the chemistry of the germination of seeds is essentially the chemistry of nuclein synthesis, and I believe the method of starting this synthesis is essentially the same as in the fertilization of the egg.

We can also understand why certain eggs can develop without fertilization or show natural parthenogenesis, while others require fertilization. The naturally parthenogenetic eggs are those in which the nuclein synthesis can be started without the addition of an outside agency. In analogy with the experience on seeds, we may assume that the acid formed in them after they have left the ovary is sufficient to bring about the necessary hydrolytic process or processes; either directly or through the activation of enzyme. Such eggs must also contain the necessary prerequisite for the normal occurrence of the process of oxidation. In the eggs which require fertilization we must probably discriminate between two groups, one for which the hydrolysis is sufficient to start the nuclein synthesis, *e. g.*, starfish, *Thalassema, Polynoë;* the second group for which in addition provisions are to be made for the processes of oxidation, by treating these eggs with hypertonic sea-water containing oxygen, *e. g.*, sea-urchin, and *Lottia.*

I am of the opinion that this mechanism of nuclein synthesis is the thread by which we can find a rational way through the maze of the otherwise bewildering mechanisms, characteristic of living matter; on one hand, the phenomena of growth, on the other, those of self-preservation.

I will illustrate this by one example. It can be proved that the nucleus itself or one of its constituents acts as a catalyzer in the synthesis of nuclein in the unfertilized egg. This follows from the fact that the velocity of the nuclein synthesis in the fertilized egg increases in proportion with the number of nuclei already present in the egg. If the mass of the original fertilization nucleus is m, the mass of nucleins increases during the first segmentation period to $2m$, during the next to $4m$, and so on, increasing with the exponent of 2; while the duration of the various periods of segmentation differs little and these differences have no relation to the mass of the nuclear material formed during the period. This behavior of a chemical reaction is characteristic for such catalytic processes in which one of the products of the reaction is itself a catalyzer for the reaction. We must therefore conclude that the nuclei themselves or one of their constituents are the catalyzer for the nuclein synthesis or one phase of it. It is possible that the nucleus catalyzes only the phenomena of oxidation, and in as much as oxidations are the conditio sine qua non of nuclein synthesis, this would explain the autocatalytic effect of the nuclei upon this reaction. A number of years ago I pointed out that the nucleus seems to act as the main (though possibly not the only) oxidizing agency of the cell. This influence of the nucleus upon the nuclein synthesis, and the rôle of this synthesis upon the preservation and continuation of living matter, explains one of the most mystifying characteristics of the latter, namely, the phenomenon of automatic reproduction of cells.

JACQUES LOEB
UNIVERSITY OF CALIFORNIA

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