C. M. Child, Factors of Form Regulation in *Harenactis attenuata*. III. Regulation in »Rings«.

Rings are formed from cylindrical pieces of the body of *Harenactis* by the union of the oral with the aboral end about the whole circumference. Their formation can be induced experimentally by removing a larger or smaller part of the mesenterial organs from the piece, and in the œsophageal region by removing the œsophagus. In general, the shorter a piece the more likely it is to form a ring in closing: when the length of the piece is less than the diameter a ring is usually formed or closure does not occur at all.

After closure the parts of the ring commonly undergo a revolution about a circular axis, so that the line of union between oral and aboral ends lies at or near the equator on the outer surface, but changes from this position may occur.

In some cases regulation goes no further, but usually one or more groups of tentacles appear at or near the line of union. The tentacle groups consist of varying numbers of tentacles — in my experiments from one to twelve — and show various degrees of approach to a perfect radial symmetry, the one extreme being a series of tentacles on each side of the line of union, the other a perfectly radially symmetrical group.

The localization of the groups along the line of union is very irregular and is apparently not determined by any factor directly connected with the original organization. Different degrees of injury to different parts of the circumference probably constitute a factor in their localization.

In some cases the groups appear on the line of union and part of the tentacles arise from the original oral end of the piece, part from the aboral. Often oral-aboral tentacles appear, i. e., tentacles in the formation of which both oral and aboral ends take part. In other cases the whole group arises on the oral side of the line of union. Differences in the degree of injury of the two ends of the piece and perhaps also certain features of the original polarity, which persist, are probably factors in determining this localization.

The formation of the tentacle groups undoubtedly involves the establishment of a new polarity and in greater or less degree of a new radial symmetry. The original polarity disappears to a greater or less extent in consequence of the union of the oral with the aboral end. The establishment of the new polarities and symmetries is undoubtedly due to local factors in the regions where the groups appear.

These cases of regulation, like axial heteromorphoses, are to be regarded as a breaking up or division of the original system in consequence of decrease or elimination of the original correlations: they are in short a kind of asexual reproduction, the differentiation of new systems being the result of the localization of regions of growth at various points in the tissues of the piece.

Personale.

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LEHAIRE

The Effects of Altering the Position of the Cleavage Planes in Eggs with Precocious Specification.

By

T. H. Morgan.

With plates VI and VII.

Eingegangen am 21. Januar 1910.

By precocious specification I mean that the character of the blastomeres is determined at a very early stage of development. In somewhat this same sense the term determinative or mosaic type of cleavage has been used by embryologists. But the word determinative may be used in a double sense, namely, to imply that the specification is determined by the cleavage; or that the cleavage of certain eggs is more fixed or definitive, i. e., less variable, than the cleavage of other types of eggs. I wish to avoid both of these implications for reasons that will appear later; and therefore I shall use the words precocious specification or precocious type to imply that the first steps towards differentiation (specification) may take place early in the egg without implying that it is the result in any sense of the cleavage process. In fact, I shall try to show that whenever a relation exists between the cleavage and specification it is the cleavage that follows the potential lines of specification rather than that the cleavage determines the specification of the blastomeres.

The following experiments with the eggs of the ascidian *Ciona intestinalis* and of the annelid *Nereis limbata* show that the results of compression of the egg with a precocious specification are different from the results reported for the plastic eggs of the frog and of the sea urchin. Our knowledge of the effects of compression has rested almost entirely on experiments with the latter kinds of eggs, which in other respects also are known to show the opposite extreme of regulative behavior from that shown by eggs of the precocious type.

The Method of Applying the Pressure.

While there is no difficulty in obtaining a graded series of eggs compressed to varying degrees it is difficult to compress to the same degree all of the eggs of a set. For a graded series a long cover-slip supported at one end by a piece of paper or cover-slip suffices. The important conditions in determining the pressure are the amount of water, the absence of foreign particles and prevention of drying. Unless precautions are taken the eggs are apt to flow over the support when the cover-slip is added, and becoming caught, make the support higher than was intended. The drop containing the eggs should be placed on the slide sufficiently far from the supports to avoid this difficulty. Immediatly after mounting, the slide should be placed in a moist chamber.

When many eggs equally compressed are required the cover should be supported at both ends. If too little water is added the cover may be drawn down between the supports by capillary action and compress the eggs more than it does near the supports. Unless the supports are broad the cover-slip may be drawn down more on one side, and unequally compress the eggs. If the supports are small four should be used — one at each corner —. Mass results obtained in this way will suffice for some purposes, especially if the preparations are carefully examined and those excluded that do not comply with requirements, but where it is necessary to make out in detail the relation between a special type of cleavage and a given result, isolation experiments must always be employed. The extent of the compression is best measured by the diameter of the egg in relation to that of the normal egg.

The Relation of the Pressure to the Axes of the Egg.

In the experiments that have been described for the sea urchin's egg little or no attention has been paid to the question of whether the egg was compressed in the direction of its primary axis, or from the sides; and while this may make little difference, so far as the location of the following cleavage planes is concerned, it may possibly make a difference as to whether normal or abnormal

embryos develop. These experiments should be reexamined, I think, from this point of view, especially in regard to the 16-cell stage, which seems to be determinative in regard to the organ-forming properties of the egg.

In the frog's egg the black and light hemispheres show approximately the position of the primary axis. After fertilization the presence of the gray area gives also the location of the right and left sides, so that it would be possible to determine accurately the direction of pressure. Whether the production of a normal embryo is independent of the position of the cleavage planes with regard to the axial relations of the egg does not seem to me to be sufficiently determined, although the results so far as published seem to indicate that normal development may follow irrespective of the alteration of the cleavage induced by compression. As will be shown for *Ciona* and for *Nereis* the position of the early cleavage planes is a matter of vital importance in the development of the embryo.

Compression of the Egg of Ciona.

The egg of *Ciona* is surrounded by a corona of follicle cells that extend far out from the surface of the egg membrane. They become flattened against the egg membrane when the egg is compressed. Within the membrane are many test cells. At the moment when the pressure is applied a curious phenomenon often occurs; — many of the eggs become flattened on one side as shown in Fig. I, Fig. III *A*, a depression that imitates the beginning of the cleavage furrow. Within a few minutes the egg expands again and fills the egg membrane, Fig. III *B*. When the egg is compressed at the two-cell stage the blastomeres, are often partly separated, Fig. I *B—F*. Sometimes an opening appears in the middle between the blastomeres and makes them assume the form of a ring, Fig. I *H, J*.

I have had some difficulty in explaining these effects. At first it seemed that the results were due to the follicle cells becoming pressed against one side of the egg and flattening it, but I found that when the follicle cells were shaken off the same result followed. It then seemed possible that the test cells might become crowed to one end of the membrane, but sections of the egg failed to reveal any special accumulation of such cells in this region. The membrane of the egg was found to be turned in, but it is not certain whether this is the result of the hardening reagents or occurred before hardening. Such a process of buckling of the membrane would however explain the phenomena.

When the eggs are compressed before or soon after fertilization there is no indication that the eggs orient under pressure, but as soon as the segmentation spindle begins to appear many of the eggs turn as the pressure is applied so that the spindle lies parallel to the compressing plates, and in consequence the first cleavage plane will appear in the same position as in the normal egg. The orientation of the egg may be due to its slight elongation in the direction of the spindle, or to its being more resistent in one plane than in another. If the elongated egg had the form of a cylinder any side would have an equal chance of lying against the compressing plates, but as a matter of fact it seems that the poles less frequently lie against the plates that do the sides of the egg, as shown by the excentric position of the spindle that can often be observed. It appears therefore that the egg may be flatter at the sides, or else, that there are differences in the resistance of the egg in different planes. This point requires a more careful study.

The eggs of *Ciona* were compressed at different stages either before the first cleavage or after that event, and released from pressure at different times. Almost without exception abnormal embryos were produced. In the earlier experiments no controls were kept, since it was assumed that eggs that have segmented normally produce normal embryos; and while this is true for the great majority of eggs from animals that have not been kept too long, it is not universally true, and is not true for eggs that have been long in the oviduct. Animals kept in running water for 48 hours, or longer, contain eggs that may fertilize perfectly, but produce abnormal embryos. Therefore it became necessary to control each set of eggs that was used and to avoid using eggs that were more than two days old. Moreover the eggs of a single individual were in each case kept apart, although sperm from several individuals was generally used.

Ciona deposits its eggs regularly at about daylight. Individuals brought into the laboratory and kept in rapidly running water will retain their eggs for several days, and larger numbers of eggs can be obtained in this way, but at the risk of producing abnormal embryos.

The following experiments give the most instructive cases observed.

Experiment I. Eggs fertilized at 3 P. M. were put under compression at 3.35 P. M., and left for 15 minutes. When taken out the eggs were beginning to divide into two cells. After 24 hours not more than 2 per cent of the eggs were normal; the rest abnormal to varying degrees. The controls were all normal.

Experiment II. Some of the same eggs compressed at 3.30 P. M. were removed from pressure at 4.05 P. M. when they were in the two cell stage. Not more than one per cent were normal.

It is evident from these two experiments that pressure applied 15 minutes before cleavage sufficed to produce an abnormal result, and the effect was more pronounced when the eggs remained under pressure until after the cleavage was completed. In both experiments the time was sufficient to affect the position of the cleavage plane.

Experiment III. Eggs fertilized at 11.20 A. M. were compressed at 11.36, i. e. after 16 minutes, and left until 11.51 (or 15 minutes under pressure). Some of the embryos were normal, others abnormal.

Experiment IV. Other eggs of this same lot were compressed at 11.50 (after 30 minutes) and released at 12 and at 12.10 or after 10 and 20 minutes respectively. In both cases normal and abnormal embryos were found later.

Experiment V. Other eggs compressed at 12 were set free at 12.10 when they were beginning to divide into two cells. More of the resulting embryos were abnormal than normal.

Experiment VI. Still other eggs compressed at 12 were set free at 12.20. Few normal embryos were produced.

The last four experiments show a progressive increase in abnormal embryos according as to whether the pressure was applied nearer to the time of division. No controls were kept, it is true, but the presence of some normal embryos indicate with probability that the set as a whole was in good condition, since as a rule all the eggs of a set are normal or abnormal.

In order to determine whether the flattening of the egg produces in itself abnormal development, or whether the effect is due to changes in the eggs while under pressure that produce the injurious effect the following tests were made.

Experiment VII. Eggs were much compressed for one minute as soon as taken from the oviduct, then set free, and fertilized after 20 minutes, in order that they might have time to recover their normal shape. All of the embryos were abnormal.

Experiment VIII. Other eggs were less compressed and treated in the same way. These too produced abnormal embryos but the degree of abnormality was less. The controls for these two sets gave nearly all normal embryos.

Experiment IX. Eggs of another individual were compressed for half a minute, set free, but not fertilized until four hours later. Many eggs were killed by the pressure, but those that developed were normal. Evidently pressure, as such, for a short interval need not produce abnormal results.

When the eggs of *Ciona* are taken from the oviduct the polar spindle is present in many eggs. It is conceivable that pressure applied during the time of polar body formation might affect the egg in a different way from pressure applied after the process had taken place. I tried therefore the effect of pressure after the egg had been allowed to ripen for one and a half hours.

Experiment X. After standing for one hour and a half the eggs were compressed for one minute, some eggs much, others were less compressed. After they were set free they were not fertilized for three hours. The embryos from the least compressed eggs were all normal, those from the more compressed eggs were slightly abnormal, the tail for instance was noticeably short and blunt.

It appears that compression in itself if extreme may injure the egg, but if not extreme the eggs may produce normal embryos.

Experiment XI. Another series of eggs from 4 individuals, studied separately, stood for half an hour in sea water and the eggs were then compressed for ten minutes, freed and fertilized 45 minutes later. Two sets produced perfectly normal embryos and the controls were normal; one set produced both normal and abnormal embryos, with normal controls. The difference in the two cases is probably to be ascribed to difference in the degree of pressure. The fourth set produced abnormal embryos both in the pressure set and in the control.

Experiment XII. Eggs were compressed at once and left under pressure for 5, 10, 15, 20 minutes, set free, and fertilized after 45 minutes. Those compressed for 5 minutes produced abnormal embryos except for one normal. Those pressed for 10 minutes produced equal numbers of normal and abnormal embryos. Those pressed 15 minutes gave abnormal embryos, while those pressed for 20 minutes were all killed by the pressure except three that were very abnormal. The controls were normal. The pressure had been extreme and appears to have been more effective than the time limit.

Experiment XIII. The last experiment was repeated with less pressure. The only difference between the two was that in the present case all the sets were fertilized at the time when the last set was released. After 5 minutes pressure both normal and abnormal embryos resulted; after 12 minutes most of the embryos were abnormal, a few normal; after 20 minutes nearly all were normal; after 25 minutes most were normal, a few abnormal. The results show again that the time of pressure before fertilization makes no difference in the results; the difference in the sets is no doubt to be put down to uncontrolled differences in the pressure.

Experiment XIV. Eggs removed from the oviduct at 2.40 were kept in plenty of water until 3.25 (45 minutes), and were then compressed. Some eggs were set free after 5 minutes, and half of these fertilized at once (A), the rest after 30 minutes (A'). Other eggs were set free after 10 minutes, half of them fertilized at once (B), the rest after half an hour (B'). A third lot were set free after 15 minutes and fertilized some at once (C), the rest after half an hour (C'). Similarly after 20 minutes, two lots (D) and (D') were obtained. The resulting embryos showed the following conditions; — A normal, A' most of them normal; B most normal, B' most abnormal; C half normal, half abnormal; C' most abnormal; D half normal, half abnormal; D' half normal, half abnormal.

The experiment shows that normal embryos may develop after unfertilized eggs have been compressed from 5 to 20 minutes. It does not seem to make any difference whether the eggs when released from pressure are fertilized at once, or after half an hour. It may appear that the proportion of abnormalities increases with the length of time of compression, but I am rather inclined to think that the results are due to difference in pressure, rather than to length of time. The controls of these sets produced normal embryos.

Experiment XV. The eggs were fertilized at 3.13 P. M. and compressed at 3.58 (i. e. after 45 minutes). Some eggs were set free after 5 (A), 14 (B), 20 (C) and 25 (D) minutes. The eggs had begun to divide at 4.15, that is, 15 minutes after the pressure was applied, so that set B was about to divide when released from pressure, and set C and D had many divided eggs. The condition of the embryos corresponds strictly to the time of compression. The A set was normal; the B set had both normal and abnormal embryos; the C and the D set were abnormal. In other words if the pressure continued long enough to affect the position of the cleavage planes the egg developed abnormally.

The injurious effect of pressure during the cleavage stages is clearly shown in the next experiment in which all of the eggs came from the same individual.

Experiment XVI. The following table shows the times at which the pressure was applied, the stage of development of the eggs when released, and the condition of the embryos after 24 hours.

Compressed at early 2-cell stage until four cell stage. Embryos abnormal a few normal.
Compressed at later 2-cell until 4-cell stage. Embryos abnormal a few normal.
Compressed at early 2-cell until 8-cell stage. Embryos abnormal.
Compressed at later 2-cell until 8-cell stage. Embryos abnormal.
Compressed at 4-cell stage until 8-cell stage. Embryos abnormal.
Compressed at 4-cell stage until later 8-cell. Embryos abnormal.
Compressed at 8-cell stage until 16-cell stage. Embryos abnormal.

The results show clearly that if the eggs are left under pressure until the next division takes place, abnormal embryos are produced, except in a few cases, when the 2-cell stage was compressed.

Experiment XVII. Eggs fertilized at 2.40 P. M. were compressed at 3.40, when three of the four sets were in the 2-cell stage; the other set was just beginning to divide. The pressure was released at 4.05 P. M. when the eggs were in the 4-cell stage; and in another set at the 8-cell stage. Both sets produced abnormal embryos.

Other eggs of these sets were compressed at the 4-cell stage, and one set then released at the 8-cell stage, the other at the 16-cell stage. Both sets produced abnormal embryos.

A number of other experiments were carried out, but as no controls were kept they are of little value, except in so far as they show that in all cases when the compression extended over one division the embryos developed abnormally. It is quite certain that in most of these cases the eggs would have gone normally unless compressed. Still other experiments in which the controls also went abnormally are also omitted, since they throw no light on the problem.

Effects of Pressure on the Egg of Nereis.

Through the kindness of Professor F. R. Lillie I obtained a number of male and female *Nereis* that were sexually mature.

Several sets of experiments were made, but a detailed study must be left for another occasion, since it was too late in the season, when I began, to carry the work very far.

In a preliminary experiment some of the eggs were put under pressure soon after fertilization and released at different times; other eggs were compressed at the 2, 4 and 8-cell stages, and released after the following division. The general effect was at once obvious, namely the formation of a few normal and a great many abnormal embryos. An examination of the slides showed that in almost every case some eggs were so little compressed that they followed the normal type of division, and it seemed not improbable therefore that the normal embryos may have come from this source. Owing to the small size of the egg of *Nereis* it is always possible that some eggs escape compression, due not infrequently to the presence of small particles of debris in the water.

It became necessary therefore to carry out isolation experiments, and since the third cleavage offers a critical stage I selected this particular division for study. The eggs were compressed moderately at various stages, and released at the 8-cell stage. Those eggs that showed an alteration in the position of the third cleavage-plane were picked out and kept separately. In one case, eggs compressed at the four-cell stage, were not released until the 16-cell stage. These too produced abnormal embryos.

Some of the eggs were compressed half-an-hour after fertilization, before the 2-cell stage; others at the 2-cell stage; and the 4-cell stage. They were released from pressure at the 8-cell stage, and 62 of them were isolated that could be seen to have produced a plate of 6 or 8-cells. Every one produced an abnormal embryo.

Seven of these 62 were not very abnormal, and in one dish there were two embryos when only one egg had been, presumeably, isolated. One was normal, or nearly so, and the other was abnormal. Probably in picking out the abnormal egg, another also got into the pipette that had not segmented abnormally; and this produced the normal embryo.

The other eggs, on the slides from which these abnormal embryos came, were also kept. Nearly all of the embryos were abnormal. A study of the abnormal and normal embryos went far towards explaining the presence of the latter. It is known that in *Nereis* the numerous oil droplets in the four endoderm cells run together to form the four oil droplets that can always be seen in the normal swimming embryo.

They are a very constant feature of the development. When the egg is compressed, so that the third cleavage is horizontal instead of vertical, producing a flat plate of eight cells, it is found later that eight oil droplets are present. Now in my cultures when more than four oil drops were present in the embryos from compressed eggs, such embryos were nearly always abnormal in some respects. It is true that if a large number of embryos are examined some will be found containing eight oil droplets and these may approach the normal condition, yet in every case that I have carefully examined something abnormal was always to be found in these embryos. WILSON has given a brief account of an experiment he made with the eggs of *Nereis* in which from a plate of eigh cells a normal embryo developed. I am far from wishing to affirm that under such conditions normal development never occurs, because some of the abnormal embryos approach closely to the normal form in my own experiments, and therefore the chance that one that was entirely normal should appear is by no means excluded. But admitting this as an extreme possibility the results of my experiments speak unanimously in favour of the view that the change in the cleavage produces abnormal development.

One reservation should always be made. The extent to which the pressure is applied may be an important factor in the results. How far an egg compressed just enough to affect the third cleavage will compare with an egg more compressed was not ascertained, but on the whole it seems that the effect is produced through the alternation of the cleavage rather than through the amount of pressure, provided that the pressure is not too great.

The characteristics of the abnormal embryos are most interesting and are deserving of more careful study. For instance, the eye specks may be entirely absent, or present on only one side of the body, or even four eyes may be present. The setae may be entirely suppressed, or be present on one side. The pigmented, trochal ring may be interrupted, or in some cases present the appearance of only half a ring. The anal pigment may be displaced to the side, or be present in two groups. One can not but be impressed by the wonderful vitality of this egg, for despite the occurence of all of these abnormalities the embryos were very alert in persuing their irregular activities. Compression and its consequent alteration of the cleavage planes has in no way stopped the development of the egg, but has interferred with the orderly differentiation and arrangement of the parts.

How far the egg of *Nereis* may orient itself before cleavage, when compressed, I do not know, but there seems to be a tendency for the egg in the two-cell stage to lie with its cleavage plane at right angles to the plates and its poles to the side. Consequently in most eggs the next cleavage plane will not pass through the poles as in the normal egg. While my experiments are not conclusive on this point, they indicate that the shifting of the second cleavage plane also acts injuriously on the subsequent development.

At the four-cell stage the eggs orient almost invariably with the poles against the compressing plates, i. e. at right angles to the position they tend to assume at the two-cell stage under like conditions. Consequently the third planes that are at right angles to the compressing plates can never happen to coincide with the normal planes of the following division.

To sum up; the evidence for *Nereis*, while not so extensive as for *Ciona*, shows very definitely that any alteration in the position of the normal early cleavage planes affects the development injuriously.

The Cause of Abnormal Development of Compressed Eggs.

The difference between the behavior after compression of these eggs with determinative cleavage and of those of the plastic type raises the question as to the cause of the difference.

The first explanation that suggests itself is that there exists in the egg of the ascidian and annelid a causal relation between the location of the cleavage planes and the production of the symmetry of the embryo, so that any disturbance of the one affects the other. In favor of this view one might argue that the strict adherence in these eggs to a very regular cleavage pattern indicates a fixed relation between the two processes. The embryos that develop from the compressed egg are not however so much characterized by the absence of planes of symmetry, as by the imperfect development and dislocation of the organs of the body, as well as by the duplication of certain organs. When it is recalled that one of the principle characteristics of eggs with a mosaic cleavage is that they produce an embryo with a comparatively small number of cells —

i. e., that differentiation begins before the egg has become much divided — we can imagine that any change that affects the size relations of the cells may bring about abnormal development. The processes of gastrulation, of formation of notochord, central nervous system etc., involve precise movements of cells and groups of cells in an orderly way. Should there exist irregularities in the sizes of the cells that interfere with these processes the embryo will be abnormal; for, at the time when these processes occur the cells have already begun to be specified in definite directions. On the other hand in eggs that divide into a large number of cells before the specification of the cells begins, — eggs for instance like those of the frog and sea urchin, — initial differences and irregularities may not cause any injurious effects on the embryos.

I am inclined to think, however, that while abnormalities in the sizes of the first formed cells may be one of the factors in causing abnormal development, it is hardly possible to ascribe the differences observed to this fact alone, but that there lies behind the observed facts effects that are more profound. The eggs with a definitive type of cleavage are characterized not only by the presence of few cells when the embryo forms, but by the early specification of these cells. If it can be shown that cell division has a definite relation to the regional differences that lead to specification, the injurious effects of compression could be traced to this source.

Two alternative and, at present, contending views are held in regard to the origin of the specification of embryonic cells. It is maintained on the one side that the specification is brought about by the sorting out of the pre-existing materials of either the nucleus or the protoplasm. On the other hand it is maintained that specification is itself the outcome of a progressive series of changes that the egg undergoes as it passes through the cleavage stages. On the former alternative we should account for abnormalities in the cleavage on the ground that with a change in the location of the cleavage planes the cells would no longer coincide with the areas of distribution of the embryo forming materials. On the latter alternative we should suppose that since the specification is a progressive change that takes place during cleavage any change in the cleavage would affect the specification.

There is a third interpretation that seems to me more in accord with the facts. On this view the specification of the developing egg is independent of the cleavage process, although in many eggs the cleavage follows largely the lines of subsequent specification, so that there is a coincidence between the two. They are interdependent only in so far as the regional relations in the unsegmented and segmenting egg give the location of the cleavage planes. This view will be further developed in the next section.

The Nature of the Cleavage Process.

The experiments with centrifuged eggs go far towards showing that the visible substances of the egg (most of them at any rate) are not concerned with the specification of the cells. Since these are the only differences apparent in the early cleavage cells it follows that if there is in reality a sorting out of protoplasmic materials these materials are invisible to us by the microscopic methods at our command. Cell-division does not separate visible materials that are of a specific or determinative character; but the substances, such as yolk, pigment, oil, that pass into special parts of the embryo have a different function to perform there.

Do the progressive stages in the direction of specification take place during cleavage, or is the specification a process that is independent of the cleavage process? At the present time this is one of the most vital questions of experimental embryology, and I am far from wishing to appear to prejudge so fundamental a problem, but so far as I can see the situation is as follows: — If the progressive protoplasmic processes go on independently of the cleavage planes then alterations in the position of the latter should cause no further changes in development than such as might be caused by the cells being of the wrong sizes, in consequence of which they either cause mechanical difficulties, or the cells contain too little or too much of a given region of differentiation. As I have said the development of abnormal embryos after pressure does not appear to be satisfactorily accounted for on the basis of size alone as a purely mechanical factor. But embryonic differentiation shows marked cell-limitations! Neighboring regions of protoplasm produce organs of entirely different characters. It has been customary to account for this fact on the assumption of interrelations between the cells. It is said that the fate of a cell is a function of its location; but the evidence on which this conclusion is based may need revision in the light of the facts here described; for, with eggs having a definitive type of cleavage it is a question of fundamental importance whether the initiation of the specification of the cells begins during the process of cleavage, or whether the process takes place after the division. The mode of development of isolated blastomeres indicates, I think, with some probability that the specification in eggs with difinite type of cleavage has already been accomplished when the division is finished. On the other hand the development of whole embryos from isolated blastomeres in the case of eggs of the plastic type of cleavage may seem to show, that, in them at least, no specification has taken place during the first divisions.

If, as seems to me probable, these advancing processes in development represent molecular changes (on their morphological side) they must be of such a kind that each change takes place in relation to the preceding conditions. The problem from this point of view resolves itself into this question — how far do the molecular rearrangements that produce specification and differentiation coincide with the cleavage process. This topic may next be considered.

Molecular Changes during Cleavage and Differentiation.

Pressure applied to the egg must bring about a condition of strain or tension of the molecular substance of the egg, and if its substance is something more than a simple mixture the pressure must affect the interrelations of the forces that give the egg its axial properties, and may if carried far enough bring about a rearrangement of the system. If on the contrary the egg is looked upon as merely a fluid, whose molecules stand in no definite relation to each other, pressure applied will be equal in all directions, and it is difficult to imagine how the pressure can have such a profound and immediate effect on the cleavage process. But if we grant that the egg has an organisation, in the sense that its molecules tend to assume a definite relation to each other, and that this relation can be best understood as a system of strains, we can understand both how the normal cleavage process stands in fixed relation to the egg symmetry, and also how the cleavage will change if the egg is put under pressure from different directions. From this point of view the position of the spindle is due, not as Hertwig supposed to its assumption of the center of the protoplasmic mass (a generalization that the formation of the polar bodies completely contradicts) but its position is determined in accordance with a system of molecular

pressure lines in the egg. If further this system is closely bound up with the process of development of the egg, as seems to be indicated by the definite relation between the egg axis and the location of the embryo, it is probable that the cleavage planes often conform to the same system that is concerned with the form-changes through which the egg passes.

On some such theory as this we can understand how the same system that underlies the development may be likewise a factor in locating the cell-divisions, and yet at the same time the process of cleavage and that of specification retain a certain degree of independence. It is this point on which I should like to lay especial emphasis, for, the facts show that however closely related the two processes, that of specification must be allowed a certain degree of independence of the process of cleavage. By referring them both to a common basis we have a point of view that meets the situation.

Applying this conception to the compressed egg we can picture to ourselves the differences in results between eggs of the two types of cleavage. In eggs with a definitive type of cleavage the molecular changes that lead to the specification take place early in development. On the other hand in eggs with a plastic type of cleavage the molecular changes that stand for specification do not take place during the early cleavages, but develop later; not along the lines of the early cleavages, except in so far as the two coincide in position, but along the lines of a pre-existing stereometry of the molecular arrangement that has not been lost or much modified during the early cleavage.

From this point of view the factor of size relation of the cells comes to have a more profound significance. In the determinative type of cleavage, specification begins early when the cells are relatively large and the cell organs are correspondingly large. Normally the cell-divisions correspond with the areas of specification. If the position of the cleavage planes is altered, some at least of the cells will contain parts of areas of different potential tendencies. Hence a conflict of interests is involved, with the result, that the development is abnormal in the sense that deficiencies and even surplusage in the organs appear. On the other hand in the case of eggs with indeterminative cleavage, large numbers of small cells are produced before specification sets in, hence there is given the opportunity for such cells each to contain one predominating influence and the result will tend towards normal development. It seems to me that this is an essential point in interpreting the difference in the behaviour of the two types of development.

In this connection it should be recalled that eggs of the definitive type show very little power of regulation, while those of the opposite type show very extensive power of adjustment. It is generally agreed that this difference is due to the early specification of the blastomeres in the former type. This difference means, on the interpretation here adopted, that the specification having taken place during the early cleavage period in the definitive type can not readily be reversed, while in the indeterminative type the specification has not yet set in, and when it does so later, it can adjust itself to the prevailing conditions.

I have intentionally left out of consideration the possibility that compression affects the distribution of the chromosomes, as in dispermic eggs. I hope soon to be able to obtain evidence on this point.

Summary.

1) The experiments were undertaken in order to find out if alterations in the position of the cleavage planes in eggs with precocious specification affect the development. Almost all of the evidence that has previously been obtained related to eggs in which differentiation begins only after a relatively large number of cells have been formed. In eggs with precocious specification, on the other hand, the fate of the cells seems to be fixed at a very early stage and consequently it seemed possible that a change in the position of the cleavage planes might affect the development injuriously.

2) In *Ciona* and in *Nereis* — the two forms used — pressure tends to cause the planes of cleavage to come in at right angles to the compressing plates, as in other eggs, so far reported. Other internal factors are also recognized that take a share in determining in eggs under pressure the particular plane selected.

3) If the eggs of *Ciona* are put under pressure during the period of dissolution of the egg nucleus, or during the period of polar-body formation, and the eggs be then freed from pressure, normal development may follow, provided the pressure has not been extreme. Pressure prior to cleavage has in itself no injurious effects.

4) If the egg is compressed before the first cleavage and kept under pressure until that division has taken place, and then freed, abnormal development occurs in a high percentage of cases. As the egg begins to elongate some little time before the first division, it will generally take at this time such a position between the compressing plates that the first cleavage plane will appear in its normal position. Such eggs should of course be expected to produce normal embryos. But if the pressure is applied before the elongation has taken place the first cleavage of the compressed egg will only very rarely coincide with that of the normal egg, and in consequence normal embryos will be correspondingly rare.

5) When compressed at the two-cell stage most eggs orient with their poles to the sides. The second cleavage is therefore equatorial in position (instead of meriodinal). If freed from pressure after this cleavage the eggs produce abnormal embryos.

When compressed at the four-cell stage, the eggs orient so that the poles are against the compressing plates, and in consequence the third cleavage planes are meriodinal (instead of equatorial). If the eggs are then released from pressure, they produce, invariably, abnormal embryos. Eggs compressed at the eight or sixteen cell stage, and kept under pressure, likewise produce abnormal embryos.

6) The experiments with the eggs of *Nereis* were less extensive consisting, in the main, in causing the third cleavage to appear in a meriodinal instead of in an equatorial plane. The embryos that developed from these eggs were abnormal in one or in many respects; the abnormalities consisting generally in defects, but sometimes in the duplication of parts. Evidently shifting the position of the third cleavage plane in *Nereis* produces as disastrous results as in *Ciona*.

7) Both in *Ciona* and in *Nereis* the effect of a change in the position of cleavage planes by moderate pressure is not to prevent the egg from developing, but to produce imperfections in the location and in the differentiation of the organs.

8) An attempt is made to analyse the results in order to discover what relation exists in eggs with precocious specification between the cleavage process and the specification of the parts of the embryo. The most probable interpretation of the facts seems to be that the specification of the different parts of the embryo is the result of differences in different regions of the egg (in a dynamic rather than in a static sense): that normally the cleavage planes follow the lines of prospective specification, and, in consequence, when differentiation begins, the cell-walls have subdivided the material into its proportionate parts in accordance with its precocious

specification. Shifting the cleavage planes not only produces cells that are of disproportionate sizes for the rôle they have to perform, but also the failure of the cleavage planes to correspond approximately to the regions of specification leads to surplusage in some organs and defects in others.

9) The interpretation of the normal cleavage process of the egg is involved in the preceding attempt to account for the results of the compression experiments. Do the prospective stages in the direction of the specification take place during cleavage, or is the specification a process that is independent of the cleavage process? It seems probable that certain cleavages are critical in the egg, and that at such times dynamic changes are initiated that determine the future course of differentiation. In normal development the cleavage planes coincide with these lines of action, so that when the process is completed the future course of development of the cells is determined. In such cases the fate of a blastomere is not determined by its relation to other blastomeres, but its fate has been determined before it was separated. Should its relation to other blastomeres be changed, further effects may it is true be induced, that may cause it to lose its specification in a given direction and acquire a new equilibrium. In a word, while specification appears before, or during cleavage the effect is afterwards maintained by the relation of the blastomeres to the others, but this relation is not initiated, but only maintained by the relation of the cells to each other. The further the specification has progressed in the early stages of development, the more difficult it appears for isolated blastomeres to retrace their steps, and, herein, lies the distinction between eggs with precocious and eggs with gradual specification.

Columbia University, Jan. 3, 1910.

Zusammenfassung.

1) Die Versuche wurden unternommen, um herauszubekommen, ob eine Lagenveränderung der Furchungsebenen in Eiern mit früher Spezifizierung auf die Entwicklung Einfluß hat. Alles früher erhaltene Beweismaterial bezog sich auf Eier, bei denen die Differenzierung erst nach Bildung einer relativ großen Anzahl von Zellen beginnt. Anderseits erscheint in Eiern mit früher Spezifizierung das spätere Schicksal der Zellen bereits auf einem relativ frühen Stadium fixiert zu sein, und es erschien infolgedessen möglich, daß eine Veränderung in der Lage der Furchungsebenen die Entwicklung schädlich beeinflussen könnte.

2) Bei *Ciona* und bei *Nereis* — den beiden Versuchsarten — zeigen die Furchungsebenen bei der Anwendung von Druck eine Neigung, sich senkrecht zu den komprimierenden Platten einzustellen, wie bei andern Eiern, soviel berichtet wird (zuerst Roux 1883, Ges. Abh. II. S. 119, 302, 976). Auch andre innere Faktoren werden anerkannt, die einen Anteil an der Determination der schließlich gewählten Furchungsebene in komprimierten Eiern haben.

3) Werden die Eier von *Ciona* während der Auflösung des Einkernes oder im Stadium der Polkörperchenbildung komprimiert und dann vom Druck befreit, so kann normale Entwicklung folgen, vorausgesetzt, daß die Kompression keine ganz starke war. Druck vor der Furchung besitzt an sich keinen schädlichen Einfluß.

4) Wird das Ei vor der ersten Furche komprimiert und unter Druck gehalten, bis die Teilung erfolgt ist, danach aber vom Druck befreit, so tritt bei einem großen Prozentsatz der Fälle abnorme Entwicklung auf. Da sich die Eier eine gewisse kurze Zeit vor dem Beginn der ersten Furche zu verlängern beginnen, so werden sie im allgemeinen zu dieser Zeit zwischen dem komprimierenden Platten eine derartige Lage annehmen, daß die erste Furchungsebene n ihrer normalen Lage erscheint. Von solchen Eiern sollte man natürlich normale Embryonen erwarten. Aber bei Anwendung der Kompression vor Eintritt der Verlängerung wird die Lage der ersten Furche beim komprimierten Ei nur sehr selten mit der des normalen zusammenfallen, und infolgedessen werden normale Embryonen entsprechend selten auftreten.

5) Bei Kompression im Zweizellenstadium orientieren sich die meisten Eier mit ihren Polen nach den Seiten. Die zweite Furche ist deshalb der Lage nach eine äquatoriale (statt einer meridionalen). Nach dieser Furche, vom Druck befreit, bringen die Eier normale Embryonen hervor. Bei Kompression im Vierzellenstadium orientieren sich die Eier so, daß die Pole den komprimierenden Platten gegenüberstehen, und infolgedessen sind die dritten Furchungsebenen meridionale (anstatt äquatoriale). Nach der Befreiung vom Druck bringen sie dann unabänderlich abnorme Embryonen hervor. Im Acht- oder Sechzehnzellenstadium komprimierte und unter Druck gehaltene Eier ergeben gleichfalls abnorme Embryonen. [Vgl. Roux vom Frosch, Ges. Abh. II. S. 926.]

6) Die Versuche mit *Nereis* waren weniger ausgedehnt, und bestanden der Hauptsache nach in der Hervorrufung der dritten Furche in einer Meridional-, statt in einer Äquatorialebene. Die aus diesen Eiern entwickelten Embryonen waren in einer oder in mehreren Hinsichten abnorm. Die Abnormitäten bestanden im allgemeinen in Defekten, aber auch hier und da in der Verdoppelung von Teilen. Offenbar führt eine Verschiebung der Lage der dritten Furchungsebene bei *Nereis* zu ebenso verderblichen Folgen wie bei *Ciona*.

7) Sowohl bei *Ciona* wie bei *Nereis* hat die Lageveränderung von Furchungsebenen unter mäßigem Druck nicht die Wirkung, die Eientwicklung ganz zu verhindern, sondern nur die, Unvollkommenheiten in der Anordnung und Differenzierung der Organe hervorzubringen.

8) Es wurde eine Analyse der Ergebnisse zu dem Zwecke unternommen, zu ermitteln, welche Beziehung in Eiern mit verfrühter Spezifizierung zwischen dem Furchungsprozeß und der Spezifizierung der Embryonalteile besteht. Die wahrscheinlichste Erklärung der Tatsachen scheint die zu sein, daß die Spezifizierung der verschiedenen Embryoteile das Ergebnis von Verschiedenheiten in verschiedenen Bezirken des Eies ist (mehr in einem dynamischen, als in einem statischen Sinne); daß normalerweise die Furchungsebenen den Linien

prospektiver Spezifikation folgen, und daß infolgedessen, wenn die Differenzierung beginnt, die Zellwände das Material in seine proportionalen Teile in Übereinstimmung mit seiner verfrühten Spezifizierung weiter zu teilen haben. Eine Verschiebung der Teilungsebenen bringt nicht nur Zellen hervor, deren Größe mit der von ihnen zu spielenden Rolle nicht im richtigen Verhältnis steht, sondern das Fehlen der annähernden Übereinstimmnng der Teilungsebenen mit den Spezifizierungsbezirken führt auch zu einem abnormen Plus bei manchen Organen, und zu Defekten bei andern.

9) Der vorstehende Versuch, eine Erklärung für die Ergebnisse der Kompressionsversuche zu finden, leitet zu der Erklärung des normalen Eifurchungsprozesses. Greifen die prospektiven Stadien in Spezifizierungsrichtung während der Furchung Platz oder ist die Spezifizierung ein von der Furchung unabhängiger Prozeß? Es erscheint wahrscheinlich, daß gewisse Teilungen für das Ei die Bedeutung von Krisen haben, und daß zu solchen Zeitpunkten dynamische Veränderungen eingeleitet werden, welche den zukünftigen Lauf der Differenzierung bestimmen. Bei der normalen Entwicklung fallen die Furchungsebenen mit diesen Aktionslinien zusammen, so daß bei der Vollendung des Prozesses auch der zukünftige Entwicklungsverlauf festgelegt ist. In solchen Fällen ist das Schicksal einer Blastomere nicht durch ihre Beziehungen zu andern Blastomeren bestimmt, sondern ihr Geschick war bereits vor ihrer Abtrennung entschieden. Sollte ihre Beziehung zu andern Blastomeren geändert werden, so können freilich dadurch weitere Wirkungen ausgelöst werden, die sie zum Aufgeben ihrer Spezifikation in einer gegebenen Richtung und zur Erwerbung eines neuen Gleichgewichtes veranlassen. Mit einem Wort: die Spezifizierung erscheint vor oder während der Teilung, die Wirkung wird durch die Beziehung der Blastomeren zu den andern unterhalten, aber diese Beziehung ist nicht eingeführt, sondern lediglich unterhalten durch die gegenseitigen Beziehungen der Zellen. Je weiter die Spezifizierung in den frühzeitigen Entwicklungsstadien fortgeschritten ist, desto schwerer erscheint es für isolierte Blastomeren, ihre Schritte wieder zurück zu machen, und hierin liegt der Unterschied zwischen Eiern mit früher und solchen mit allmählicher Spezifizierung.

(**W. Gebhardt,** Übersetzer.)

Pressure Experiments on the Egg of Cerebratulus lacteus.

By

Pauline H. Dederer.

With 7 figures in text.

Eingegangen am 21. Januar 1910.

The following is a record of the results obtained by compressing the eggs of *Cerebratulus lacteus* during maturation and early cleavage stages. The material is excellent for this purpose, since division is regular, and the egg may be oriented both by means of the polar bodies, and the stalk of the egg membrane at the vegetative pole. The experiments were undertaken with a view to determining how the maturation and early cleavage stages might be modified by pressure, and to discover if possible whether any modifications so produced would have any permanent effect upon later development.

I am indebted to Professor T. H. Morgan for the suggestion of the problem, and for assistance in the course of the work, which was carried out at the Biological Laboratory of Tufts College at Harpswell Maine, where the privileges of the Laboratory were accorded me by Prof. J. S. Kingsley and Prof. H. V. Neal.

A brief review of normal cleavage is necessary, in order to make clear how the compressed eggs differ in their development. For this I have referred to the papers of C. B. Wilson, and E. B. Wilson, in connection with my study of the living eggs.

Normal Cleavage of Cerebratulus.

When the eggs are freed from the body, each one within its egg membrane, the large conspicuous germinal vesicle is still intact and lies eccentrically between the center of the egg and the animal