INVESTIGATIONS IN PHYSIOLOGICAL MORPHOLOGY. III.

EXPERIMENTS ON CLEAVAGE.

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1. In the second part of my *Untersuchungen zur Physiologischen Morphologie*¹ I showed that regeneration and growth in animals is, as in plants, a function of the amount of water contained in the cells. When I increased the amount of water in the cells of hydroids by bringing these organisms in more diluted sea-water than that in which they usually live, the rate of growth increased with the decrease of the concentration of the sea-water. When I diminished the amount of water in the tissues of hydroids by bringing these animals into a more concentrated solution than the normal sea-water, the rate of growth diminished too. We know that seedlings of plants need water in order to develop. It is the same in the animal egg, as recent investigations concerning the development of sea-urchins, starfish, arthropods, and fish showed me. If we reduce the amount of water contained in the egg of the sea-urchin by bringing it into more concentrated sea-water, the process of segmentation is retarded only, as long as the increase in the concentration is small. As soon as the concentration is greater, however, the fertilized egg does not segment at all. In one case the eggs had been fertilized at 10.40 A.M. A few minutes after the impregnation, one part (a) of the eggs were put into sea-water to which 1 g. NaCl to 100 ccm. had been added. A second part (b) was put into sea-water to which I had added 1.3 g. NaCl to 100 ccm. A third part (c) was brought into sea-water, the concentration of which was increased by the addition of 2 g. NaCl to 100 ccm., and a fourth part (d) remained in normal sea-water. At 10.50 nearly all the eggs which had remained in normal sea-

¹ Würzburg, 1892. Hertz, publisher.

water were in the two-cell stage, whilst none of the eggs in the other solutions were yet segmented; in part *a* the first egg was segmented at 10.55; in *b* the first segmentation took place at 11.45, nearly an hour later than in normal sea-water; and in *c* no segmentation took place at all. That the amount of water and the intracellular pressure in these experiments, varied with the concentration could be seen from the form of the cleavage spheres. In normal sea-water, and still more in sea-water which was a little diluted by the addition of 10 to 20 per cent of fresh water, the first two cleavage spheres were nearly perfect hemispheres. In sea-water of higher concentration the first two cleavage spheres became ellipsoidal in shape, approaching more the sphere the higher the concentration was. When I added more than 2 g. NaCl to 100 ccm. sea-water, in a few hours plasmolysis took place, and the surface of the protoplasm began to shrink irregularly. But by bringing the eggs back into normal sea-water the normal form was restored in a few minutes.

2. Further investigations concerning this subject led me to another series of facts, which, as I believe, give the physiological explanation of some of the phenomena of cleavage. In my investigations concerning the regeneration and growth of hydroids, I found that a salt solution which is just concentrated enough to prevent regeneration and growth by no means kills the hydroids, or even annihilates the power of growth and regeneration. Hydroids which had been in such a solution for several days when brought back into normal sea-water began to regenerate and to grow. When I made the same experiments on fertilized eggs, the results were the same. A salt solution which is just concentrated enough to prevent segmentation does not annihilate the power of segmentation at once. But when I brought such eggs back into normal sea-water, I found that the manner of segmentation changes in a remarkable way, according to the time the eggs had been in the concentrated sea-water.

3. I fertilized eggs of sea-urchins at 9.30 in the morning, and at 9.43 a part of these eggs were put into sea-water to which 2 g. NaCl to 100 ccm. had been added. The rest of the eggs remained in normal sea-water. I will call the sea-water to which 2 g. NaCl to 100 ccm. had been added the concentrated solution, and the eggs which had been exposed to it the plasmolyzed

eggs. At 10.20, before any segmentation, even in the normal sea-water, had taken place, I took a lot of eggs out of the concentrated solution and brought them back into normal sea-water. At 10.33 these eggs began to segment. The segmentation was a normal one, as only segmentation into two cells took place. At the same time segmentation had taken place in nearly all of the normal eggs. The only difference between the normal eggs and the plasmolyzed eggs was that the former at 10.33 were nearly all segmented, whilst of the latter only a small part had undergone segmentation. Ten minutes later, however, every second one of the plasmolyzed eggs was segmented mostly into two, exceptionally into four, segments. But now the situation began to change. By this time, the normal eggs began to reach the four-cell stage, and now many of the plasmolyzed eggs which had not yet segmented into two cells began to segment into three or four cells at once, without going through the two-cell stage at all. The cleavage took place in this way, — that at the same time, or shortly after each other, spherical projections appeared on the surface of the egg, which at first were coherent, but which soon, at the same time or in quick succession, were separated. This kind of segmentation seems to be identical with that kind which O. and R. Hertwig observed under other circumstances, and have described as "Knospenfurchung."¹ The further segmentation was the same in the plasmolyzed and in the normal eggs.

At 11 o'clock I brought a second lot of eggs back from the concentrated solution into normal sea-water. These eggs did not show the slightest trace of segmentation. At 11.22 the eggs began to segment, but in hardly any case did the egg divide into two, but nearly all of them segmented into more cleavage spheres at once. The number and size of the cleavage spheres was not quite regular. There were mostly about four spheres in one egg, sometimes, however, five to eight. The size of the single cleavage spheres of the same egg varied, the smallest spheres being about the size of a cleavage sphere of the eight-cell stage, the largest that of a two-cell stage. At 11.44 the first segmentation was finished, and from now the segmentation was perfectly regular. At 11.40 the normal eggs were in the eight-cell stage.

¹ O. a. R. Hertwig, *Ueber den Befruchtungs- und Teilungsvorgang des tierischen Eies unter dem Einfluss äusserer Agentien*. Jen. Zeitschr., XX, 1887.

At 2.40 I brought another lot of eggs from the concentrated solution back into normal sea-water. Not one egg showed segmentation. At 2.50 the segmentation began. Just as in the 11 o'clock lot, hardly one egg segmented into two cleavage spheres. But whilst most of the eggs of the 11 o'clock lot segmented into from four to eight cells, most of the eggs segmented now into from eight to sixteen cleavage spheres at once. The number and size of the cleavage spheres varied again in the different eggs, but the striking feature this time was the prevalence of cleavage spheres of the size of the sixteen-cell stage. The normal eggs by this time came into the morula stage. At 4.05 another lot of eggs was brought back from the concentrated solution into normal sea-water. Not one egg had segmented. Twenty minutes later, however, nearly all the eggs were in cleavage. But this time they did not divide into sixteen, but into many more segments at once. I think that most of the eggs showed about thirty cleavage spheres. Of course in this lot, just as in the foregoing lots of the same kind, I found cleavage spheres of very different sizes in the same egg. At 6.50 I repeated the same experiment, taking out a lot of eggs from the concentrated solution, and bringing them back into normal sea-water. Not one egg showed any trace of segmentation, but in a very short time — about twenty minutes — the eggs segmented at once into a great number of small cleavage spheres, the smallest and most numerous having the size of a cleavage sphere of about the sixty-four-cell stage. I repeated this experiment about twenty times, always with the same result, which in a few words may be expressed as follows: *If we bring impregnated eggs into sea-water of a certain higher concentration, no segmentation takes place; but if we bring them back into normal sea-water, they divide in about twenty minutes directly into nearly but not quite so many cleavage spheres as they would contain by that time if they had remained in normal sea-water all the time.* It must be added, however, that the normal eggs in this experiment are always ahead of the plasmolyzed eggs in regard to their stage of segmentation, and that their advance becomes the more obvious the farther they develop.

Eggs, after having been in the concentrated solution from twelve to twenty-four hours, do not segment at all if brought back into common sea-water. All these experiments are the more satisfactory the better the material is.

4. I varied these experiments by sometimes bringing the impregnated eggs into the concentrated solution immediately after impregnation, and sometimes later. The result remained the same on the whole, and I will not dwell upon the details of these experiments. But the following fact may be of interest: I impregnated eggs in normal sea-water and left them there until they were all in the two-cell stage. Then I brought them into the concentrated solution. The cleavage stopped directly. After having been there for three hours, I brought them back into normal sea-water; and now every cleavage sphere divided at once into more than two pieces, sometimes into eight or even more.

5. I concluded from the foregoing experiments *that in the concentrated solution a segmentation of the nuclei might take place without any segmentation of the protoplasm*. Eggs which had been impregnated in normal sea-water were brought into the concentrated solution and watched carefully. No segmentation of the protoplasm took place; but the nucleus divided, indeed, into two, and then further divisions followed. I tried, moreover, to see whether the protoplasm of such eggs, if brought back into normal sea-water, divided into as many cleavage spheres as there were nuclei pre-formed. I saw, indeed, that every nucleus becomes the centre of one of these projections, which later on become cleavage spheres. Dr. Conklin was kind enough to stain some of the eggs which had been in the concentrated solution for some time and which showed no trace of segmentation. Some of these stained eggs showed very distinctly from four to about thirty distinct nuclei. In other eggs the segmentation of the nucleus was not so perfect. The nucleus, extremely enlarged, seemed to consist of several parts which, however, were still connected. These eggs had been killed at a time when the eggs of the same lot which had remained in normal sea-water all the time were in about the sixty-four-cell stage.

6. Fol and O. and R. Hertwig found that in the case of polyspermia the egg at once divides into about as many cells as there are asters. We know that for the segmentation of the protoplasm it does not make any difference whether the nuclei are derived from the male pronuclei exclusively, as in the case of the impregnation of an enucleated egg, or from the conju-

gated nuclei, as in the normal case, or from both conjugated nuclei and male pronuclei together, as in some cases described by Fol. In my experiments the eggs were impregnated under normal conditions and cases of polyspermia were very rare indeed. Nearly all the eggs which remained in normal sea-water segmented quite normally. But I thought of the possibility that new spermatozoa might enter the impregnated egg in the concentrated solution. I knew that such a supposition was in contradiction with all known facts, but these facts are still meagre. If a polyspermia in my experiments took place, it could only happen in the concentrated solution, as here the increase of the number of the nuclei was observed. But I found that the spermatozoa were perfectly paralyzed as soon as they were brought into the concentrated solution; that is, in the sea-water to which 2 g. NaCl to 100 ccm. had been added. I could show, moreover, that in this concentrated solution no impregnation is effected. I brought unfertilized eggs into this concentrated solution and added spermatozoa. When I brought them back into normal sea-water, it took more time from that moment until segmentation began than it took in normal eggs and in normal sea-water from the moment of impregnation to the moment of segmentation. The spermatozoa contained in the concentrated salt solution became active again a few minutes after being brought back into normal sea-water and then entered the eggs. Polyspermia in this case could be observed, but not as a rule. Most of these eggs segmented into two cells. But it was astonishing how soon the spermatozoa lost their power of impregnating under these circumstances. Spermatozoa which had been in the concentrated solution only a few hours, when brought back into normal sea-water fertilized only a thousandth part, or still less, of the normal eggs; whilst spermatozoa of the same animal which had remained in normal sea-water fertilized at the same time practically all the eggs of the same female. When I tried to fertilize eggs in normal sea-water which had been in the concentrated solution for a few hours with spermatozoa that had been under the same conditions, only about one egg in a million began to show some trace of segmentation, and as a rule this segmentation remained *in statu nascendi*, but was not accomplished. All these observations are totally different from the phenomena described above. Eggs which had been fertil-

ized in normal sea-water and which were put in the concentrated solution, after being brought back into normal sea-water for from ten to twenty minutes segmented without any exception and were able to develop into normal planulæ and plutei. Eggs of this kind were still able to develop into normal larvæ after having been in the concentrated solution for four or six hours. But eggs which *before* impregnation had been put into the concentrated solution together with spermatozoa, and which four or six hours later were brought back into normal sea-water, reached only the first stages of segmentation, if they segmented at all, and then stopped developing. I never got a living larva from these eggs. From all these facts I conclude that the continual increase of the nuclei of the impregnated eggs in the concentrated solution was due not to polyspermia, but simply to segmentation of the nucleus. In these experiments somewhat bacteriological precautions are necessary, as the water of the aquarium is liable to contain quantities of spermatozoa.

7. From the above I believe to have shown that, by bringing fertilized eggs of sea-urchins into more concentrated sea-water, — we added 2 to 2.4 g. NaCl to 100 ccm. sea-water, — the segmentation of the nucleus proceeds, although more slowly than under normal conditions, whilst no segmentation of the protoplasm is possible. The fact in itself is of some technical value, as it enables us to separate two processes which nature generally produces together, or which hitherto we had not the power to separate at desire. In regard to our knowledge of segmentation, we see from this that the physiological conditions for segmentation of the nucleus are different from the physiological conditions of the segmentation of the protoplasm. We now can be positive in this regard, as under the same conditions the nucleus continues segmenting, whilst the protoplasm does not show the slightest trace of segmentation. But these experiments allow us to go one step farther and to make clear one element in the complex called segmentation, namely, the physiological cause for the segmentation of the protoplasm. We saw that in the concentrated solution the protoplasm did not segment, whilst as soon as it was brought back into the normal sea-water it segmented at once into about as many cleavage spheres as nuclei were formed. All further inferences depend upon our knowledge of the effect of salt solutions on proto-

plasm. I have investigated this point myself, and have caused others also to take up this question. The result of all investigations hitherto carried on is as follows: Raising the concentration of the salt solution in which an animal or a tissue lives has qualitatively and quantitatively the same effect as lowering the temperature; lowering the concentration has qualitatively and quantitatively the same effect as raising the temperature. I will mention two cases to illustrate this. First, one example to show the parallelism of the mentioned effect of the temperature and the concentration in qualitative regard. I have recently succeeded in making animals belonging to different classes — larvæ of *Polygordius*, Copepods, etc. — positively heliotropic by bringing them into low temperatures, and making them negatively heliotropic by raising the temperature of the water. In water from 0° to about 10° larvæ of *Polygordius*, for instance, are exclusively positively heliotropic. In water above 25° they are exclusively negatively heliotropic. But by adding a certain amount of NaCl to normal sea-water I was able to make them just as well positively heliotropic, and by adding a certain amount of fresh water to the normal sea-water I could make them negatively heliotropic. The same was the case in Copepods, only the absolute figures differ, as was to be expected. To show the *quantitative* parallelism, I refer to the fact that lowering the temperature diminishes the irritability of the tissues. This can easily be shown in the heart. As every one knows, cooling off the heart makes it beat more slowly, while heating it increases the rate of beating. I asked Miss Schively to investigate how the rate of the heart-beat would depend on the concentration of the solution in which the isolated heart or the whole animal is put. Her investigations were extended over the heart of ascidians, crustaceans, embryonic and adult vertebrates, and even the rhythmical motions of medusæ; and she found that the rate decreases with astonishing regularity, especially in the cut-out heart of ascidians, in the measure the concentration of the sea-water by adding NaCl increases; and that the rate increases with the same regularity by adding fresh water to the normal sea-water. *By bringing living tissues into a solution of higher concentration, we therefore reduce their irritability by reducing the amount of water contained in them.* By reduction of irritability we mean that the effect determined by

the same cause is quantitatively less. That explains how the segmentation of the protoplasm is generally determined, why in a solution of a certain concentration no segmentation of the protoplasm takes place, and why when brought back into normal sea-water the protoplasm segments at once into about as many spheres as there are nuclei pre-formed. *The segmentation of the protoplasm is the effect of a stimulus which the nucleus applies to the protoplasm, and which makes the protoplasm close around the nucleus.* If we bring the fertilized egg in the concentrated salt solution (2 g. NaCl; 100 sea-water) the nucleus divides and every nucleus applies the stimulus to the protoplasm with which it is in contact. But the protoplasm of the egg, on account of its containing too little water, is in the condition of a cooled-off muscle, which does not answer to the stimulation of the nerve, and no segmentation of the protoplasm takes place. But as soon as we bring the egg back into normal sea-water, the protoplasm takes up water very fast and regains its irritability; and now, of course, it answers to the stimuli from the nuclei, and closes around every nucleus or segments. If we add a smaller dose of NaCl, namely, 1.3 g. NaCl to 100 ccm. sea-water, the irritability is only little less than it is normally, and the whole effect is that the reactions of the protoplasm are somewhat slower and retarded. Of what kind the stimulus is and from which part of the nucleus it is exercised, we cannot tell. From other facts I am inclined to believe that this stimulus is a chemical one, and caused by certain substances produced in the nucleus which also may be effective if separated from the nucleus.

8. The physiological causes of the segmentation of the nucleus are not directly touched by these experiments. But two points ought to be mentioned: first, that the segmentation of the nucleus in the concentrated solution (2 g. NaCl; 100 ccm. sea-water) was retarded, and at last ceased entirely after from twelve to twenty-four hours; secondly, that the segmentation of the nucleus was extremely irregular when the protoplasm did not take part in segmentation. We see in these facts some of the influences which the protoplasm exercises on the segmentation of the nucleus. This influence may be exercised in this way, — that by the high intracellular pressure which normally exists in the cleavage spheres these spheres press and flatten each other. The form of the cell, however, determines as Sachs showed

long since, the orientation of the plane of division, and as Hertwig believes, in such a way that the longitudinal axis of the Kernspindel is put in the longest diameter of the cell. Therefore we ought to expect that, within certain limits, with increasing intracellular pressure, Sachs's law of the rectangular division of the plains of cleavage would become more obvious. I found, indeed, that in normal, or still more in somewhat diluted, sea-water, where the turgor, and consequently the flattening of the cleavage spheres, was the greatest, Sachs's law was the most exactly realized. Therefore this geometrical regularity in the segmentation of the nucleus which is so striking under normal conditions must disappear at once if the protoplasm does not take part in segmentation.

9. Our observations concerning the dependence of irritability of the protoplasm upon the water contained in the tissues add one more fact to those given already to explain the importance of water for all processes of growth and development. If we reduce the amount of water in a regenerating or growing tissue, we not only retard or prevent these processes by reducing the volume of the cells and the mechanical effects of the intracellular pressure, but we reduce also the irritability of the protoplasm. This irritability, as we saw, plays an important rôle in the process of cleavage, and as regeneration and growth is a function of processes of cleavage we at once understand why regeneration and growth must be retarded or accelerated by bringing hydroids in more concentrated, or more diluted, sea-water. But if this inference is right, our experiment not only holds good for the process of cleavage in eggs, but in cells in general.

The experiments which are mentioned in this paper were all made on sea-urchins (*Arbacia*). The other experiments will be published later.

The chief result of these investigations is, shortly, as follows :

If we reduce the irritability of the protoplasm of the egg by reducing the amount of water contained in it, the nucleus can segment without segmentation of the protoplasm. If we increase again later the amount of water, and consequently the irritability of such an egg, the protoplasm at once divides into about as many cleavage spheres as there are nuclei pre-formed. The segmentation of the protoplasm in the egg, and probably in every cell, is only the effect of a stimulus exercised as a rule by the nuclei.