EXPERIMENTS IN REGENERATION AND IN GRAFTING OF HYDROZOA

PEEBLES

Experiments

in

Regeneration and in Grafting of Hydrozoa.

A Dissertation

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for the Degree of Doctor of Philosophy

by

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ERRATA.

Page 14, line 22, for *gread* read *great.*

Page 19, line 37, insert comma after *began.*

Page 23, line 3, for *often* read *after.*

Page 25, line 38, for *Hydranths* read *hydranths.*

Page 26, line 37, for *have* read *has.*

Page 27, Fig. 41, for *T*, *M*, *M′*, *T′*, and *P*, read *t*, *m*, *m′*, *t′*, and *p*. Line 20, for *P* read *p*.

Page 28, line 12, for *B*. read *b*.

Page 36, line 33, for *4 mm.* read *1 mm.*

Page 39, Fig. 60, for *P*. read *p*.

Page 39, line 6, for *α* read *a;* line 9, omit semi-colon after *end* and insert after (*a*); line 20, for *6.11 mm.* read *.79 mm.;* line 23, for *3-5 mm.* read *.4-.6 mm.*

Page 46, Fig. 77, for lower *c* read *c′*.

1. Introduction.

Material for the experiments described in the following pages was obtained first at Wood's Holl, where the experiments were begun in the summer of 1896. Hydractinia, Podocoryne, Pennaria, Eudendrium, Bougainvillia, Parypha, and Clava, were experimented upon. The work was resumed at Wood's Holl for a few weeks in the following summer.

In September 1896 a supply of Cordylophora was obtained from the Chesapeake Bay, not far from Baltimore. By repeated addition of fresh supplies of salt water the polyps remained in good condition and showed great capacity for regeneration until December.

During the winter of 1896/97 Hydra viridis, and Hydra grisea furnished material for experiments, some of which I (Peebles 31) have already described. These two species of Hydra were found in ponds in the vicinity of Bryn Mawr College.

In the autumn of 1898 I had an opportunity to continue these studies at the Zoological Station in Naples. Large supplies of Eudendrium, Pennaria, Tubularia, and Bougainvillia were readily available. Unfortunately Podocoryne and Hydractinia are rare in the Bay of Naples, and Antennularia is difficult to obtain.

In the summer of 1899, at the University of Halle, a series of experiments in grafting were made on Hydra grisea, and Hydra fusca.

The methods used in the experiments are so simple that no description of them is necessary. Where any new method was employed it is mentioned in connection with the experiment in which it was used.

It affords me great pleasure to have this opportunity to express my gratitude to those who have given me valuable suggestions and assistance in obtaining material, and in carrying on these experiments. I wish especially to make known my appreciation of the courtesies of the Marine laboratories of Wood's Holl, and Naples, extended by Professor Whitman and Professor Dohrn. I wish also to thank Professor Grenacher and Dr. Brandes of the University of Halle for their assistance, and Professor T. H. Morgan, whose guidance and interest in my studies have been of great value.

2. Regeneration in Colonial Hydroids: Hydractinia and Podocoryne.

Hydractinia, commonly found on the shells of snails, inhabited by hermit-crabs, consists of several kinds of individuals, and these individuals show, to a marked extent, the power of regeneration.

The colony, as described by Agassiz (1), consists of four different kinds of polyps. The first kind, the reproductive individuals, are the male and female polyps, they bear short spherical tentacles on a rounded head, in the center of which a small mouth is present. The individuals of the second class are especially adapted for protecting the colony, they are capable of contracting rapidly, they have small heads, but the stalk is much longer than that of the other polyps. The third and fourth classes Agassiz calls the sterile forms with long or short probosces. These are found in great numbers, and are adapted for nourishing the colony, they possess a mouth surrounded by long tapering tentacles.

Bronn (6) does not classify the different individuals exactly as Agassiz does. He distinguishes four classes of individuals, the reproductive, the nutritive, the protective or spiral, having no mouth-opening; and a fourth class, the short cone-shaped bodies rising from the branched network or hydrorhiza.

Allman (2) also refers to four classes, the spiral zooids, destitute of mouth, but having a hollow body, the hydranths or nutritive zooids, the reproductive zooids, and the tentacular individuals, which he says are not always present; he considers them abnormal, or a malformation of the spiral zooid.

Collcutt (7) has studied Hydractinia echinata with special reference to the anatomy. She describes the members of the colony as follows: the nutritive polyps or Gasterozooids consist of an elongated stalk bearing a cone-shaped head with hypostome surrounded by two rows of tentacles numbering from ten to thirty, and increasing in number as the animal grows. The second class includes the reproductive zooids or Blastostyles; these are shorter and more slender than the Gasterozooids, and have a small mouth at the summit of a conical head bearing two or more closely approximated rows of tentacles which are rudimentary and knob-like. The third class is composed of the spiral, protective zooids, or Dactylozooids, which bear ten to sixteen knob-like rudimentary tentacles, crowded with nematocysts. The tentacular polyps comprise the fourth class, they have long slender stalks tapering anteriorly to a point; no mouth opening is present.

The species upon which most of my experiments were made was found at Wood's Holl. It corresponds to the description that Collcutt gives for Hydractinia echinata. The nutritive, reproductive, protective, and tentacular polyps were present, and also the low cone-shaped individuals described for this species by Bronn.

The experiments were made on individuals from each class, but the results from those made on the first three classes were more satisfactory than the others. I am inclined to agree with Allman in considering the tentacular polyps abnormal. When isolated they soon die, and pieces of the stalk show no power of regeneration.

In consideration of the fact that the different kinds of individuals of a colony differ from each other in form, while they are organically united, a comparison of the way in which they regenerate is of interest. If the stalk of a reproductive polyp is deprived of its hydranth will it regenerate a new hydranth of its own kind or will a nutritive or protective hydranth take the place of the original one?

For the experiments the reproductive, nutritive, and protective polyps were cut from the hydrorhiza and isolated in small dishes containing sea water. The first set of experiments was so simple that it is unnecessary to give a detailed description of the behavior of each individual. The head was removed from each polyp and the portion of the stalk posterior to the cut, which in all cases bore a cut surface at each end, was isolated and its subsequent behavior carefully observed at regular intervals. In the course of twenty-four to thirty-six hours each individual, showing any sign of growth, produced a new head similar to the one previously removed. An exception to this rule was never seen. If a head regenerated it was nvariably identical in kind with the original head. Hundreds of individuals were tested, always with the same result. The long tapering tentacles of the nutritive polyp rendered it easily distinguishable from the protective individual with its short knob-like tentacles. The tentacles of the reproductive polyp are also short and knob-like, but the constriction in the stalk just back of the hydranth is peculiar to the reproductive polyps and they can be recognized by this before the reproductive organs develop.

Having determined that each individual invariably regenerates parts of its own kind, another series of experiments was undertaken in order to discover if the power of regeneration in each polyp is the same at different levels of the stalk. On account of the extreme shortness of the reproductive polyp, and the presence of the reproductive organs, it is difficult to divide the stalk into several pieces, therefore the experiments were confined to the protective and nutritive polyps.

In the first experiment the stalk of the nutritive polyp was divided by transverse cuts into five pieces as shown in Fig. 1. The pieces with the exception of the most anterior piece (*1*) were isolated, and the rate and character of regeneration watched. There was a slight variation in the rate but no definite conclusion could be drawn for in one experiment a new hydranth formed on the fifth piece before any tentacles appeared on the second piece, and in another experiment a new hydranth developed on the third piece before the fifth piece showed any new tentacles. The experiment was repeated eight times and each time the new hydranths without exception resembled the original hydranth in kind.

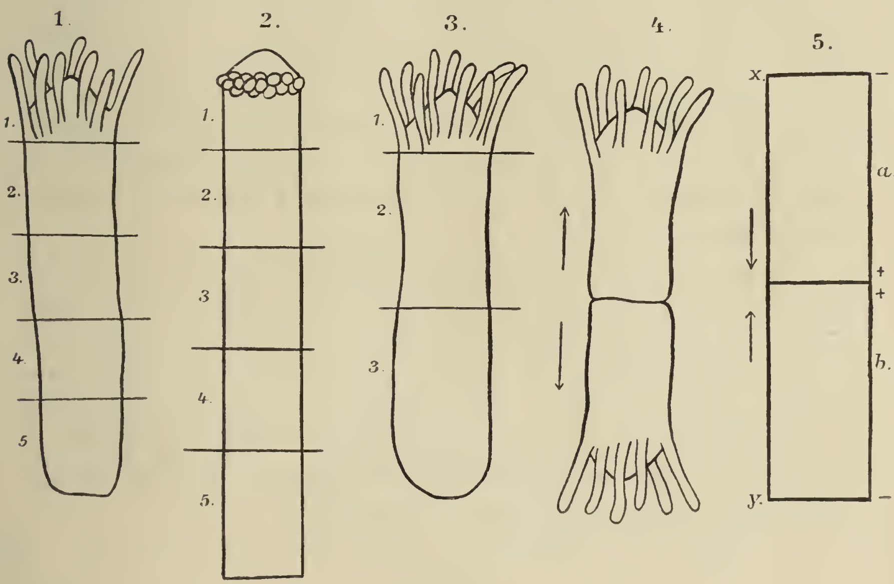
A similar experiment was repeated five times on the protective polyps as seen in Fig. 2. The rate of regeneration in the different pieces varied slightly but with no regularity. The new hydranths were similar in kind to the original hydranth of the polyp from which the pieces were cut.

The experiment was varied by making two transverse cuts through the stalk as indicated in Fig. 3. The first piece was not watched as the object of the experiment was to discover if there was any difference in the rate of regeneration in the anterior (*2*) and posterior (*3*) halves of the stalk. The experiment was repeated twelve times; no regularity in the variation of the rate was observed.

The posterior half (*3*) regenerated new tentacles and hypostome as rapidly as the anterior half (*2*).

In all of the experiments new hydranths frequently developed on both ends of the piece. This was true for all four regions of the stalk, in the protective and also in the nutritive polyps. This heteromorphosis was more frequent in occurrence in small pieces than in those consisting of the greater portion of the stalk [1]. In one experiment fifty-six out of eighty-eight pieces formed hydranths

Figs. 1—5.



on both ends. In another six out of eighteen pieces developed a head at each end.

In many of the experiments on Hydractinia and Podocoryne, especially the latter, when portions of the stalk of a nutritive polyp were left in a dish, undisturbed, and without having any fresh water added, the new growth was limited to sending out stolons instead of developing hydranths. These stolons grew out spreading in all directions; they appeared first at the cut ends of the piece but later growth began from several points along the stalk. The stolons increased in number and in length until a delicate network covered

[1] This result has been observed in similar experiments made by Bickford (4) on Tubularia and Morgan (28) on Parypha.

the bottom of the dish, and it became impossible to distinguish the original piece of the stalk from the stolons. After several days new individuals began to appear, growing up at right angles to the stolons, just as the different individuals of a colony grow from the hydrorhiza. This formation of new polyps continued for several weeks when the experiments were brought to an end. As many as ten to fifteen new polyps were produced by one piece of the stalk. These polyps were invariably nutritive individuals, characterised by their long slender tentacles.

Pieces from the stalk of the protective polyps were isolated in the same way but the spreading of the stolons was exceedingly slow, and the formation of new individuals was never seen.

In one piece out of many that were cut from the stalks of the reproductive polyps, one new individual developed after the formation of a short stolon. The hydranth on the new polyp seemed to be a nutritive hydranth, but the stolon disintegrated before its character was definitely determined.

Owing to lack of time the colonies, resulting from the experiments described above, were not kept longer than three weeks; it is possible that if they were nourished, and growth continued, the network of stolons would eventually give rise to other kinds of individuals thus forming a complete colony. The spreading of the stolons was much more rapid, and the growth more luxuriant in Podocoryne than in Hydractinia. This was observed in the behavior of those colonies found both at Wood's Holl and at Naples.

Grafting.

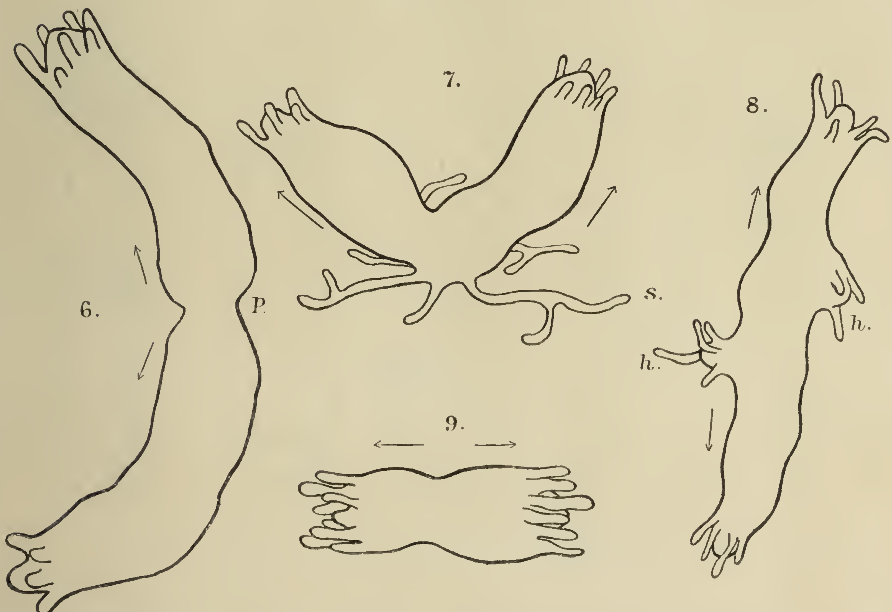
In another series of experiments I tried to determine if pieces of individuals, of the same and of different kinds, could be grafted together, and if so whether the subsequent development would be modified. First, I tried to unite individuals of the same class; for this, nutritive polyps were selected as they are larger and more easily handled than the other individuals.

a. Union of nutritive polyps in opposite directions.

For convenience it may be well to speak of the ends of the stalk as poles, the anterior or distal end as the positive (+) or oral pole, the posterior or proximal end as the negative (—) or aboral pole. In all experiments in grafting arrows are used to indicate the direction of the positive pole, therefore they point toward the anterior or hydranth end of the piece. The union of similar poles may be indicated as shown in Figs. 4 and 5. In Fig. 4, the negative poles are united, in Fig. 5, after the removal of the hydranth the pieces are united at their positive poles.

Exp. 1. Six pairs of nutritive polyps were united at their aboral surfaces as shown in Fig. 4. The polyps had been cut from the hydrorhiza near the base of the stalk. After isolation they are no longer as active as when united with the colony. The shock

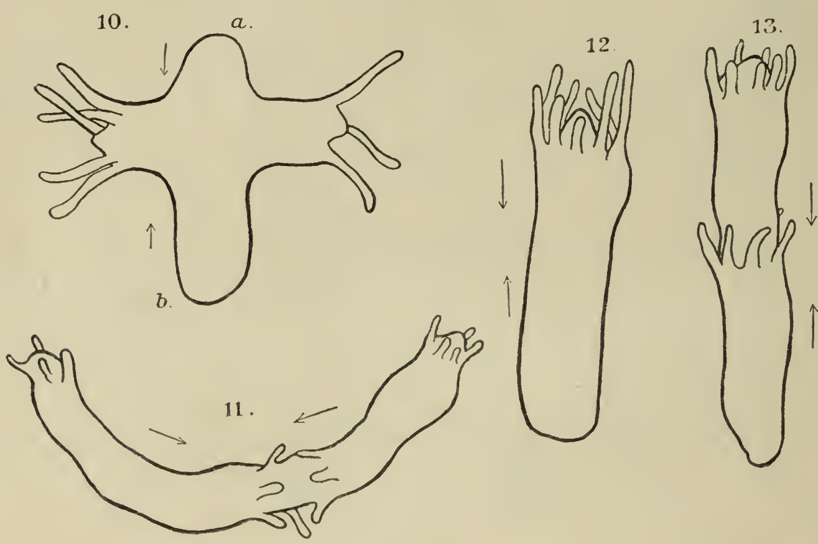
Figs. 6—9.



of separation seems to make them inactive so that it is not difficult to keep the pieces in contact until the tissues are firmly united. This complete union results in a few hours, and the line where the pieces are joined soon disappears. Twenty-four hours after the graft was made four of the polyps appeared as seen in Fig. 6, the hydranths forming with each other an obtuse angle, and the common proximal end (*p*) taking a posterior direction. On the following day they were firmly attached at the point (*p*) and at the end of a week had sent out stolons (Fig. 7 *s*) from the region where they were united. The polyps lived for several weeks but no separation of the components took place. The remaining two pairs, instead of forming stolons and becoming fixed at the line of union, developed two hydranths in this region (Fig. 8 *h*) so that each component bore one hydranth on each end.

Exp. 2. The experiment described above was modified by cutting each hydranth from its stalk just back of the ring of tentacles. The anterior pieces were united as in Exp. 1, but the extent of the common stalk between the two hydranths was much reduced (Fig. 9). After complete union was effected the hydranths remained directly opposite to each other as shown in Fig. 9. In twenty-four hours food was taken and the body cavity was seen to be continuous. The

Figs. 10—13.



stalk increased slightly in length but there was no subsequent attachment in the region where they were united.

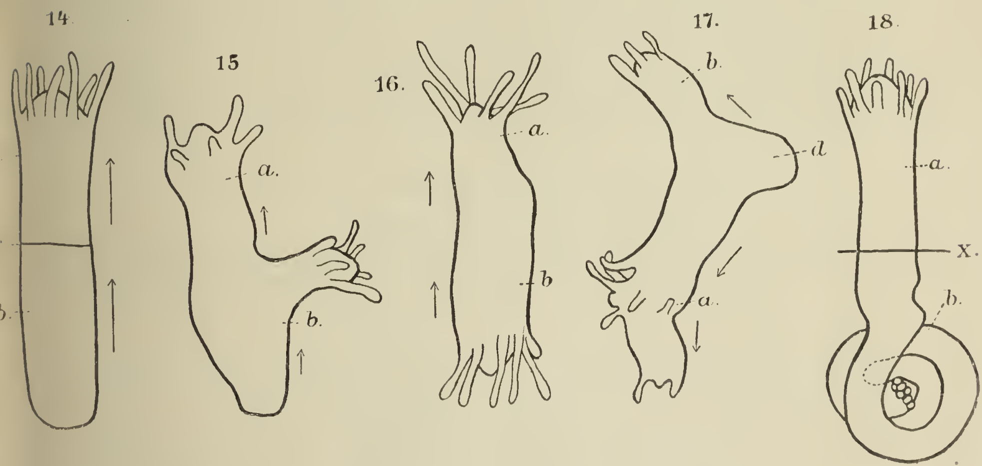
Exp. 3. In a third experiment the hydranths were removed by a transverse cut just back of the tentacles. The posterior pieces of two individuals were united as seen in Fig. 5. The negative poles, x and y, are the regions where the polyps were cut from the hydrorhiza. Sixteen pairs were united in this manner. Several hours after the grafts were made no trace of the line of union of the two components could be detected. At the end of two days new hydranths began to appear but not always in the same place. Six pairs formed new hydranths in the middle, near the line of union (Fig. 10). Three pairs developed mouth and tentacles not only in the middle but also at the aboral ends (Fig. 11). Five pairs formed a hydranth at the aboral end of one of the components (Fig. 12). The remaining two pairs produced one hydranth at x (Fig. 5), and another at the line of union (Fig. 13).

The great variation in these results can scarcely be due to the region where the cuts were made, for hydranths were produced at both cut surfaces and in regions where no opening was visible, regardless of the position of the components.

b. The union of nutritive polyps in the same direction.

On July 2nd eight pairs of nutritive polyps were united in the manner indicated in Fig. 14. The anterior individual (*a*) was not deprived of its hydranth, but merely cut from the colony near the base of the stalk, and grafted on the anterior end of *b* after the

Figs. 14—18.



removal of the hydranth from the latter. The aboral pole of *a* was thus grafted on the oral pole of *b*. In the course of six to eight hours the union at *c* was complete, the aboral end (*d*) closed in, and one individual of unusual length having a hydranth at the anterior end was formed. On six pairs at the end of two to three days tentacles began to appear near *c* and on July 5th the polyps appeared as shown in Fig. 15. A new hydranth had been produced at the anterior end of *b* but no separation of the two components took place. The two remaining pairs developed as seen in Fig. 16, a new hydranth appearing at *d*, while no new growth was observed in the region where the two individuals were united.

In another experiment two polyps were united in the same way. Three new hydranths were formed in addition to the one already present. Two of these new hydranths developed at the anterior end of *b* and one at the posterior end of *a* (Fig. 17). The component *a* gradually became cut off from *b* but never completely freed itself. After two weeks it was impossible to determine the relation of the new hydranths to the original components.

On July 7th two pairs were united in the manner described in the preceding experiment. On the 8th the union was complete; no new formation was observed. The polyps lived two weeks in the condition shown in Fig. 12; no new hydranths developed, although the cut end (Fig. 14 *d*) healed and the polyps appeared active.

Summary. It is shown, by the experiments described above, that portions of the stalks of nutritive polyps may be grafted upon each other; that the union of the tissues is complete, and that new hydranths may develop at the oral and aboral ends or in the middle near the line of union of the two components. Polyps united as described in these experiments may produce as many as four new hydranths or they may remain alive for one to two weeks without producing new parts.

c. The union of different members of the colony.

Exp. 1. Nutritive and protective polyps.

The complete union of the nutritive and protective polyps was finally effected after many unsuccessful attempts due to the activity of the protective individuals. The experiment was limited to the union of like poles, and the tentacles at first were not removed. A few hours after the polyps were united it was impossible to detect the line of union, but as soon as the nutritive component had taken food its body cavity enlarged while the slender stalk of the protective component retained its normal size. In Fig. 18 the two individuals *a* and *b* are shown. They remained in this condition several days; the nutritive polyp increased slightly in size but no new parts developed. Finally the anterior two-thirds of the nutritive polyp was removed by a cut at the line *x* Fig. 18, leaving a small portion still attached to *b* in order to see if a new hydranth would form at the cut surface, and if so, whether this hydranth would resemble that of the nutritive or protective polyp. As the portion of *a* that was left attached to *b* was exceedingly small I hoped that the new structure would bear some of the characters of the protective polyp. A new hydranth developed at the cut oral surface of *a* but it resembled the original head. The tentacles were long and slender and the hypostome surrounded a large mouth opening. This experiment was repeated five times with the same result.

Exp. 2. Nutritive and reproductive polyps.

A series of experiments similar to those just described was made with nutritive and repruductive polyps. The reproductive organs were removed before the graft was made, as their presence often causes disintegration of the material. As the nutritive polyps possess apparently the greater capacity for regeneration, and are in every way stronger, than the reproductive, it seemed probable that some influence might be exerted on the reproductive polyp by the nutritive individual. The anterior two-thirds of the reproductive polyp was removed leaving a small portion attached to the base of the nutritive component. A new hydranth formed at the cut surface, resembling that originally borne on the reproductive polyp; the presence of the large nutritive polyp in no way modified the new part. The experiment was repeated four times with the same result.

Summary. The experiments on Hydractinia and Podocoryne show beyond doubt that the individuals of each class retain, throughout the process of regeneration and grafting, the characteristics of the class to which they belong. Although the different kinds of polyps are organically connected, the tissue and structure of each kind is especially modified to perform its individual function, and this modification is never lost.

3. Variations in Regeneration dependent upon the Region from which the Piece is taken.

The experiments made on Hydractinia where the stalk was cut into five pieces (see page 6) seem to prove that in this species the nature and extent of regeneration in each piece is not dependent upon the region from which the piece is taken.

In other forms it has been demonstrated that the character and also the size of the new part is to some extent dependent upon the region where the cut is made. Morgan (27) has found in Allolobophora foetida that short pieces from the anterior end rarely regenerate posteriorly, yet they show great capacity to regenerate in an anterior direction. Very short posterior pieces do not regenerate anteriorly, but pieces from the middle of the worm sometimes regenerate both anteriorly and posteriorly.

I have shown in experiments on Hydra (31) that the rate of regeneration varies according to the region from which the piece is cut. Pieces from the anterior end develop new heads more rapidly than those from the foot region.

In the light of these results it is not possible to accept Weismann's hypothesis of latent cells, because it fails to explain the variation in the rate of regeneration in different parts of the body.

In order to determine if the embryonic tissue is qualitatively differentiated in different parts of the stalk, Davenport (9) cut pieces from the branches of Obelia commisuralis at three different levels, and carefully compared the subsequent regeneration. He was lead to conclude that the embryonic tissue was capable at all levels of producing the same thing.

Driesch (11) has shown that the hypostome of Tubularia when cut off just back of the distal row of tentacles never regenerated a proximal row of tentacles, while the proximal piece soon replaced the lost hypostome and distal tentacles.

In Hydra I have found that pieces of the hypostome, cut off distal to the tentacles, die without further growth. This result was also obtained when the hypostome of the Scyphistoma of Aurelia was isolated. The pieces showed gread activity but never completed themselves in a proximal direction.

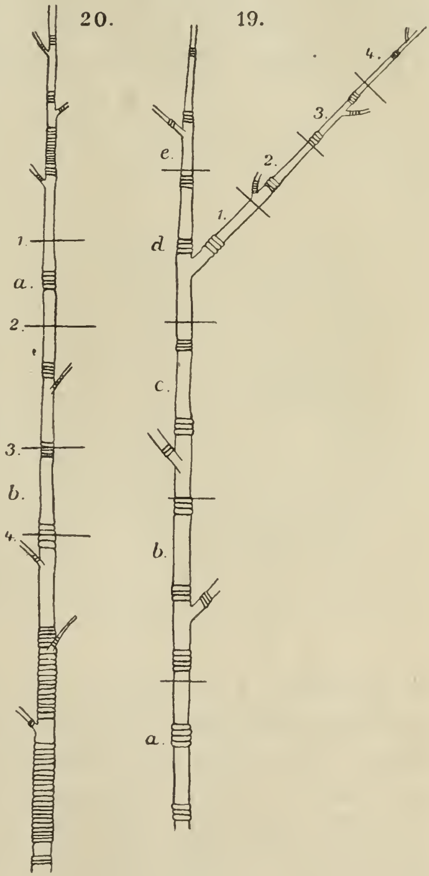
Morgan (28) has observed that small pieces of Planaria maculata cut from the anterior end beyond the eyes do not regenerate new parts, while pieces including the eyes show a capacity for regenerating the entire worm.

Driesch (12) has recently shown that when »not too small« pieces of the stalk of Tubularia are isolated, the extent of the space occupied by the anlage of the new hydranth depends upon the original position of the piece in the stalk. The length of the area occupied by the new hydranth during development decreases from the oral to the aboral end of the stalk.

In order to determine whether the different regions of the stalk of Pennaria showed variation in regeneration I made the following experiments.

In the large species, Pennaria cavolini, found in the Bay of Naples, the stalks measure from twelve to fifteen cm in height and bear strong branches. The longest stalks were selected and cut into short pieces about one cm long. The pieces from each region were isolated and their behaviour observed each day. Three days after the pieces were cut no change was seen in those taken from the region *a* Fig. 19. In those from *b* six out of seven pieces showed a slight protrusion of the coenosarc beyond the perisarc. The following day five out of seven from the region *c* bore new hydranths, the pieces from *d* and *e* after slight protrusion of the coenosarc died, and those from *a* showed no sign of growth.

Figs. 19—20.



This experiment was repeated twice; in each series twenty-five to forty pieces were observed, and the results from each section (*a*—*e*) showed that the pieces from the center of the stalk regenerated more rapidly than those cut from regions near the base and tip, while pieces from the extreme ends failed to show any growth.

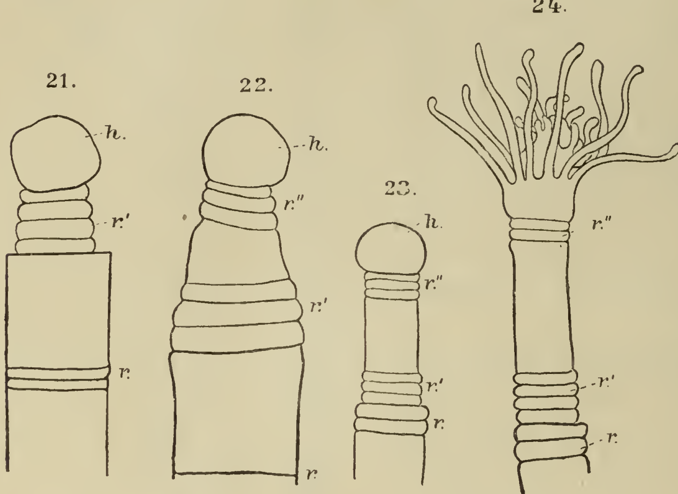
The same experiment was repeated with the branches (Fig. 19 *1*—*4*). Each piece measured from 7—10 mm in length. The pieces from regions *1*, *2* and *3* showed growth and formation of hydranths; those at the extreme tip died soon after isolation.

The perisarc of the stalk and branches of Pennaria is annulated at regular intervals. Allman (2) describes the annulation as occuring at the origin of the branches and on the branches themselves; those bearing terminal hydranths are often ringed along their entire length. The species Pennaria cavolini found in the Bay of Naples has groups of three rings just above the origin of the branches.

Agassiz (1) found in Pennaria eight to twelve narrow rings, without any intervals between them, lying at the base of the stalk, while the region above is only ringed at intervals, and each group is composed of three or four rings. The branches, according to Agassiz, bear three to twelve rings at the base, just beyond the origin of each stalk, and nearer the tip of the branch only three lying below the terminal hydranth.

In the long stalks of Pennaria cavolini, upon which my experiments were made, the annulation of the main stalk was generally limited to groups of three to four constrictions just above the origin of the branches. Stalks were frequently found upon which a region

Figs. 21—24.



of annulation 2—3 cm long was seen. No unsegmented region interrupted these rings. The color of the most anterior segments was slightly lighter than the more posterior segments. Such a stalk is represented in Fig. 20. These accumulations of rings suggested that the stalk might have been broken off at this level, and the new tissue lighter in color had formed above the original rings, thus lengthening the annulated area.

In order to test this view I made a series of cuts along stalks upon which the annulated regions were marked by groups of three or four rings. In Fig. 20 the lines *1—4* represent the position of the cuts. In *a* the piece lying between the lines *1* and *2* was cut so that an unsegmented portion was exposed at each end of the piece, and a group of rings included between the cut surfaces. In *b* the unsegmented region lay in the center of the piece while the extremities bore two to three rings. The method of regeneration in these pieces was carefully observed in order to determine whether new rings always developed at the exposed surfaces. After recovery from the injury the coenosarc began to grow out beyond the perisarc in the usual manner and the anterior end became round and knob-like prior to the formation of the new hydranth. In a series of three experiments in which thirty-five pieces were cut as indicated by *a* Fig. 20, fifteen pieces developed new hydranths. Fig. 21 shows one of these pieces five days after the operation. At *r* the original rings are seen, at *r′* the new ones forming at the cut surface. As the region between the hydranth and the old perisarc became longer a short unsegmented region appeared between *r′* and the rings immediately below the hydranth (Fig. 22).

In another series of experiments fifty pieces were cut as shown in Fig. 20 *b*. Eighteen of the pieces formed new hydranths. In Fig. 23 one of these pieces is shown four days after the operation. The new rings (*r′*) lie just beyond the cut; the hydranth (*h*) is beginning to develop. On the following day the pieces completed their new hydranths; in Fig. 24 such a piece is shown. Two segmented regions (*r′ r″*) have developed in the new tissue, the one at the cut surface (*r′*) has remained at the opening and the new hydranth has a group of rings at its base (*r″*). All of the eighteen pieces that developed new hydranths showed the same regularity in the segmented and unsegmented regions. No great accumulation of rings anterior to the cut surface was observed.

4. The Regulative Influence of External and Internal Conditions on Regeneration.

I. External Conditions.

By modifying the external conditions that surround the organism Loeb (21) has demonstrated a corresponding modification in the rate and character of regeneration.

a) Light. In experiments on Eudendrium Loeb (23) found that changes in the intensity of light produced a marked effect upon the regeneration of hydranths. The greatest number of new hydranths appeared on the branches near the side of the aquarium that was most brightly lighted. Also when cut ends of stalks were placed in great depths of water, where the light was dim, the new formation was slow and imperfect. By shutting out all light the regeneration of new hydranths was delayed, but when the light was again admitted the growth began at once. In complete darkness Loeb found that the formation of hydranths on the stalks of Eudendrium racemosum rarely took place. In the light, roots grew as luxuriantly as in the dark.

I have attempted through experiments similar to those made by Loeb, to ascertain the effect of light and darkness on regeneration in Tubularia, Pennaria, Bougainvillia, and Podocoryne, and have also repeated Loeb's experiments on Eudendrium.

Small pieces of the stalk were placed in glass dishes containing sea water. All light was excluded by covering the dishes with black boxes. Control experiments were carried on at the same time. In Eudendrium regeneration was delayed, and in ninety per cent of the pieces long stolons formed at the cut ends, while the remaining ten per cent developed hydranths. The majority of the pieces in the control experiment formed hydranths instead of stolons.

Tubularia produced new hydranths as rapidly in the dark as in the light. After the first hydranths formed they dropped off and were replaced by new ones. This process took place slowly in the dark, more rapidly in the light. In the dark, many of the pieces formed long stolons, and in some cases a new hydranth formed at the end of a long stolon-like outgrowth. After the loss of the second hydranths the pieces in the light sometimes produced new hydranths again, but this was never observed in those pieces in the dark.

As the process of hydranth formation in Tubularia is exceedingly rapid, taking place in 24—36 hours, it is not improbable that the darkness has not had sufficient time to modify the formation of the first hydranths, and its influence is exerted only on the subsequent growth.

The production of new hydranths on the cut ends of Pennaria takes place slowly in the light; there was no perceptible change in the rate when the pieces were subjected to darkness, but the percentage of hydranths formed was not so large as in the light.

Bougainvillia shows a marked tendency to produce hydranths at each end of a piece of the stalk. This tendency is as strong in the dark as in the light. In one experiment, in diffuse daylight, sixty-six out of one hundred and seven pieces were biapical. As a control experiment thirty-eight pieces were subjected to total darkness; every piece formed a new hydranth at each end.

The tendency of pieces of Podocoryne to become attached and to form stolons is so strong that it is difficult to determine whether or not light affects the process of regeneration. Tentacles and hypostome formed as often on pieces in the dark as on those in the light.

Hydra viridis and Hydra grisea, as I have already shown (32), regenerate with equal rapidity in light and in darkness.

Loeb (23) observed that in Eudendrium racemosum blue light has, in a measure, the same effect upon regeneration as darkness. I have placed complete branches and parts of branches of these Hydroids in either red, blue, yellow, or green light. A slight variation in the rate of regeneration was observed, but it was not possible in any one experiment to trace this variation to the character of the light, for as great and even greater variation was seen in the control experiments.

b. Temperature. Lillie and Knowlton (20) have measured the rate and extent of regeneration of Planaria torva, and have proved that cold retards growth while a rise of temperature up to 31.5° C. increases the rate.

The effect of temperature upon regeneration in Hydroids has not been demonstrated, but in Hydra viridis and Hydra grisea I (32) found a rise of temperature up to 32° C. produced a marked increase in the rate of regeneration, and in Hydra viridis under a reduction of temperature to 12° C. the formation of new parts was delayed twenty-four to ninety-six hours.

c. Gravity. According to Loeb, Antennularia reacts to the force of gravity in the following way: Pieces of the stalk suspended vertically in water develop roots on the lower end whether the oral or aboral extremity is directed downward. He found that pieces placed in a horizontal position produce roots on the lower side and branches on the upper side of the stalk.

In order to determine the result produced by constantly changing the relation of the pieces to gravity, I made the following experiment.

Pieces of the stalk of Antennularia, measuring 3—4 cm in length, were fastened in different positions on a large cork. Through this cork a hard-rubber bar was passed, and the bar was revolved at the rate of three revolutions in an hour. The apparatus was placed in a large aquarium through which salt water was constantly running. Five days after rotation of the pieces began new tips measuring 4—5 mm had formed; this tissue grew out in a straight line, but the experiment unfortunately was abruptly brought to an end, so the results were entirely negative.

d. Contact with foreign bodies is an important factor in the determination of the nature of the new part. In Tubularia LOEB (21) found that the regeneration of a new hydranth on either the oral or aboral end of the stem could be prevented by submerging that end in sand. After removal from the sand the hydranth immediately developed.

DRIESCH (11) sealed the cut ends of pieces of Tubularia to see if in this way by the prevention of new growth at the ends, branches would appear along the sides. The closure of the ends prevented the new formation, and although some of the pieces remained alive, and showed circulation within the perisarc for twelve days, no lateral buds were produced. In one piece a hydranth formed at one end, pushing the wax off and finally emerging from the perisarc.

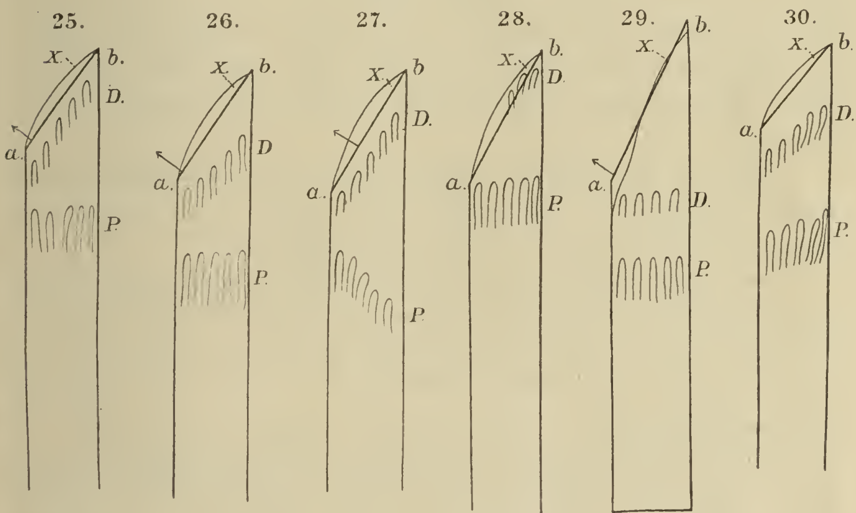
LOEB (22) has demonstrated in Pennaria that when pieces of the stem are placed so that the cut end touches the bottom of the dish in which it is kept, a stolon is produced regardless of whether the cut surface of the piece is a distal or a proximal end. He says that »in Pennaria the nature of the contact not only determines the place of origin of the various organs, but also the direction of growth«. If out-growing hydranths are placed in contact with a solid body, the hydranths grow away from the body, and the new stalk soon becomes nearly perpendicular to the surface with which it comes in contact.

The formation of stolons from small pieces of Hydractinia and Podocoryne, which I have mentioned in a previous section, also demonstrates the effect of contact with a solid body: In one experiment when the pieces were left undisturbed on the bottom of the dish, sixty-six per cent of the pieces formed only stolons; in another experiment twenty-five per cent of the pieces produced stolons. Later all of these stolons sent up new individuals perpendicular to the bottom of the dish. On account of the delicacy of these Hydroids it was impossible to suspend the stalks in the water so that the ends should not touch any fixed surface. Dishes containing the pieces were suspended and swung back and forth, but this change of position was not sufficient to keep the pieces from becoming attached to the bottom of the dish.

A series of experiments on Tubularia have led me to think that the direction of growth of the new hydranth, produced when the stalk is cut obliquely, may be due in part to contact. DRIESCH (10) cut the ends of stalks of Tubularia obliquely as shown in Fig. 25.

The line *a—b* indicates the surface exposed to the water. The position of the ridges which are laid down prior to the formation of the two rows of tentacles, he observed, was altered. The distal row lay nearly parallel to the line *a—b*, the proximal appearing somewhat later, and generally parallel to the cut surface, but sometimes it assumed the usual transverse position (see Fig. 25). The new hydranth, according to DRIESCH, grows out of the perisarc at right angles to the cut surface and never comes to lie in the long axis of the piece.

Figs. 25—30.



My own results, obtained from cutting the stalks of Tubularia obliquely, show that the hydranth grows out approximately at right angles to the surface *a—b* (Fig. 25) as DRIESCH has already demonstrated, but this direction of growth does not bear, as far as I can determine, any direct relation to the position of the ridges that form the tentacles. The hydranth grows out in the direction of the arrow (Fig. 25) whether the tentacles are laid down parallel to the cut surface or in a transverse position.

The position of the tentacles when they first appear may be due to the shape of the coenosarc in the distal end of the piece, and this seems highly probable in consideration of the fact that the position of the distal row of tentacles is generally more modified than that of the proximal row. In one experiment I cut twenty-four pieces at the angles indicated in Figs. 25—30. In each piece the coenosarc extended as far as the point x, and in eight pieces the distal row of tentacles appeared almost parallel (Fig. 26 *D*) to the oblique surface; the proximal row (*P*) remaining straight. In one piece (Fig. 27) the distal row (*D*) appeared parallel to the cut, while the line of the proximal row sloped obliquely in the opposite direction (*P*). In another (Fig. 28) the distal row formed far out in the acute angle near the tip of the coenosarc in a position very slightly oblique (*D*). The proximal row (*P*) lay in a transverse direction. Two pieces cut in an exceedingly oblique direction developed both distal and proximal tentacles parallel to each other (Fig. 29) but not parallel to the cut surface. This may have been due to the fact that only a very small amount of the coenosarc lay in the oblique tip, and therefore the tentacles developed further back. The remaining twelve pieces formed ridges almost parallel to the oblique surface (Fig. 30).

Barfurth (3) observed in tadpoles that regeneration at an oblique surface took place at right angles to the cut, but later the new part came to lie in the same line with the original piece.

Hescheler (16) and Morgan (25) have seen a similar phenomenon in the earthworm, and King (19) has found in the star-fish that after the removal of an arm by an oblique cut the new arm grows out at right angles to the cut, but later comes to lie in the long axis of the original arm.

Morgan (28) cut pieces of Planaria maculata obliquely at each end. A new head and tail formed; these new parts were »turned at first obliquely to the median axis of the old part«, but later the worm became straight, and head and tail were directly opposed.

I have found that when the heads of Hydra are removed by an oblique cut, the new tentacles form along the line of the cut, but when the new hypostome develops and the new tentacles become longer, the head assumes the normal position in the long axis of the body.

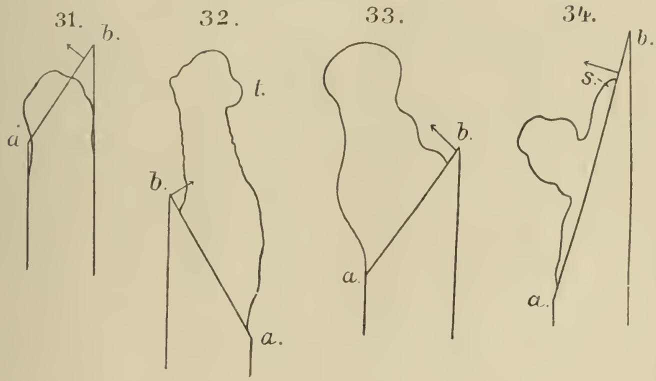
The new parts formed in Planaria and Hydra and other animals such as the earthworm and tadpole, are produced without the limitation of a surrounding perisarc, but Tubularia forms its new hydranth while it is still enclosed in the perisarc. In order to test the effect of cutting the stalks obliquely in species where the hydranth does not form within the perisarc, the following experiments were made.

The stalks of Pennaria and Eudendrium were cut at the angle indicated by the line $a—b$ Figs. 31—38. In Fig. 31 a stalk of

Pennaria is shown twenty-four hours after the cut was made. The coenosarc (x) is not modified in shape as in Tubularia (Figs. 25—30) but is rounded at the anterior end exactly as it is often a cut is made transversely across the stalk.

A later stage may be seen in Fig. 32 where the tentacles are beginning to form (t). The new stalk is slightly crooked but the hydranth lies in the line of the original piece. A small per cent of the pieces cut in this way showed a tendency to grow out at right angles to the cut surface (Fig. 33) but the new hydranth with its stalk soon came to lie in the line of the piece.

Figs. 31—34.

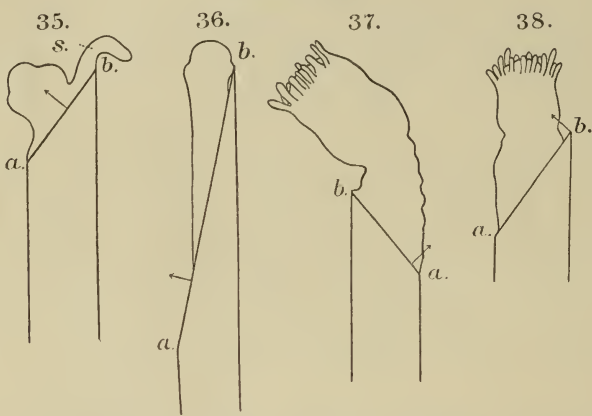


In Figs. 34—38 pieces of the stalk of Eudendrium are shown. Some of these pieces were cut more obliquely than the stems of Pennaria; the difference in the result is probably due to the formation of stolons in front of the hydranths. The line $a—b$ indicates the angle of the cut, the arrow is perpendicular to the line $a—b$, and is used in comparing the variation in the direction of growth of the hydranths.

In four pieces out of fifteen used in one experiment the development of the hydranth took place as shown in Figs. 34 and 35. The direction of growth in the hydranth is almost at right angles to the cut surface, but this seems to be due to the formation of the stolon (s) near the point b. In three pieces cut so obliquely that the angle at b measured not more than 30° the hydranth grew out in the long axis of the stalk as seen in Fig. 36. In the remaining eight pieces the hydranth developed in a line almost directly in the long axis of the stalk (Figs. 37 and 38), but sometimes slightly inclined in the opposite direction from that indicated by the arrow.

In consideration of the fact that in these forms where the hydranth develops outside of the perisarc, there is little connection between the direction of the cut and the position of the resulting hydranth, I am inclined to consider that in Tubularia there is some other factor that influences the direction of growth. The weight of the perisarc in Eudendrium and Pennaria and the branches from each side of the piece enable it to retain the position in which it is placed. In such a piece as that illustrated in Fig. 37 the surface $a-b$ might have been directed toward the bottom of the dish while the same surface in Figs. 34 and 35 lay in the opposite position.

Figs. 35—38.



It may be safely assumed then that the direction of growth in the new hydranth is not controlled by the obliqueness of the cut but is dependent upon some other factor, in this case probably the position ef the piece.

c. Slight injuries at any given point may be included among the external conditions influencing regeneration. LOEB (24) cut small slits in the side of Cerianthus, and by preventing the wound from closing, tentacles were produced on the aboral side of the wound.

Having noticed the strong tendency shown by Hydractinia to produce new hydranths at any point along the stalk, I tried to discover if after injuring the stalk in different places by the insertion of a red-hot needle, new growth would take place at the injured region. The wounds made in this manner healed immediately without causing the production of new hydranths. The experiment was repeated with the Scyphistoma of Aurelia with the same negative result.

II. Internal factors regulating regeneration.

a. The size of the piece used in all experiments controls to a certain extent the character of regeneration. I have found in Hydra that under a certain definite size the piece does not regenerate the typical number of organs present in the adult from which it was taken. Pieces averaging $^1/_6$ mm in diameter produced a hypostome and one tentacle, larger pieces measuring $^1/_5$ to $^1/_3$ mm in diameter produced two tentacles, while still larger pieces produced a greater number.

Rand (34) has recently demonstrated that Hydra viridis generally regenerates fewer tentacles than those borne on the adult. This would not account, however, for these small pieces forming only one, and sometimes two tentacles.

b. The shape of the piece. Not only the size but the shape of the piece that is taken from any part of the body modifies the regeneration of the piece. Long narrow strips cut from the wall of Hydra, close in, forming a tube; sometimes they round into balls from which tentacles and hypostome develop. If pieces of the hypostome bearing two or three tentacles are removed, each piece closes in and forms a new hypostome. In this manner small individuals of abnormal shapes are produced. Such pieces, I found, often produce new tentacles around the hypostome, one of the older tentacles in some cases assuming an abnormal position and frequently disappearing. Later, Rand (33) isolated the complete ring of tentacles and found that out of the ring a new Hydra developed, sometimes bearing one of the old tentacles far back on the body. This tentacle usually disappears.

Morgan (28) cut pieces from Planaria maculata in the shape of isosceles triangles, and right-angled triangles, and also long narrow strips. Such pieces are at first abnormal in shape but finally assume the normal form by a rearrangement of old tissue and the addition of new tissue.

The experiments on Tubularia, described on page 32, show that the position of tentacles bears a definite relation to the shape of the coenosarc at the cut end.

c. Modifications produced by interrupting the process of regeneration.

Driesch (11) has found that after interrupting the process of regeneration of Hydranths in Tubularia different methods of repairing the injury and finally completing the hydranth are followed. After cutting the stalks of Tubularia into pieces 2—3 cm long he waited until the anlage of the two rows of tentacles was visible (Fig. 39 *a* and *b*); then a second cut was made (*x*) parallel to the end, separating *a* and *b* so that each piece bore a row of tentacles. The tentacles on *a* developed rapidly into a hypostome, the piece showed great activity, living for several days, but never formed a complete hydranth. The proximal piece (*b*) grew and completed itself distally but this result was reached by different methods. The first method Driesch calls »Regenerationsmodus«; here the tentacles pinch off rapidly, the coenosarc pushes out from the perisarc, and the knob-like anterior end forms a new mouth and distal tentacles by a process of budding.

The second method he terms the »Ersatzanlagemodus«. This occurs usually when the cut (Fig. 39 *x*) is made very near the base of the distal tentacles, leaving a long space between the cut surface and the distal end of the proximal tentacles. A new distal row of tentacles appears in this space and the hydranth develops as if no interruption in the normal process had occurred.

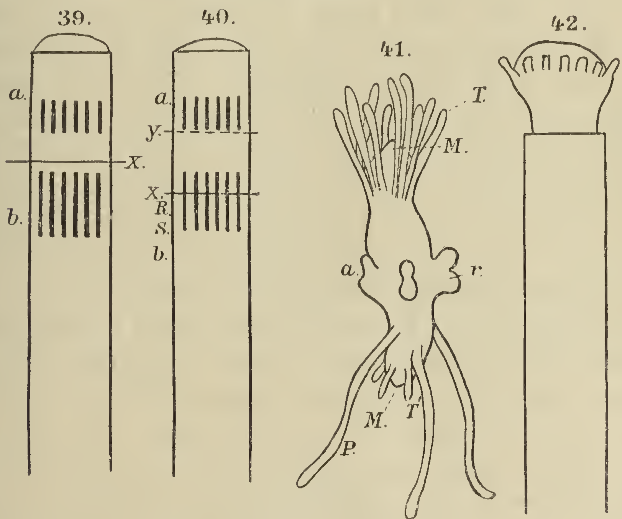
In the third method, the »Auftheilungsmodus«, the proximal ridges divide transversely into two portions, the anterior ends becoming the distal tentacles and the posterior portion the proximal tentacles.

According to the fourth method, the »Auflösungsmodus«, the proximal tentacle ridges disappear by a process of absorption, new distal and proximal ridges are produced and the hydranth develops in the normal manner. The »Regenerationsmodus« was the method most frequently observed by Driesch.

In order to determine if the distal piece (*a* Fig. 39) would regenerate in a proximal direction, thus completing a hydranth, I made a transverse cut at *x* Fig. 40, leaving the anterior ends of the proximal tentacle ridges on the anterior piece (*a*). The region where the cut was made was further back on the piece than that in Driesch's experiment (Fig. 39 *x*). More than a hundred stalks were cut and from these only one showed any development in a proximal direction. In Fig. 41 this piece (*a*) is shown twenty-four hours after the separation from *b* (Fig. 40). The distal row of tentacles (*t*) have developed, a mouth (*m*) has formed and the reproductive organs (*r*) have begun to appear. The three tentacles (*p*) back of the reproductive organs are the fully developed distal ends of the original proximal tentacles (Fig. 40 *p*). At *m'* a new mouth has formed on the proximal end of the piece; this mouth bears three oral tentacles of its own (*t'*); the original proximal tentacles (*p*) do not become oral tentacles, but remain at the base of the reproductive organs. The tentacles (*t'*) grew longer; no reproductive organs developed between the new mouth and the proximal tentacles (*p*). The hydranth showed great activity but died without completing the usual number of tentacles.

From this experiment it is evident that when the cut (*x* Fig. 40) is made through the tips of the proximal tentacles just after the

Figs. 39—42.



ridges are definitely formed, it is possible for the distal piece to regenerate new structures in a proximal direction. The fact that Driesch did not obtain this result may be due to the time of development at which the experiment was made or to the position of the cut.

In connection with this experiment it is of interest to determine which end of the proximal ridges (Fig. 40) on *a* becomes the tip and which end the base of the tentacles. As they pinch off from the wall does the end *R* or *S* become the tip? As they usually lie in the position indicated in Fig. 41 *P*, it would seem probable that the original tip (*R*) had become the base and *S* the tip. To determine this several stages in the development should be studied.

The material that I have preserved is unfortunately insufficient for this determination; the tentacles have developed and their position is so irregular that no conclusion can be drawn as to the probable relation of tip and base to the original anlage. The fact that the base of each tentacle in the older pieces lies near the proximal end is in favor of the view that they develop in the usual manner, *S* becoming the base and *R* the tip.

The later development of the proximal piece of the stalk is also modified by the experiment just described (Fig. 40 *b*). If the tentacle ridges are cut apart, as soon as they are visible through the perisarc as indicated in the figure the basal ends of the proximal row on *B* disappear as Driesch has described in the »Auflösungsmodus«, and two new rows form finally producing a new hydranth. If, however, the cut (*x* Fig. 40) was not made until the red ridges were definitely marked off from the surrounding tissue they remained in the position shown in Fig. 42. The coenosarc has pushed out from the perisarc, bearing the basal stumps of the proximal tentacles. The material lying just anterior to the tentacles finally increases in size, and forms a knob-like hypostome upon which new distal tentacles develop. The method of development in this piece corresponds to the »Regenerationsmodus« observed by Driesch. The variations in his results are due, I believe, to the stage of development of the ridges when the cut (*x* Fig. 39) was made, rather than to a different kind of regulative force within the organism.

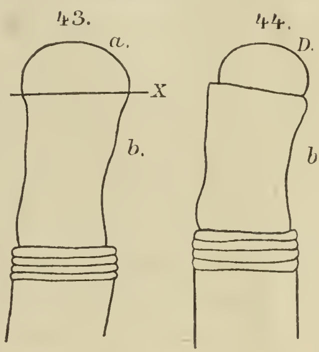
It is not without interest to note that the basal ends of the proximal tentacles retain their original character instead of becoming oral tentacles for the new hydranth, and that instead of a new row of proximal tentacles developing behind them, a new distal row forms in front (Fig. 42).

Driesch also cut some pieces close to the base of the proximal tentacles at *y* Fig. 40. I have repeated this experiment on forty-nine pieces; out of these thirty-nine formed a new row of distal tentacles on *b* in front of the proximal row, the remaining pieces lost all trace of the proximal tentacle row but never produced new hydranths.

In such forms as Pennaria and Eudendrium where the formation of the new hydranth takes place outside of the perisarc a similar experiment demonstrates a different method by which an injury is repaired.

Pieces were cut from the stalks of Eudendrium and Pennaria and isolated until the coenosarc had pushed out beyond the perisarc and the end had rounded slightly as seen in Fig. 43. The distal end was then removed by a cut at x as nearly as possible between the regions where the distal and proximal rows of tentacles would appear. As no trace of the tentacles was visible the cut was made without definite knowledge of its position in relation to the tentacles. After the operation, the distal piece (a) rounded into a ball and died in a few days. The cut end of the proximal piece (b) closed in, and the coenosarc remained apparently passive for several days without further development of the proximal tentacles. On the fourth or fifth day a slight prominence began to appear (D Fig. 44). This knob grew larger and larger until it could no longer be distinguished from the anterior surface of b and the end appeared just as it did before the operation. Development after this proceeded as usual. There was no apparent rearrangement of the old material; all growth and development posterior to the line x seemed to cease until the new tip (D) reached the size of the original tip and the symmetry of the piece was restored.

Figs. 43—44.



5. Grafting.

Trembley (35) succeeded in uniting Hydras of the same species. Marshall (24) repeated these experiments, holding the different individuals together by means of a hair, until the tissues united. Ischikawa (17) united polyps of the same species, holding the components together by means of bristles run through the body. This method was adopted later by Wetzel (37) who has made extended and important experiments on various species of Hydra.

Born (5) has demonstrated the possibility of grafting tadpoles and Joest (18) has made similar experiments on the earthworm. Harrison (14) and Morgan (29) have continued the experiments on the frog larva.

The question whether or not animal tissue of the same or even of different species can be grafted, has been settled affirmatively.

Another problem arising in connection with these experiments is whether the united tissues exert any influence upon each other, and, if so, whether this influence is shown in the subsequent regeneration of either or both of the components, or will it appear in the offspring of the united individuals?

Born's experiments show that in the union of parts of two tadpoles of different species each part retains the characteristics of its own species. Joest also found in the earthworm that even when one of the components was much smaller than the other, the larger piece exerted no apparent influence whatever on the small piece.

Vöchting's (36) results show that in the majority of plants experimented upon no characteristics were transmitted from one part to the other, although modification resulted through the union of annual and biennial varieties.

Harrison (14) interchanged the tails of young tadpoles of different species, and found that in subsequet regeneration the tissues maintained their specific characters.

Morgan (29) has recently made experiments similar to Harrison's in order to discover if the new tissue formed after perfect union of the parts, bears any of the characteristics of the old tissues. In order to determine this the larvae of frogs differing greatly in color were selected. He was able to follow, in this way, the growth of each tissue. After careful observation of the regeneration he found that no modification whatever resulted; each tissue produced new tissue of its own kind.

In the summers of 1896 and 1897, among other experiments I made an attempt to discover if there was any influence exerted by the different individuals of colonial Hydroids when they were grafted together. The experiments have been described in section 3 so that it is unnecessary to give them in detail. They were, I think, conclusive; each individual regenerated tissue of its own kind under all conditions of the experiment.

Later (1898) I tried to bring about some modification in the regeneration of the hydranths of Tubularia by injecting masses of cells from a different species into the body cavity in the following way. After the removal of the hydranth of Tubularia, small quantities of the coenosarc of Pennaria were injected by means of a pipette into the body cavity near the distal end where the new hydranth of Tubularia would develop. Owing to the transparency of the perisarc of Tubularia the mass of Pennaria could easily be seen. Although rapid circulation soon began in the piece of Tubularia the injected mass remained the same size and apparently none of the cells changed their position. Twenty-four hours after the operation there was no trace of the tentacle ridges and the coenosarc of the Tubularia had grown out beyond the perisarc. The protruded end of the coenosarc was covered with Pennaria cells[1]. On the following day evidence of growth was seen, the coenosarc having extended as much as 1 cm beyond the perisarc, but the mass of Pennaria cells was in the same position. On the fifth day as no hydranth had appeared on either end of the stalk, the anterior end containing the injected mass was cut off. On the following day a new hydranth formed on the aboral end of this piece. The hydranth developed in the usual manner, and was in no way modified by the presence of the cells of Pennaria which remained at the base of the hydranth. The only modification in the result was the first outgrowth of the coenosarc from the anterior end of the piece. This outgrowth was probably due to the effort of the piece of Tubularia to rid itself of the foreign substance. A repetition of this result has proved that the formation of the hydranth on the end of the stalk of Tubularia may be prevented by the injection of cells of another species.

Pieces of the stalks of Tubularia were grafted on the cut ends of branches of Pennaria. Union of the coenosarc lasted for a short time but after the formation of hydranths the pieces disintegrated.

Having failed in the attempt to permanently unite these very different species, a number of experiments in grafting were made with individuals of two species having a close resemblance in form and manner of living. Eudendrium and Pennaria were selected and pieces of the stalks were tied together by means of a cotton thread. The grafted stalks were then placed in a large aquarium where they lived for a much longer period than small pieces live in dishes of salt water. The method of binding the stalks together by a thread along a slender splint of wood was found exceedingly satisfactory. The thread soon wears away in the salt water and drops off taking the splint with it. This does not happen until the tissues have had time to unite. The pieces in this way are supported long enough for them to unite.

When Eudendrium and Pennaria are united together a union of the perisarc does not take place, and the union of the coenosarc is imperfect and only temporary.

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[1]) These cells were probably lying around the edge and were carried out by the coenosarc of Tubularia.

When two or more pieces of Pennaria stalks ware grafted together in the same and in different directions, union of the coenosarc results; this union lasts for several weeks, but the perisarc never unites.

Hargitt (13) has recently published the result of experiments made at Wood's Holl on Eudendrium, Pennaria, Parypha, and Campanularia. He succeeded in uniting pieces of the same species after contact of eighteen to thirty-six hours. He held the pieces together and in place by means of small bits of lead until permanent union of the tissues was established. Immediately after the two pieces were placed with their cut ends together, he observed a receding of the coenosarc of each piece within the perisarc, and a closing of the enteric cavity, but later there was a forward extension and final union of the coenosarc of the two pieces, the layer of cells at the ends where the cavities had closed were absorbed, and the two cavities thus rendered continuous.

The perisarc, according to Hargitt, secretes over the line of union a delicate sheet of tissue which may be formed entirely from the perisarc of one end or from both pieces [1]).

He has brought about temporary union between pieces of Eudendrium and Pennaria. The perisarc united but the coenosarc did not. This result is exactly the opposite from that which I obtained from similar experiments on the species found at Naples.

Experiments on Tubularia.

Pieces of equal length united in the same and in opposite directions.

Pieces measuring from 1—2 cm in length were cut from different stalks of Tubularia, and were held together for a short time until the coenosarc of their oral ends was united. Soon after the union was established rapid circulation between the two parts began, and in twenty-four hours the ridges, indicating the position of the new tentacles, were definitely laid down. In nineteen pairs united in this way the following results were obtained.

In five pairs perfectly united two new hydranths formed, one

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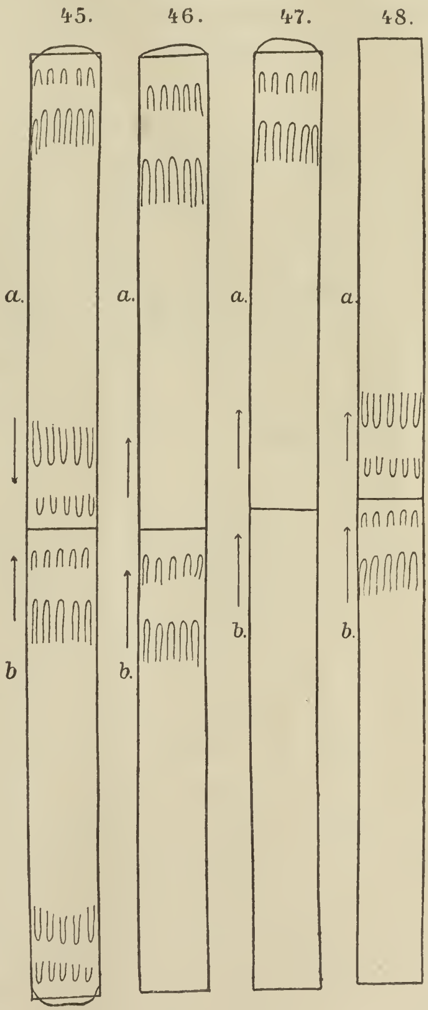
[1]) I can not discover whether the thickened ring around the region of the graft in Hargitt's figures is intended to represent the perisarc or the lead. I have observed such a thickening of coenosarc but never of perisarc.

on each side of the line of union, and later two more appeared, one on each of the aboral free ends (Fig. 45). As soon as the hydranths at the oral ends were fully developed they pushed out from the perisarc but remained attached by a common hypostome until the pieces died.

Figs. 45—48.



Six pairs produced hydranths on each side of the line of union, but the pieces separated before the oral hydranths were fully developed.

Eight pairs, after perfect union at the oral ends was established, produced aboral hydranths on the free ends first, and later in five cases the oral hydranths appeared.

From these results it seems evident that the union of two pieces at their oral ends does not prevent subsequent regeneration of hydranths at the closed ends. The closing of the end by means of another mass of the same kind of substance does not produce a retarding effect on the formation of new hydranths. The perisarc never unites.

When pieces of equal length were united in the same direction, their unlike poles lying together, the following results were obtained.

In fifteen pairs five rapidly produced new hydranths, one at the oral end of each piece (Fig. 46). As soon as the hydranths were fully developed the pieces separated. Four of the fifteen pairs showed no further growth after they united. In five, a new hydranth was produced on the oral end of *a* (Fig. 47) while *b* showed no trace of the rows of tentacles. In this way a single individual was formed by the union of the two pieces. The last pair (see Fig. 48) produced hydranths on each side of the line of union, one on the oral end of *b* and one on the aboral end of *a*. The growth of the coenosarc pushed the hydranths, which were united by a common hypostome, out of the perisarc, and they remained in this condition until the pieces died. It is possible that one of the pieces was reversed, as this result was obtained only once in this experiment.

The union of a long piece with an extremely short one.

Driesch (11) has shown that very small pieces of the stem of Tubularia show great variation in the regeneration of new structures. Out of eighty-two pieces five formed one row of tentacles nearer one end of the piece than the other, twenty-six formed a row at each end; in twenty pieces all the material was used in the formation of a complete hydranth which remained within the perisarc. In twenty-eight pieces one row of tentacles appeared in the middle of the piece, and later the tip of the hydranth and the distal tentacles formed by a process resembling budding. The hydranth finally freed itself from the perisarc.

These variations must be caused by some internal factors regulating the production of the new parts. Recently Driesch (12) has in part accounted for the variation in the behavior of these small pieces. He found that the character of the result frequently depends upon the region of the stalk from which the piece is taken, and also upon the amount of red »hydranth-forming« material present in the piece. This substance increases toward the distal end of the stalk. The smaller the quantity of this red substance, the slower the hydranth is in forming. He says: »Das Schicksal kleinster Stammstücke der Tubularia hängt nicht nur von ihrer Größe, sondern auch von ihrem Ort im ursprünglichen Individuum ab.«

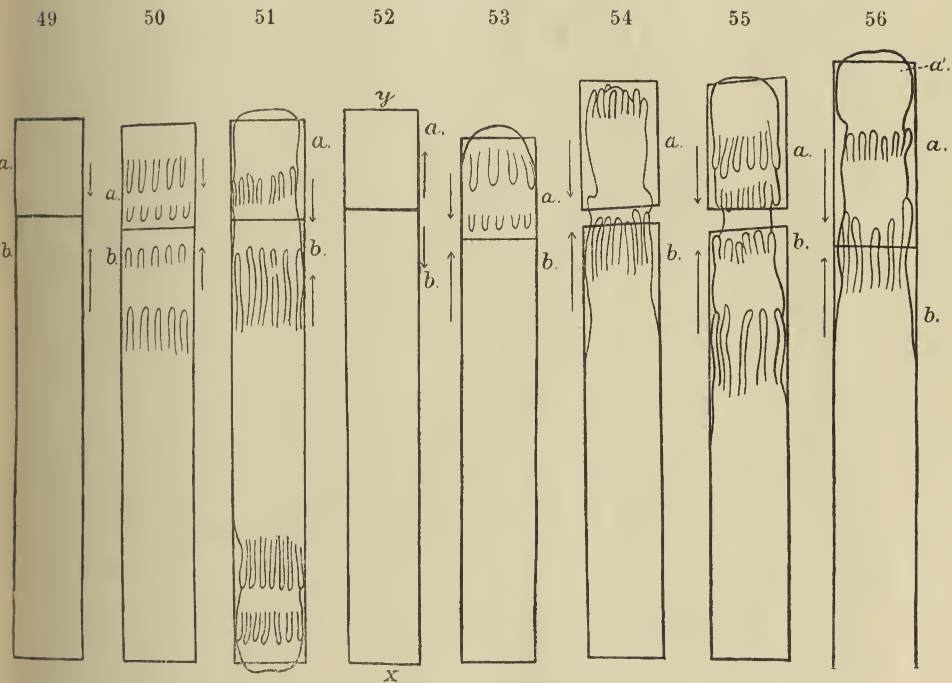
This regional variation led me to carry out the following experiments. A small piece from the region near the base of the stalk was grafted, in a reversed direction, on the oral end of a long piece cut out just below the hydranth. My problem was to determine whether a piece from the basal part of the stalk, when grafted on a distal and more anterior piece, would delay or in any way modify regeneration in the longer piece.

Exp. 1. Grafting a short basal piece on the oral end of a long piece.

From a stalk measuring 3—4 cm in length, the hydranth was removed and a piece (Fig. 49 *b*) about 2.5 cm long was cut from the anterior end.

A small piece (Fig. 49 *a*) of the same stalk, taken from the region near the base, was grafted in the opposite direction on the oral end of the long piece (*b*)[1]. The coenosarc of the oral ends united; thus the aboral surface of the two components were exposed.

Figs. 49—56.



On Nov. 3rd five pairs were grafted in this manner; four remained permanently united until the pieces died. The first pair produced two complete hydranths simultaneously, one on the oral end of each piece (see Fig. 50). The subsequent growth of the hydranths pushed aside the perisarc of the short piece and the hydranth *a* freed itself form its perisarc, but remained attached by its hypostome to the hydranth on *b* (see Fig. 57). No hydranth developed on the aboral end of the longer piece (*b*).

The second pair formed hydranths simultaneously at the exposed

[1] The arrows used in all figures point toward the hydranth end.

surface regardless of the reversed direction of the short piece. The hydranth on the end bearing the short piece was not formed entirely from the material in *a* but, as Fig. 51 shows, the distal tentacles formed in *a* and the proximal row in *b*.

The two remaining pairs formed hydranths first on the end to which the short piece was united, the distal row of tentacles lying in the short piece and the proximal in the longer piece. Twelve hours later a hydranth developed on the aboral end of the longer piece (*b*).

The number of times that this experiment was repeated is not sufficient to warrant any conclusion as to the probable influence in the rate of development that either piece exerted on the other. The results indicate a slight delay in the regeneration when the short and long pieces combine to produce one complete hydranth.

An experiment similar to this was recently made on the earthworm. Hazen (15) has clearly demonstrated that the small piece from the anterior end of the worm, when grafted in a reversed direction on the anterior surface of the posterior segments of the body, produced a head instead of a tail, although the aboral surface of the piece was exposed. The result is not directly comparable with that obtained in the same experiment on Tubularia for in the earthworm the larger piece takes no direct part in the formation of the new head although it nourishes the small piece and thus enables it to accomplish what it could not do alone. In Tubularia, as the figures show, the long piece actually contributes part of its material to the formation of the new hydranth.

Exp. 2. Grafting a short anterior piece on the posterior end of a longer piece.

In order to test the effect of grafting a short piece from the anterior or distal end of the stalk upon the posterior end of a longer piece, the following experiment was made. A piece 1,5 to 2 cm long was cut from the proximal end of the stalk; on the aboral end of this piece was grafted a shorter piece measuring about 4 mm in length, taken from the stalk just below the original hydranth. In Fig. 52 a graft of this description is shown. The aboral ends of *a* and *b* are united, and the oral ends exposed, thus the position of the pieces in the grafts described in the preceding experiment is reversed.

Twelve out of eighteen grafts were successful. Four of the twelve formed a new hydranth at the oral end of the long piece and

Experiment	Length of perisarc a short piece	Length of Regeneration Area
1	.78 mm	1.00 mm
2	1.00 -	.80 -
3	.90 -	1.10 -
4	.80 -	1.03 -
5	.71 -	.90 -
6	.78 -	.90 -
7	.78 -	.60 -
8	.59 -	.80 -
Mean.	.79 mm	.89 mm

none at the oral end of the short piece. Eight formed a hydranth at *y*, one row of tentacles appearing in *a* and one in *b*. Later, after the hydranth at *y* was completed, another developed at *x*, the oral end of the long piece. This seems to indicate that the presence of the small piece from the anterior region of the stalk hastens regeneration at the aboral end of *b*. This may be due to the fact that *a* contributes some of its material to the formation of the new hydranth, although the greater part is developed from the cells lying at the basal end of the stalk. A simultaneous appearance of hydranths at both ends was not observed.

Exp. 3. Grafting a short piece in reversed direction on the oral end of the same piece.

For this experiment pieces of the stalk measuring 2—3 cm in length were cut from the region lying immediately under the hydranth. From the distal end of the piece a short portion was cut and after reversing the position of this short piece it was grafted on the oral end of the same piece from which it was cut.

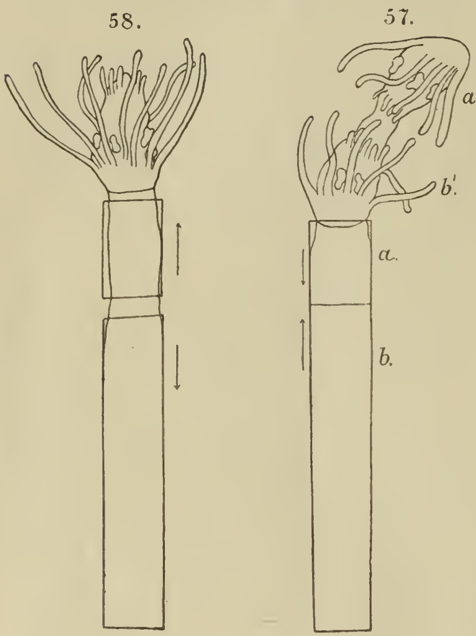
This experiment was performed eighty-eight times; out of this number fifty-one remained permanently united. Thirty-six formed a complete hydranth at one end, the two pieces acted as one, the distal row of tentacles appeared on *a*, the proximal row on *b*, the hydranth was completed, and pushed out from the perisarc passing through the perisarc of *a* as shown in Fig. 58. Careful observations were made at frequent intervals and measurements were taken which prove that the line of union of these pieces lay between the two rows of tentacles. The length of space occupied by the anlage of the hydranth was compared with the length of the perisarc of the short piece. The following table gives these dimensions; the average length of the long piece was 3.5 cm.

Experiment	Length of perisarc a short piece	Length of Regeneration Area
1	5.0 mm	8.5 mm
2	6.5 -	5.6 -
3	5.4 -	9.0 -
4	10.0 -	16.5 -
5	4.5 -	8.0 -
6	6.0 -	7.5 -
7	7.0 -	6.0 -
8	4.5 -	7.5 -
Mean.	6.11 mm	8.6 mm

In experiments 2. and 7. where the length of the perisarc was greater than the length of coenosarc in which the tentacles developed, the two rows of tentacles lay entirely within the perisarc of the short piece as indicated in Fig. 53.

In six of the fifty-one grafts the tips of the proximal tentacles projected beyond the line of union (Fig. 54) so that they seemed to be formed partly in the short piece and partly in the longer one.

Figs. 57—58.



In five pairs the two pieces developed hydranths independent of each other. The hydranth of the small piece, as seen in Fig. 55, always remained in contact with the hydranth on the oral end of *b*.

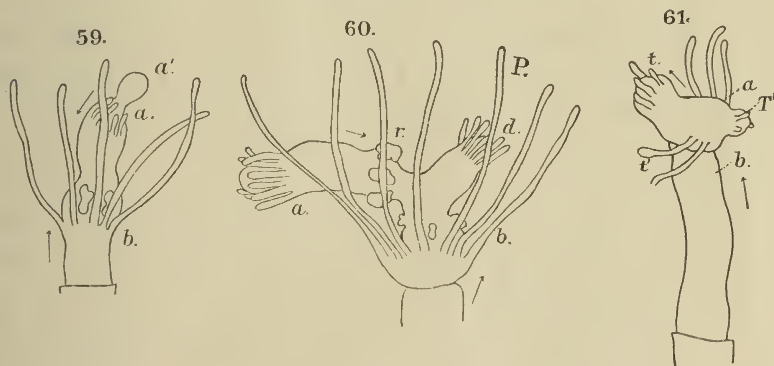
The remaining four grafts are shown in Figs. 56, 59, 60 and 61. In Fig. 56 the new hydranth is nearly complete. It has formed from material in both *a* and *b*, but all the material in *a* has not been used as is generally the case when the piece is very short. The coenosarc *a'* remained attached to the hypostome of the hydranth, preventing the mouth from breaking through. The hydranth finally pushed out through the perisarc of *a* but died at the end of five days.

In Fig. 59 another graft of the same kind but somewhat older is shown. The hydranth has emerged from the perisarc bearing the extra material (*a'*) on the tip of the hypostome. The piece *a'* decreased in size from day to day until on the fifth day it reached the size shown in Fig. 59 *a'*.

The third graft five days after the union of the two pieces is seen in Fig. 60. The pieces were not evenly united; the result of this is shown in the peculiar relation existing between *a* and *b*. The longer piece (*b*) has produced a complete hydranth with proximal (*p*) and distal tentacles (*d*). No proximal tentacles are present on *a*. The reproductive organs (*r*) have developed near the base where it is united with *b*.

The fourth and last graft is shown in Fig. 61. The long piece (*b*) has produced a complete hydranth which is concealed by the short piece (*a*). The original distal end of a bears a row of tentacles (*t*) surrounding a hypostome which is united with the hypostome of *b*. Two long tentacles (*t'*) lie in the middle of the short piece (*a*) at the exposed surface of a the original proximal end; a new mouth has developed around which three short tentacles (T'') lie. The perisarc originally surrounding *a* has been lost.

Figs. 59—61.



Although great care was taken to reverse the direction of the short pieces it is barely possible that in these cases they were grafted in the same direction as the long pieces. In consideration of the fact that such a result has occurred only several times, it is possible that the pieces were grafted in opposite directions.

The behaviour of isolated pieces of the same length should be compared with that of the short components in the experiments just described. In the table (page 37) the mean length of the short component is 6.11 mm. In nearly all of the pieces used in the experiments given in the table only one row (distal) of tentacles formed in the short piece. Isolated pieces of this length and even shorter (3—5 mm in length) are capable of forming a complete hydranth. This is seen in those grafts where a complete hydranth forms in the short piece.

6. Heteromorphosis.

In the course of experiments made on different species of Hydroids heteromorphosis was observed in Hydractinia, Podocoryne, Cordylophora, Pennaria, and Bougainvillia, and also, as already demonstrated, in Eudendrium and Tubularia.

Experiments on Hydra.

1. Heteromorphosis.

The formation of a new head on the cut aboral surface of the body of Hydra, so far as I can discover, has never been seen. Rand (34) attempted to fasten a Hydra, in an inverted position, to a fixed surface, thinking that a head might form on the free aboral end if the development of a new head on the oral end was prevented. After many unsuccessful attempts he resorted to a method of preventing development at the oral end by grafting pieces of the foot on the open oral surface.

Before describing my experiments in grafting it may be of interest to mention similar attempts which I made (June 1899) to fasten Hydras by the oral end although my object was not so much to prevent development at the oral end, as to hold the polyp so that the cut aboral surface could not come in contact with any fixed object, and develop the characteristic foot.

Large polyps of Hydra grisea or Hydra fusca were selected for the experiment, and were fastened by plunging the oral end in Agar. The Agar was first dissolved in warm water; as soon as it reached a consistency as nearly as possible resembling that of the polyp, the water was poured off and the Agar allowed to cool so that all danger of injury from heat was avoided. The hydra was then lifted by means of fine forceps and plunged head first in to the Agar. Cold water was added, the Agar became hard and the anterior end of the polyp was held firm while the foot protruded from the surface. As soon as the body extended, the foot was cut off thus leaving the aboral surface free.

The experiment was repeated a number of times but was not successful; when the polyps survived the operation they tore themselves from the Agar and soon replaced the lost parts in the usual manner.

2. Heteromorphosis in Grafts.

Having failed in all attempts to produce heads on the aboral surfaces of polyps, a second attempt was made to reach this result through grafting.

Experiments made by Wetzel (34) seem to indicate that it is possible to produce heteromorphosis in Hydra. In one experiment the aboral or foot ends of two polyps (H. grisea) were removed and the individuals held together by means of a bristle until the aboral surfaces united. As soon as perfect union was brought about a transverse cut was made through one of the components just back of the tentacles. Five days later two short hook-like processes formed on the exposed oral surface. These hooks finally formed a foot by which the polyp became attached to a plant. The hydra was killed and sectioned, and the glandular cells of the foot were found at this surface which originally lay just back of the tentacular ring.

A second experiment was carried out by Wetzel in a manner similar to the first, but no cut was made in either of the polyps after their aboral surfaces were united. After the components were firmly united a process began to appear on one side of the body in the region of the line of union. This process soon formed a rounded eminence resembling a bud and later mouth and tentacles developed on its summit. One of the components then began to dwindle into a short hook-like process and the other component with the new bud-like portion remained.

It seems highly probable that this development of a new head at the side of the graft was a bud and that it remained in contact with the body of the mother instead of separating as is usual. Rand (34) has shown by experiment that when nearly mature buds are grafted back into the trunk of the parent, they do not continue to constrict off.

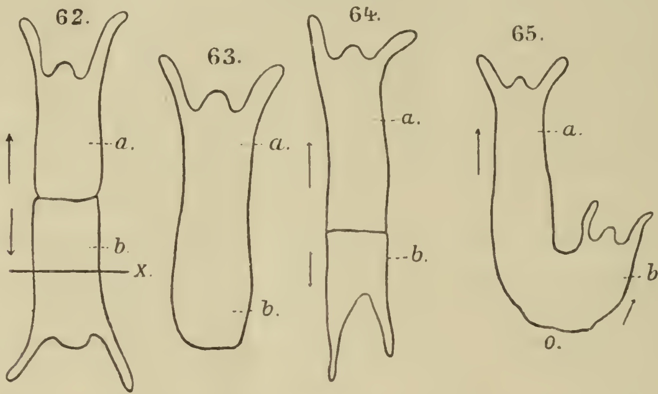
The following experiments on Hydra grisea and Hydra fusca confirm in part, and also extend the experiments made by Wetzel.

Grafting pieces in opposite directions.

In the first experiment the posterior ends of two individuals were removed and the cut surfaces united so that the negative poles were in contact, and the free positive poles bore the original heads (Fig. 62). The results of seven sets of experiments were as follows.

Exp. a. On June 20th two individuals were united as shown in Fig. 62; after complete union a cut was made through *b* at the line *x*. The original head end of *b* was not further observed as the experiment was made for the purpose of discovering the behavior of the cut oral surface of *b*. Three days later the polyp appeared as seen in Fig. 63; the point of union could not be detected, no sign of mouth or new tentacles was visible on the oral surface of *b*, neither was a foot developed instead of a head. Two weeks later, however, the polyp was observed again and was found resting on a leaf but not firmly attached. Sections of the graft were made but no glandular cells were found.

Figs. 62—65.



Rand (34) succeeded in producing a foot on the free oral surface of a very short lateral graft; this he says may be »a possible case of Heteromorphosis«.

Exp. b. On June 21st two pairs were united and cut at *x* in the manner described above (Fig. 62). No tentacles or foot appeared on *b* but in one of the grafts two hook-like processes on the oral surface, such as Wetzel describes, were observed (Fig. 64).

Exp. c. On June 23rd five pairs were united and the anterior end of *b* removed. Four of the five pairs developed a new hypostome and tentacles on *b* (Fig. 65). On the 28th the graft became attached in the region of union (*o*). One pair showed no new structures on *b* and the polyps died without attaching themselves at the closed end.

Exp. d. Two pairs were united on June 28th. No new parts were formed. On July 5th *b* disintegrated and *a* was attached by a new foot on the aboral surface where it was united with *b*.

Exp. e. One of two pairs, united in the same way, showed a similar disintegration of *b* and the formation on *a* of a new foot. The other graft became attached at the oral surface of *b* although no true foot formed.

Exp. f. Two pairs under the same conditions were united on July 7th. On the 13th one pair was attached in the region of the graft as in Exp. c, and b had formed a new mouth and tentacles. The second pair showed no regeneration at the anterior end of *b*. On the 17th the polyps were killed.

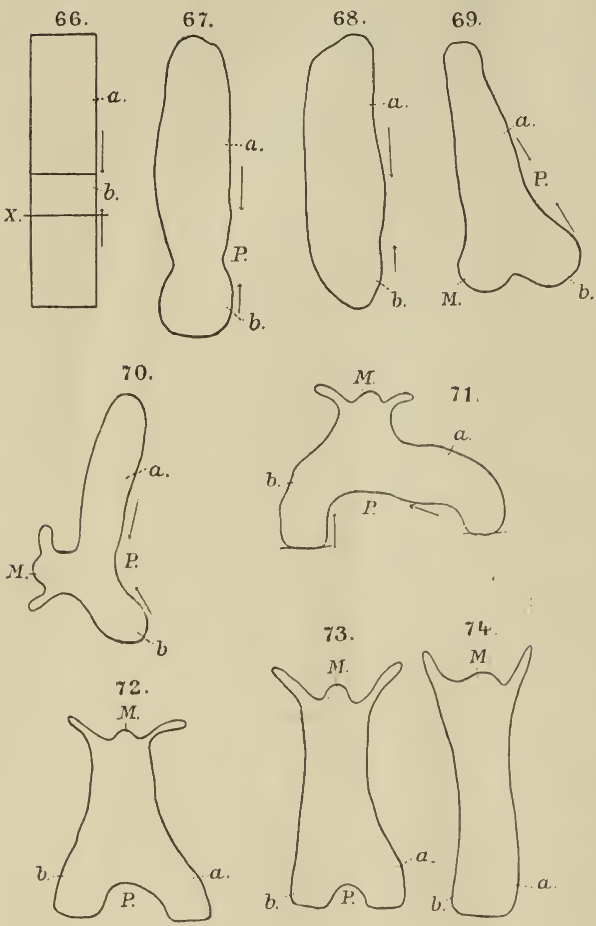
Exp. g. On July 13th two polyps were united in the same way. On the 17th a small bud began to form on *a* but no new structures developed on *b*.

Summary. Fifteen pairs were successfully united at their aboral ends. After the removal of the anterior end of one component, five formed a new head at the cut surface, replacing the lost one and becoming attached at the line of union. Two more seemed to become attached in the same way but showed no regeneration at the cut surface of *b*. Five pairs did not regenerate like or unlike structures after the removal of the anterior half of *b*. Three became attached at the oral surface of *b* but none of the pieces developed the characteristic foot in place of the original head.

2. In the second series of experiments the anterior or oral surfaces of the polyps were united after the removal of the tentacles and hypostome. The direction of the pieces and their relation to each other is indicated by the arrows in Fig. 66. Eight pairs were united in this way, in some the union was perfect, the two individuals forming together a hollow bag-like body, with no visible break in the walls, in others the union was not complete; in places the edges of the two components were open, forming slits. In the eight pairs a new hypostome and tentacles formed invariably at the line of union which represented the anterior regions of the individuals. That the head produced at this point did not belong entirely to one component could be easily seen, for not only a greater number of tentacles were formed than normal for one individual, but in a few cases two mouth-openings appeared. The later fate of such double individuals will be described in the following section.

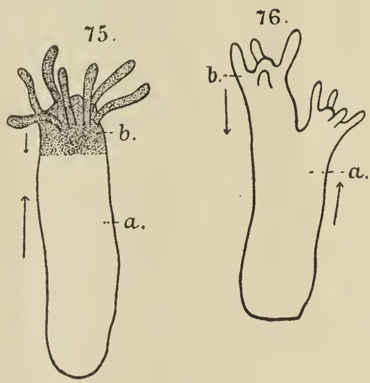
It has been shown by the experiments just mentioned that although no cut surfaces were exposed, new structures were produced, and there was a decided effort on the part of the double organism to complete itself by the formation of one or two heads in the region of the union. By another series of experiments I have tried to discover if new heads always form in the region of union if a large surface on one of the components is exposed. The experiments were repeated thirteen times with the same general results. After the complete union of the two components (*a* and *b* Fig. 66) the posterior half of *b* was removed by a transverse cut at *x*; thus the aboral surface of *b* was exposed. A typical result will suffice to describe the behavior of the grafts.

Figs. 66—74.



On June 21st two pairs were united at their oral surfaces as seen in Fig. 66; on the following day the posterior half of *b* was removed. On the 26th the cut surface had healed; a new mouth with its surrounding tentacles appeared near the line of union. On the 27th *a* and *b* were firmly united at their aboral ends while the new head was directed forward. The two bodies were then gradually transformed into one individual having two feet and one head, and finally one head and one foot. This union was brought about in the manner shown in Figs. 67—74. At the point *P* the body of *a* begins to merge into that of *b* by a process which seems to be a forward growth in the direction of the arrow, and a gradual absorption of the wall between *a* and *b*. In Fig. 67 a slight constriction at *P* renders the region of union distinguishable. The component *b* is less than half the length of *a*; the cut surface of *b* has closed in. In Fig. 68 the constriction has disappeared and the two components form one continuous bag-like body; *a* and *b* can be no longer distinguished unless they differ in color. Fig. 69 shows the new mouth forming on one side (*M*). In Fig. 70 the new head is completed. Up to this time the posterior end of *a* has been firmly attached to the bottom of the dish and the healed aboral surface of *b* has floated freely in the water. In Fig. 71 the new mouth has taken the anterior direction, *b* has bent around until its aboral surface has also become attached and has developed a new foot. The growth or merging of the two individuals into one begins as soon as the head is fully developed; it can be traced by comparing Figs. 71—74. The angle *P* grows smaller and smaller, the feet gradually approach each other, the body back of the ring of tentacles increases in length until, finally, one complete individual is formed; this individual has one mouth and one foot, but generally a much larger number of tentacles than would be normal for one polyp. Twenty-six of the forty grafts merged into one polyp in this way.

Figs. 75—76.



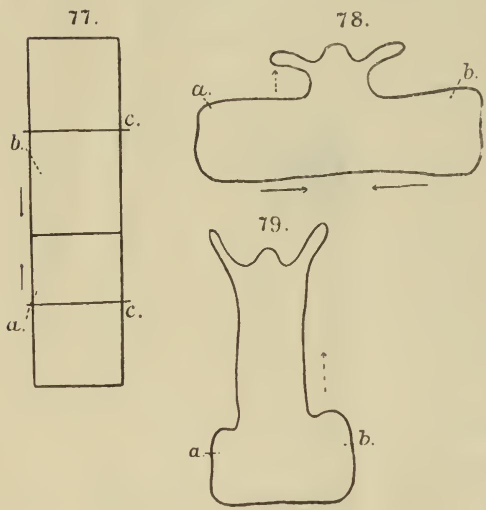
The method described above was also followed when the pieces were united without the subsequent removal of the oral end of *b*. When two distinct heads form at the line of union the two components seem to struggle to free themselves and through this effort many abnormalities result.

On June 19th two individuals were united as described above (Fig. 66); and on the following day a transverse cut was made through *b* close to the line of union of the two components. The cut end closed and for five days no change was visible. On the seventh day mouth and tentacles appeared on the aboral end of *b*, and the new head was composed only of the material in *b* as the line of union was easily seen on account of difference in the color of *a* and *b*.

Fig. 75 shows without doubt that this is a clear case of heteromorphosis, or the formation of a head instead of a foot. This result was obtained only when the cut through *b* was made near the line of union. Five out of twenty-two grafts formed a head instead of a foot. In a sixth graft a head formed on the aboral end of *b* and also on the oral end of *a* as seen in Fig. 76. In the remaining sixteen grafts, if new heads formed at all, they appeared in the region of union.

In another series of experiments where the polyps were united at the oral ends, two transverse cuts were made in order to see if the further development of the new heads would be modified by the presence of two exposed surfaces (see Fig. 77). Thirteen pairs were cut in this way (*c*, *c*′); they developed as follows: The ends healed over but no new structures were developed at either surface; the new head invariably appeared in the middle as shown in Fig. 78. In two grafts this head proceeded to grow forward in the direction indicated by the dotted arrow, at right angles to the original long axis of the body. The forward growth of the new head continued until *a* and *b* became small eminences at the side of the new body (see Fig. 79). The polyps remained in this condition for several weeks but never showed any tendency to become attached by the aboral surface of *a* or *b* or to form a new foot on the lower end of the new polyp.

Figs. 77—79.



In nine of the thirteen grafts a head appeared in the middle at the original line of union; the polyp became attached by the two feet at the posterior ends of *a* and *b*; the two components finally merged into one as described on page 44 where one cut was made instead of two.

Two pairs formed two heads, one on the anterior end of *a* and one on *b*. The two individuals became attached but no separation of the components resulted.

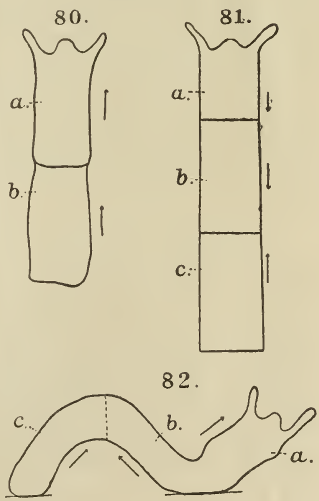
Wetzel (37) has described the merging of two individuals into one. He grafted two polyps together at their oral surfaces after removing the tentacles. The new head appeared near the line of union, the two bodies formed an acute angle with each other, then finally united by a fusion from the apex of the angle.

I have not observed the »wandering« of the smaller component of the graft which Rand (34) describes. The two foot ends seem to be brought together by the forward growth of the new body, not by any migration of the smaller piece.

The formation of one polyp from two pieces united in the same direction.

The experiments just described seem to prove that as a general rule, no matter what the position of the pieces is, or how they are related to one another, the end result is the formation of one complete individual, although the process by which this result is reached may differ with different individuals.

Figs. 80—82.



After the removal of the foot end of one polyp and the head of another the two individuals were grafted so that the aboral surface of *a* was united with the oral surface of *b* (Fig. 80). In a few of the experiments *a* and *b* showed no further growth, the polyp became attached at the base of *b* and, as far as could be seen, the two components formed one individual; however, the body of this individual possessed two budding zones and one reproductive zone (see Fig. 80). In one pair a new head formed at the line of union.

Two single individuals of unusual length were made from the parts of three different polyps; the components were united as seen in Fig. 81. A few days after the experiment the polyp was attached at two points, at the foot end of *c* (Fig. 82) and the combined aboral surfaces of *a* and *b*, the bodies of *b* and *c* forming an arch. In one graft the foot at *x* soon freed itself and the polyps took the normal position indicated in Fig. 81. In the other the component *a* tore apart from *b* and *c*, a new head formed at the line of union of the oral surfaces of *b* and *c*, and these two components merged into one in the manner described in a previous section.

Summary. The experiments made upon Hydra grisea and Hydra fusca show that no matter in what direction pieces are grafted together they generally form, sooner or later, one complete individual. This result is, in a few instances, brought about by the rapid formation of new structures at the cut surface (Fig. 75) irrespective of the surface exposed. In others the process is exceedingly slow, several weeks elapsing before the two components lose their identity and merge into one individual. These results show a strong tendency on the part of the two components to bring about the normal form but this tendency does not manifest itself in the final separation of the components; on the contrary they seem to strive toward a perfect union by the further fusing of their tissues.

It is of interest in connection with these results to note that when an unusually large number of tentacles develop, in the line of union of the two components, some of them generally disappear as the new polyp is formed. In cases where the new tentacles form a complete ring around the middle of the double body those lying in the angle *P* Fig. 71 are absorbed. Rand (33) has also observed this disappearance of the tentacles when as a result of an experiment they came to lie in abnormal positions.

The later history of these double polyps should be studied. I regret that I could not keep them longer than three or four weeks. It would be of interest to see how and where buds and new reproductive organs appear, for such polyps are composed of a double budding zone, a double foot and no reproductive zone. If no new tissue except that forming the hypostome and tentacles is produced, will the animals remain in an asexual condition sending out buds, but developing no testes and ovaries, or will the new organs regenerate as they do in Tubularia? The process of union between two polyps, forming in the end a single individual, lasts from one to three weeks, ample time for the development of either buds or reproductive organs, but not one of all the polyps showed traces of reproduction by either of these methods.

Summary.

1) Pieces of the stalk of Hydractinia and Podocoryne, when isolated, regenerate a new hydranth on the oral end and a stolon or hydranth on the aboral end; a large per cent of the pieces produce a hydranth on each end.

2) When the stalks of the different individuals of Hydractinia are deprived of their hydranths and are isolated, each individual retains the characteristics of its kind throughout the process of regenerating new hydranths.

3) The rate and frequency of hydranth formation on short pieces of the stalk of Hydractinia is approximately the same at all levels of the stalk. The hydranth forms as soon on the anterior half of the stalk as on the posterior half.

4) Pieces of the stalk of a nutritive polyp of Hydractinia, and especially of Podocoryne, if left undisturbed send out stolons in all directions, and from these stolons new individuals arise, but these individuals always retain the characteristics of the nutritive polyp. Colonies of this sort were kept for several weeks but none of the polyps of the other classes were produced.

5) Two individuals of the same or of different colonies of Hydractinia may be grafted upon each other; the union of the tissues is perfect and lasting. Nutritive polyps may be united with protective or reproductive individuals. In such grafts no influence is exerted by one component upon the other; each retains its own characteristics throughout subsequent regeneration.

6) When two pieces of the stalks of nutritive polyps are united in the same or in opposite directions the subsequent regeneration of new hydranths is rarely ever delayed. New heads appear on aboral and oral surfaces and frequently in the region where the pieces are united. As many as four new hydranths may be produced on one graft.

7) Pieces from the stalk and branches of Pennaria show slight variation in their capacity for regenerating new hydranths. Those cut near the base of the stalk do not regenerate new tissue; the extreme tips show a slight growth of coenosarc but no new hydranths are produced. The most vigorous and complete regeneration takes place in pieces near the middle of the stalk. The pieces from the base and middle of the branches regenerate hydranths, those from the extreme tip show a little new growth but no hydranths develop.

8) The formation of annular constrictions at regular intervals along the perisarc of the stalk takes place in the regeneration of new hydranths. Rings generally appear at the cut surface and later through growth of the coenosarc an unsegmented area forms beyond which another series of rings develops at the base of the new hydranth.

9) Absence of light delays regeneration in Eudendrium and reduces the percentage of new hydranths on Pennaria. Regeneration of new hydranths on Bougainvillia and Tubularia is not affected in the least by darkness. The development of second hydranths on Tubularia may be slightly delayed. Red, blue, yellow and green light do not influence hydranth formation in Pennaria, Tubularia, Podocoryne and Bougainvillia.

10) Contact with foreign bodies produces modification in the regeneration of new tissue. Pieces of the stalk of Hydractinia and Podocoryne form stolons instead of hydranths if left undisturbed in contact with some hard substance.

11) When pieces of the stalk of Tubularia are cut obliquely the new hydranth grows out at right angles to the cut surface regardless of the position of the tentacle anlage.

12) When the heads of Hydra are removed by an oblique cut the new tentacles form along the line of the cut but as soon as the new head is completed it grows into line with the posterior part of the body.

13) If pieces of the stalk of Pennaria and Eudendrium are cut obliquely the new hydranth forms in the normal position; if the growth at first is at right angles to the cut surface the hydranth soon comes to lie in the long axis of the piece.

14) If the anlage of the proximal tentacles of Tubularia is removed from the distal row by a cut just back of the distal end of the proximal tentacles, the anterior piece may regenerate in a proximal direction, forming a proximal mouth surrounded by tentacles.

15) The character of the further development of the proximal piece of Tubularia after separation from the distal piece by a cut between the developing tentacle rows, is dependent upon the stage of development at the time the cut is made. If the tentacle rows are separated as soon as they are laid down the proximal row usually disappears; if the cut is made after the rows are well defined the new hydranth is completed by Driesch's »Regenerationsmodus«.

16) If the tip of the hydranth of Eudendrium or Pennaria is

removed before the tentacles form, the tip dies and the proximal piece regenerates a new tip before the new proximal tentacles develop.

17) When a mass of Pennaria cells was injected into the body cavity of Tubularia, the new hydranth forming on the end of the stalk of Tubularia was in no way modified by the presence of the Pennaria. The injected mass usually prevented hydranth formation.

18) Pieces of Pennaria when grafted on Eudendrium show temporary union of the coenosarc but no union of the perisarc. When pieces of Pennaria are grafted upon each other the coenosarc unites and the pieces remain together for several weeks. No union of the perisarc takes place.

19) Pieces of the stalk of Tubularia may be permanently united. The coenosarc of the two components becomes continuous but the perisarc never unites. After union of two pieces of equal length hydranths appear on each side on the line of union or on the exposed surfaces. Pieces united in the same and in opposite directions generally retain their identity, forming separate hydranths.

20) When short pieces are grafted in a reversed direction on longer pieces of the stalk of Tubularia, the short piece frequently takes part in the formation of a new hydranth, the hydranth taking the direction of the long piece, the distal tentacles developing in the short component and the proximal tentacles in the longer component.

21) The experiments seem to prove that when short pieces from the anterior end of the stalk of Tubularia are grafted on the aboral end of a posterior piece the development of the new hydranth on the aboral surface of the longer piece is hastened.

22) Heteromorphosis occurs in Hydractinia, Podocoryne, Cordylophora, Pennaria and Bougainvillia.

23) When two bodies of Hydra grisea or Hydra fusca are united at their aboral surfaces, after the removal of the foot ends, and a second cut is made through one of the components leaving an oral surface exposed, this oral surface may become attached. No characteristic foot forms. A new head usually appears at the cut surface.

24) If two individuals of Hydra grisea or Hydra fusca are united at their oral ends after removal of the heads, a new head develops at the line of union and the two components merge into one. If after the union of two individuals a cut is made through one component, close to the line of union, a new head may form on the aboral surface of the small piece.

25) A single Hydra will form from two pieces grafted in the same or in opposite directions. This process may take place rapidly by the production, at the cut surface, of a head instead of a foot, or a foot instead of a head; or it may take place slowly by a gradual merging of one component into the other.

Bryn Mawr, Pa., May 11th 1900.

Literature.

1. Agassiz, L., Contributions to the Natural History of the United States. Vol. IV. 1862.
2. Allman, Geo. J., Report on the present state of our knowledge of the Reproductive system in the Hydroidea. Report of the Brit. Assoc. for the Adv. of Science. 1864.
3. Barfurth, D., Zur Regeneration der Gewebe. Archiv f. mikr. Anatomie. Bd. 37. 1891.
4. Bickford, E. E., Notes on Regeneration and Heteromorphosis of Tubularian Hydroids. Journal of Morphology. Vol. IX. 1894.
5. Born, G., Über Verwachsungsversuche mit Amphibienlarven. Archiv f. Entwickelungsmech. Bd. IV. 1897.
6. Bronn, Klassen und Ordnungen des Thierreichs. Leipzig 1897. Bd. II.
7. Collcutt, M. C., On the structure of Hydractinia echinata. Quart. Journ. of Mic. Sc. April 1897. Vol. 40 Pt. 1.
8. Dalyell, J. G., Rare and remarkable animals of Scotland. London 1847.
9. Davenport, C. B., Studies in Morphogenesis. II. Regeneration in Obelia, and its Bearing on Differentiation in the Germ Plasma. Anatom. Anz. IX. 1894.
10. Driesch, H., Zur Analyse der Reparationsbedingungen bei Tubularia. Vierteljahrsschrift d. Naturforsch. Gesellsch. in Zürich. XLI. 1896.
11. Driesch, H., Studien über das Regulationsvermögen der Organismen. 1. Von den regulativen Wachsthums- und Differenzirungsfähigkeiten der Tubularia. Archiv f. Entwickelungsmech. Bd. V. 1897.
12. Driesch, H., Studien über das Regulationsvermögen der Organismen. 2. Quantitative Regulationen bei der Reparation der Tubularia. Archiv f. Entwickelungsmech. Bd. IX. 1899.
13. Hargitt, C. W., Experimental Studies upon Hydromedusae. Biological Bulletin. Vol. I. No. 1.
14. Harrison, R. G., The Growth and Regeneration of the Tail of the Frog Larvae. Archiv f. Entwickelungsmech. Bd. VII. 1898.
15. Hazen, A. P., The Regeneration of a Head instead of a Tail in an Earthworm. Anat. Anz. XVI. 1899.
16. Hescheler, K., Über Regenerationsvorgänge bei Lumbriciden. Jenaische Zeitschr. Bd. 30. 1896.
17. Ischikawa, C., Trembley's Umkehrungsversuch an Hydra. Zeitschr. f. wiss. Zool. Bd. 49. 1890.

18. Joest, Ernst, Transplantationsversuche an Lumbriciden. Archiv f. Entwickelungsmechanik. Bd. V. 1897.
19. King, H. D., Regeneration in Asterias vulgaris. Archiv f. Entwickelungsmechanik. Bd. VII. 1898.
20. Lillie and Knowlton, On the Effect of Temperature on the Development of Animals. Zool. Bull. I. No. 4. 1897.
21. Loeb, J., Untersuchungen zur physiologischen Morphologie der Thiere. I. Über Heteromorphose. Würzburg 1891. II. Organbildung und Wachsthum. Würzburg 1892.
22. Loeb, J., On some Facts and Principles of Physiological Morphology. Wood's Holl Lectures. 1893.
23. Loeb, J., Über den Einfluss des Lichtes auf die Organbildung bei Thieren. Archiv f. d. ges. Physiologie. Bd. 63. 1896.
24. Marshall, W., Lebenserscheinungen der Süßwasserpolypen und eine neue Form von H. viridis. Zeitschr. f. wiss. Zool. Bd. 37. 1892.
25. Morgan, T. H., A Study of Metamerism. Quart. Journ. Mic. Sc. Vol. 37. 1895.
26. Morgan, T. H., Some Problems of Regeneration. Biological Lectures Wood's Holl. 1897—98.
27. Morgan, T. H., Regeneration in Allolobophora foetida. Archiv f. Entwickelungsmechanik. Bd. V. 1897.
28. Morgan, T. H., Experimental Studies of the Regeneration of Planaria maculata. Archiv f. Entwickelungsmech. Bd. VII. 1898.
29. Morgan, T. H., Regeneration of Tissue composed of Parts of two Species. Biol. Bull. I. 1899.
30. Morgan, T. H., A Confirmation of Spallanzani's Discovery of an Earthworm Regenerating a Tail in place of a Head. Anat. Anz. XV. 1899.
31. Peebles, F., Experimental Studies on Hydra. Archiv f. Entwickelungsmech. Bd. V. 1897.
32. Peebles, F., The Effect of Temperature on the Regeneration of Hydra. Zool. Bull. II. 1898.
33. Rand, H. W., Regeneration and Regulation in Hydra viridis. Archiv f. Entwickelungsmech. Bd. VIII. 1899.
34. Rand, H. W., The Regulation of Graft Abnormalities in Hydra. Archiv f. Entwickelungsmech. IX. 1899.
35. Trembley, A., Mémoires pour servir à l'histoire d'une Gendres de Polypes. Paris 1744.
36. Vöchting, H., Über Transplantation am Pflanzenkörper. Tübingen 1892.
37. Wetzel, G., Transplantationsversuche mit Hydra. Archiv f. mikr. Anat. Bd. 45. 1895—96.
38. Wetzel, G., Transplantationsversuche mit Hydra. Archiv f. mikr. Anat. Bd. 52. 1898.

Life.

I was born in Kentucky, June 3, 1874. I was prepared for College by private study, and by The Girl's Latin School, Baltimore. I entered the Woman's College of Baltimore in the fall of 1892, and after three years of study, received the degree of Bachelor of Arts. During the summer of 1895 I held the Woman's College Table in the Marine Biological Laboratory at Wood's Holl. In 1895—96 I held the Mary E. Garrett Scholarship in Biology at Bryn Mawr College, where I studied under Prof. Thomas Hunt Morgan, and Dr. J. W. Warren. In the summer of 1896 I occupied the Bryn Mawr room at Wood's Holl. In 1896—97 I was given the resident fellowship in Biology in Bryn Mawr College, and returned to Wood's Holl for part of the summer of 1897. I continued my studies at Bryn Mawr College until the spring of 1898 when I was appointed Mary E. Garrett European Fellow for the year 1898—99. I held the American Woman's Table in the Zoological Station at Naples from September to November, and for a part of the winter semester heard lectures and worked in the laboratory of the University of Munich. During the summer semester of 1898—99 I attented the University of Halle, where I continued my investigations in Biology.

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