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REGENERATION

Columbia University Biological Series.

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REGENERATION

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To My Mother

PREFACE

THIS volume is the outcome of a course of five lectures on "Regeneration and Experimental Embryology," given in Columbia University in January, 1900. The subjects dealt with in the lectures are here more fully treated and are supplemented by the discussion of a number of related topics. During the last few years the problems connected with the regeneration of organisms have interested a large number of biologists, and much new work has been done in this field; especially in connection with the regenerative phenomena of the egg and early embryo. The development of isolated cells or blastomeres has, for instance, aroused widespread interest. It has become clearer, as new discoveries have been made, that the latter phenomena are only special cases of the general phenomena of regeneration in organisms, so that the results have been treated from this point of view in the present volume.

If it should appear that at times I have gone out of my way to attack the hypothesis of preformed nuclear germs, and also the theory of natural selection as applied to regeneration, I trust that the importance of the questions involved may be an excuse for the criticism.

If I may be pardoned a further word of personal import, I should like to add that it has seemed to me that far more essential than each special question with which the biologist has to deal is his attitude toward the general subject of biology as a science. Never before in the history of biology has this been more important than at the present time, when we so often fail to realize which problems are really scientific and which methods are legitimate for the solution of these problems. The custom of indulging in exaggerated and unverifiable speculation bids fair to dull our appreciation for hypotheses whose chief value lies in the possibility of their verification; but those who have spent their time and their imagination in such speculations cannot hope for long to hold their own against the slow but certain advance of a scientific spirit of investigation of organic phenomena. The historical questions with which so many problems seem to be connected, and for which there is no rigorous experimental test, are perhaps responsible for the loose way in which many problems in biology are treated, where fancy too often supplies the place of demonstration. If, then, I have tried to use my material in such a way as to turn the evidence against some of the uncritical hypotheses of biology, I trust that the book may have a wider bearing than simply as a treatment of the problems of regeneration.

I wish to acknowledge my many obligations to Professor H. F. Osborn and to Professor E. B. Wilson for friendly criticism and advice; and in connection with the revision of the text I am greatly indebted to Professor J. W. Warren, to Professor W. M. Wheeler, to Professor G. H. Parker, and to Professor Leo Loeb.

Bryn Mawr College, Pennsylvania,
    June 11, 1901.

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REGENERATION

CHAPTER I

GENERAL INTRODUCTION

ALTHOUGH a few cases of regeneration were spoken of by Aristotle and by Pliny, the subject first attracted general attention through the remarkable observations and experiments of the Abbé Trembley. His interest was drawn to certain fresh-water polyps, hydras, that were new to him, and in order to find out if the organisms were plants or animals he tried the effect of cutting them into pieces; for it was generally known that pieces of a plant made a new plant, but if an animal were cut into pieces, the pieces died. Trembley found that the polyp, if cut in two, produced two polyps. Logically, he should have concluded that the new form was a plant; but from other observations, as to its method of feeding and of movement, Trembley concluded that the polyp was an animal, and that the property of developing a new organism from a part must belong to animals as well as to plants. "I felt," he says, "strongly that nature is too vast, and too little known, for us to decide without temerity that this or that property is not found in one or another class of organized bodies."

Trembley's first experiments were made in 1740, and the remarkable results were communicated by letter to several other naturalists. It came about in this way that before Trembley's memoir had

appeared, in 1744, his results were generally known, and several other observers had repeated his experiments, and extended them to other forms, and had even published an account of their own experiments, recognizing Trembley, however, as the first discoverer. Thus Réaumur described, in 1742, a number of other forms in which regeneration takes place; and Bonnet, in 1745, also described some experiments that he had made during the four preceding years. Widespread interest was aroused by these results, and many different kinds of animals were experimented with to test their power of regeneration. Most important of these new discoveries were those of Spallanzani, who published a short preliminary statement of his results, in 1768, in his *Prodromo*.

Trembley found that when a hydra is cut in two, the time required for the development of the new individuals is less during warm than during cold weather. He also found that if a hydra is cut into three or four parts, each part produces a new individual. If these new hydras are fed until they grow to full size, and are then again cut into pieces, each piece will produce a new polyp. The new animals were kept in some cases for two years, and behaved in all respects as do ordinary polyps.

Trembley also found that if the anterior, or head-end, with its tentacles, is cut off, it also will make a new animal. If a hydra is cut lengthwise into two parts, the edges roll in and meet, and in an hour, or less, the characteristic form may be again assumed. New arms may appear later on the new individual. If a hydra is split lengthwise into four pieces, each piece will also produce a new polyp.

If the head-end only of a hydra is split in two, each half becomes a new head, and a two-headed hydra results. If each of the new heads is split again, a four-headed hydra is produced; and if each of the four heads is once more split in two, an eight-headed hydra is formed. A hydra of this kind, in which seven heads had been produced in this way, is shown in Fig. 1, *A*. Each head behaves as a separate individual, and all remain united on the same stalk. If the foot-end of a hydra is split, a form with two feet is produced.

One of the most ingenious and most famous experiments that Trembley made consisted in turning a hydra inside out (Fig. 1, *B*, 1 and 2). The animal tends to turn itself back again, but by sticking a fine bristle through the body, Trembley thought that the turning back could be prevented, and that the inner surface of the hollow body remained on the outside, and the outer surface of the body came to line the new central cavity. Each layer then changed, he thought, its original characteristics, and became like that of the other layer. The details of these experiments will be described in a future chapter, as well as more recent experiments that have put the results in quite a different light.

Réaumur repeated Trembley's experiment of cutting a hydra into pieces, and obtained the same results. He found also that certain fresh-water worms, as well as the terrestrial earthworm, regenerated when cut into pieces. At his instigation two other naturalists[1] examined the starfish and some marine polyps, and they concluded that it was highly probable that these forms also could regenerate. Réaumur pointed out that regeneration is more likely to occur in fragile forms which are more exposed to injury.

Bonnet's experiments were made on several kinds of fresh-water

Fig. 1.—*A-B*. After Trembley, *C-G'*. After Bonnet. *A.* Seven-headed hydra made by splitting head-ends lengthwise. *B.* Illustrating the method of turning hydra inside out by means of a bristle: 1, foot being pushed through mouth; 2, completion of process. *C.* Middle piece of an earthworm (cut into three pieces) with new head and tail. *D.* Anterior part of an earthworm regenerating a new "delicate" tail. *E.* Posterior third of a worm (lumbriculus) that regenerated two heads. *F.* Middle piece of a worm (another species) cut into three pieces. It made a tail at each end. *F'.* Anterior, enlarged end (tail) of last. *G.* Small piece of a worm. *G'.* Regeneration of head and tail of same.

worms, one of which, at least, seems to have been the annelid lumbriculus. His first experiments (1741) showed that when the worm is cut in two pieces, a new tail develops at the posterior end of the anterior piece, and a new head at the anterior end of the posterior piece. He found that if a worm is cut into three, four, eight, ten, or even fourteen pieces, each piece produces a new worm; a new head appearing on the anterior end of each piece, and a new tail on the posterior end (Fig. 1, *G, G'*). The growth of the new head is limited in all cases to the formation of a few segments, but the new tail continues to grow longer, new segments being intercalated just in front of the end-piece that contains the anal opening. In summer the regeneration of a new part takes place in two to three days; in winter in ten to twelve days, this difference not being due to the time of year, but to the temperature. Bonnet found that if a newly regenerated head is cut off, a new one regenerates, and if the second one is removed, a third, new one develops, and in one case this occurred eight times: the ninth time only a bud-like outgrowth was formed. In other cases a new head was produced a few more times, but never more than twelve. He thought that the capacity of a part to regenerate is in proportion to the number of times that the animal is liable to be injured under natural conditions.

Bonnet found that short pieces from the anterior or posterior end of the body failed to regenerate, and usually died in a few days. Occasionally two new heads appeared at the anterior end of a piece (Fig. 1, *E*), and sometimes two tails at the posterior end.

Another kind of fresh-water worm[2] was found that gave a very remarkable result. If it was cut in two pieces, the posterior piece produced at its anterior end, not a new head, but a new tail. Thus there is formed a worm with two tails turned in opposite directions, as shown in Fig. 1, *F, F'.*

Spallanzani made many experiments on a number of different animals, but unfortunately the complete account of his work was never published, and we have only the abstract given in his *Prodromo* (1768). He made a large number of experiments with earthworms of several kinds, and found that a worm cut in two pieces may produce two new worms; or, at least, that the anterior piece produces a new tail, which increases in length and may ultimately represent the posterior part of the body; the posterior piece, however, produces only a short head at its anterior end, but never makes good the rest of the part that was lost. A short piece of the anterior end fails to regenerate; but in one species of earthworm, that differs from all the others in this respect, a short anterior piece or head can make a new tail at its posterior end.[3] Spallanzani also found that if much of the anterior end is cut off, the development of a new head by the posterior piece is delayed, and, in some species, does not take place at all.

If a new head is cut off, another is regenerated, and this occurred, in one case, five times. If, after a new head has developed, a portion only is cut off, the part removed is replaced, and if a portion of this new part is cut off it is also regenerated. If a worm is split longitudinally into two pieces, the pieces die. If only a part of the worm is split longitudinally and one part removed, the latter will be regenerated from the remaining part.[4] Several contemporaries of Spallanzani also made experiments on the earthworm.[5]

Spallanzani found that a tadpole can regenerate its tail; and if a part of the new tail is cut off, the remaining part will regenerate as much as is lost. Older tadpoles regenerate more slowly than younger ones. If a tadpole is not fed, it ceases to grow larger, but it will still regenerate its tail if the tail is cut off.[6] Salamanders also regenerate a new tail, producing even new vertebræ. If a leg is cut off, it is regenerated; if all four legs are cut off, either at the same time or in succession, they are renewed. If the leg is cut off near the body, an imperfectly regenerated part is formed. Regeneration of the legs was found to take place in all species of salamanders that were known to Spallanzani, but best in young stages. In full-grown salamanders, regeneration takes place more promptly in smaller species than in larger ones. Curiously enough, it was found that if the fingers or toes are cut off, they regenerate very slowly. If the fingers of one side and the whole leg of the opposite side are cut off at the same time, the leg may be regenerated as soon as are the fingers of the other side. A year is, however, often insufficient in some forms for a leg to become fully formed. If an animal is kept without food for two months after a leg has been cut off, the new leg will regenerate as rapidly as in another salamander that has been fed during this time. If the animal is kept longer without food, it will decrease in size, but nevertheless the new leg continues to grow larger. Occasionally more toes or fewer toes than the normal number are regenerated; but as a rule the fore leg renews its four toes, and the hind leg its five toes.

In one experiment, all four legs and the tail were cut off six times during the three summer months, and were regenerated. Spallanzani calculated that 647 new bones must have been made in the new parts. The regeneration of the new limbs was as quickly carried out the last time as the first. Spallanzani also found that the upper and lower jaws of salamanders can regenerate.

If the tentacles of a snail or of a slug are cut off, they are renewed; and Spallanzani found that even if the entire head is cut off a new one is regenerated. Also other parts of the snail, as the foot, or the collar, may be regenerated. The head of the slug, it was found, regenerates with more difficulty than does that of the snail.

These justly celebrated experiments of Trembley, Réaumur, Bonnet, and Spallanzani furnished the basis of all later work. Many new facts, it is true, have been discovered, and in many cases we have penetrated further into the conditions that influence the regeneration, but many of the important facts in regard to regeneration were made known by the work of these four naturalists.

SOME FURTHER EXAMPLES OF REGENERATION

So many different phenomena are included at the present time under the term "regeneration," that it is necessary, in order to get a general idea of the subject, to pass in review some typical examples of the process.

The regeneration of different parts of the salamander shows some characteristic methods of renewal of lost parts. If the foot is cut off a new foot is regenerated; if more than the foot is cut off, as much is renewed as was lost. For instance, if the cut is made through the fore leg, as much of the fore leg as was removed, and also the foot, are regenerated; if the cut is made through the upper part of the leg, the rest of that part of the leg and the fore leg and the foot are regenerated. The new part is at first smaller than the part removed, although it may contain all the elements characteristic of the leg. It gradually increases in size until it has grown to the same size as the leg on the other side of the body, and then its growth comes to an end.

Other parts of the body of the salamander also have the power of regeneration. If a part of the tail is cut off, as much is renewed as has been removed; if a part of the lower or upper jaw is cut off, the missing part is regenerated; if a part of the eye is removed, a new eye is formed from the part that remains; but if the whole eye is extirpated, or the whole limb, together with the shoulder girdle, is removed, neither structure is regenerated.

In other vertebrates the power of regeneration is more limited. A lizard can regenerate its tail, but not its limbs. A dog can regenerate neither its limbs nor its tail.

It has been stated that the new limb of the salamander is at first smaller than the one removed, but it may contain all the elements of the original limb. We find this same phenomenon in other forms, and since it is a point of some theoretical interest, a few other examples may be given. If the tail of a fish that has a bilobed form is cut off near the base, as indicated in Fig. 40, *G*, there appears over the exposed edge a narrow band of new material. The new part

Fig. 2.—*A. Allolobophora fœtida.* Normal worm. *B-F.* Anterior ends of worms, which, after the removal of one, two, three, four, and five segments, have regenerated the same number. *G.* Anterior third cut off. Only five head-segments regenerated. *H.* Worm cut in two in middle. A head-end of five segments regenerated. *I.* Worm cut in two posterior to middle. A heteromorphic tail regenerated at anterior end.

now begins to grow faster at two places than at intermediate points, as shown in Fig. 40, *H*. The new tail, although very short, assumes, as a result, the characteristic bilobed form. The point of special interest is that the new material that appears over the exposed edge does not first grow out at an equal rate at all points until it reaches the level of the original fork, and then continue to grow faster in two regions to form the lobes of the tail, but the two regions of most rapid growth are very soon established in the new tail. Subsequent growth in all parts of the new tail enlarges it to the full size.

Fig. 3.—*A*, *B*. Short head-ends of *A. fœtida* that did not regenerate at posterior surface. *C*, *D*, *E*. Longer anterior pieces, that made new segments at their posterior ends. *F*. After Hazen. A piece consisting of five (3 to 7) anterior segments grafted, in a reversed position, upon the anterior end of another worm. A heteromorphic head of about two segments regenerated at the free end, which is the posterior end of the piece.

In some cases of regeneration, in which the new part is at first smaller than the part removed, the new part represents at first only the distal portion of the body, and although the new part may grow to the full size, the whole of the part removed may never come back. This is illustrated in the regeneration of the anterior end of the earthworm; for example, in the red-banded earthworm, or brandling (*Allolobophora fœtida*).[7] If one segment of the anterior end is cut off, one segment is very quickly regenerated (Fig. 2, *B*); if two segments are cut off, two come back (Fig. 2, *C*); if three segments are cut off, as many are regenerated (Fig. 2, *D*); if four are cut off, generally four come back (Fig. 2, *E*); when five are cut off, four or five come back (Fig. 2, *F*); but if six or more are cut off, only four or five are regenerated (Fig. 2, *G*). It is found in this case that a limit is soon reached beyond which fewer segments are produced than have been removed. The new segments form the anterior end or head that enlarges to the characteristic size; but the missing segments behind the new head are never regenerated, and the worm remains shortened throughout the rest of its life. If the reproductive region has been removed with the anterior part, new reproductive organs are never formed and the worm remains incapable of reproducing itself.

This same relation between the number of segments cut off from the anterior end and the number that is regenerated seems to hold good throughout the whole group of annelids, although the maximum

number that comes back may be different in different species. Thus in lumbriculus six or seven or even eight new segments come back if more than that number have been removed.

If we examine the method of regeneration from the posterior end of a piece of an earthworm, we find that when several or many posterior segments have been removed a new part comes back, composed at first of a very few segments. The terminal segment contains the new posterior opening of the digestive tract. New segments are now formed just in front of the terminal segment, the youngest being the one next to the end-segment. The process continues until the full complement of segments is made up (Fig. 3, *C*, *D*, *E*). Comparing these results with those described above for the anterior end, we find, in both cases, that only a few segments are at first formed, but in the posterior regeneration new segments are intercalated near the posterior end. This process of intercalation is the characteristic way in which many annelids add new segments to the posterior end, as they grow larger and longer.

Amongst the flatworms the fresh-water planarians show remarkable powers of regeneration. If the anterior end is cut off at any level, a new head is produced (Fig. 4, *C*). The new worm is at first too short, *i.e.* the new head is too near the pharynx, but changes take place in the region behind the new head that lead to the development of new material in this part. The new head is, in consequence, carried farther and farther forward until the typical relations of the parts have been formed, when the growth in the region behind the head comes to an end (Fig. 4, *C'*). Similar changes take place when the posterior end is cut off, as shown in Fig. 4, *B, B'*. The new part contains the new pharynx that is proportionately too near the head, but the pharynx is carried farther backwards by the formation of new material in front of it, until it has reached its typical distance from the head. In these planarians the results are somewhat complicated, owing to the old part changing its form, especially if the piece is not fed; but the main facts are given above, and a more complete account of the changes that occur will be given in another place.

Fig. 4.—*A-E. Planaria maculata. A.* Normal worm. *B, B¹.* Regeneration of anterior half. *C, C¹.* Regeneration of posterior half. *D.* Cross-piece of worm. *D¹, D², D³, D⁴.* Regeneration of same. *E.* Old head. *E¹, E², E³.* Regeneration of same. *F. P. lugubris.* Old head cut off just behind eyes. *F¹.* Regeneration of new head on posterior end of same.

LATERAL REGENERATION

Not only does regeneration take place in an antero-posterior direction, but in many animals also at the side. The regeneration of the limb of the salamander is, of course, a case of lateral regeneration in relation to the animal as a whole, but in a longitudinal direction in regard to the limb itself. Lateral regeneration of the limb would take place if the limb was split lengthwise into two parts and one of the parts removed. If the entire salamander were cut in two lengthwise, each half would most certainly die without regeneration, if for no other reason than that the integrity of the median organs is necessary for the life of the different parts. If, however, a planarian is cut lengthwise into a right and left half, each piece will complete itself laterally and make a new worm (Fig. 13½, *A-D*). Even a narrow piece cut from the side will produce a new worm by regenerating laterally, as shown in Fig. 19, *a*, *b*, *c*. In hydra, also, a half-longitudinal piece produces a new animal, but in this case not by the addition of new material at the side, but by the cut-edges meeting to make a tube of smaller diameter. Subsequently the piece changes its form into that characteristic of hydra.

REGENERATION OF TERMINAL PORTIONS OF THE BODY

In most of the preceding examples the behavior of the larger piece of the two that result from the operation has been described; but there are some important facts in connection with the regeneration of the smaller end-pieces. The leg, or the tail, that has been cut from the salamander soon dies without regenerating. The life of the leg can be maintained only when the part is supplied with certain substances from the body of the animal. It does not follow, of course, that, could the leg or the tail be kept alive, they would regenerate a salamander. In fact, there is evidence to show, in the tail at least, that, although it may regenerate a structure at its anterior end, the structure is not a salamander, but something else. This has been definitely shown in certain experiments with the tail of the tadpole. It is possible to graft the tail of one tadpole in a reversed position, *i.e.* with its anterior end free, on the tail of

another tadpole ([Fig. 54](), *A-D*), or even on other parts of the body. Regeneration takes place from the free end, *i.e.* from the proximal end of the grafted tail. The new structure resembles a tail, and not a tadpole. If it be objected that the experiment is not conclusive because of the presence of the old tail, or of the use of the newly developing part, the objection can be met by another experiment. If, as shown in [Fig. 56](), *A*, a triangular piece is cut out of the base of the tail of a young tadpole, the cut being made so deep that the nerve-cord and notochord are cut in two, there develops from the proximal end of the tail a new tail-like structure that is turned forward, or sometimes laterally. In this case the objections to the former experiment do not apply, and the same sort of a structure, namely, a tail, is produced.

Fig. 5.—*Hydra viridis. A.* Normal hydra. Lines indicate where piece was cut out. *B*, 1-4.
Changes in a piece of *A*, as seen from the side. *C*, 1-4. Same as seen from the end. *D, E,*
F. Later stages of same piece, drawn to same scale.

In the earthworm also we find some interesting facts connected with the regeneration of the terminal pieces. If one, two, three, four, or five segments are cut from the anterior end, they will die without regenerating. Pieces that contain more segments, six to ten, for example, may remain alive for a month or longer, but do not regenerate (Fig. 3, *A*, *B*). That this lack of power to regenerate at the posterior end is not due to the smallness of the piece can be shown by removing from a piece of five segments one or two of its anterior segments. These will be promptly regenerated. Another experiment has shown, however, that if these small pieces can be kept alive for a long time, and also supplied with nourishment, regeneration will take place at the posterior end. If, for instance, a small piece of eight or ten segments has its anterior three or four segments cut off, and is grafted by its anterior end to the anterior end of another worm, as shown in Fig. 3, *F*, the piece will begin, after several months, to regenerate at its exposed posterior end, but in the one instance in which this experiment has been successfully carried out, a new head, and not a tail, appeared on the exposed free end. The result is not due to the grafting, or to the anterior position of the posterior end, but to some peculiarity in the piece itself. We find the converse of this result in an experiment with the tail region of the earthworm, where the outcome is more clearly seen to be connected with the nature of the piece itself. If a piece less than half the length of the worm is cut off from the posterior end, there is generally formed from its anterior cut-surface, not a head, but another tail (Fig. 2, *I*). The result is similar to that described by Bonnet for one of the fresh-water annelids. A parallel case to that of the head of the earthworm is found in one of the planarians. If the head of *Planaria lugubris* is cut off just behind the eyes (Fig. 4, *F*), there is produced, at the posterior cut-edge of the head, a new head turned in the opposite direction, as shown in Fig. 4, *F¹*.

REGENERATION BY TRANSFORMATION OF THE ENTIRE PIECE

In the regeneration of some of the lower animals, the transformation of a piece into a new animal of smaller size is brought about by a change in form of the piece itself, rather than through the production of new material at the cut-ends. If a ring is cut from the body of hydra, as shown in Fig. 5, *A*, the open ends of the ring are soon closed by the contraction of the sides of the piece, and in the course of a few hours the ring has become a hollow sphere; or, if the piece is longer, a closed cylinder. After a day or

two, the piece begins to elongate, and four tentacles appear near one end (Fig. 5, *B, C, D*). The piece continues to elongate until it forms a small polyp, having the typical proportions of length to breadth (Fig. 5, *E, F*). It has changed into a new cylinder that is longer than the piece cut off, but correspondingly narrower. In this case there cannot be said to be a replacement of the missing parts, but rather, through the transformation of the old piece, the formation of a new whole. In planarians also the formation of a new worm from a piece involves a change in the form of the old part, as well as the addition of new material at the cut-end. If a cross-piece is cut out, as shown in Fig. 4, *D*, new material appears at the ends, but the old piece also becomes narrower and longer (Fig. 4, *D¹-D⁴*). If the old head is cut off, it produces new material at its posterior end (Fig. 4, *E, E¹*), and also becomes smaller as the new part grows larger (Fig. 4, *E², E³*). In a land planarian, *Bipalium kewense*, a piece is transformed into a new worm, as shown in Fig. 6, *A, B*. In this case the old pigment stripes of the piece are carried directly over into the new worm, the piece elongating during the transformation.

Fig. 6.—*A.* Piece of *Bipalium kewense*. Middle pigment stripe injured at two points (see circles in *A*). *B.* Regeneration of same piece.

Fɪɢ. 7.—*Stentor cœruleus. A.* Normal, fully expanded individual. *A¹.* Same contracted. Line *a-a* indicates where it was cut in two. *B, C.* Pieces after division. *B¹, B², B³.* Regeneration of three distal pieces (*B*) containing old peristome. *C¹, C².* Regeneration of two proximal or foot pieces (*C*).

A similar change takes place in pieces of unicellular animals, as best shown by cutting off pieces of stentor. If *Stentor cœruleus* is cut in two pieces, as indicated in Fig. 7, each piece makes a new individual of half size, but of proportionate form. The old peristome remains on the anterior piece, but becomes reduced in size as the piece changes its shape, and although it may be at first too large for the length of the new piece, it ultimately reaches a size about proportionate to the rest of the animal. The posterior piece is at first too long for the size of the new peristome that is formed, but the latter becomes larger, until the characteristic form has been reached. The change in form of the stentor may take place in a few hours, and the result is brought about, not by the development of new protoplasm over the cut-end, but by a change of the old protoplasm into the new form. A similar experiment is shown in Fig. 8, in which a stentor was cut into three pieces, each piece containing a part of the old nucleus.

Fig. 8.—After Gruber. *Stentor cœruleus. A.* Cut into three pieces. *B.* This row shows regeneration of anterior piece. *C.* This row shows regeneration of middle piece. *D.* This row shows regeneration of posterior piece.

REGENERATION IN PLANTS

In the higher plants the production of a new plant from a piece takes place in a different way from that by which in animals a new individual is formed. The piece does not complete itself at the cut-ends, nor does it change its form into that of a new plant, but the leaf-buds that are present on the piece begin to develop, especially those near the distal end of the piece, as shown in Fig. 32, *A*, and roots appear near the basal end of the piece. The changes that take place in the piece are different from those taking place in animals, but as the principal difference is the development of the new part near the end, rather than over the end, and as in some cases the new part may even appear in new tissue that covers the end, and, further, since the process seems to include many factors that appear also in animals, we are justified, I think, in including this process in plants under the general term regeneration.

Fig. 9.—After Vöchting. *A*, *A¹*, *A²*. Pieces of thallus of *Lunularia communis* regenerating at the apical end. *B.* Piece of thallus cut in two in the middle line. *B¹.* Same split at side of middle. *C.* An oblique piece extending to middle line. *C¹*, *C².* Oblique pieces not extending to middle line. *D.* Fruiting stalk stuck into sand, producing new thallus above sand. *D¹.* Same laid horizontally regenerating near base. *E.* Same with fruiting head cut off. Regenerating at base. *E¹.* Twisted piece regenerating at two points. *F.* Piece of ray of head regenerating near base. *F¹.* Same with distal end of ray cut off. Also regenerating at base.

In the lower plants, such as the mosses, the liverworts, the moulds, and the unicellular forms, regeneration also takes place. Vöchting has shown that pieces from any part of the thallus of a liverwort[8] produce new plants. If a cross-piece is cut off, there appears a small outgrowth from the middle of the anterior cut-edge, as shown in Fig. 9, *A*, *A²*, that gradually enlarges to form a new thallus. It will be seen from the figures that the whole anterior edge does not grow forward, but a new thallus arises from a group of cells at, or near, the anterior edge. These cells are the least-differentiated cells in the piece, and have softer cell walls than have the other cells.

Fɪɢ. 10.—After Pringsheim. *A.* A piece of seta of sporophore of *Hypnum cupressiforme*, sending out protonema-threads. *B.* Longitudinal section of a piece of the seta of sporophore of *Bryum cæspitosum*. *C.* Piece of same of *Hypnum cupressiforme*. Moss-plant arising from new protonema. *D.* Piece of same of *Hypnum serpens* with protonema and moss-plant arising from it.

Pringsheim has shown that if a piece of the stalk of the sporangium of certain mosses is cut off, it produces at its ends thread-like outgrowths which are like the protonema-stage of the moss, and from this protonema new moss-plants may arise (Fig. 10, *A*, *B*, *C*, *D*).

Braefeld has obtained a somewhat similar result in one of the moulds, in which a piece of the sporangium stalk gives rise to a mycelium from which new sporangia may be produced.

REGENERATION IN EMBRYOS AND EGGS

Regeneration takes place not only in adult organisms, but also in embryos, and larvæ of many animals. It is often stated that the power of regeneration is more highly developed in embryos than in adults, but the facts that can be advanced in support of this view are not numerous. One of the few cases of this sort known to us is that of the leg of the frog, that does not regenerate, while the leg of the tadpole is capable of regenerating.

Fig. 11.—*A.* Blastula of Sea-urchin. Dotted lines indicate where pieces of wall were cut off. To the right are shown stages in the development of these pieces. *B.* Two-cell stage of egg of sea-urchin. One blastomere isolated. Its development shown in figures to right of *B*. *C.* Fertilized but unsegmented egg. Dotted line indicates where it was cut in two. Upper row of figures to right shows development of nucleated piece; lower row shows the fertilization and development of non-nucleated piece.

The early stages in the development of the sea-urchin, or of the starfish, may be taken to illustrate the power of regeneration in embryos. If the hollow blastula of the sea-urchin is cut into pieces (Fig. 11, *A*), each piece, if not too small, may produce a new blastula. The edges of the piece come together, and fuse in the same way in which a piece of hydra closes. A new hollow sphere of small size is formed, which then passes through the later stages of development as does the whole normal blastula.

Still earlier stages of the sea-urchin, or of the starfish, have the power of producing embryos if they are cut into pieces. If the segmenting egg is separated into a few parts, each part will continue to develop. Even the first two blastomeres or cells will, if separated, produce each a whole embryo (Fig. 11, *B*). The power of development of a part does not even end here, for, if the undivided, fertilized egg is cut into pieces, the part that contains the nucleus will segment and produce a whole embryo (Fig. 11, *C*, upper row). If the egg is cut in two or more pieces before fertilization, and then each part is fertilized, it has been found that not only the nucleated, but even the non-nucleated fragments (if they are entered by a single spermatozoon) may produce embryos (Fig. 11, *C*, lower row).

It may be questioned whether the development of parts of the embryo, or of the egg, into a whole organism can be included in the category of regenerative processes. There are, it is true, certain differences between these cases and those of adult forms, but as there are many similarities in the two cases, and as the same factors appear in both, we cannot refuse, I think, to consider all the results from a common point of view.

PHYSIOLOGICAL REGENERATION

Finally, there are certain normal changes that occur in animals and plants that are not the result of injury to the organism, and these have many points in common with the processes of regeneration. They are generally spoken of as processes of physiological regeneration. The annual moulting of the feathers of birds, the periodic loss and growth of the horns of stags, the breaking down of cells in different parts of the body after they have been active for a time, and their replacement by new cells, the loss of the peristome in the protozoon, stentor, and its renewal by a new peristome, are examples of physiological regeneration. This group of phenomena must also be included under the term "regeneration," since it is not sharply separated from that including those cases of regeneration after injury, or loss of a part, and both processes appear to involve the same factors.

DEFINITION OF TERMS

The older writers used such terms as "replacement of lost parts," "renewal of organs," and "regeneration" to designate processes similar to those described in the preceding pages. The term regeneration has been for a long time in general use to include all such phenomena as those referred to, but amongst recent writers there is some diversity of opinion as to how much is to be included in the term, and the question has arisen as to the advantage of applying new names to the different kinds of regeneration. There can be little doubt of the advantage, for the sake of greater clearness, of the use of different terms to designate different phenomena, but I think that there is at the same time the need of some general term to cover the whole field, and the word regeneration, that is already in general use, seems to fulfil this purpose better than any other.

Roux[9] points out that Trembley, and later Nussbaum, showed that a piece of hydra regenerates without the formation of new material. Roux adds that since during development the piece takes no nourishment, the *regeneration* must be brought about by the rearrangement of the cells present in the piece.[10] The change may, or may not, involve an increase in the number of the cells through a process of division. In consequence of this method of development a re-differentiation of the cells that have been already differentiated takes place. This process of regeneration, Roux points out, is very similar to the "post-generation" of the piece of the blastula of the sea-urchin embryo, and he concludes that "regeneration may be brought about entirely, or very largely, through the rearrangement and re-differentiation of cells without any, or with very little, proliferation taking place." In the adults of higher animals regeneration by proliferation preponderates, but rearrangement and re-differentiation of cells occur in all processes of regeneration, even in higher vertebrates. The two kinds of regeneration that Roux distinguishes are, he says, essentially quantitative.[11]

Barfurth[12] has defined regeneration as "the replacement of an organized whole from a part of the same." If the part is given by nature, there is a process of physiological regeneration; if the part is the result of an artificial injury, the process is one of pathological regeneration. Barfurth includes in the latter category the production of a new, entire individual from a piece, as in hydra; regeneration by proliferation, as in the earthworm; and also the development of pieces of an egg or of an embryo.

Barfurth's definition of regeneration is unsatisfactory, since an egg is itself a portion of an organism that makes a new whole, and this sort of development is not, of course, as he himself points out, to be included in the term regeneration. Nor does the use of the word "replacement" save the definition, since in many cases the kind of part that is lost is not replaced. The use of the word "pathological" to distinguish ordinary regeneration from physiological regeneration is, I think, also unfortunate, since it implies too much. There is nothing necessarily pathological in the process, especially in such cases as hydra, or as in the development of a piece of an egg where the piece is transformed directly into a new organism. Furthermore, in those cases in which (as in some annelids and planarians) a new head is formed after or during the process of natural division, there is little that suggests a pathological process; and in this instance the regeneration takes place in the same way as after artificial section.

Driesch, in his *Analytische Theorie*, states that Fraisse and Barfurth have established that during regeneration each organ produces only its like. Driesch defines regeneration, therefore, as the re-awakening of those factors that once more bring into play, by means of division and growth, the elementary processes that had ceased to act when the embryonic development was finished. This is regeneration in the restricted sense, but Driesch also points out that this definition must be enlarged,

since, when a triton, for example, regenerates its leg, not only does each tissue produce its like, but later a reconstruction and differentiation takes place, so that a leg and foot are formed, and not simply a stump containing all of the typical tissues. Driesch holds that regeneration should include only those cases in which a *proliferation* of new tissue precedes the development of the new part, and suggests that other terms be used for such cases as those of pieces of hydra, pieces of the egg, etc., in which the change takes place in the old part without proliferation of new tissue. It seems to me unwise to narrow the scope of the word regeneration as Driesch proposes, for it has neither historical usage in its favor, nor can we make any fundamental distinction between cases in which proliferation takes place and those in which it does not. As will be shown later, the factors that are present in the two cases appear to be in large part the same, and while it may be convenient to put into one class those cases in which proliferation precedes the formation of the new organs, and into another class those cases in which the change takes place without proliferation, yet, since the distinction is one of subordinate value, it is necessary to have one word to include both groups of cases; and no better word than regeneration has, I think, been as yet suggested.

Driesch has made use of two other descriptive terms. The word "reparation" is used to describe the development of the hydranth of tubularia. The new hydranth is formed in this case out of the old tissue at the end of the piece (Fig. 20, *A*). The change appears to be the same as that which takes place in a piece of hydra, etc. The word "reparation" does not seem to me to express very satisfactorily this sort of change, or sharply separate it from those cases in which the animal is *repaired* by adding what has been taken away; but in this latter sense Driesch does not use the term. I have not made use of the word, in general, except as applied to Driesch's work.

Another term, "regulation," used by Roux,[13] and also by Driesch and others, is used in a sort of physiological sense to express the *readjustments* that take place, by means of which the typical form is realized or maintained. By inference we may extend the use of the word to include the changes that take place in the new material, that is proliferated in forms that regenerate by this method. Driesch uses this term, regulation, to include a much more general class of phenomena than those included in the term regeneration, as for instance, the regulation of metabolism and of adaptation, etc. One of the subdivisions of the term regulation is called "restitution." This word also is used where I should prefer to use the word regeneration as a general term, and the word reorganization when reference is made to the internal changes that lead to the production of a typical form.

Both Roux and Driesch also speak of "self-regulation," by which is meant, I suppose, that the changes taking place are due to readjustments in the part itself, and are not induced by outside factors. The expression "self-regulation" is not, I think, a very happy one, since all change is ultimately dependent upon a relation between inside and outside conditions.

Hertwig[14] defines regeneration as the power of replacement of a part of the organism. He states that in all cases the beginning of the process is the same, viz. the appearance of a small protuberance composed of cells, that is the rudiment of the new part. It is evident that Hertwig has taken into account only one side of the process. Those cases in which a rearrangement or reorganization takes place in the old part are not even considered.[15] Goebel[16] points out that in plants the fully formed cells are, as a rule, incapable of further growth after they have once served as a basis of an organ of the body, but often some of the cells may remain in a latent condition, and grow again, when the intercellular interactions are disturbed. This is the case, he thinks, in regeneration. Goebel speaks of regeneration by means of adventitious buds in those cases in which the buds had not previously existed before the removal of the part. In those cases in which the buds are in existence before the piece is removed, as in the leaves of Asplenium, Begonia, etc., the development is not the result of regeneration, Goebel thinks, but the buds represent a stage in the development of the species. It may be pointed out, however, that it is certainly a remarkable fact that often the conditions that lead to the unfolding of an existing bud are the same as those that lead to the development of a new bud.

The preceding account will suffice to illustrate some of the principal ideas that are held in regard to the process of regeneration. Since many new facts have come to light in the last few years, it may not be amiss to point out what terms will be used in the following pages to include each kind of process.

The word "regeneration" has come to mean, in general usage, not only the replacement of a lost part, but also the development of a new, whole organism, or even a part of an organism, from a piece of

an adult, or of an embryo, or of an egg. We must include also those cases in which the part replaced is less than the part removed, or even different in kind.

At present there are known two general ways in which regeneration may take place, although the two processes are not sharply separated, and may even appear combined in the same form. In order to distinguish broadly these two modes I propose to call those cases of regeneration in which a proliferation of material precedes the development of the new part, "epimorphosis." The other mode, in which a part is transformed directly into a new organism, or part of an organism without proliferation at the cut-surfaces, "morphallaxis."

In regard to the form of the new part, certain terms may be used that will enable us to characterize briefly different classes. When the new part is like that removed, or like a part of that removed, as when a leg or a tail is regenerated in a newt, the process is one of

Fig. 12.—After Herbst. Diagram showing brain, eye, and "heteromorphic" antenna (in place of eye of one side) of palæmon. The animal had lived in a dark aquarium for five months.

"homomorphosis."[17] Under this heading we may distinguish two cases, in one of which the entire lost part is at once, or later, replaced—holomorphosis; in the other the new part is less than the part removed—meromorphosis. When the new part is different from the part removed the process has been called by Loeb "heteromorphosis," but there are at least two different kinds of processes that are covered by this definition. In one case the new part is not only different from the part removed, but is also an organ that belongs to a different part of the body (or it may be unlike any organ of the body). This we may call "neomorphosis." As an illustration of this process may be cited the development of an antenna, when the eye of a crab or of a prawn is cut off near the base (Fig. 12); and as an example of an organ different in kind from any organ of the same animal, may be cited the case of *Atyoïda potimirum*, in which the new leg is unlike any other leg on the body. The name "heteromorphosis" can be retained for those cases in which the new part is the mirror figure of the part from which it arises, or more generally stated, where the new part has its axes reversed as compared with the old part. As an example of this may be cited the development of an aboral head on the posterior end of a piece of the stem of Tubularia (Fig. 15, *B*), or the development of a tail at the anterior end of a posterior piece of an earthworm (Fig. 2).

The term "physiological regeneration" I shall use in the ordinary sense to include such changes as the moulting and replacement of the feathers of birds, the replacement of teeth, etc.,—changes that are a part of the life-cycle of the individual. In some cases it can be shown that these processes are closely related to ordinary regeneration, as when a feather pulled out is formed anew without waiting for the next moulting period, and formed presumably out of the same rudiment that would have made the new feather in the ordinary moulting process.

It is sometimes convenient to contrast the process of physiological regeneration with all other kinds. The use of the term "pathological regeneration" for the latter seems to me, as has been said, unsatisfactory. The two terms proposed by Delage,[18] viz. "regular regeneration" and "accidental regeneration," have certain advantages, although there is nothing accidental, or at least occasional, in regard to the process itself, as it is entirely regular, although it may only occur after an accident to the animal. The term "regular regeneration" is, I think, more satisfactory than "physiological regeneration," but the latter has the advantage that it has come into current use. For what is known as pathological or

accidental regeneration, I propose the term "restorative regeneration," and I shall continue to use the term "physiological regeneration" as generally understood.

CHAPTER II

THE EXTERNAL FACTORS OF REGENERATION IN ANIMALS

THERE is a constant interchange of material and of energy that takes place between a plant or an animal and its surroundings, and this interchange may be influenced by such physical conditions as temperature, light, gravity, etc., or by such chemical conditions as the composition of the atmosphere or of the water surrounding the organism. We can study the process of regeneration either by keeping the regenerating organism under the same conditions that it is subject to in its natural environment, or else we can change the surrounding physical or chemical conditions. In this way we can determine how far the regeneration is affected by external changes, and how far it is independent of them. If a change in the external conditions produces a definite change in the regeneration, then the new condition is called an external factor of regeneration.

TEMPERATURE

That the rate at which regeneration takes place can be influenced by temperature has been shown by Trembley, Spallanzani, Bonnet, and by many more recent writers. In fact, so familiar is the process to every one who has studied regeneration, that it is usually taken for granted that such is the case.

In general it may be stated that the limits of temperature under which normal growth may take place represent also the limits of temperature for regeneration. Lillie and Knowlton ('97) have determined the limits of temperature within which regeneration takes place in *Planaria torva*. The worm was cut in two transversely through the pharynx, and the time required at different temperatures to produce a new head on the posterior piece was recorded. The lowest temperature at which regeneration was found to take place was 3°C. Of six individuals kept at this temperature only one regenerated at all, and in this one the eyes and brain were still incomplete after six months. The optimum temperature, or at least that at which regeneration takes place most rapidly, was found to be 29.7°C.; a new head developed in 46 days at this temperature. At 31.5°C. regeneration was slower, requiring 8.5 days to make a new head. At 32°C. incomplete regeneration sometimes took place, but death occurred in about six days. At 33°C. regeneration was very slight, and the animals died within three days. At 34°C., and above this point, no regeneration took place, and death soon occurred.

In *Hydra viridis*, Peebles ('98) has found that regeneration is quicker at 26°-27°C. than at 28°-30°C. At the former temperature regeneration takes place in 48 hours. If kept at 12°C. pieces may regenerate in 96 hours, but not all the pieces had regenerated in this case until 168 hours.

INFLUENCE OF FOOD ON REGENERATION

While the growth of an animal or of a plant is, in most cases, and, of course, within certain limits, directly connected with the amount of food that is obtainable, nevertheless extensive regeneration may take place in an animal, or part of an animal, entirely deprived of food. In this case the material for the new part is derived from the excess of material in the old part, and not only surplus food material, but even the protoplasm itself appears to be drawn upon to furnish material to the new part. The relation between regeneration and the amount of food present in the old part is well shown by experiments with planarians. If a planarian is kept for several months without food, it will decrease very much in size. In fact, the volume of a starved worm of *Planaria lugubris* compared with that of a fully fed individual may be only one-thirteenth of the latter (Fig. 13, *A*, *B*). If a starved worm is cut in two pieces, each piece will regenerate, although less quickly than in a well-fed worm. The new part will continue to increase in size at the expense of the old piece that is already in a starved condition. On the other hand, an excess of food does not necessarily produce a hastening of the regeneration, for, as Bardeen ('01)

has shown, worms that have been for several days without food may regenerate more quickly than worms that have been fed just before they were cut into pieces.

The growth of the new part at the expense of the old tissues is a phenomenon of the greatest importance, an explanation of which will involve, I think, the most fundamental questions pertaining to

FIG. 13.—Drawn by N. M. Stevens. *A.* Large well-fed individual of *Planaria lugubris*. *B.* Same after being kept without food for 4 mos. 13 days. Both drawn to same scale.

FIG. 13½.—*Planaria lugubris.* Dotted line indicates where the worm was cut in two lengthwise. Upper three figures show how a half, that is being fed, regenerates. Lower three figures show other half kept without food.

growth. The results show that growth is connected with a structural factor, and is not simply a physiological phenomenon, although no doubt physiological factors are involved. But the physiological factors that are here at work seem to be different from what is ordinarily understood; for the fact that a tissue that is slowly starving to death should be reduced still further, and at a more rapid rate, in order to supply material to a new part, is certainly a remarkable phenomenon. At present we are not in a position to offer any explanation that rests on observation, or experiment, as to how the transfer of material takes place, or as to how the new tissue manages to get hold of the material from other parts. It is possible to protect the old part to a large extent by keeping the regenerating piece well supplied with food. If a well-fed planarian is cut in two along the middle line of the body as indicated in Fig. 13½, *A*, there develops, in the course of five or six days after the operation, new material along the cut-side of each piece, and a new pharynx appears at the border between the old and the new parts. If one of the pieces is fed at intervals, it is found that the new part grows more rapidly than does the new part in the piece without food. The old tissue in both pieces has shortened somewhat after the operation, and has also decreased somewhat in size as the first new material developed along the cut-side, but in the piece that is fed the old half begins to increase again until it reaches its former size, and may even surpass the latter. A large full-sized worm is produced from this piece, as shown in Fig. 13½, *B*, *C*, *D*. In the starved piece the old part continues to grow small, due to the lack of food and also to the increase in the new side. This increase takes place very slowly, but ultimately a small symmetrical worm may be produced, as shown in Fig. 13½, *E*, *F*, *G*. It will be seen that the starved piece needs to produce relatively less and less new material in order to become symmetrical, because as the old material diminishes, the pharynx comes to lie nearer to the middle line.

EFFECT OF LIGHT ON REGENERATION

Although few experiments have been made to test the effect of light on regeneration, it is certain that in many cases light has no effect on the process, neither as to the quality nor the quantity of the result. In one form, a tubularian hydroid, *Eudendrium racemosum*, it has been shown by Loeb that the regeneration of the hydranth takes place only when the animal is exposed to light. When a colony of eudendrium is brought into the laboratory and placed in an aquarium, the hydranths soon die; but if the colony is kept in a lighted aquarium, new hydranths are regenerated in a few days. If, on the other hand, the colony is kept in the dark, new hydranths do not appear; but if it is brought back again into the light the hydranths appear. In one experiment one lot of pieces was kept in diffuse daylight, and another lot in the dark. The former produced fifty new hydranths in a few days; those in the dark had not made any hydranths after seventeen days. They were then brought into the light, and in a few days several hydranths had developed on each piece.

Loeb also tried the effect of different colored light on the regeneration of eudendrium. Dishes containing pieces of the hydroid were put into a box that was covered by colored glass plates. Pieces subjected to dark red and to dark blue light gave the following results. The old hydranths, as is generally the case, were absorbed in the course of three days. The first new hydranths appeared in the blue light on the fourth day, and during the following days the hydranths in this lot steadily increased. Eight days after the beginning of the experiment there were eighty hydranths under the blue glass, but not one had developed in the red light. On the ninth day the red glass was replaced by a dark blue one. Two days later hydranths began to appear, and on the following day thirty-two hydranths had appeared, and in a few days more as many as sixty had developed.[19] Loeb concluded that only in the more refrangible (blue) rays does the regeneration of the hydranth take place, while the less refrangible (red) rays act as darkness does.[20] This hydroid is the only animal yet found that shows the effect of light on regeneration, and it is interesting to find that it is one of the few animals known in which light has an influence on the growth, if the heliotropism, or turning towards the light, of the hydranth is looked upon as a phenomenon of growth.

There is another series of experiments made to test the effect of light on regeneration, which gave, however, negative results. Herbst observed that when the eye of certain crustacea[21] is cut off, sometimes an eye and sometimes an antenna is regenerated. A number of individuals from which the eyes had been removed were kept in the light, and others in the dark, in order to see if the presence or absence of light is a factor in determining the kind of regeneration that takes place. It was found that as many individuals regenerated eyes in the dark as in the light. It was discovered later by Herbst and myself, independently, that, when the end only of the eye-stalk is cut off, an eye regenerates, but when the eye-stalk is cut off at the base, an antenna regenerates. The difference in the result has therefore no connection with the presence or absence of light.

GRAVITY

The only case known amongst animals, in which regeneration is influenced by the action of gravity, [22] is that of the hydroid *Antennularia antennina*. This hydroid lives attached to the bottom of the sea several metres below the surface. The hydroid consists of a single, vertical, central stem, or axis, with two or four series of lateral branches along which the hydranths arise (Fig. 14, *A*). The stem is attached by so-called stolons, or roots. In its normal growth at the free end the hydroid has been shown by Loeb to exhibit marked geotropic changes. If, for instance, the stem is bent over to one side the new growth that takes place at the apex of the stem directs the new part upwards in a vertical direction.

If pieces are cut from the stem of antennularia and suspended in

FIG. 14.—After Loeb. Normal stalk of *Antennularia antennina. B.* Piece regenerating in vertical, normal position. *C.* Piece regenerating in inverted position. *D.* Piece regenerating in inclined, vertical position. *E.* Piece regenerating in inclined, inverted position. *F.* Piece regenerating in horizontal position.

the water, regeneration takes place at the cut-ends. If a piece is suspended with its apical end upwards (Fig. 14, *B*), a new stem develops at the upper cut-end, and new roots from the lower cut-end. If a piece is suspended with its basal end upwards (Fig. 14, *C*), there is formed at its upper (basal) end a new stem with its branches also slanting upwards as shown in the figure. Roots appear at the lower (apical) end. Since gravity is the only force that acts in a vertical direction under the conditions of the experiment, Loeb concluded that it plays an important rôle in determining the kind of regeneration that takes place. Its action is of such a nature that a new stem develops from the upper cut-end, and roots from the lower end, regardless of whether the upper end is the basal or the apical end of the piece. Similar results are also obtained, according to Loeb, if the pieces are suspended obliquely. In a piece of this sort, it is found that new stems arise along the upper surface of the old stem, and roots from the lower surface as well as from the lower cut-end (Fig. 14, *D*, *E*). If a piece of the stem is placed horizontally on the bottom of an aquarium, the branches that come off from the under surface of the stem begin to grow downwards at their ends, and where they come in contact with a solid body they fasten themselves to it, thus showing that they are true roots (Fig. 14, *F*). One or more stems may arise from the upper side of the main stem. These stems grow vertically upwards, and produce lateral branches. Only in one case did a new stem, or stem-like structure, arise from one of the vertical branches, as shown to the left in Fig. 14, *F*.

Loeb found it also possible to change the character of the growth of the apex of the normal stem and to transform it into a root. A long piece of the hydroid was cut off and suspended vertically with the basal end upwards. From the upper end a new stem began to grow, and then the entire piece was reversed, so that the new stem pointed downwards. Under these circumstances the young stem did not bend around and begin to grow upwards, as a young plant might have done, but it ceased to grow as a stem, and at its apex one or more roots developed. Loeb concludes: "I cannot imagine by what means

the place of the formation of organs in antennularia is determined in connection with the orientation of the animal except by means of gravity."

The response of antennularia to the action of gravity is, I think, conclusively demonstrated by Loeb's results, but that the phenomenon may be complicated by other factors is shown, I think, by the following experiments. Driesch found that if pieces of antennularia are cut off and placed between horizontal plates, so that both ends are free, roots are produced by the basal end.[23] If the basal end with its new roots is cut off, new roots may appear, but sometimes a thin stem also. If the end is again cut off, a larger stem, and also one or two roots, may appear, and if the operation is repeated again only a stem is formed. The factor that brings about this change is not shown by the experiment. The piece had been kept in a horizontal position throughout the whole time. The apical end died in most cases without producing roots, but it is not stated whether or not roots appear on the stem between the plates of glass. If they develop they may affect the result, as certain experiments that I have made seem to show.

In my experiments, made at a different time of year from that at which Loeb's experiments were made, pieces of the stem were suspended vertically,—some with the apical end upwards, others with the basal end upwards. In nearly all cases roots were formed by both the upper and lower ends. In a few cases, in which the apical end was upwards, a new stem developed at that end. Pieces suspended in a horizontal position also produced roots at both ends. After removing the ends with their new roots from the pieces suspended vertically, I found that roots again appeared at both ends in nearly every case. The difference between these results and those of Loeb may be due to the time of the year at which the experiments were made, or possibly to some other difference, but the results show that the response to gravity is not always so constant as Loeb's results indicate.

In a few cases in my experiments the basal end of the hydroid was left attached to the stem on which it had grown, and the piece was put into the same aquarium used for the preceding experiments. In those pieces that lay on the bottom of the aquarium, with the stem standing vertically, a new shoot, and not new roots, appeared on the upper end. Other pieces were hung at the top of the water of the aquarium with the stem turned downwards, and the basal, attached end of the piece upwards. These pieces produced neither a stem nor roots from the apical end. The results show that the presence of roots at one end has an influence on the regeneration at the other end. The same thing was shown in one case in which a short piece sank to the bottom of the dish and, developing roots at its basal end, became fixed: a stem grew out of the apical end.

A number of other experiments that I made, in which pieces of antennularia were fixed to a rotating wheel, gave negative results, since neither roots nor stems appeared on the pieces. The rubbing of the ends of the piece against the water as the wheel turned round, or else the agitation of the water, prevented, most probably, the regeneration from taking place.

How gravity acts on antennularia has not as yet been determined. The only suggestion that we can offer at present is that it brings about a rearrangement of the lighter and heavier parts of the tissues. A rearrangement of this sort has been demonstrated when the egg of the frog is inverted, and in consequence certain changes are brought about in the development that will be described in another chapter.

EFFECT OF CONTACT

The contact of a newly forming part with a solid body has been shown by Loeb in a few cases, at least, to be a factor in regeneration. If a piece is cut from the stem of the tubularian hydroid *Tubularia mesembryanthemum*, and the piece held so that its basal end comes in contact with a solid body, a root develops at that end. If a piece is held in a similar position, but with its apical end in contact with a solid body, a root does not develop from this end. Evidently the development of a root in this form is also connected with an internal factor; but that there is in reality a reaction in this case, and not simply the development of a root at the basal end, is shown by the following experiment: If a piece is cut from the stem and suspended so that both ends are surrounded by water—it makes no difference whether the piece is vertical or horizontal—a hydranth develops first on the apical end, and then another on the

basal end ([Fig. 15](), *B*). When the apical end of a piece is stuck in the sand, leaving the basal end free, a hydranth develops on the latter, but not on the end in the sand.

FIG. 15.—After Loeb. *A.* A piece of the stem of margelis placed in a dish. Roots come off where stem touches dish, and polyps at other points. *B.* Piece of the stem of tubularia producing a hydranth at each end. *C. Cerianthus membranaceus.* Piece cut from side producing tentacles only on oral side of cut.

In another hydroid, *Margelis carolinensis*, studied by Loeb, the effect of contact is more easily demonstrated. If a branch of margelis is put into a dish of water and is kept from all motion, the parts that come in contact with the dish produce roots that attach themselves. Even the apical end of the stem may grow out as a root, as shown in Fig. 15, *A.* Those parts of the branch that are not in contact with any solid object give rise to new hydranths. Another hydroid, *Pennaria tiarella*, also shows, according to Loeb, the same response to contact. In this connection it is interesting to find that a growing hydranth of pennaria, if brought in contact with a solid body, turns away from the region of contact and bends at right angles to the body which it touches. We find, once more, that a factor having an influence on the growth of the animal has also a similar influence on the regeneration.

Loeb has found that if pieces of the hydroid *Campanularia* are cut off and placed in a dish filled with sea water, all the hydranths that touch the bottom of the dish are absorbed and transformed into the substance of the stem. The cœnosarc may creep out of the stem wherever it comes in contact with the glass, and produce stolons that give rise to new polyps on their upper surfaces. Loeb shows that growth takes place at the end of the stolon that pushes out of the perisarc, and this growing region draws the rest of the cœnosarc after it. If a new hydranth appears along the old piece, the cœnosarc is drawn towards the hydranth.

EFFECT OF CHEMICAL CHANGES IN THE ENVIRONMENT

Temperature, light, gravity, and contact are the most familiar kinds of external physical agencies that have a direct influence upon the growth of organisms. Food, though coming from the outside, yet acts only after it has entered the body. Organisms that live in water may be affected by the quantity and the kinds of the salts contained in the water, and also by the dissolved gases. The only experiments that have been made to show the influence of this last class of agents on animals are those made by Loeb. He placed pieces of the stem of tubularia in sea water of different degrees of concentration. After eight

days the pieces, that had meanwhile produced hydranths, were measured. It was found that the maximum growth in length takes place, not in normal sea water, but in a much diluted solution. Loeb interprets this result to mean that the cells of tubularia must have a certain amount of turgidity in order to grow, and this is possible so long as the concentration does not pass a certain limit. This limit is reached by the addition of 1.6 grams of sodium chloride to each 100 c.c. of sea water. With a decrease in the concentration, the cells become more turgid, the maximum point corresponding to the maximum amount of growth. Below this point the solution is supposed to act as a poison. The most important result of this experiment is to show that the maximum growth does not take place in sea water in which the animal is accustomed to live, but in a much more dilute solution. Normal sea water contains about 3.8 per cent of salts; the maximum growth takes place in a solution containing only 2.2 per cent. Not only is the length of the stem greater in the latter solution, but the thickness of the stem is also greater. The stem is smaller in a solution containing more salt than that contained in ordinary sea water.

There is another variant in these solutions which Loeb takes into account. With the increase in concentration of the solution its power of absorbing oxygen decreases, but the difference is too slight to affect the main result.

Not only does the amount of salts in solution affect the osmotic condition of the cells, but the salts also play a part in the metabolism of the animal. As the result of a series of experiments, the details of which may be here omitted, Loeb has shown that the regeneration of tubularia takes place only when the salts of potassium and of magnesium are present. A very little of the potassium salt is necessary, too much retards, and still more prevents regeneration.

There must be also a certain amount of oxygen dissolved in sea water in order that regeneration may take place. If a piece of the stem of tubularia is cut off and one end pushed into a small tube that fits the stem closely, and if the tube is then stuck into the sand at the bottom of an aquarium, a hydranth develops only at the free end of the piece, and none at the end in the tube. The result appears to be due to the lack of oxygen. If the piece is then taken from the tube, a hydranth may appear at the end that has been in the tube.

Another experiment shows the same result even more clearly. If a piece of the stem is suspended freely in the water, so that its lower end is almost in contact with the surface of the sand, but does not quite touch it, no regeneration takes place at the lower end. This result is interpreted by Loeb as due to the lack of oxygen in the water near the surface of the sand.[24]

GENERAL CONSIDERATIONS

In connection with the action of external factors on regeneration it is evident that in some cases they may not be in themselves necessary for the growth of a new part, yet when growth takes place they may determine what sort of a part is produced. For instance, if gravity determines the kind of regeneration in antennularia, it is possible that if the regenerating piece were placed on a rotating wheel, the piece might still produce a new stem at the apical end, and roots at the lower end. In an experiment of this sort that I made, the pieces did not, it is true, regenerate at all, but this was probably due not to the change of position in regard to gravity, but to agitation of the water, or to the rubbing of the cut-end against the water. It is also possible that in this form the attachment of the piece at one end may be a factor that may counterbalance the action of gravity. Other factors, such as food, or temperature, or oxygen, appear not to determine the kind of product that results, but only the rapidity with which the change takes place. The salts in solution seem also to act on the rate and extent of the new growth, but possibly other cases may be found in which the kind of regeneration may also be affected by the salts.

It is important to find that those animals whose growth and regeneration are influenced by such external factors as light, gravity, and contact are attached animals that stand in a constant relation to these physical agents. They form only a very small part of the entire number of animals in which regeneration takes place. Animals that constantly move about are not, as a rule, influenced during their growth and regeneration by gravity and contact, and under natural circumstances they are always changing their position in regard to these agents. Temperature, and food, and substances in solution act alike on fixed and free forms, and they are, it appears, both influenced in the same way by these agents.

The most significant fact that has been discovered in connection with the influence of external factors on regeneration is that the same factors that influence the normal growth of the organism also affect in the same way the regeneration.

As yet an analysis of the external factors that influence growth has not been made out as completely for animals as for plants, especially in those cases in which the result is determined by several factors at the same time. An examination of the factors that influence regeneration in plants will be made in a later chapter. First, however, the internal factors of regeneration in animals will be considered.

CHAPTER III

THE INTERNAL FACTORS OF REGENERATION IN ANIMALS

THE comparatively few cases in animals in which regeneration has been shown to be influenced by external factors have been given in the preceding chapter. In all other cases that are known the factors are internal. By this is meant that we cannot trace any direct connection between the result and any of the known external agents that have been shown in other cases to have an influence on regeneration. Certain external conditions must, of course, be present, such as a supply of oxygen, a certain temperature, moisture in some cases, etc., in order that the process may go on, but they are without influence on the kind of regeneration, and are necessary for all parts alike.

POLARITY AND HETEROMORPHOSIS

Trembley, Spallanzani, and Bonnet knew that, in general, at the end of a piece of an animal from which a head has been cut off a new head develops, and from the posterior cut-surface of a piece a new posterior part is regenerated. Allman was the first to give the name "polarity" to this phenomenon.[25]

In several animals regeneration takes place more readily from one end than from the other of the same cut, and this difference seems to be connected with the kind of new part that is to be regenerated, and not with the actual power of regeneration of the region itself. For instance, if a short piece is cut from the anterior end of an earthworm, a new anterior end is quickly regenerated from the anterior cut-surface of the posterior piece, but no regeneration takes place, or only after a long time, from the posterior cut-surface of the anterior piece. These relations are reversed if the posterior end of a worm is cut off. There regenerates very quickly a new posterior end from the posterior cut-surface of the anterior piece, but no regeneration takes place, or only after a long time, from the anterior cut-surface of the posterior piece. The new structures that develop after a long time from the posterior surface of a short anterior piece, and from

FIG. 16.—*A*. Head of *Planaria lugubris* with line indicating level at which *A¹* was cut off. *A¹*. Head of last regenerating a new head at its posterior end. *B*. Piece of *P. maculata* regenerating head at each end. *C*. Posterior end of *Allolobophora fœtida* regenerating a new tail at its anterior end. *C¹*. Enlarged anterior end of last with new tail. *C²*. Tip of new tail. *D*. Anterior end of one individual of *A. fœtida*, grafted to anterior end of another worm, leaving posterior end of piece exposed. This has begun to regenerate. *E*. After Hazen. Similar experiment in which a *new head* regenerated at posterior end of grafted piece. *F*. Two longer pieces of *A. fœtida* united by anterior ends. One end was subsequently cut off and a new tail regenerated. *G*. End of a developing piece of *Tubularia mesembryanthemum* that had been cut off; it has regenerated, at its proximal end, another proboscis.

the anterior surface of a short posterior piece, correspond to a different part of the worm from that which would be expected to develop, if the polarity of the piece is taken into account. Another reversed head develops on the posterior cut-surface of the anterior piece, and another tail on the anterior end of the posterior piece. The polarity of the new part is in this case reversed, as compared with that of the piece from which it arises. In the earthworm there is a marked delay in the regeneration of these heteromorphic parts. Even in tubularia in which heteromorphosis takes place, there is usually a delay of twenty-four hours in the formation of the reversed head. In *Planaria lugubris*, in which a reversed head develops, if a piece is cut from the anterior end just behind the eyes, the delay in the formation of the reversed head is very slight, if indeed there is any delay at all.

In the earthworm and in the planarian the production of reversed structures appears to be connected with the part of the body through which the cut is made, and to be due to internal factors. The question arises whether the presence of certain organs at the exposed surface can account for the result. It is conceivable that if such organs are present, and produce new cells that go into the new part, the presence of such cells may be the factor that determines what the new part will become; and in consequence the polarity of the part may be reversed. For example, the presence of the cut-end of the œsophagus or of the pharynx at the posterior surface of the anterior piece of the earthworm may determine that a new pharynx develops at the cut-end, and this may in turn act on the rest of the new

tissues in such a way that a head rather than a tail is formed. When a posterior piece is cut off, the presence of the stomach-intestine at the cut-end may influence the new part, so that a tail is produced. It can be shown, however, that a new head may arise at the anterior end of a piece that contains only the stomach-intestine, as sometimes occurs when the worm is cut in two anterior to the middle; and it is not improbable that a tail can be produced from the posterior end of a piece that contains the old œsophagus, and perhaps even the old pharynx. In the planarian I have especially examined this point, but I have not yet found that the result can be referred to the cut-surface passing through any particular organ, or to the absence of any organs at the cut-end.

If, instead of referring the result to any one organ, we assume that the tissues near the cut-ends are specialized in such a way that they can only produce their like, and that the sum total of tissues of this sort making up the new part determines the result, we can only suggest that this may be so, but we cannot show at present that it is so, or that the result could be brought about in this way.

We might make an appeal to the hypothesis of formative stuffs, and assume that there are certain substances present in the head, and others in the tail, of such a sort that they determine the kind of differentiation of the new part; but this view meets also with serious objections. In the first place, it gives only the appearance of an explanation because it assumes both that such stuffs are present, and that they can produce the kind of result that is to be explained. Until such substances have been found and until it can be shown that this kind of action is possible, the stuff-hypothesis adds nothing to the facts themselves, and may withdraw attention from the real solution of the problem.

Bonnet, who first proposed the hypothesis of specific stuffs, went further and assumed also that they move in definite directions in the body, the head-stuff flowing forward and the tail-stuff flowing backward. It was necessary to assume definite movements of the stuffs in order to account for the development of the head at the anterior end of a piece and of a tail at the posterior end. In cases of heteromorphosis of the sort described above, these stuffs, if they brought about the results, would have to move in *opposite* directions from those assumed in the hypothesis; or else that part of the hypothesis that postulates the movement of the substances must be dropped, and in its place there must be substituted the idea of the excessive amount of such substances in the ends accounting for the heteromorphosis. An hypothesis that must be changed in this fundamental way to explain both classes of facts cannot be given very serious consideration. Of these possible ways in which it has been attempted to account for the phenomenon of heteromorphosis, the first one suggested seems to me simpler and more probable, but which organs are to be made responsible for the result cannot at present be stated. The fact that both Bardeen and I have obtained heteromorphosis in planarians in other regions than in the head indicates at least that other factors than the presence of head tissues or of head substances may bring about the development, and if it can be discovered what produces the result in regions remote from the head we may be in a position to explain the result in the head region in the same way, although it may be, of course, that the same result may be brought about by different factors, when the internal conditions are somewhat different.

Another phenomenon connected with the polarity of a piece is shown by *Cerianthus membranaceous*. When a triangular piece is cut from the side of the body, a half circle of tentacles appears around the lower edge of the cut, as shown in Fig. 15, *C*. The presence of a free distal edge on the lower side of the opening is a sufficient stimulus to call forth the development of tentacles.

A somewhat similar result is obtained when an incision is made in the side of the body of a planarian. A lateral head may grow out from the anterior edge of the cut-surface, as shown in Fig. 17.

Fig. 17.—After Voigt. Planarian with three oblique cuts at side. The most anterior cut (left side), directed forward, produced a tail. The one on the right side, directed backwards, produced a head. The most posterior cut (left side) made a head with pharynx, and also a tail-like outgrowth.

FIG. 18.—*A.* After Loeb. Anterior end of *Ciona intestinalis* with oral-siphon partially cut off. Eye-specks regenerate, both on oral and aboral edge. *B.* Same (after T. H. M.), showing similar result on excurrent siphon.

It has been shown by Loeb that if the incurrent siphon of the ascidian *Ciona intestinalis* be partially cut off, new eye-specks develop around the margin of the cut, as shown in Fig. 18, *A*. I have repeated this experiment and obtained the same result, and found, as had Loeb also, that the same holds true for the excurrent siphon (Fig. 18, *B*). In these cases the new eyes appear both on the anterior and posterior edges of the cut. Most probably the result is connected with an external stimulus, rather than with an internal one. This may be true also for cerianthus, but probably not for the planarian.

LATERAL REGENERATION

Since the most familiar cases of regeneration are those that take place at the anterior and posterior ends, we not unnaturally come to think of polarity as a phenomenon connected only with the long axis of the animal; but there are also many cases of lateral regeneration in which a similar relation can be shown. In such a case as the regeneration of the leg of a salamander, or of a crab, we find instances of lateral regeneration, but since the development takes place in the direction of the long axis of the leg, the polarity of the leg may be thought of as substituted for that of the body. In other animals, however, the regeneration is strictly lateral. I have found that if the anterior end of an earthworm, or even of lumbriculus, is split lengthwise in halves, and then one of the half-pieces is removed, the missing half is replaced by the half left attached to the rest of the worm. Trembley split a hydra lengthwise into two pieces, and each piece bent inwards to make a new tubular body. Bickford, Driesch, and I have obtained similar results with pieces of the stem of tubularia.

In planarians which have a flat, broad body, lateral regeneration takes place readily. If a worm is split in two along the middle line of the body (Fig. 13½, *A*), each half regenerates the missing half. This is brought about by the development of new tissue along the cut-side, and the extension into the new part of outgrowths from the digestive tract. Lateral regeneration also takes place if the worm is split lengthwise into two unequal parts. In this case the larger piece produces new material along the cut-side, and into this new part the branches of the old digestive tract extend. The smaller piece also produces new material along the cut-side, a new pharynx appears along the line between the old and the new tissue, and a new digestive tract is formed out of the remains of the old one (Fig. 19, *a*, *b*, *c*). New branches grow out of the fused part into the new tissues at the side. The new worm that develops from a piece that is less than half the width of the old worm is about as wide as the piece that was cut off, for what is gained at the cut-side is lost in the old part. The piece loses in length also during regeneration. If the new worm is fed, it increases in size, gaining in breadth both on the old side, as well as on the new side, and in time it becomes a full-grown, symmetrical worm.

In the formation of the new part in these cases of lateral regeneration it is not difficult to understand how some of the old organs, as the digestive tract, grow out laterally into the new part; but it is more difficult to see how longitudinal organs, such as the nerve-cord and genital ducts, are formed anew. Bardeen, who has examined the development of the new nerve-cord in lateral pieces, thinks that the new nerve-cord grows backwards in the new part from the brain that develops at the anterior end, either out of the old brain, if it, or any part of it, is left, or out of the new brain that develops from the anterior end of the lateral cord that is present in the piece. What takes place in pieces cut so far to one side that none of the old cord is present in the piece he did not make out; but I can state that a new brain develops even when none of the lateral cord is present.

The development of a new head in pieces cut to one side of the old median line offers some facts of interest. A piece may be cut from the side of a planarian of such a shape that it has no anterior surface at all (Fig. 19, *A*); yet a head develops at the anterior end of the new material that appears at the side. It stands at first to one side, later it assumes an anterior position. In this case an axial structure arises in a lateral position, unless we look upon the new head as arising at the anterior end of the new part, rather than at the side of the old, but there is no evidence in favor of such an interpretation, since the head arises at the same time as does the rest of the new material at the side. In a small piece all of the new

material at the side may be used to form the new head (Fig. 19, *d*). Sometimes two heads develop (Fig. 19, *e*).

Fig. 19.—Indicating how a piece is cut off from side of *Planaria maculata*. *a, b, c.* Regeneration of last. *d.* Regeneration of single head at side. *e.* Regeneration of two heads at side.

REGENERATION FROM AN OBLIQUE SURFACE

There are also certain important facts connected with the regeneration from an oblique surface. The first case of the sort was described by Barfurth. He found that if the tail of a tadpole is cut off obliquely, as shown in Fig. 20, *B*, the new tail that develops stands at first at right angles to the oblique surface. The angle that the new tail makes with the axis of the old tail will be in proportion to the obliquity of the cut-surface. The notochord that occupies the centre of the new tail begins at the end of the old notochord, and extends to the tip of the new tail, dividing it in the same proportionate parts as does the notochord of the normal tail. The other organs occupy corresponding positions. As the new tail becomes larger it slowly swings around into line with the old part. This phenomenon of regeneration from an oblique surface has been found in a number of other forms. It has been described by Hescheler, and by myself

Fig. 20.—*A, A¹.* After Driesch. *A.* Piece of stem of tubularia cut off obliquely, showing oblique position of tentacles. *A¹.* Same, later stage. *B.* After Barfurth. Tail of tadpole regenerating from oblique surface. *C.* Tail of fundulus regenerating from oblique surface. *D.* After Hescheler. Anterior end of allolobophora regenerating from oblique surface. *E.* Piece of planaria, cut off by two oblique cuts, regenerating new head and tail. *F, F¹, F².* Three stages in the development of a new head (of a piece of bipalium) at anterior end of oblique surface.

in earthworms (Fig. 20, *D*), both for the anterior and posterior ends. I have shown that it also takes place in the tail of a teleostian fish, fundulus (Fig. 20, *C*), and have offered the following explanation of the phenomenon. The new material that is first laid down is, to a certain extent, indifferent as regards its axes. A symmetrical structure is then formed, with the old edge as a basis. The median point of the cut-edge connected with the median point of the outer surface of the new edge, gives the axis of symmetry of the new tail. The other regions assume corresponding positions. In the tail of the tadpole the position of the new notochord is determined by the cut-end of the old notochord and the median, outer point of the new material, and since the new material is at first equally developed along the cut-edge, or at least symmetrically developed, the new tail must stand at right angles to the cut-edge. This explanation will cover, I think, all cases of regeneration from an oblique surface. It assumes a law of symmetry in the new material that is in accordance with the observed position in which the new structure appears. The hypothesis makes no pretence to explain why the new structures *should* assume a symmetrical position, but given that they do, the observed result follows.

Fig. 21.—Planaria lugubris. Upper row. *A*. Part of head cut off obliquely; *a-a*⁴. Regeneration of new head. Lower row. *B*. More of head cut off obliquely; *b-b*⁴. Regeneration of same.

There are certain peculiarities connected with the regeneration from an oblique surface in planarians that may be considered in this connection. If the worm is cut in two by means of an oblique cut, as shown by the oblique line in Fig. 21, *B*, the new head that appears on the anterior cut-surface of the posterior piece appears *at one side* and not in the middle of the oblique surface (Fig. 21, *B*, *b*). The new head stands at right angles to the cut-surface. The anterior piece of the worm produces a new tail at the side of the posterior cut-surface, in the same way that the tail is formed in Fig. 20, *E*. The tail also stands at right angles to the cut-surface. The new pharynx that develops in a piece of this kind appears in the middle of the posterior cut-surface, between the old and the new parts. It may extend somewhat obliquely in the new part, and point toward the new tail.

Fɪɢ. 22.—Two upper rows *Planaria lugubris*. Lower row *Planaria maculata*. Upper row. Tail-piece cut off obliquely in front of genital pore. Figures show mode of regeneration. Middle row. Piece including old pharynx cut off by two cross-cuts, regenerating head and tail. Lower row. Piece cut off as last, regenerating head and tail.

If a piece is cut from the anterior part of a worm by two oblique and parallel cuts, the new head appears at one side of the anterior cut-surface, and the new tail at the other side of the posterior cut-surface. The new pharynx appears in the new material of the posterior part in the middle line. Thus the middle lines of the new head and tail and pharynx lie in different positions, yet these parts are subsequently brought into the same line. This is done by the head extending more forward and becoming broader, the tail growing backward and also becoming broader. The old piece becomes narrower at the same time. These three changes going on simultaneously produce a new symmetrical worm. In one form, *Planaria lugubris*, the symmetrical form is reached largely by the forward growth and the enlargement of the head, and the growth backward and the enlargement of the tail (Fig. 22, *B*). In *Planaria maculata* the old part shifts, so that it forms a new median line connecting the median line of the new head and new tail. This is best shown when the piece includes the old pharynx (Fig. 22, *C*). The pharynx is also shifted, so that its anterior end points towards the side at which the new head lies, and its posterior end towards the new tail. The result is that a new symmetrical worm is formed, as shown by the series of figures in Fig. 22, *C*. In *Planaria maculata* the changes take place largely in the old part, and the old material extends throughout the entire length of the new worm. In *Planaria lugubris* the change takes place largely in the new parts (Fig. 22, *B*). The general method in the latter species by which the symmetry is attained can be best shown by cutting the worm in two by an oblique cut just in front of the genital pore (Fig. 22, *A*). The posterior piece produces a new head at the side, and a new pharynx appears along the border between the new and the old parts, as shown in these figures. Its posterior end touches the middle line of the old part, and from this point it extends obliquely across the new tissue towards the middle of the new head. As regeneration goes on the new head is carried farther forward, it becomes larger, and the main region of new growth is found to be, in the figure, to the left side of the new part. As a result of these changes the new head turns forward, and comes to lie nearer the middle line of the old part. The pharynx is also turned more forward, and finally, as the new parts enlarge, the symmetrical form is produced. The internal factors that are involved in the development of these oblique pieces are very difficult to analyze. The position of the new head and tail

at one side of the cut-edge is the most difficult phenomenon of all to explain. We may, I think, safely regard the first new material that is proliferated along the cut-edge as totipotent, and our special problem resolves itself into discovering what factor or factors determine that the new head is to form at the most anterior end of the new material, and the new tail at the most posterior end. If we assume that the result is in some way connected with the influence of the old part on the new, and that this influence is of such a sort that the more anterior part of the old tissue determines that one side of the head must be at the most anterior edge, we have at least a formal explanation of the position of the head at the side. Given the position of the new head fixed at one side, its breadth will be determined by the maximum breadth possible for the formation of a new head. This is also in part an assumption, but it has at least certain general facts of observation in its favor. The oblique position of the new head is the result of its symmetrical development in the new material in the same way that the position of the tail of the fish or of the tadpole is the result of the symmetrical formation of the new tail on the oblique surface. The subsequent changes, by means of which a symmetrical worm is developed, are the result of different rates of growth in the different parts. In this connection the most important fact is that the growth takes place most rapidly where it will bring about the new form. This problem, which is one of the most fundamental in connection with the phenomena of development and of regeneration, will be more fully discussed in a later chapter.

A number of assumptions have been made in the above attempt to give an analysis of the formation of a head at the side of an oblique surface. That these assumptions are not entirely arbitrary, but have a certain amount of evidence in their favor, can, I think, be shown. The new material that first appears is supposed to be totipotent, in the sense that any part of it may produce any part of the structure that develops from this material. That this is probable is shown by the following experiment. If a cross-piece is cut from a worm, and then split lengthwise into halves, each half will produce a new head at the anterior edge of the piece. This result shows, at least, that from the tissue lying to the right or to the left of the middle line new material may be formed from which a whole head may develop. The new head does not stand at first with its middle axis in line with the middle of the old piece, *i.e.* it does not stand squarely at the anterior end of the half-piece, but more towards the inner side of the piece. It may appear that the old part has sufficient influence on the new part to shift the axis of the latter toward the old middle line, but while some such influence may be present, it is probable that the position of the head is in part the outcome of another factor, viz. the presence at the inner side of the piece of an undeveloped new side, with which the explanation of the less development of the inner side of the head is also connected.

If a cross-piece is cut from a worm and kept until a small amount of new tissue appears over the anterior and posterior cut-surfaces, and if then the piece is split in two lengthwise, there will develop from each piece a new head out of the new material over the anterior surface. The result shows that the new material is at first totipotent, in the sense that it may still produce one or more heads according to the conditions. It is possible, of course, that the formation of the new head may have begun at the time of the experiment, but if it had, the development had not gone so far that a new arrangement was impossible. If, however, the piece is not cut lengthwise until just before the formation of a head ([Fig. 23](), *A*), then each half-piece produces at first a half-head, that completes itself later at the cut-side.

FIG. 23.—*Planaria maculata. A.* Cross-piece, allowed to regenerate, then cut in two lengthwise, as indicated by line. *a-a*[5]. Regeneration of left half.

Another experiment shows even more satisfactorily that the material over an anterior cut-edge may produce one or more new heads according to the conditions, and that the result is not connected with the region from which the new material is derived. If the anterior end of a planarian is cut off and then an oblong piece is removed from the middle of the worm, as shown in [Fig. 24](), *A*, it will be found, if the side parts are kept from fusing together in the middle line, that a new head develops at the anterior end of each part, as shown in [Fig. 24](), *c, c¹*. If, on the other hand, the two sides come together and fuse in the middle line, as shown in [Fig. 24](), *a, b*, the new material that appears over their anterior ends becomes continuous and produces a single head. In this case, although the middle part of the old tissue has been removed, a single head develops that is normal in all respects, and the eyes are not nearer together than when the middle part is present, as when regeneration takes place from an anterior cross-cut surface.

Fɪɢ. 24.—*Planaria lugubris. A.* Showing where a piece, 4. was removed from middle of a worm. *a, b.* Regeneration of a single head. *c, cᶦ.* Regeneration of two heads. *D, E, F.* Regeneration of small piece, 4. that was cut out.

The assumption that the lateral position of the head on an oblique surface is connected with the more anterior region of the old material that is found at that side, can be made at least more intelligible by the following experiment: If the head of a planarian is cut off obliquely, as indicated in Fig. 21, *B*, so that one of the "ears" is left at one side, the new head arises at the side in connection with the part of the old head that lies at that side. The new head does not extend over the entire cut-surface, which is longer of course than a cross-cut would be, but lies at one side, as in the other cases just described. In this case we can see that if the new head cannot, on account of certain conditions, extend over the entire cut-surface, one side of it may be determined by the presence of a part of the old head, and this influence may be stronger than any other that might tend to locate the new head in the original middle line. If we suppose that similar conditions prevail in all cases when oblique surfaces are present in these worms, we have a formal solution of the problem. The argument cannot be convincing unless we can give a further explanation of the nature of this influence that the old part has upon the new.

In other cases, as in the regeneration from an oblique surface in the tail of the tadpole and of a fish, we must assume that the factor that determines the middle of the new part has a stronger influence on the new material than has the most posterior part of the old tissue.

The influence of an oblique cut-surface on the position of the new parts is shown in a different way in the hydroid, tubularia. The conditions are different in this case inasmuch as there is no proliferation from the cut-end, but the old part produces the new hydranth. Driesch found that if the stem of tubularia is cut in two obliquely, the new tentacles, that develop as two rings around the tube near its cut-end, stand obliquely on the stem,[26] as shown in Fig. 20, *A*. In most cases, both the distal and the proximal circles of tentacles lie obliquely to the long axis of the stem, but there is some variability in the result, and occasionally one or the other, especially the proximal circle, may be squarely placed, although, as a rule, the influence of the oblique cut-end can be seen. It can be shown, I think, that the oblique position of the rings of tentacles in tubularia is the outcome of factors different from those that are found in the regeneration of the tail of the tadpole and of the head and tail of the planarian. Driesch suggested that the distance of the tentacle-rings from the cut-end is the result of some sort of "regulation" that determines their position at a given distance from the region at which the surrounding water acts on the exposed end. Hence, if the exposed surface is an oblique one the rings will also be formed in an oblique position. On the other hand, I have suggested that we can imagine the regulation to result from other factors. At the beginning of the development, and before the tentacles appear, there is a withdrawal of tissue from the cut-end that leaves the region from which the proboscis develops quite thin. If this material withdraws at a uniform rate and to the same distance at all points from the end of the piece, as observation shows to be the case, and if, as appears also to be true, the outer end of the distal ring of tentacles lies at the inner end of the proboscis region, then it too will assume an oblique position if the cut-end is oblique. If we imagine a similar series of regulations taking place throughout the piece, we can account for the results. On this hypothesis the action of the water on the free end need not be a

factor in the result, but the oblique end is itself sufficient to determine the series of regulations, or mass-relations, that lead to the laying down of an oblique hydranth.

When the hydranth protrudes from the stem it assumes an oblique position, as shown in Fig. 20, *A¹*. Driesch supposed the oblique position of the hydranth to be due to an oblique zone that develops behind the hydranth, but the result can best be explained, as certain other experiments that I have made seem to show, as due to the negative thigmotropism of the hydranth at the time it protrudes from the old perisarc. It turns away from the projecting side of the oblique end of the perisarc, as it does from any solid body with which it comes in contact. That this is the case is best shown by splitting the stem lengthwise into halves. In this case, although the two circles of tentacles may be laid down squarely (Fig. 25, *A*), the new hydranth protrudes at right angles to the old perisarc, as shown in Fig. 25, *B*.

Fig. 25.—Piece of stem of *Tubularia mesembryanthemum* split in two lengthwise. Formation of whole hydranth that turned away from contact with old perisarc.

THE INFLUENCE OF INTERNAL ORGANS AT THE CUT-SURFACE ON THE NEW STRUCTURE

In a few cases it has been discovered that the presence of certain organs at the exposed surface is necessary in order that regeneration may take place. The following experiment that I have recently carried out shows, for instance, the influence of the nerve-cord on the regenerating part. A few of the anterior segments of the earthworm are cut off, as shown in the left-hand figure in Fig. 26, and then a piece of the mid-ventral body wall of the worm is cut out, a part of the ventral nerve-cord being removed with the piece. The cut-edges meet along the mid-ventral line and fuse, closing the wound. As

a result of the operation there is left exposed, at the anterior end of the worm, a cut-surface with all of the internal organs present except the nervous system. The anterior end heals over, but I have not observed the development of a new head at this level, although the exposed end is in a region at which, under ordinary circumstances, a new head readily regenerates. In several cases a new head developed at the point where the cut-end of the nervous system is situated, *i.e.* at the level *B* in the figure.

A variation of the same experiment shows still more conclusively the importance of the nervous system for the result. A few anterior segments are cut from the anterior end as before. A cut is made, as shown in the right-hand figure in Fig. 26, to one side of the mid-ventral line (indicated by the black line in the figure at the level *A*). Then, at the posterior end of this cut a piece is removed from the mid-ventral line as in the former experiment (shown by the stippled area in the figure). A portion of the ventral nerve-cord is removed with the piece. As a result of this operation, two anterior ends of the nervous system are left exposed (shown by the black dots in the figure). At the anterior end of the worm, *i.e.* at *A*, there is one exposure, and at the posterior end of the region from which the piece was removed there is another. Two heads develop in successful cases, one at the anterior end of the anterior cut-surface, *i.e.* at *A*, and the other at *B*.

The results show that in the absence of the cut-end of the nervous system at an exposed surface a new head does not develop; and conversely, the development of a new head takes place when the anterior end of the nervous system is present at a cut-surface, even when such a surface is not at the anterior end of the worm. We may perhaps be able to extend this statement, and state that as many heads will develop as there are exposed anterior ends of the nervous system.

FIG. 26.—Left-hand figure X shows how, after cutting off the anterior end of *Allolobophora fœtida*, a piece of the ventral wall (including a part of the nerve-cord) is cut out. Right-hand figure *Y* illustrates a more complicated operation, in which the piece of the ventral wall that is cut out is a little behind the anterior end.

In two other cases, at least, a somewhat similar conclusion may be drawn, although it appears that in these cases other organs than the nervous system may be the centres around which the new parts develop. Tornier has shown that when the vertebræ of the tail of the lizard are injured, the new material proliferated by the wounded surfaces serve as centres[27] for the regeneration of new tails; and Barfurth has found that the notochord in the tail of the tadpole plays a similar rôle in the formation of a new tail. These experiments will be more fully described in connection with the formation of double structures, but from what has been said it will be seen that the cases are parallel to that of the earthworm.

Until more has been discovered in regard to the internal factors of regeneration, it would be venturesome to make any general statement based on these few cases, but there is opened here a wide field for experimental work. By eliminating one by one the different organs that are present in the old part, it may be possible to discover much more in regard to the internal conditions that are necessary in order that the process of regeneration may take place.

THE INFLUENCE OF THE AMOUNT OF NEW MATERIAL

There are certain results connected with the amount of new material which is produced during regeneration, that should be considered in connection with the question of internal factors. It has been pointed out that when one segment only is removed from the anterior end of the earthworm only one new one returns; when two are cut off two come back, and this holds good up to five segments. Beyond this, no matter how many are removed, only five at most come back. The latter result seems to be connected with the amount of material that is formed over the cut-surface before differentiation begins. When only one or two segments have been cut off, the new material that is formed is soon sufficient in

amount for the production of one or two new segments, but when three to five are cut off somewhat more material is formed before differentiation begins. When more than five are cut off the new material is at best only sufficient to produce five new ones, and in some cases even a smaller number is formed. This hypothesis assumes that there is a lower limit of size for the formation of new segments below which a segment cannot develop. The interpretation is fully in accordance with what we know to be the case for small pieces of hydra and of other forms that, below a certain minimal size, do not regenerate. The question as to how many segments are formed out of the new part is determined, not only by the amount of new material, but also by the number of segments to be replaced, at least up to five segments. Beyond this limit we may think of the maximum possible number of segments appearing in the new material. That a relation of some sort obtains between the old and the new parts, that may have an influence on the number of the new segments which are formed, is shown by the fact that, when one, two, three, four, or five are cut off, just this number comes back. A sort of completing principle exists as a factor in the result, but when so much has been cut off that the old part cannot complete itself in the new material that is formed, then other factors must determine how many segments will be produced.

In planarians we find a similar phenomenon. If much of the anterior end is cut off, only a head is formed at the anterior cut-surface of the posterior piece, and the intermediate region is absent. I interpret this in the same way as the similar case in the earthworm. As soon as enough new material has been formed for the anterior end to appear, it begins to develop, and since it cannot develop below a certain minimal size, or rather, since the tendency to produce a head approaching the maximum size is stronger than the tendency to produce as much as possible of the missing anterior end, all the new material goes into the new head. In the planarian the possibility of subsequently replacing the missing region behind the head exists, and the intermediate part is later produced, the head being carried farther forward. The same is true of the new posterior end of the earthworm, in which a growing region is established at a very early stage in front of the tip of the tail, but no such growing region is present at the anterior end in the earthworm. These differences appear to be connected with the general phenomena of growth in these forms. In the planarian interstitial growth can take place in any part of the body, hence the possibility of producing a missing region is present in all parts of the worm; but in the earthworm we never find new segments intercalated at the anterior end during normal growth, nor does this take place during regeneration. At the posterior end of the earthworm we find a region of growth in which new segments are produced, and we find the same thing is true in the regeneration of the posterior end. In other words, the growing region in front of the last segment is also regenerated.

It has been found in several forms that pieces below a certain size do not regenerate. In those cases in which a small piece dies soon

Fig. 27.—*Tubularia mesembryanthemum. A.* Minimal-sized piece that produced a hydranth. *B, C.* Pieces below minimal size. *D.* Ring produced by closing of small piece.

after its removal from the rest of the body we have no direct means of knowing whether or not the piece has potentially the power to regenerate, but in some other cases, in which small pieces may be kept alive for some time, they may not regenerate. Furthermore, the regeneration of small pieces that are just above the minimal size is often delayed and is sometimes imperfect. These small pieces seem to meet with a greater difficulty in regenerating than do larger pieces. Peebles has shown that pieces of hydra that measure less than ⅙ mm. in diameter (= about $1/200$ of the volume of hydra) do not regenerate, although if very small pieces are taken from a developing bud they may regenerate, even when only a $1/9$ mm. in diameter. Very small pieces that are, however, just above the minimal size, while they may assume a hydra-like form, produce only one or two tentacles. The failure of the smallest pieces to regenerate is not due to their dying, since they may live for a much longer time than would suffice for larger pieces to regenerate. Isolated tentacles of hydra do not produce new hydras, although they may remain alive for some time. A single tentacle is larger than the minimal piece, so that its failure to regenerate is probably connected with the differentiation of the tentacle, rather than with its size. The lack of power to regenerate in the smallest pieces of hydra cannot be connected with the absence of any special organ, since these pieces contain both ectoderm and endoderm. In tubularia also, Driesch and I have found that pieces below a certain size do not

regenerate (Fig. 27). There is likewise in planarians a lower limit of regeneration, even for pieces that contain all the elements which, being present in larger pieces, make regeneration possible. Lillie has found that nucleated pieces of the protozoon stentor fail to regenerate if they are below the minimal size. He places this minimal size at 80 μ. diameter, which he calculates as $1/_{27}$ of the volume of the stentor from which the piece has come. I have obtained a slightly smaller piece that regenerated, and since it came from a larger stentor it represents about $1/_{64}$ of the whole animal. The lack of the power of development of these smallest pieces seems to be due to the absence of sufficient material for the production of the typical form. We can give no other explanation of the phenomenon at present, especially since the pieces contain material that we know from other experiments has the power of producing any part of the organism. The superficial area of small pieces is relatively greater than that of larger pieces, but there is no evidence that this relation can in any way influence the result. Whether the difference in surface tension could prevent the small piece from assuming the typical form and hold it, as it were, in a spherical form is not known, but there is little probability that this is the explanation of the phenomena.

The regeneration of small pieces of animals and of plants may often fail to take place, because, as Vöchting has pointed out, the injury caused by the cutting may extend so far into the small piece that its repair may be impossible. In other cases there may be an insufficient reserve supply of food stuff, although, if a proportionate form of any size could be produced, it is difficult to see how this could be the case. There can be no doubt, however, that pieces taken from parts of the body that are dependent on other parts for their food, oxygen, etc., will die for lack of these things, and even if they can live for some time their further development may not take place in the absence of sufficient food to carry on the process. After these possibilities have been given due weight, there remain several cases in which there can be little doubt that the failure of a small piece to regenerate is owing to the lack of sufficient material to produce even the smallest possible form for that sort of material, *i.e.* for the organization to be formed on so small a scale.

There are some facts in connection with the regeneration of small pieces of tubularia that have an important bearing on this question of organization size. If long pieces of the stem are cut off, the new hydranth, that develops out of the old tissue at the end of the piece, occupies, within certain limits, a region of definite length. If pieces of the stem are cut off that are only twice the length of the hydranth-forming region, the length of the latter will be reduced to half the length that it has in longer pieces, and if still smaller pieces are cut off, the hydranth-forming region may be reduced, as Driesch has shown, to seventy per cent of the normal length. The hydranths that develop from the smaller pieces have also a reduced number of tentacles, as I have found. It was first shown by Bickford, and later by Driesch, and by myself, that in many cases very short pieces of the stem of tubularia produce *only the distal parts of a hydranth*. This happens most often when the length of the piece is less than the average normal length of the hydranth-forming area, but it may also take place in pieces that are much longer than the minimal size of the least hydranth-forming region. Driesch made the further discovery, which I have confirmed, that pieces from the distal end of the stem are more likely to produce these partial structures than are pieces from the more proximal part. Some of these partial structures are represented in Fig. 28, *C-G*. Sometimes the inner tube, or cœnosarc, which is composed of the two layers of the body, ectoderm and

Fɪɢ. 28.—*Tubularia mesembryanthemum.* Products of regeneration of short pieces. *A.* Piece that regenerated a hydranth in same way as do longer pieces, but with fewer tentacles. *B.* Pieces whose stem drew away from wall of old perisarc (cylinder in figures). *C.* Hydranth with almost no stalk. *D.* Hydranth without stalk. *E.* Distal part of hydranth with one long proximal tentacle. *E¹.* Similar, but more reduced. *E².* Similar, with two tentacles at side. *F.* Proboscis with reproductive organs. *G.* Proboscis without reproductive organs.

endoderm, draws away from the chitinous perisarc, as shown in Fig. 28, *B*. A hydranth with a short stalk is then produced. In other cases, Fig. 28, *C*, almost all of the cœnosarc is used up to form the hydranth, and only a short, dome-shaped knob represents the stalk. In still other cases there may be no stalk at all (Fig. 27, *D*), but only the hydranth. Forms like the last two are more often produced from pieces of the distal end of the stalk. From very small pieces, forms like those shown in Figs. 28, *E-E²*, that represent only proboscides with a reduced number of tentacles, are sometimes formed. Reproductive organs may be present at the base of these pieces. A further reduction is shown in Figs. 28, *F*, *G*, that are proboscides with only the distal circle of tentacles; in one of these, reproductive organs are present around the base. Partial forms more reduced than these have not been found.

If we examine the factors that determine the production of the partial structures, we find, in the first place, that the size of the piece is of the greatest importance. The reduced forms appear most often in pieces that are shorter than the average length of the hydranth-forming area. A second factor is connected with the region of the stem from which the piece is taken. Larger pieces from the distal end produce partial structures, especially hydranths with very short stalks (Fig. 28, *C*), or with none at all (Fig. 28, *D*). There are certain facts connected with this distal region, which lies just behind the hydranth, that should be mentioned in this connection. It was first discovered by Dalyell that a hydranth-head lives for only a limited time, and that when it dies a new head is regenerated from the region behind the old one. The stalk of the new hydranth continues to elongate for some time after the new hydranth has been formed. Whether this continuous growth in the distal end, or the normal formation of a new hydranth by it from time to time, can in any way be connected with the development of partial structures from this region cannot at present be stated. The distal part of the stem contains more of the red-pigment, that gives color to the stem and to the hydranth, than does any

other part. Loeb first advanced the view that the red-pigment in the stem acts as a formative substance in Sachs' sense, and determines the production of a new hydranth by accumulating near the cut-end of the piece. Driesch also assumes the red-pigment to be a factor in the result, but supposes that it acts quantitatively, rather than in determining the quality of the result. If this red-pigment acted in the way supposed either by Loeb or by Driesch, it might act as one of the factors in the production of these partial structures. This red-pigment is contained in the form of reddish granules in the cells of the endoderm. The granules are of various sizes, the largest being easily seen even with low powers of the microscope. When a piece of the stem is cut off, the ends close by the drawing in of the cut-edges over the open-end. A circulation of the fluid contained in the piece then begins. In the fluid, globules appear very soon that contain red-pigment granules like those in the endoderm. The globules appear to be endodermal cells, or parts of cells, that are set free in the central cavity. The circulation continues for about twenty-four hours. At about this time one end of the stem becomes reddish, owing to the presence in it of a larger number of red-pigment granules than before. The ridges that are the rudiments of the tentacles appear (Fig. 30, *A*), and a new hydranth very rapidly develops. At the time when the hydranth begins to appear the globules in the circulating fluid disappear. They disappear at the time when the red-pigment of the forming hydranth is rapidly increasing in quantity, and not unnaturally one might suppose that the pigment of the circulating fluid had been added to the wall where the hydranth is produced. The globules disappear in the region of the new hydranth, but, I think, it can be shown that they do not form any essential part of the hydranth. They may be found stuck together in a ball that lies in the digestive tract of the new hydranth, and when the hydranth is fully formed the pigment is ejected, as Stevens has shown, through the mouth.

The development of the new hydranth begins several hours before the red-pigment globules have disappeared from the circulation. The walls in the region of the future hydranth begin to thicken, and, later, pigment develops in the endoderm of this region. The new pigment is formed in the new cells of the endoderm, and does not come from the circulating globules, as shown by the development of very short pieces of the stem. In these the amount of new pigment that develops in the new hydranth may be far greater than that in the whole original piece (Fig. 30, *D*), and in this case there can be no question but that new pigment is made in the endodermal cells of the hydranth. The formation of a hydranth, that usually takes place after another twenty-four hours, from the basal end of a long piece, shows that a hydranth may develop when there are no granules in the circulating fluid. These basal hydranths may contain as much pigment as do the distal ones.

Driesch suggested that the red-pigment in the circulating fluid determines quantitatively by its presence how much of a hydranth is formed, or the size of the hydranth in relation to the rest of the piece. There seems to be no evidence in favor of this view and much against it. Loeb has not stated specifically whether he means that it is the pigment in the circulating fluid or that in the walls which acts as a formative stuff; the presumption is that he meant the latter. An examination of the piece during regeneration gives no evidence in favor of the view that the pigment moves into the region of the new hydranth. On the contrary, it remains constant in amount at all points except where the new hydranth is developing, and there is in this region unquestionably a large development of new pigment.

The evidence for and against the idea that the red-pigment of tubularia is a formative stuff, or even building material, has been considered at some length, because it is the only case in which the hypothetical formative stuff has been definitely located in a specific, recognizable substance that can be followed during the process of regeneration. It is well, I think, to give the question full consideration, especially as the hypothesis often appears to give an easy solution of some of the problems of regeneration. In a later chapter the subject will be more fully treated.

FIG. 29.—*Tubularia mesembryanthemum. A.* Short piece with hydranth at each end. *B.* Double piece with one circle of proximal tentacles. *C.* Double piece with only two proximal tentacles. *D.* Double proboscis with two sets of reproductive organs. *E-E³.* Double proboscis.

Since the red-pigment hypothesis does not explain the phenomenon of the formation of the partial structures in tubularia, we must look for another explanation. As the matter stands at present we can only assume that there is a *predisposition* of a very small piece to form a larger partial structure than a smaller whole one. This problem of the method of development of small pieces of the stem of tubularia is further complicated by the development in many cases of double hydranths, or double parts of hydranths, as shown in Fig. 29, *A-E.* The first form (Fig. 29, *A*) shows two hydranths turned in opposite directions, that are united at their bases. Another form has only a single circle of proximal tentacles between the two proboscides (Fig. 29, *B-C*). In other forms there are only two proboscides, each with its reproductive organs (Fig. 29, *D*), and often there are simply two proboscides united at the base (Fig. 29, *E-E³*). It is the rule, even in longer pieces, that a hydranth appears at each end of the piece, if the piece is suspended or even lies on the bottom of the water; but

FIG. 30.—*Tubularia mesembryanthemum. A.* Short piece with reduced hydranth-region. *B.* Piece from distal end of stalk producing a hydranth without a stalk (see Fig. 27, *D*). *C.* Piece producing hydranth as outgrowth of end. *C'.* Later stage of last. *D.* Short piece producing double proboscis (see Fig. 28, *E*).

in all these cases the basal hydranth develops about twenty-four hours after the apical one. In the short pieces, however, the two ends develop at the same time, although the development of all the short pieces, whatever structures they may produce, whether single or double, is delayed, and the hydranths may not appear until after the long pieces have produced their basal hydranths. In these double structures both ends develop at the same time (Fig. 30, *D*). If we suppose the influences that start the development of the piece begin first at the distal end, the region affected will lie so near to the proximal end of the piece that the development at this end may be hastened, and under these circumstances the region of new formation will be shared by the two hydranths. The factors that determine that a larger, partial structure is formed in preference to a smaller whole one will no doubt be found to be the same in these double structures and in the single ones.

THE INFLUENCE OF THE OLD PARTS ON THE NEW

One of the most striking and general facts connected with the phenomenon of regeneration is that the new part that is built up on the exposed surface is like the part removed. This suggests that an influence of some sort starts from the old part and changes the part immediately in contact with it into a structure that completes the old part in that region. We can imagine that the new part that has been changed in this way may act on the new part just beyond it, and so step by step the new part may be differentiated. It is not difficult to show that the phenomenon is really more complicated than this, and that other factors are also acting on the new part; but, nevertheless, that the old part has some such influence is probable. Under certain conditions, however, this influence may be counteracted by other factors, and something different from the part removed may be formed. One example of this sort has already been discussed, namely, that in which after the removal of much of the anterior end of the earthworm or of a planarian, only the distal end comes back. Another case is that in which something different from the part removed is regenerated. If the tip of the eye of the hermit-crab or of other crustaceans is cut off a new eye is regenerated, but if the eye-stalk is cut off near its base an antenna-like organ develops. Herbst has suggested that the presence of the ganglion at the end of the stalk accounts for the regeneration of a new eye, when only the tip of the stalk is cut off. In the absence of the ganglion at the cut-edge the stalk does not produce an eye, but an antenna, as is shown when the eye-stalk is cut off near the base. The factors that determine the development of an antenna instead of an eye have not been discovered. Przibram has shown that when the third maxilliped of portunas, carcinas, or of other crustaceans is cut off near the base, the new appendage that develops is different from the one removed, and resembles a leg in many ways, but if the animal is kept until it has moulted several times the appendage becomes more and more like the part removed. Another remarkable case has also been described by Przibram for *Alpheus platyrrhynchus*. In this decapod, the claws of the first pair of legs are different from each other, one being much larger than the other and having a different structure.[28] If the larger claw is thrown off at its breaking-joint, and the smaller one left intact, the latter at the next moult (or sometimes after two moults) changes into the characteristic larger claw and

the newly regenerated claw is like the smaller one. If the experiment is repeated on this same animal, *i.e.* if the newly acquired large claw is removed, then at the next moult the smaller claw becomes the larger one and the new claw becomes the smaller one—the conditions now being the same once more as at the beginning. If both claws of an animal are thrown off at the same time, two new claws regenerate that are both of the same size, and each is a small copy of the claw that was removed. As yet no experiments have been made that show what factors regulate the development of each kind of claw.

Returning again to the question of the regeneration of parts similar to the ones removed, there are some interesting results that Peebles has obtained in the colonial hydroids, podocoryne and hydractinia. These colonies consist of three principal sorts of individuals: the nutritive, the reproductive, and the protective zooids. Peebles has found that if the stalks of these zooids are cut into pieces, each produces the same kind of zooid as was originally carried by that stalk. Pieces of the stem of the nutritive zooid produce new nutritive zooids at the anterior end of the piece, and sometimes also at the basal end. A similar statement may be made for each of the other kinds. Another method of regeneration sometimes takes place, when, for instance, a piece of the stalk of a nutritive individual is left undisturbed without being supplied with fresh water. It sends out root-like stolons instead of producing a new zooid. The stolons appear first at the ends of the piece, but may later also appear at several points along the piece. They make a delicate network, and the original piece may entirely disappear in the stolons. After several days new feeding zooids grow out at right angles to the stolon network. Pieces of the stalk of protective zooids may also produce stolons, but they spread less slowly, and the formation of new individuals was not observed. In one case a piece of a reproductive zooid made a short stolon, and from it arose a new individual that seemed to be a nutritive zooid. If the latter result proves to be true, we see that a piece may produce a new part that is of a different kind from that of which the piece itself was once a part, but this is brought about by the formation of a stolon that is itself one of the characteristic structures by means of which these colonial forms produce new nutritive zooids. In this case there is a return of the piece to a simpler form, the stolon, and, acting on this, the factors that produce nutritive zooids may bring about new nutritive zooids. The influence of the old structure is lost when the piece assumes a new character.

Another series of experiments gives an insight into an internal factor of regeneration that may prove, I think, to be one of some importance and help in interpreting certain phenomena. If the head-end of a planarian is cut off, the posterior piece split along the middle line, and one side cut off, just above the lower end of the longitudinal cut, as shown in Fig. 31, *A*, it will be found that, if the long and the short sides are kept from uniting along the middle line, each half will produce a new head on its anterior surface (Fig. 31, *C*). If the two halves grow together, and the anterior surface of the shorter piece becomes connected with the anterior surface of the longer piece by means of the new tissue that develops along the inner side of the latter (Fig. 30, *B*), then a head appears only on the anterior half. The development of a head on the shorter half is prevented by the establishment of a connection with the new side. Sometimes an abortive attempt to produce a head is made, but the posterior surface fails to produce anything more than a pointed outgrowth. If we attempt to picture to ourselves how this influence of the new side on the posterior surface is brought about, we can, I think, most easily conceive the influence to be due to some kind of tension or pull of the new material which is of such a sort that it restrains the development of a head at a more posterior level. We can picture to ourselves the same kind of process taking place in the regeneration of the tail of a fish from an oblique surface. The maximum rate of growth is found over that part of the cut-surface that is nearer the base of the tail (Fig. 40). At all other points the growth is retarded, or held in check, and it can be shown that the suppression is connected with the formation of the typical form of the tail in the new part. If we cannot actually demonstrate at present that this is due to some sort of tension between the different parts which regulates the growth, we find, nevertheless, that it is by means of some such idea as this that we can form a clearer conception of how such a relation of the parts to each other is established. In a later chapter this subject will be dealt with more fully.

Fig. 31.—*Planaria lugubris. A.* Showing how worm was operated upon. *B.* A single head regenerated at anterior cross-cut. It was united by a line of new tissue along the side of the long half-piece with the new tissue at the anterior end of the short half-piece. The two half-pieces reunited along the middle line. *C.* Two heads regenerated, one from each half cross-cut. The two half-pieces were kept apart along the middle line.

THE INFLUENCE OF THE NUCLEUS ON REGENERATION

The influence of the nucleus on the process of regeneration has been shown in a number of unicellular forms. It was first observed by Brandt in 1877 that pieces of *Actinosphærium eichhornii* that contain a nucleus assume the characteristic form, but pieces without a nucleus fail to do so. Schmitz (’79) found that when the wall of the many-celled siphonocladus is broken, the protoplasm rounds up into balls, some of which contain one or more nuclei, while others may be without nuclei. The nucleated pieces produce a new membrane, and later become typical organisms, but non-nucleated pieces do not form a new membrane, and soon disintegrate. Nussbaum (’84, ’86) cut into pieces the ciliate infusoria, oxytricha and gastrostyla. Those pieces that contained a nucleus quickly regenerated a new whole organism of smaller size, that had the power of further reproduction, while the pieces that did not contain a part of the nucleus showed no evidence of regeneration; and, although they continued to move about for as much as two days, they subsequently disintegrated. Gruber obtained the same result on another ciliate infusorian, *Stentor cœruleus*. He found that, although the non-nucleated pieces close over the cut-surface, and move about for some time, they eventually die. He further showed that a non-nucleated piece containing a portion of a new peristome in process of formation will continue to develop this new peristome, although a new peristome is never produced by a non-nucleated piece under other circumstances. He believes that if the new peristome has begun to be formed under the influence of the old nucleus, it may continue its development after the piece is severed from its connection with the nucleus. A non-nucleated piece containing a part of the *old* peristome does not produce a new peristome from the old piece. Gruber observed that a non-nucleated piece of amœba behaves differently from a nucleated piece, and dies after a time.

Klebs found that when certain algæ are put into a solution that does not seriously injure them, but causes the protoplasm to contract into balls, some of these contain nuclei, others not. If, for instance, threads of zygnema, or of spirogyra, are placed in a 16 per cent solution of sugar, the protoplasm of each cell breaks up into one or more clumps, some with nuclei, others without. Both kinds may remain alive for a time; some of the non-nucleated pieces may live for even six weeks. The nucleated pieces surround themselves at once, when returned to water, with a new cellulose wall, but the non-nucleated pieces remain naked. The latter can, nevertheless, produce in the sunlight new starch that is used up in the dark and is made anew on the return to light.[29]

Balbiani ('88) found that non-nucleated pieces of cytrostomum, trachelus, and protodon failed to regenerate, and Verworn ('89 and '92) obtained similar results on several other protozoa. Similar facts have been made out by Hofer ('89), Haberlandt and Gerassimoff ('90). Palla ('90) found that in certain cases non-nucleated pieces, especially those from cells in growing regions, can produce a new cell wall; while more recently Townsend ('97) has shown in several forms that non-nucleated pieces do not produce a new cell wall unless they are connected by protoplasmic threads with nucleated pieces. The most delicate connection suffices to enable a non-nucleated piece to make a cell wall, even when the nucleated piece lies in one cell and the non-nucleated in another, the two being connected by a thread of protoplasm that passes through the intervening wall.

If we examine somewhat more in detail some of these cases, we find that when a form like stylonychia is cut into three pieces, the two end-pieces without a nucleus fail to regenerate, while the central piece makes a new entire organism of smaller size. If stentor is cut into three pieces, each piece containing one or more nodes of the macronucleus, each produces a new stentor. If, however, a piece is cut off so that it does not contain a part of the macronucleus, it fails to regenerate. Verworn ('95) succeeded in removing the central capsule with its contained nucleus from the large radiolarian, *Thallasicolla nucleata*. The non-nucleated animal remained alive for some time, but eventually died. The nucleated capsule developed a new outer zone with processes like those in the normal animal. If the nucleus is taken from the capsule, the capsule dies, but shows some traces of the formation of an outer zone. If the protoplasm is removed as far as possible from around the nucleus, the latter does not regenerate new protoplasm, but dies after a time. Verworn concludes that the protoplasm cannot carry on all its normal functions without the nucleus, or the nucleus without the protoplasm.

These experiments sufficiently demonstrate that non-nucleated pieces are unable to regenerate. If we attempt to examine further into the meaning of the phenomenon, we find a few things that appear to have a bearing on the result. The behavior of the non-nucleated pieces shows that the metabolism of the cell has been changed after the removal of the nucleus. In some cases the protoplasm is not able to carry out the process of digestion of the included food substances. This process may be due to some interchange that goes on between the nucleus and the protoplasm, which is stopped by the removal of the nucleus, and, in consequence, the metabolism of the cell is changed. The lack of regenerative power may be due to this change in the metabolism. It cannot be claimed, however, that the result is due to a lack of energy in the pieces, for the incessant motion of the cilia in some kinds of pieces, that goes on for several days, shows that a large store of energy is present. Unfortunately, we do not know enough of the relation that subsists between the nucleus and the protoplasm to be able to state to what the lack of regenerative power is due.

Loeb ('99) has suggested that the lack of power of non-nucleated pieces may be due to a lack of oxidation. The nucleus contains substances which, according to Spitzer, are favorable to the process of oxidation. When the nucleus is removed, the oxidation is supposed by Loeb to be too low to allow the process of regeneration to take place. In support of this view, he points out that while non-nucleated pieces of infusoria live for only two or three days, non-nucleated pieces of plants containing chlorophyl may be kept alive for five or six weeks. Non-nucleated pieces containing chlorophyl can obtain a supply of oxygen, owing to the breaking down of carbon dioxide in the chlorophyl-bodies, and the consequent setting free of oxygen. It should be pointed out, on the other hand, as opposed to Loeb's view, that non-nucleated pieces of amœba have been kept alive for fourteen days; and that despite the better oxidation that may take place in non-nucleated pieces of plants, regeneration does not take place.

It has been found that non-nucleated pieces of the egg of the sea-urchin do not segment or develop, and the result is the same whether the pieces come from fertilized or unfertilized eggs. If, however, a spermatozoon enters one of these pieces, the piece will segment, and, as Boveri and later Wilson have shown, it will produce an embryo.

Boveri also tried fertilizing a non-nucleated piece of the egg of one species of sea-urchin with a spermatozoon of another species. He found that the embryo that develops is of the type of the species from which the spermatozoon has come, and he concluded that the nucleus determines the character of the larva, and that the protoplasm has no influence on the form. The evidence from which Boveri drew his conclusion is not beyond question. It has been shown by Seeliger ('95) and myself ('95) that if whole eggs of the species *Sphærechinus granularis*, used by Boveri, are fertilized by the spermatozoa

of the other species, *Echinus microtuberculatus*, there is great variability in the form of the resulting larvæ. Most of them are intermediate in character between the types of larvæ of the two species, but a few of them are like the paternal type. Vernon ('99) has more recently shown that the character of hybrids is dependent upon the ripeness of the sexual products of the two parents. If, for instance, the eggs (sphærechinus) are at the minimum of maturity, the hybrids are more like the male (strongylocentrotus).

It remains, therefore, still to be shown whether or not the protoplasm has any influence on the form of larva that comes from a non-nucleated piece, fertilized by a spermatozoon of another species. That the nucleus of the male does have an influence on the form of the animal is abundantly shown by the inheritance of the peculiarities of the father through the chromatin of the spermatozoon.

THE CLOSING IN OF CUT-EDGES

One of the most familiar changes that takes place when a cut-edge is exposed involves the rapid covering over of the exposed tissues. This takes place from the margin of the wound, and a layer of cells, usually the ectoderm at first, covers the surface. The closing in is brought about in many forms by the contraction of the muscles of the outer wall of the body. This seems to be the case in the earthworm and in the planarian, as well as in other animals, such for instance as the starfish, holothurian, etc. But in addition to this purely muscular contraction another process takes place, that is less conspicuous in forms in which the muscles bring about the first closing, but which is evident in forms in which the muscles are absent or little developed. I am able to cite two striking cases that have come under my own observation. When a piece is cut from the stem of tubularia, the ends close in twenty minutes to half an hour. The body wall, the cœnosarc, composed of the two layers of ectoderm and endoderm, withdraws a little from the cut-edge of the outer hard tube, or perisarc, that covers the stem, and then begins to draw across the open end. A perfectly smooth, clean edge is formed that advances from all points to the centre, where the final closing takes place. The closing is not due to an arching over of the cœnosarc, but the thin plate is formed standing nearly at right angles to the outer tube. This plate is composed of two layers of cells, of which there are a number of rows arranged concentrically between the centre and the outer edge. In the absence of muscle-fibres in the stem, the result cannot be due to a muscular contraction, and even if short fibres existed the transportation of cells entirely across the open end would speak against this interpretation.[30] Since the closing over takes place without any support, we cannot suppose the process to be due to any sort of cytotropic effect. The closing takes place equally well in diluted sea water and in stronger solutions. The method of withdrawal of the cells, as best seen when longitudinal pieces are studied, resembles very much the withdrawal or contraction of protoplasmic processes in the protozoa, and so far as one can judge from resemblances of this sort, the two processes appear to be the same.

This closing in of the cut-surface, while a preliminary step in the process of regeneration, cannot, I think, be regarded as a part of the regeneration in a strict sense. That the two processes are not dependent on the same internal factors is shown by the following experiments: If a bunch of tubularia is kept in an aquarium, it will produce new heads two or three times and then cease, and if after the last-formed heads have died, pieces of the stem are cut off, they close as readily as do pieces from fresh hydroids. Moreover, at certain times of year the species *Tubularia (Parypha) crocea* lose their heads, and only the stalks remain. Pieces of these stalks will not regenerate new heads, at this time, although they close in as quickly as do pieces at other times of the year when the heads are present and when new ones regenerate.

Another equally good illustration of what seems to be the same phenomenon is found in the closing in of wounded surfaces in the young tadpole embryos. If embryos are taken from the jelly membranes, or even after they have been set free, and cut in half, each piece quickly covers over the wounded surface by means of the ectodermal cells. A much more striking illustration of this closing over in the young tadpole is obtained by cutting, with a pair of small scissors, a large piece from the side. The area may be a fourth or more of the entire side, and yet it may be closed over in an amazingly short time. Half an hour or an hour often suffices to cover a large exposed surface. In this case also the wound is covered not by individual cells wandering over the exposed surface, but by a steady advance of the

smooth edge of the ectoderm toward a central point. The process is so similar to that which takes place in tubularia that little doubt can remain as to the two being due to the same factors. As there are no muscle fibres present in the part of the frog's embryo from which the piece is cut off, the result cannot be due to muscular contraction, but appears to be a contractile phenomenon similar to that in tubularia. Even the small piece that is cut from the side of the body shows the same phenomenon. At first it suddenly bends outwards owing to some physical difference between the inner and the outer parts of the piece. Then the edges thicken, bend in, and begin their advance over the inner tissues. The process is seldom completed, since there appears to be a limit to which the ectoderm can be stretched as the edges advance. A most striking phenomenon both in pieces of tubularia and of the frog's embryo is the entire absence of dead material at the wounded surface. No sooner is the operation performed than the advance begins; and there is not a trace of dying cells or parts of cells to be seen.

CHAPTER IV

REGENERATION IN PLANTS

THE series of experiments that Vöchting has carried out on the regeneration of the higher plants are so much more complete than all previous experiments, and his analysis of the problems concerning the factors that influence regeneration is so much more exact than any other attempts in this direction, that we may profitably confine our attention largely to his results. Many of his experiments were made with young twigs or shoots of the willow (salix), which, after the removal of the leaves, were suspended in a glass jar containing air saturated with water. Under these circumstances the pieces produced new shoots from the buds (leaf-buds) that are present near the point at which the leaves were attached, and new roots, in part from root-buds, that are also present on the stem.

If the piece is suspended in a vertical position with its *apex upward* (Fig. 32, *A*), small swellings appear after three or four days near the lower, *i.e.* the basal, end of the piece. These break through quickly and grow out as roots. If a leaf-bud is present near the basal end of the piece, the first roots appear at the side of or under this; later others appear around the same region. The first roots to appear under these conditions come from pre-formed root rudiments, the others are, in part at least, new, adventitious roots. If the lower end of the cut is made through the lower part of a long internode, *i.e.* just *above* a bud, the roots appear as a rule only near the cut-end, and few if any of the roots develop at the first bud above this region. In many cases there is formed over the basal cut-surface, in the region of the cambium, a thickening, or callus, and not infrequently from this also one or more roots may develop. The direction taken by the new roots is variable, being sometimes downward, sometimes more or less nearly at right angles to the stem.

While these changes have been taking place at the base, the leaf-buds at the apical end have begun to develop. One, two, three, four, or even five of the higher buds begin to elongate, the number and extent of development depending on the length of the piece. The topmost or apical bud grows fastest, and the others grow in the order of their position. In the region below the lowest bud that develops there may be one or more buds that do not grow; but if the piece is cut in two just above these buds, they will then grow out.

Fɪɢ. 32.—After Vöchting. *A.* Piece of willow cut off in July, suspended in moist atmosphere with apex upward. *B.* Older piece of willow (cut off in March) suspended in moist atmosphere with apex downward. *C.* Piece of willow with a ring removed from middle. Apex upward. *D.* Piece of root of *Populus dilatata*. Basal end upward. Shoots from basal callus. *E.* Piece of root of same with two rings removed. New shoots develop from basal callus, and from basal end of each ring.

The results show that at the base of the piece the same factors that bring about the development of the rudiments of preëxisting roots also cause the development of new roots, if the lower end is in a region in which there are no rudiments of roots present. The influence that produces the new roots is confined to the basal part of the piece. In the apical part of the piece there are no adventitious structures produced, but a longer region is active, and several pre-formed leaf-buds begin to elongate. The topmost shoot grows faster than the others, showing that the influence that produces the growth is stronger near the apical end than at points further removed.

If another piece of a willow stem be placed under the same conditions, but suspended with the basal end uppermost, results that are in many resp