

THE
AMERICAN NATURALIST

VOL. XXXV.

December, 1901.

No. 420.

REGENERATION IN THE EGG, EMBRYO,
AND ADULT.

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THE term "regeneration" has come to mean not only the replacement of a lost part, but also the production of a whole organism from a piece of an animal or of a plant. There are even other phenomena that must be included under the same term, such as, the production of a part different in kind from the one lost, and also, as I shall try to show, those cases in which a part of an egg or of an embryo produces a whole or a part of an organism. It appears that there are two principal modes by which regeneration takes place. The first and more familiar process is by means of the formation of new tissue at the exposed region, followed by the development of new organs out of this new tissue. In order to distinguish this mode from the other, we may call it "epimorphosis." The second mode of regeneration is brought about by the transformation of the entire piece into a new form. For this process I have proposed the term "morphallaxis." These two modes of regeneration are not sharply separated, for we find in some forms — noticeably in the planarians — both processes combining to produce a new organism.

It has been only within the last few years that zoölogists have discovered that parts of the embryo, or even of the unsegmented egg, have also the power of producing new, whole organisms, and while this process has been here and there brought into relation with the process of regeneration, — especially by Roux and by Barfurth, — yet up to the present time no systematic analysis has been carried out in order to see how far the regeneration of pieces of an adult organism and of pieces of the egg are similar or identical processes. It is the special object of this paper to examine further into this question.

I.

In order to make clear our subsequent comparison between the development of pieces of the adult and of the egg and embryo it will be necessary in the first place to review briefly a few well-known facts. It has been stated that there are, in general, two modes by which a piece of an adult may regenerate: (*A*) either by the development of new tissue at the exposed regions, — epimorphosis; or (*B*) by transformation of the old part into a new form, — morphallaxis.

(*A*) *a*. Furthermore, we find under the first category that when a small piece of the organism is removed (1) the organism regenerates as much as is lost; but (2), on the other hand, the small piece does not generally make the whole organism, if in fact it regenerates at all. As examples of these different processes I may cite the following cases. If the leg is cut from a newt, the larger piece — the newt — makes a new leg, but the leg does not make a new newt. If the head is cut from a snail, the snail makes a new head, — if the cut has not been made too far back, — but the head does not make a new snail. If it be objected that in both cases the smaller piece dies before regeneration can begin, I may cite another experiment made on the earthworm. If a few anterior segments are cut off, they do not make a new worm, although the piece may remain alive for a long time without showing any signs of regeneration, and during this time the larger piece, from which the anterior end had been cut off, may have

completely replaced the lost smaller part. It may also be stated that the result is not dependent upon a lack of material in the small piece, for the process of regeneration does not even begin, and, as other experiments have shown, there is certainly enough material in the small piece for regeneration to have been carried out to some considerable extent.

b. The other case in this category is that in which the large piece produces only the distal end of the part removed and, therefore, less than the part cut off. For instance, if more than five segments are taken from the anterior end of the earthworm, still only five are regenerated, and the intermediate region is never regained. In the planarian, if more than the head is cut off, only the head is *at first* regenerated, but in this case the intermediate, or missing region, is made good by subsequent changes that take place in the region behind the new head and in front of the old part. A similar thing happens when the posterior end is cut from an earthworm. At first only a small part comes back, consisting of the end-piece — the telson — and a few segments, but by subsequent growth new segments are intercalated near the posterior end just in front of the telson. Again, if the tail of a fish is cut off near its base, there appears at first only *a small new part that assumes the characteristic form of the tail, even before the new part has grown out to the level at which the tail was cut off.* Subsequently, by a process of continuous growth, the entire tail is replaced.

(*B*) *a.* The second mode in which the process of regeneration takes place is, as I have said, by the transformation of the entire piece into a new organism. This is known to occur only in those forms that we speak of as the lower animals, but in which, nevertheless, a high degree of specialization may exist. The most familiar case, and the one longest known, is that of hydra. Trembley saw that small pieces may change into new, whole animals, and subsequent investigators have observed the same thing. They have not, however, pointed out specifically that new tissue is not added at the cut-ends, but from personal observation I can state that there is no special development of new tissue in these regions. The entire piece changes over

into the new form. Another example of the same process, first made known by Bickford, is that of Tubularia. In this form it is even more evident that a piece of the stem may be transformed into a new hydranth without new tissue forming beyond the level of the cut-end.

In planarians we find not only new tissue developing at the cut-end, but also a transformation taking place in the old parts. The Protozoa also regenerate by changing over the old part into a new form. From the figures given by Gruber and others there can be little question that the piece is transformed directly into a new whole, although this important fact has not been emphasized by those who have written on the subject. A piece of Stentor, for example, if it contains one or more nodes of the macronucleus, produces a new Stentor having the characteristic form, but on a smaller scale. I have repeated this experiment on Stentor, and have found that small pieces produce new organs that are of proportionate size.

In these cases of morphallaxis, just described, the small pieces change over into the characteristic form of the species, *i.e.*, they are new wholes of smaller size. They may subsequently grow to the full normal size, and even produce sexual organs. On the other hand, there is known at least one case in which very small pieces produce not a whole form, but only a part of a whole. It has been shown that very short pieces of the stem of Tubularia may produce only the distal end of the hydranth, *viz.*, a proboscis. Other experiments show that, nevertheless, the material out of which the proboscis develops is totipotent. This problem will be more fully discussed later in another connection. The result also suggests direct comparison with those cases in which only a part of what has been removed is regenerated out of the new tissue that appears at the cut-end, as when only a few anterior segments regenerate in the earthworm after the removal of a much greater number. In fact, I think it may be shown that there is something more than a mere similarity between the two cases, and that they both result from the action of the same factors.

We may next examine some of the cases in which a part of the egg, or of the embryo, produces an entire organism. The

earliest observation of this sort was made by Haeckel in 1870. Pieces of the embryo of a jellyfish were found to produce each a new smaller embryo which gave rise to an entire organism. This discovery attracted little or no attention at the time, because the important theoretical questions that are involved were not appreciated. Without treating the facts in the order of their historical sequence I shall describe a few other results that have a direct bearing on the questions before us.

The development of isolated blastomeres is one of the simplest cases of the kind. If the first two blastomeres or cells of the egg of the sea-urchin be separated from each other, each continues to segment, in most cases as though it were still a part of a whole. There is produced a half blastula open at the side that corresponds to the region at which the other blastomere lay at the two-cell stage. The half sphere next closes in to become a whole sphere of half size, and from this time onwards the embryo continues to develop as a symmetrical whole, passing through the later stages as does the normal, whole embryo.

These results show that after separation of the blastomeres each continues to develop for a time in the same way that it would have done had it remained in contact with its fellow; but the process of half development is only carried through the cleavage stages, and then a new change takes place, initiated by the closing of the open side, and a new whole of half size is established. It is this process of closing of the opening and the subsequent whole development of the half piece that resemble the changes that take place in the regeneration of pieces of adult animals.

In other species of sea-urchins the isolated blastomeres behave in a somewhat different way. In *Sphærechinus* the cleavage is not so obviously a half cleavage, or at least while the first divisions may be more or less like those of a blastomere that remains in contact with its fellow, yet there is not formed an open half sphere, but the blastomeres close in as they are formed. A whole embryo of half size is also produced in this case.

In another sea-urchin, *Toxopneustes*, it has been found that after separation of the blastomeres the cleavage may proceed

at once as a whole, *i.e.*, the isolated blastomere divides in the same way that the whole egg divides, and not as it would have divided had it remained in contact with its fellow. The blastula is closed from the beginning. Later, as in the other cases, a whole embryo develops.

These and other experiments seem to show that the form of cleavage of the egg is the result of an arrangement or structure of the protoplasm, and that in some cases this same arrangement is retained after the separation of the blastomeres; while in other cases the protoplasm may rearrange itself at once into a new whole, in which the arrangement of the parts is symmetrical and like that of the whole egg. The blastomere divides in consequence in the same way as does the egg.

The closing in of the half blastula of *Echinus* presents certain obvious resemblances to the closing in of pieces of adult animals. Several writers have supposed that in both cases this change is due to a simple physical rearrangement of the material, but it is, I think, not altogether improbable that at least one other factor is also present. I base my opinion on the study that I have made of the closing in of pieces of *Tubularia*, of hydra, and of the closing of large wounds made in the side of very young tadpoles. While the closing in seems to be the result, in part, of the physical property of the semifluid substance to become round, or more symmetrical, it seems also to involve certain contractile phenomena of a different sort. The cells that are in contact draw towards each other as a result of their own movement or contractility. There may be also certain tactive phenomena present. At least, if we cannot show positively that some such factors may be at work we have at least no right to exclude them in the present state of our knowledge as possible factors in the result.

After the closing of the half blastula has taken place we find that the piece no longer behaves as a part, but as a new whole. We must assume that it has become a symmetrical or whole structure. Let us look somewhat further into this question.

We have seen that in the case of the egg the change that takes place in a piece, making the piece symmetrical, may be brought about by a simple physical rearrangement of the

substances of the protoplasm; but after the piece has been divided into parts or cells the contents of each cell is separated from that of its neighbor by the cell-walls which we have come to look upon as barriers preventing free interchange between the cells. In many cases, however, an actual continuity of the protoplasm from cell to cell has been demonstrated, and it has been recently shown that connections may be established during the cleavage period between neighboring cells. We shall not go far wrong, I think, if we assume that the protoplasm throughout the embryo is a continuous structure, and that it is not shut up in protoplasmic-tight compartments. How, then, from this point of view can we look upon the changes that take place in an unsymmetrical piece so that it becomes symmetrical? Can we regard the first step to be the same as in the piece of an egg, *i.e.*, to be the outcome of a symmetrical rearrangement of the material or substances contained in the egg? We may think of this as possible, but the change in itself would not be sufficient to account for the subsequent events, unless we assume that it leads to the symmetrical organization of the living substance itself, for on this rather than on the gross contents of the protoplasm the subsequent changes seem to depend. That this must be so is shown, I think, by the following considerations.

Numerous experiments have demonstrated that any part of the egg may produce a whole embryo—provided, of course, that it contains the nucleus and is large enough. The substances contained in the different parts of the protoplasm are very similar throughout the egg, although there may be more or less of one or of another sort in each region. Since any part of the egg can produce an embryo we cannot regard the presence or absence of any of these substances as of fundamental importance; hence it is probable, I think, that the result depends on the protoplasm rather than on the substances it contains.

The structure of the egg is a symmetrical one, as its development shows; a piece of the egg retains at first its old structure in most cases as its cleavage demonstrates, but it may later become also symmetrical, as the later changes

prove, and we are led to ask, What is the nature of this symmetry¹ or organization that exists in the protoplasm of the egg, and which may appear in the protoplasm of a part of the egg? The problem is all the more difficult to understand when we find that an egg that has divided on a radially symmetrical plan may produce a bilateral embryo. It is probable, however, in such cases that a bilateral structure is really present from the beginning of the cleavage, but that the factors that are at work during the cleavage are not necessarily those that determine the bilateral organization of the embryo. In some cases, as in the frog's egg, a careful examination even of the early cleavage stages shows that a bilateral organization is present in the protoplasm, although the form of the cleavage may appear in many cases to be radially symmetrical.

In order to answer our question in regard to the nature of the organization we should have to know more of the nature of development itself. All we can do at present is to examine some of the implications that are involved in the assumption, and at least attempt to make clear our position. The new axial relations that are established in the piece present certain interesting relations. If the original organization was a bilateral one that corresponded, let us assume, more or less with the plane of the first division, then an isolated blastomere has at first only the organization of a half, or rather contains the factors that lead to a half development. In other words, it has no median plane of symmetry, yet later such a median plane is established. It is this change, taking place in the isolated part, that we are forced to assume, that gives us one of the most interesting and also important problems with which the student of experimental embryology has to deal. We know of nothing similar taking place in inorganic nature. The most obvious change that may seem to approach this is in the formation of an entire crystal from a piece, but in this case there is never any change in the position of the original axes that the piece has inherited from the old crystal; there is no rearrangement

¹ The term "symmetry" does not, perhaps, express the idea entirely, since an egg with an asymmetrical cleavage must be regarded as having also a definite organization.

and, hence, the essential condition is absent. Our conception of the structure of a magnet must be also fundamentally different from the ideas involved in the organization of the egg. It is true that any piece of a magnet at once becomes a whole magnet of smaller size, and this occurs in the smallest pieces that it is possible to obtain. On the other hand, the change in the organization of a piece of the egg is a relatively slow one, and it can take place only in a relatively large piece of the substance. If it be objected that these differences are only trivial ones, and not essential, still it can be shown, I think, that we must hold entirely different views of the nature of the polarity of a magnet and the organization of the egg. Our conception of the polarity of the magnet rests on the idea that it is the sum total of the polarities, or, perhaps, of the orientation of the minutest elements, the molecules, of which the magnet is made up, while our conception of the organization of the egg is exactly the reverse (or at least I shall try to show that we must really believe this to be the case), and we must think of the entire egg as a whole and not the sum total of an infinite number of smaller wholes. We may claim, I think, that this property of the egg substance of forming itself into a new whole is peculiar to the living protoplasm and is a property that we do not find, or have not found as yet, in inorganic, or perhaps we may go further and say in dead, matter. If we choose to call this property of living matter a vital factor in the sense that it is not found in matter that is dead there can be, I think, little objection to so doing. If the statement seems to be arguing in a circle, we may state more simply that those properties of living things that are not shown by non-living things we shall call vital properties. We may add that we cannot be sure, at present, whether these vital factors will conflict with our present ideas of causality or not; they seem rather to be, however, new causal phenomena peculiar to certain organic substances or compounds, but it would be out of place here to examine further into these difficult questions.

I have dwelt somewhat at length on this topic because, as I shall try to show, an analysis of the phenomenon that takes place when a piece of an adult animal (hydra, for

example) changes over into a new, whole organism, leads us directly to the same conclusion.

A ring cut at any level from the body of hydra closes its open ends quickly, and in the course of a few days it elongates and assumes the typical proportions of this form. At the time of the operation the piece was a part of a definite organization, yet it afterwards becomes itself a whole structure. The material of which the piece is composed must be totipotent, since any piece may make a whole structure. The results demonstrate that although the piece must be regarded as a part of a whole organization before it was removed, yet after its removal it becomes itself a whole organism. This fact shows that there is no contradiction in our regarding the entire egg as also having a definite structure, or as being an organized whole, and yet any piece of it may become a new whole. This conclusion is of some importance because, at first, students of experimental embryology were inclined to go too far and assume that, since any piece of the egg could become a new whole, therefore, the egg itself must be regarded as a very simple structure. The experiments with hydra show that the egg *may be* as highly organized as is an adult animal, and yet a piece produce a new whole. It is scarcely necessary to add that the results do not show that the egg *is* so highly organized but only that it may be thought to be so without contradicting the results of the experiments.

I pointed out in my Woods Hole lecture of last year (1899) that from whatever part of the body a piece may be cut it will still be different in its different parts, in so far as one part was nearer the anterior end and another nearer the posterior end of the animal. And similarly for the sides of pieces of a bilateral animal, one part will always have been nearer than another to the median plane, etc. These differences suffice, I think, for us still to form a *causal* conception of how the new axial relations are attained, since the differences always present will be the basis on which the subsequent rearrangements take place, and the results of observations show, in fact, that the anterior end of the new organism comes from the anterior end of the pieces, etc. We may infer that a similar change takes place

in the reorganization of the egg; and we can form here also a causal conception of the change, although we may admit that it is a change different from anything that we know of in inorganic matter.

We may next examine some other results connected with the development of pieces of the adult and of the egg. It has been stated that a small piece of the stem of *Tubularia* sometimes produces only a partial form, — a proboscis, or a proboscis with reproductive organs, or a hydranth without a stem. The development of these partial structures occurs only in small pieces. The production of a proboscis, for instance, takes place only in pieces that are much smaller than the average size of the hydranth-forming region of a long piece of the stem. An entire hydranth without a stem may, however, develop from pieces that are much longer than the minimal size of the hydranth-forming region of short pieces. The point of special interest is that the development of the proboscis takes place in pieces whose material is totipotent, *i.e.*, the material can produce any part of a hydranth or of a stem. We may restate the result by saying that there is a stronger tendency, especially in the more distal parts of the stem, to form the full-sized organs than to form the whole structure in a reduced form.

These results with small pieces of *Tubularia* suggest at once a comparison with those cases in which a part of an egg develops into a part of an embryo, and not into a whole one of smaller size. This is most strikingly shown in the ctenophore egg and also in the egg of a snail, *Ilyanassa*. It has been found that, if the first two or first four blastomeres of the ctenophore egg are isolated, each not only divides as a part, but each develops into an incomplete embryo that lacks, in the case of the one-half blastomere, a half or nearly half of the entire structure, and in the isolated one-quarter blastomere lacks about three-fourths of the entire structure, etc. If pieces are cut from the fertilized but unsegmented egg of the same animal the embryos that develop from the nucleated pieces are also often incomplete. The results indicate that the phenomena are due to a lack of regulative power, or power of rearrangement

in the egg; perhaps to a lack of lability in the protoplasm, so that it fails to reorganize itself into a new whole. The result is not due to the smallness of the piece, for under certain conditions, not yet understood, a smaller piece than a half or a fourth of the egg may make a whole structure. We must, therefore, look upon the egg of the ctenophore as totipotent in all its parts, in the same way that the sea-urchin's egg is totipotent, but an incomplete embryo develops from a piece, since the egg *after* fertilization has not the power, under ordinary circumstances, of rearrangement or regulation. It is conceivable that at a different temperature or in a slightly different medium the protoplasm of the piece might rearrange itself so that an entire embryo would develop.

If we think of the embryo as organized in the protoplasm of the egg in the sense of there being present a definite structural arrangement of the protoplasm, then when the blastomere of the ctenophore egg is isolated there is simply a lack of change. If, on the other hand, we think of the egg at the beginning of cleavage as being organized only in so far as the cleavage is concerned, the later organizations appearing afterwards, then we must think of the half organization of the embryo as being induced by the half form of the cleavage, or the half form of the isolated part. The half development would, in this case, not be due to a lack of change, but to the appearance after cleavage of a half organization. If we adopt the latter alternative we can compare the development of the one-half ctenophore embryo with the production of a part of a hydranth in a small piece of Tubularia. In both cases a part of a structure is formed on account of the form or size of the piece in material that is totipotent. If, on the other hand, we adopt the first alternative we find the two processes different, unless it could be shown that towards the distal end of the piece of Tubularia a new hydranth may be organizing before the old hydranth is thrown off. The removal of a piece taking place at this time, each piece continues to develop in the determined direction. Since, however, only distal organs develop from such pieces at several levels we see that this view is insufficient by itself to give a satisfactory solution.

In regard to this latter point it seems that the development of an incomplete structure in *Tubularia* is not such an isolated phenomenon as it appears to be at first sight, but can be brought into harmony with certain other known results. For instance, when more than five segments are cut from the anterior end of the earthworm only five, as a rule, come back at whatever level of the anterior end the cut may be made. The amount of material that is formed at the cut-end, before differentiation begins, is about the same at all levels, and this gives, I believe, an insight into the phenomenon. There is for the earthworm, also, a lower limit of organization for the formation of the head, or of a ring of the body, and the same new part is formed at each level, because the amount of material that is at first formed over the exposed end is the same. We must look upon this material as totipotent, but the number of rings that it produces is limited, on account of the amount of material present and of the necessary connection that exists between the lower limit of organization and the volume of matter.¹ The analysis leads us in both cases to the supposition that the results are somehow dependent on the ultimate structure of the protoplasm from which arises the structure that we can see. The development of a proboscis from a small piece of the stem of *Tubularia* indicates that there is a quantitative element that comes into the problem, and this conclusion is directly in line with the conclusion that we reach from a study of pieces of minimal size of both adult organisms and of eggs. Below a certain point the organization of the protoplasm cannot take place, and it is this result that shows, I think, that we cannot look upon the whole as simply the sum total of similar parts, but as itself having a single plan of organization or structure.

If we next proceed to an examination of the method of development of pieces of older embryos we find some further facts that bear on our problem. It has been found that if the hollow blastula of the sea-urchin be cut in two, the opening in

¹ The form of the new part must also be a factor in the problem, for the same amount of material, if of a different shape, might give rise to more or less rings. The part of the body from which the new substance arises may be still another factor.

each piece soon closes, and a new sphere of smaller size forms from each piece. These spheres then proceed to pass through the later stages of development characteristic of the sea-urchin development. Driesch has found that if the embryo is cut in two at a later stage, when the process of gastrulation has begun, not only the piece that contains the part about to invaginate will continue to develop and produce a whole embryo, but the piece from the opposite side will also form a gastrula and embryo. If, however, the same experiment is carried out after the gastrulation has been finished, these pieces of the outer wall fail to produce a new gastrula. A similar result was obtained in still later stages in regard to the formation of the two pouches that pinch off from the inner end of the archenteron of the starfish embryo. If the inner end of the archenteron is cut off before the two pouches have formed there, they will be formed again from any part of the more proximal portion of the archenteron; but if we wait until the pouches have once been completely formed by the archenteron, and then cut off the inner end of the latter, the two pouches are not made anew at the cut-end. In other words, after the archenteron has once produced the two pouches it seems to lose *throughout* its entire length the power of repeating the process, although at an earlier stage all the parts possessed this power.

Another somewhat similar result has been recently obtained by Spemann. If a thread be tied around the middle of the embryo of the frog at the time when the medullary folds are appearing, the egg being partially constricted by the thread into a more anterior and a more posterior part, the latter will produce at its anterior end (where the string constricts the nerve plate) a new head, and there is produced in this way a double embryo with two heads, one at the anterior end, and one behind this at the middle where the constriction is present. The result shows that the material of the dorsal nerve plate is totipotent in so far as the formation of all the structures of the brain, eyes, and nerve cord are concerned, and that the position of any part in relation to the rest determines its differentiation. The brain forms at the anterior end of the nerve-plate, but it

may also appear in the middle of the plate if the middle comes to represent the anterior end, as is the case in the experiment just described.

A most important theoretical question is involved in these results, although it has not been as yet sufficiently considered by those who have carried out the experiments, *viz.*, that a stage once passed through by the embryo cannot be repeated again by the other parts of the body that may not have been directly concerned in the operation, although these parts possessed this power at an earlier stage. A thorough discussion of this important question would lead us too far from our present subject, but I may point out that the result indicates that at each stage in the development the ultimate structure seems to change its organization, so that it no longer repeats a stage once completed. The organism has not, however, lost the power of forming a new individual, or at least certain parts have not, since eggs are produced by the adult animal, each of which may make an entire organism, and the adult animal has also the power in many cases of replacing lost parts, although it does not repeat the embryonic processes in doing so.

When we examine the method of development of pieces of still later stages of the embryo we find the process more and more like that which takes place in the adult. In the higher forms (echinoderms, mollusks, annelids, and vertebrates) the regeneration of the late stages of the embryo, or larvæ, and of the adult takes place by the development of new material at the free end, while in the earlier stages in certain embryos in these groups a new whole is established by the entire structure changing over into the new form. In other words, the method of development of pieces of the early embryo is like that followed by the lower animals, *i.e.*, by a process of morphallaxis, while in the later stages of development the method of regeneration is more like that in the higher animals. The difference seems to be connected with what we call the specialization of the tissues in the later stages of development and in the adults of the higher forms, but what is meant by this specialization is a question that needs to be further examined, although it will not be possible to do so at the present time.

We have seen that there is both for pieces of the adult animal and for pieces of the egg, or of the isolated blastomeres, a lower limit of size below which a piece does not produce the typical form. Even in a form like *Tubularia*, in which a very small piece may produce an incomplete structure, there is still a lower limit below which the pieces do not produce any structure. We find this lack of power to develop in small pieces in which we know from other experiments that the material is totipotent, and the only clue we have to account for the facts is that the result is in some way directly connected with the *amount* of material present. We find for each animal a lower limit of size—a limit that may vary at different stages of development—for producing a miniature copy of the type form; or, in other words, the organization must have a certain amount of material in order to develop. We find this same law to hold for pieces of eggs, of embryos, and of adult Metazoa, and it is equally true for adult unicellular Protozoa. It has been found that very small pieces of the egg may continue to divide, although they may be much too small to produce an embryo; but as several other results have also indicated, the factors that determine the cleavage are not necessarily connected with those that relate to the organization of the embryo. Isolated blastomeres that are below a certain size also fail to produce an embryo. The one-eighth blastomeres and even the one-sixteenth blastomeres produce in some few cases the early stages of development, but many of them fail to do so, and beyond this point it is doubtful if a blastomere can develop past the gastrula stage. The results show that the lower limit is soon reached in these eggs. It is certain that for adult animals pieces much smaller than one-eighth or one-sixteenth of the body can produce new animals of half size, but we have at present no data for comparison between the absolute minimal size of a piece of the egg and of the adult of the same species that can produce a whole animal. It has been found that pieces of planarians that contain less than $\frac{1}{100}$ of the volume of the adult may produce the typical form. Pieces of hydra $\frac{1}{200}$ of the adult produce a new hydra; and pieces of the stem of *Tubularia* about $\frac{1}{245}$ of the length of the stem can also make

a new hydranth and stem.¹ The eggs produced by these forms are much smaller—hydra perhaps is to be excepted—than these minimal-sized pieces, and as the egg produces the typical form we cannot expect to find any agreement between the ultimate size of an isolated blastomere and of a piece of the adult that produces a whole structure.

Peebles found that a smaller piece of a young bud than of the wall of hydra would produce a whole organism. The result is interesting inasmuch as the region where the bud appears is also a part of the wall. We find in a growing region of this sort that the protoplasm is denser than elsewhere, and may contain at first less water than other parts of the body, so that the absolute amount of living substance may be the same in a small piece of such a bud as is contained only in a much larger piece of the wall elsewhere. If this is true, and if the egg can also be shown to contain relatively less water in its structure than do other parts of the adult animal, it may still be possible that the amount of living substance necessary to give rise to the typical form may be found to be the same in the piece of the egg and of the adult. At present, however, we lack the data necessary to make the comparison. It is certain that in many forms—as for instance in the starfish—a piece of the adult that can regenerate the entire structure must be at least several thousand, perhaps million, times greater than the egg and must contain a much vaster amount of living substance. Many other factors than that of mere size must come into play in such cases, and it seems to me that the comparison is not, after all, of much theoretical importance, since the method of development of a piece of an egg and of an adult may be entirely different.

¹ The length of the stem is so variable in *Tubularia* that a comparison is difficult. If we assume the length of the stem to be 35 mm., then a piece of the stem measuring $\frac{1}{7}$ mm. in length is about $\frac{1}{243}$ of the whole; and pieces of this size produced a new hydranth and stem.

II.

A year ago¹ I reviewed the different hypotheses that have been advanced to account for the phenomena of regeneration and I pointed out that if the development of a part of an egg, or of an embryo, is also a process of regeneration, as I believe to be the case, the same hypotheses ought to apply also to the development of these parts. It may be, therefore, worth while to see how far these hypotheses may account for the development of pieces of the egg or embryo.

Bonnet extended his theory of præformed germs, primarily invented to explain the development of the embryo from the egg, to include the phenomena of regeneration. At that time the development of a part of an egg was not known, but there is no obstacle at present to applying the same interpretation to the development or regeneration of parts of the egg and embryo. In fact, Weismann, who also believes in a theory of præformation both for egg-development and for development of pieces of adult organisms, has applied the same view to the development of parts of an egg. The theory of præformed germs as held during the seventeenth and eighteenth centuries need no longer be seriously discussed in the form in which it was then expressed, since we have abundant evidence to disprove the view; but the same conception has appeared again in a more insidious form in our own time, and has been applied not only to development of the egg, but also to the regeneration of both the adult and embryo. I shall not attempt to repeat here the arguments in favor of or against this view, but shall confine what I have to say to those points that bear on our present examination. The modern form of the doctrine of præformation as held by Weismann and his school is as follows. There exists in the nucleus of the egg præformed germs that correspond part for part to the later structures that develop from the egg. The process of development is a process of sorting out of the nuclear germs to different parts of the embryo, and later, by the action of the germs contained in each nucleus on the protoplasm of each cell, they

¹ Woods Hole Lectures, 1899.

determine the differentiation of the cell. In other words, the protoplasm is looked upon as a kind of indifferent medium on which the nuclear germs feed and multiply, and, replacing the protoplasm of each cell, produce there their particular product.

In order to account for the regeneration of parts of the adult it is assumed that there has taken place during development not only an unraveling of the qualities of one germ, but at the same time at each or many of the divisions of the nucleus a quantitative division of another original whole germ, or later of a part of that germ, into like parts. This second germ is a sort of reserve stuff for future contingencies, and during regeneration this reserve stuff is supposed to become active and to do what is required of it. Pieces of the egg or of the embryo are also supposed to be able to regenerate, owing to the presence of this *reserve idioplasm* or germ in each nucleus, that is awakened into activity by the injury to the whole and then proceeds by qualitative division of *exactly the right sort* to set free the necessary germs to complete the embryo.

I need not repeat here the many objections that have been brought forward in opposition to Weismann's view, or stop to point out how the upholders of this view have been obliged again and again to add subsidiary hypotheses to meet the objections that have appeared, but I may indicate at least some of the difficulties that this view meets with from the point of view of regeneration in the egg and embryo.

The hypothesis assumes that at each division of the egg a sorting out of the qualities of the embryo takes place. Observation shows, however, that at each division there is an equal division of the chromatin, and not an unequal division, as would necessarily take place in some of the divisions, if the hypothesis is correct. The experiments with the ctenophore egg indicate that the protoplasm is an important factor in the early divisions. The experiment of compressing the frog's egg during its period of division, so that a distribution of the nuclei in the protoplasm, different from that in the normal egg, is brought about, indicates that the early nuclei are equivalent; and the development into whole embryos of pieces of the egg in which

the segmentation nucleus passes through fewer divisions than in the whole egg also points in the same direction.

The regeneration of pieces of Hydra, Tubularia, and Planaria shows that at every level of the body the cells have the power of producing any part of the organism,¹ and we must regard them as totipotent, or nearly so. The same conclusion is arrived at from a study of the development of isolated blastomeres. These facts, and others that might be given, furnish, I think, a strong argument against Weismann's hypothesis, and I believe we must look in a different direction for a solution of the problem of development and of regeneration.

We should be careful to distinguish between the idea of pre-determination and that of præformation. The former includes the latter, but also much more, and is, in fact, an almost necessary idea in connection with the phenomenon of development, at least in one sense. Every egg of the sea-urchin is pre-determined to give rise to a sea-urchin *so long as the conditions remain as we find them at present*, but it by no means follows that the hypothesis of *præformation* is a necessary consequence of this view. It is true that since each egg passes through the same stages to reach the same goal we must regard the series of changes as predetermined in the structure or composition of the egg, and in the external conditions surrounding the egg, but the way in which the development takes place may be in no way connected with the presence of a præformed germ of the embryo or with the presence of præformed germs of any sort in the nucleus. In fact, a process of pure epigenetic development, as generally understood nowadays, may also be predetermined in the egg. It is well, I think, to be clear on this point, since otherwise we grant too readily the necessity of assuming some sort of præformation hypothesis.

In my Woods Hole lecture of last year I considered the hypothesis of formative stuffs and reached the conclusion that it is insufficient to explain many of the phenomena of regeneration. Let us see if this view will apply to the development of parts of the egg and embryo. In the first place, it should be noticed that the hypothesis was primarily invented to account

¹ At least so far as the different layers are concerned.

for those kinds of regeneration in which a new part is added at the end of the old part, and, as I have pointed out, it is not well suited to explain those cases in which a piece changes over entirely into a new whole. Since the development of parts of the egg and of isolated blastomeres and pieces of the embryo takes place by a process of morphallaxis, it is clear that the hypothesis is equally inapplicable to such cases. The kind of rearrangement or regulation that takes place in a piece of an egg, or of an isolated blastomere, or of a piece of a protozoön, so that a symmetrical whole is formed, cannot be made any clearer, I think, by the assumption of specific fluids or stuffs in the different regions that determine the later differentiation.

The idea that there is a similarity between the process by which a broken crystal completes itself and that by which an animal or a plant may make good a lost part has often been suggested. The comparison rests, I think, on a superficial resemblance, and a careful examination of the nature of the two processes shows them to be the outcome of different factors. It may, however, be asked in what respects is the modern view of crystallization different from the view which I have advocated in regard to the reorganization of the protoplasm in a part of an egg or in a piece of hydra. The two conceptions are in reality entirely different. A piece of a crystal does not rearrange its parts, much less its axes, to form a new whole of smaller size, but has deposited on its surface, from the saturated solution in which it lies, new material that conforms in every respect to the original axes and planes. In a piece of an egg or of an animal, on the contrary, the entire old structure changes over into a new whole involving in some cases a change of axes.

Pflüger's conception of the process of regeneration has many points of resemblance to the idea of the recompletion of a crystal. He applies the conception, however, only to those cases in which the regeneration is by a process of addition to the old part (epimorphosis). Pflüger's hypothesis is that at the cut-end the old material attracts from the blood new material, which is deposited over the cut-surface and is there organized at its region of contact with the old part. He speaks of the new molecules being organized at the region of contact with the

new ones, and those that have thus been organized are supposed to attract others which are then deposited on the outer end and in turn become living structures. This idea is not fundamentally different from that held at present as to the growth of a crystal, except in so far as the molecules are supposed to be organized by those in the old part or by those that have previously been altered, etc. But on this view we have no explanation of the process of morphallaxis, for, in this case, the change takes place only in the old part and throughout the old part. If we attempt to extend Pflüger's idea so that it may include these phenomena, we must suppose that a sort of recrystallization takes place in the old tissue or isolated piece; but this is really a very different conception from the former, for the process is not reorganization by apposition, but by complete working over of the entire piece into a new whole. This idea is entirely foreign to our conception of crystal growth, and also to the conception entertained by Pflüger. I have also pointed out that even in some cases in which a new part is added to the old one there are grave difficulties in the way of accepting Pflüger's view, especially in the case of the planarian, in which the new head does not appear in the middle of an anterior oblique exposed edge, but to one side of the middle line, where the influence of the old part would produce, on Pflüger's view, a very different result.

Finally, I wish to discuss a problem that is at present one of the most difficult questions in connection with the process of regeneration. I mean our idea of polarity or polarization of the living material of the animal and plant. The term was first used by Allman in 1864 to mean that a new anterior end appears on that part of a piece that lay nearest to the old anterior end, and a posterior end appears on that part that lay nearest the old posterior end. This rule is followed in a great many cases, but it is not invariable. In certain forms an organ different in kind from the one removed has been found to develop. The first case of the kind was described by Bonnet, who found when certain fresh-water worms were cut in two that the posterior piece produced at its anterior end, not a new head, but another new tail. Loeb also has described

several other instances of the same phenomenon in hydroids, and has made the additional and important discovery that in these forms the external conditions may determine the kind of regeneration that takes place. However, even in these forms differences are present in the two ends of a piece, for, even in the most favorable forms it is found that, as a rule, a new head or hydranth forms sooner on the anterior end of the piece than on the posterior, and from the posterior end of the piece a posterior end is more easily developed than from the anterior end. In other and higher forms the factors that determine the kind of regeneration seem to come from within the piece itself. In some of these forms we also find occasionally that the new part may be different from the part removed (heteromorphosis), and in some cases, as in Bonnet's fresh-water worm, a posterior end develops on an anterior surface. Other cases of the same sort are also known; for instance, in the earthworm, *Allolobophora*, a short posterior piece (less than half the length of the worm) generally produces at its anterior end another tail; a very short piece of the head-end may produce a new head on its posterior surface, and the same holds for short pieces of the head of a planarian.¹

Sachs has vigorously combated this idea of polarity, that he refers to as a metaphysical notion of the morphologists, and offers as a substitute his hypotheses of formative stuffs. I have attempted to show that the idea of formative stuffs is insufficient to explain the phenomena of regeneration, and at present I see no escape from the idea of a polarity of some sort existing in the organism. On the other hand, we cannot be too careful in clearly defining what we intend to include in this idea of polarity, for I think we can see even at present that we must form a conception of the polarity different from that in which the term is used by the physicist.

The term "polarity" when used in connection with phenomena connected with the magnet means that there is a difference in the two ends of the magnet. The idea also involves the further one that any piece of the magnet also shows this same

¹ We may call that kind of heteromorphosis in which the new part is more or less a mirror figure of the old part "amphimorphosis."

difference at the ends, and since this is found to be true for every smallest piece of the magnet, it is assumed to be a property of each molecule or atom of which the magnet is made up—the magnet being the sum total of the action of all its molecules. We speak also of a crystal showing polarity in the sense that it has a definite form that can be referred to definite *poles* and *axes*, and it is supposed that this same property is possessed by the smallest “crystal molecules” of which the crystal is made.

On the other hand, in an animal or in a plant in which we find the two ends of a piece behaving differently, yet always showing the same constant difference, although we have a result resembling in many respects the polarity of the magnet, we find also in several essential respects differences and, I think, fundamental differences (in the sense that they are the outcome of different factors) between this phenomenon and that in the magnet. For instance, we find in the pieces of *Tubularia* that external conditions may reverse entirely, after a time, the polarity of the piece, so that a head may appear on the posterior end even while another head is present on the other end. We know of nothing similar in the case of the magnet, unless we consider the change that can be brought about in it by re-magnetization; but there are quite obvious differences in the two results. The most fundamental difference, however, between the two conceptions is, I believe, connected with the kind of structure that we must suppose to be present in the two cases. The polarization of the magnet is the sum total of a vast number of smaller units, each unit being itself a magnet. On the other hand, we have at present clear evidence to show that while the polarity of the organism is the outcome of its structure or organization, this is not the effect of the presence of a large number of units of which the organization is only the sum total, but, on the contrary, the organism is itself the ultimate unit that makes the polarity, and while we must believe that this same organization may develop in a piece of the original whole, the evidence goes to show that the reorganization is of a different nature from that by which a piece of a magnet becomes *at once* a new magnet.

In the first part of this paper I have given my reasons for looking upon the organization of living things as a phenomenon *sui generis*, and I have wished to bring here into connection with this consideration the conception of polarity as applied to the organism. It is a phenomenon, I think, that is different from the one bearing the same name applied to inorganic substances. We are therefore, I think, justified in looking upon the reorganization of pieces of the egg, embryo, and adult as a phenomenon peculiar to the living structure of the egg, and without a parallel in inorganic nature. It seems to be one of the peculiar physical properties connected with the matter that we call living substance, and in the last analysis we find it to be not simply the outcome of a complex of known physical principles. We are therefore, I believe, also justified in calling the organization of living things a vital property in the sense, to repeat what I have just said, that it is peculiar to this kind of substance or structure, and not the result of a complex of known physical principles; or, in other words, it is a physical phenomenon as fundamental as the polarity shown by crystals or the magnetism of the magnet, and just as the latter are associated with certain kinds of matter, so is the organization associated with the substance protoplasm.