THE PRODUCTION OF NEW HYDRANTHS IN HYDRA BY THE INSERTION OF SMALL GRAFTS

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WITH SIX PLATES

INTRODUCTION

During the winters of 1906–1908, I carried on some experiments in grafting Hydra viridis for the purpose of throwing more light on the factors concerned in regeneration. The work was done at the suggestion of Professor Morgan, whom I sincerely thank for his kind interest and help.

In my first series of experiments, which were done with the ordinary green hydra, I tried to discover what material when grafted would give ,the necessary stimulus to call forth the development of a new hydranth. It was found by experiment that if a tentacle with a small bit of peristome tissue at its base was inserted into the body of another hydra, the stock would regenerate a whole new hydranth at the place of grafting, the grafted tentacle remaining as one of the new circlet of tenacles. The question arose whether the regeneration was due merely to the presence of foreign tissue of any kind or whether it was initiated by some special kind of material; and if so, what kind of tissue this was. To solve this problem, tissue was taken from different regions of the body of Hydra viridis and grafted into different regions of the body of other hydras of the same species.

In my second series of experiments, I endeavored to find out the exact origin of the regenerating material. An excellent opportunity to decide this question was offered by the discovery of Mr. D. D. Whitney that the green color can be entirely removed from Hydra viridis by putting the animals in a .5 per cent glycerine solution and leaving them for about three weeks. These artificial white hydras form perfect grafts with normal green hydras and the two parts remain distinct in color.

In all my experiments, I used for operation a small watch glass coated over with paraffine on the bottom and filled with spring water. The operations were all done under a binocular microscope which has the advantage of giving considerable enlargement and plenty of working distance. After operation, the animals were kept in watch glasses filled with either spring water or aquarium water, renewed daily.

Part I

GRAFTS TO PRODUCE A NEW HYDRANTH FROM THE STOCK

The object of this series of experiments was to determine, first, what tissue when grafted into the body of a normal hydra would cause a new head to regenerate in the region of the graft; and, secondly, in what region of the stock the graft must be made in order that the new hydranth develop. To test these questions, material was taken from various regions of the body and grafted at different levels along the bodies of other hydras. All these experiments were done with Hydra viridis as both graft and stock.

Group A

Tentacle with Peristome at Base as Graft

For operation in the present group of experiments, two hydras were put into the paraffine-coated watch glass and one was cut just below the circle of tentacles. This circlet was cut in one radius so that the tentacles extended out from the peristome in a straight line or slight curve. From this line of tentacles I cut off one, being careful to leave some peristome tissue attached at its base. As quickly as possible I made a small transverse slit in the body wall of the other hydra with a small scalpel or sharpened needle, and inserted the prepared tentacle into this slit by means of a needle. If the operation was successful, the raw surfaces healed so that after a few hours there resulted a perfectly normal hydra with the exception of a tentacle projecting from some part of the body. In this way I inserted a tentacle with a small bit of peristome tissue at its base in different regions of the stock hydras with varying results.

Series I Graft Made in Middle of Stock

Result 1. The usual result following the graft of a tentacle with a bit of peristome tissue at its base into the middle region of a hydra was the outgrowth of a new hydranth from the region of graft, the grafted tentacle persisting as one of the new circlet. The result can be best shown by a specific instance.

On February 19, a tentacle with peristome tissue at its base was grafted in the middle of the body of a healthy green hydra. After a few hours, the wounded surfaces had healed and there resulted a hydra perfectly normal except for the protrusion of a tentacle from the middle of its body (Fig. 1). On the next day, a slight outpushing of the body wall around the tentacle was observed. On February 21, two days after operation, this outpushing could be distinctly recognized as a new hydranth. The new hydranth consisted of a short body protruding from the body of the stock, having at its distal end the large grafted tentacle and three very short tentacles (Fig. 2). On the following day these new tentacles were distinctly longer but not so long as the grafted tentacle. On February 23, the fourth day after operation, a small fifth tentacle had appeared and the grafted tentacle was still distinctly larger than the regenerated ones (Fig. 3). The difference in length of the tentacles was gradually lost by the further growth of the regenerated ones until on February 28, the hydra appeared as a double-headed hydra, one head bearing six tentacles and the other, the regenerated head, five; the original head was still considerably longer than the regenerated one (Fig. 4). That this new hydranth was functional and similar in all respects to the old hydranth was proved by the fact that it could capture and ingest food with as great ease as the old one. This double-headed hydra remained in the condition described without further elongation of the new hydranth until it died on March 14. Similar results have been obtained in ten other similar grafts. In some cases, however, the new hydranth grew longer than in the case described, so that it was of about the same length as the original head.

The axial relations assumed by the new head in reference to the common foot were not constant for even the same hydra at different times. The new head was sometimes at right angles to the stock hydra, it sometimes formed a right angle with the foot and a straight line with the original head, it sometimes formed an obtuse angle with the foot making a Y-shaped structure, and it was sometimes at an acute angle with the foot forming a λ-shaped figure. The new hydranth never showed any tendency to travel toward the aboral end of the stock hydra. This is of interest in connection with Miss King's experiments in grafting whole heads into the side of stock hydras. As a result of her work, she found that the graft either separated from the stock in from 14 to 22 days, or migrated toward the foot region and separated in from 5 to 7 weeks. These two modes of separation are due, she concludes from her experiments, to the "axial relations assumed by the components of the graft." If the graft remains at right angles to the trunk, separation takes place without migration; if the graft forms a Y-shaped structure with the stock, it migrates toward the aboral end before separating. The new hydranth that is formed in my experiment seems to act quite differently. It has no definite axial relations with the stock and does not migrate toward the foot. As to the final separation of the regenerated hydranth from the old stock, I can say nothing, for I have not succeeded in keeping these grafts more than a month after operation, and they have not separated within that time.

Result 2. In one case grafted February 19, after a slight outpushing of the body wall around the grafted tentacle and the formation of three additional tentacles (Fig. 5), a process of absorption set in. On the seventh day after operation, the projection of the body wall to form a new hydranth was no longer visible, the four tentacles emerging directly from the body of the stock (Fig. 6). Four days later, one of the four tentacles was absorbed, and just below the place where the tentacles were present, a small protrusion on the body wall was noticed (Fig. 7) which on the following day could be definitely determined to be a bud (Fig. 8). The other three tentacles were absorbed one by one, while the bud was developing, until on March 12, three weeks after grafting, the whole regenerated material had been absorbed and the bud had pinched off leaving the stock as a normal hydra.

The departure from the usual result may be accounted for in this case by the formation of the bud. It may be that the stimulus for regeneration was present and excited the growth of new tissue as is evidenced in the outgrowth of three new tentacles, but when the bud developed, the available material was used for its development, thus depriving the regenerating hydranth of any means of growth.

Result 3. In only two instances, no regeneration was stimulated by the graft of a tentacle with its basal tissue into the side of the stock hydra. But a gradual process of absorption set in, so that after two weeks no trace of the tentacle was left. It is possible that this result was due to the fact that none of the peristome tissue was left at the base of the tentacle, this bit of tissue being accidentally broken off from the tentacle in the process of grafting; or, the results obtained in these two instances may have been an exception to the usual result.

Series II Graft Made in Foot of Stock

Result 1. The usual result following grafting a tentacle with a small bit of peristome tissue at its base into, or in the region of, the foot of a normal hydra, was the outgrowth of a diminutive hydra at the point of grafting. The grafted tentacle was partially absorbed as regeneration took place, till it assumed the proportion proper for the small hydra. This small hydra pinched off from the stock in three or four days after grafting, sometimes with only the grafted tentacle present, sometimes with one or two regenerated ones.

Following the history of one of these grafts, we find it as follows: On March 5, I inserted a tentacle with a bit of peristome tissue at its base into the foot of a normal green hydra (Fig. 9). On the next day, there was a very slight outpushing of the body wall of the stock carrying the grafted tentacle with it. On March 7, this outpushing had increased and two new tentacles were just beginning to grow out from its distal end beside the grafted tentacle (Fig. 10). The grafted tentacle had decreased in size noticeably. This outgrowth was distinctly different from that observed when the tentacle was grafted in the middle of the stock, being much smaller in circumference. On the next day, March 8, the outgrowth could be clearly recognized as a minute hydranth with two short tentacles and one longer one, the grafted tentacle, which had become still further reduced in size by absorption (Fig. 11). In the afternoon of the same day, this small hydranth pinched off from the stock. On March 11, owing to the further absorption of the grafted tentacle and the further growth of the two regenerated tentacles, this hydra appeared as a typical hydra of very minute size, with three tentacles of about equal length arranged about the hypostome (Fig. 12). The volume of the small hydra was not more than one-tenth that of the stock from which it pinched off. In several other similar experiments, the same history followed except that the small hydra separated with only two tentacles present, the grafted one (reduced in size) and one regenerated one. In these cases, a third tentacle usually appeared after the hydra had pinched off. In still other similar experiments, there were no new regenerated tentacles formed, but the small hydra pinched off with only one tentacle, the grafted one (Fig. 13). One or two tentacles were formed, however, after the small hydra had separated.

Result 2. In two out of about twenty grafts in the foot region, the tentacle grafted was slowly absorbed.

Result 3. In two other cases, abnormal hydras resulted from the graft of a tentacle in the foot region. An outgrowth from the foot carried the grafted tentacle along with it, as though to form a new hydranth (Fig. 14). But the tentacle was absorbed while the outgrowth enlarged, and no separation took place before the end of two weeks when these abnormal hydras died (Fig. 15).

Series III Graft Made in Circlet of Tentacles of Stock

The insertion of a tentacle into the circlet of tentacles of a normal hydra in no instance induced the formation of a new hydranth. The tentacle either remained as grafted or instigated the outgrowth of one or two additional tentacles. In one case two new tentacles grew out two days after grafting a tentacle into the circlet, making ten tentacles in all (Fig. 16). A week later, three of these became fused at the base (Fig. 17). The process of fusion spread until the three tentacles formed a single tentacle, leaving eight in the circlet, one more than the original number. Unless, however, as in this case, an abnormally large number of tentacles was present, no later absorption took place after regeneration.

Series IV Graft Made Below Circlet and Above Middle of Stock

Result 1. In the majority of cases, this graft instigated the outgrowth of a new hydranth. The history of these cases is similar to that which followed the graft of a tentacle in the middle of the body. A double-headed hydranth resulted from the graft, but the two heads were considerably shorter in proportion to the body than in the case of the middle-region grafts (Fig. 18).

Result 2. In one case, after a slight outpushing of the body wall and the regeneration of two new tentacles (Fig. 19), this new hydranth coalesced with the old hydranth a week after grafting (Figs. 20, 21). There thus resulted a single-headed hydra with an extra number of tentacles.

Result 3. In another case, no new tentacles were regenerated, but the grafted tentacle was included in the old hydranth by the shifting downward of the other tentacles (Fig. 22).

Result 4. In one case, the grafted tentacle was absorbed slowly so that after two weeks, no trace of the graft was left.

Series V Graft Made Between Foot and Middle of Stock

Result 1. In most cases, this graft followed the same history as a foot-region graft. A minute hydra developed from the body wall of the stock, and pinched off in three or four days after grafting, when possessing only one or two tentacles (Fig. 23).

Result 2. In several cases this graft gave rise to a new hydranth similar to that which is found in the middle region.

Result 3. In one case, the grafted tentacle itself became a small new hydranth by enlarging; and on this former tentacle small tentacles grew out (Figs. 24, 25). By further increase in size and the formation of additional tentacles, there resulted a double-headed hydra, one of whose heads was somewhat longer and larger than the other (Fig. 26).

Result 4. In one case, the grafted tentacle was slowly absorbed without any regeneration.

From the foregoing group of experiments (Group A), it is evident that in every region of the body, the graft of a tentacle with a bit of peristome tissue at its base may cause regeneration on the part of the stock. Moreover, the regeneration in every region except in the circlet of tentacles takes the form of a new hydranth, of normal size in the anterior and middle regions of the stock, and of minute size in the posterior and foot regions.

Group B

Two Tentacles with Peristome at Base as Graft in Opposite Sides of Stock

In this set of experiments, I endeavored to find out whether a second tentacle with peristome tissue at its base inserted soon after the first in the same region of the body on the opposite side would cause a second new hydranth to regenerate. In some cases the second tentacle was inserted about two hours after the first, and in others it was inserted a day later, but both methods gave practically the same results.

Result 1. In two out of six cases, both grafted tentacles caused the outgrowth of hydranths. One of these was grafted on March 20 (Fig. 27); two days later, there was a slight outpushing of the body wall of the stock at the base of each tentacle, a new tentacle having developed on one of the outgrowths (Fig. 28). On March 25, the hydra had two quite well developed but small hydranths in the middle region, each having two tentacles, a grafted and a regenerated one (Fig. 29). The smaller one of these was gradually absorbed, till on March 30, no trace of it was left, the hydra having only two heads, one with seven tentacles and the other, the regenerated one having four tentacles, one longer grafted one and three shorter regenerated ones (Fig. 30). By April 1, these four tentacles were of the same length, and by April 5, a fifth tentacle had appeared, so that the hydra was a typical double-headed animal (Fig. 31). In the other similar graft, after the outgrowth of two small hydranths at the base of each grafted tentacle, they migrated from opposite sides so as to be adjacent (Fig. 32). A process of fusion then set in, so that ten days after grafting, the two regenerated hydranths, each with three tentacles, were separate only at their distal ends (Fig. 33). This fusion was complete two days later, resulting in the formation of a double-headed hydra.

Result 2. In two other cases of similar grafts, only one of the grafted tentacles gave rise to a new hydranth, the other one being gradually absorbed (Fig. 34).

Result 3. In the other two cases, both tentacles were absorbed without any regeneration.

From this group of experiments it is evident that each of two tentacles with peristome tissue at their base, when grafted into the middle region of a hydra, may cause the regeneration of a new hydranth. However, a process of fusion or absorption sets in sooner or later, so that only one of the regenerated hydranths remains.

Group C

Tentacle Without Peristome at Base as Graft

Having obtained so definite a response on the part of the stock to a grafted tentacle, I next tried to find out what part of the grafted tentacle was responsible for the regeneration. In this group of experiments I grafted just the tentacle without any peristome tissue at its base into the stock. This operation was somewhat difficult as the raw surface of the tentacle healed over rapidly and would not then adhere to the cut surface of the stock. About eight times, however, by performing the operation very quickly, I was successful. All the experiments gave the same result, whether the graft was made in the middle or foot region of the stock. There was no outpushing of the body wall of the stock, no regeneration whatever, but, on the contrary, the grafted tentacle was slowly absorbed so that after about ten days no trace whatever of the graft was left.

These experiments show that the graft of just the tentacle without peristome tissue at its base does *not* stimulate the regeneration of a hydranth.

Group D

Peristome at Base of Tentacle Without Tentacle as Graft

Methods

In one set of these grafts, I cut off a tentacle with the peristome tissue at its base and inserted it into the body of the stock as described under Group A. Then after the raw surfaces of the stock and graft had healed, I cut off the grafted tentacle close to the body wall, thus leaving grafted in the stock the bit of peristome tissue that was at the base of the tentacle. In another set, I cut off a circlet of tentacles, then cut off a few tentacles at their base, close to the circlet, and used a small piece of the remaining ring of peristome tissue. This I grafted quickly into a previously prepared cut in the body wall of the stock. Both methods gave the same result.

Series I Graft Made in Middle of Stock

Result 1. In three out of five cases tried, the graft in the middle region of the body gave rise to a new hydranth. The outgrowth was similar to that initiated by the graft of a whole tentacle and peristome tissue at its base. The body wall of the stock at the region of graft pushed out and new tentacles were formed on it (Figs. 35–37).

Result 2. In two other similar experiments, absorption took place and no regeneration occurred.

Series II Graft Made in Foot Region of Stock

Result 1. In two out of three cases in which the peristome tissue at the base of the tentacle was grafted into the foot region of the stock, there grew out a minute hydra, similar in formation and appearance to that instigated by a graft of a tentacle and basal tissue into the foot (Figs. 38, 39).

Result 2. In the other case, the tissue was absorbed and no regeneration took place.

CONCLUSION

This group of experiments shows that the whole tentacle is not necessary for the production of a new hydranth on the part of the stock. But merely the peristome tissue at the base of the tentacle is sufficient, when grafted, to instigate the outgrowth of a new hydranth.

Group E

Tissue Anterior to Circlet of Tentacles as Graft

The amount of tissue anterior to the circlet of tentacles is very small and it was found very difficult to cut it off without getting into the region of the tentacles. By waiting, however, until a large hydra was fully expanded and very quickly bringing the scalpel just anterior to the tentacles, I found it possible in five cases to get this small bit of tissue cut off. This was grafted into the middle region of the stock.

In none of the five cases was any regeneration instigated. The grafted tissue was absorbed, so that the day after grafting no trace of the graft was to be seen.

The tissue anterior to the circlet of tentacles will *not* give the stimulus necessary for the outgrowth of a new hydranth, when grafted into the body wall of a stock hydra.

Group F

Tissue From other Regions of the Body as Graft

In this set of experiments, I endeavored to find out whether tissue from any region of the body other than at the base of the tentacle, would, when grafted, give rise to a new hydranth. For this purpose, I cut off a small ring of tissue from various regions of the body, including the region just beneath the circlet of tentacles and grafted it into the middle region of a hydra. These rings in no case gave rise to a new hydranth, but were soon absorbed.

If, however, instead of a small ring of tissue, a large ring was grafted, the result was different. The experiments were done in the following manner: A hydra was cut in two beneath the circlet of tentacles; the foot was then cut off from the lower part. The aboral end of this band of tissue was then grafted into the side of a normal hydra. On the following day, the graft had developed tentacles. In this case, however, it must be noted that the regeneration is entirely on the part of the graft and not of the stock, and the stock takes no part in the formation of new tissue.

The conclusions drawn from these experiments is that no other tissue than that at the base of the tentacles is capable of so stimulating the stock as to cause it to produce from its body wall a new hydranth.

Group G

Regenerating Tissue as Graft

This series of experiments was undertaken for the purpose of finding out whether tissue which has begun, in the process of regeneration, to be differentiated into tentacle-forming material, would, when grafted, influence the body wall of the stock to regenerate a new hydranth. The method adopted was as follows: A green hydra was cut in two at about the middle of the body. The posterior half was then left till the following day when the wound had healed and the process of regenerating a new hydranth had started, although no tentacles had formed. A very small piece was cut off from the oral surface, and this was grafted into the side of a normal hydra. On the next day an outpushing of the body wall had occurred and a day later two tentacles had formed on the new hydranth (Figs. 40, 41). By leaving the regenerating piece different lengths of time before grafting part of it into the stock, it was found that about ten hours was the minimum that would give regeneration. If left only seven hours before grafting, the graft was absorbed and no regeneration took place. The length of time necessary for regeneration to be far enough along for a piece of the regenerating tissue to call forth a hydranth from the stock when grafted, would of course depend on the rate of regeneration from an exposed surface, and this has been found to vary in different regions of the body. If, therefore, the original cut were made just below the tentacles instead of in the middle of the body, less than ten hours would probably suffice for the regenerating tissue when grafted to call forth a new hydranth from the stock.

Group H

Tissue of Bud as Graft

In this experiment, a small piece of the anterior end of a young bud whose tentacles had not yet been formed, was cut off and grafted into the middle of the body of a stock hydra. There followed as a result of the graft an outgrowth of a new hydranth similar to that instigated by the insertion of regenerating tissue into the stock (Figs. 40, 41). That this outgrowth was a new hydranth and not an ordinary bud is shown by the length of its tentacles. These were long like those of a hydranth and not short like those of a bud (cf. Figs. 41 and 8.)

General Conclusions to Part I

The conclusions to be drawn from the whole set of experiments are as follows. A new hydranth can be formed by a hydra in any part of its body except in the tentacle region, when the necessary stimulation is given by a grafted piece. The stimulus can be given by no other grafted piece than the material that lies at the base of the free tentacles, or that lies in a regenerating hydranth or a bud. The transformation of body wall material into hydranth material depends therefore not on the size of the piece grafted into it, but entirely on the differentiation of the grafted material. If the material grafted has been entirely differentiated into material lying at the base of a tentacle, or if it has been sufficiently differentated by regeneration or budding into this kind of material, this tissue will cause the body wall tissue of a normal hydra to change its differentiation and function and become tentacle and hypostome tissue.

Part II

ORIGIN OF REGENERATING TISSUE AND FATE OF ABSORBED TISSUE

In the second series of experiments, I tried to find out the source of the regenerating material and the fate of absorbed material in the foregoing and other experiments. Attempts have been made to solve these questions in some cases by grafting Hydra fusca and Hydra viridis, a brown and a green hydra, but these attempts have proved unsuccessful, for the two species do not graft well. Although the graft has been made to stick for a day or so, it always pulls away from the stock before any results can be obtained. Miss King has attempted to solve the question in her experiments by using light and dark green individuals of Hydra viridis. She states that she is able to distinguish the two shades for two or three weeks, at the end of which time they fuse. In combinations between the artificial white hydras produced by Whitney's method and the normal green one the contrast between the tissue of the stock and that of the graft is very distinct and remains so for about a month.

Group A

Regeneration of Hydranth

In order to determine the exact source of the material forming the new hydranth in the preceding set of experiments (Part I), the following experiments were done.

Series I White Tentacle with Base Grafted in Middle of Green Hydra

The result of this graft was in six cases the outgrowth of green tissue from the stock carrying the white tentacle with it, and the later regeneration of green tentacles (Fig. 42). The new hydranth material, then, must come from the body wall of the stock, while the grafted tentacle remains as one of the new circlet.

Series II White Tentacle with Base Grafted in Foot of Green Hydra

The result of this graft was in four cases the outgrowth of a small amount of green tissue at the base of the white tentacle to form a minute hydranth (Fig. 43). This pinched off as a small green hydra with one white tentacle (Fig. 49). Four days after operation a second tentacle appeared on it, composed of green tissue; some of the white material of the grafted tentacle seemed to have been absorbed into the anterior part of its body (Fig. 50).

From these experiments the conclusion must be drawn that it is principally the material of the body wall of the stock and not the hydranth material of the graft that forms the new hydranth. The ectoderm and endoderm cells that have been body wall cells are therefore changed over into cells composing tentacles and hypostome.

Group B

Absorption

In order to find out the fate of the material grafted into the stock hydra when no regeneration took place; whether the material was incorporated into the tissue of the stock hydra, or whether it was so absorbed that it no longer existed as such, the following experiments were performed.

Series I Green Tentacle Without Base Grafted into White Hydra

As the green tentacle was absorbed, the green material spread along the body wall of the white hydra for a small area at the union of stock and graft (Figs. 44, 51). The two hydras on which the experiment was performed were unfortunately lost before complete absorption. But it was evident that the green tentacle material was being transformed into body wall material and incorporated in the stock.

Series II Green Circlet in White Hydra

After the graft of a green circlet of tissue from a normal hydra into the middle region of the body of a white hydra, this tissue remained in the white body as a patch of green (Fig. 45).

We conclude then, that when a piece of tissue is grafted into a normal hydra and does not cause regeneration, the grafted tissue is incorporated into the body wall of the stock. The cells that have formed tentacle or body wall tissue are made over in the stock into body wall cells. The tissue is absorbed, not in the sense that it disappears, but in the sense that it becomes one with the tissue of the stock.

Group C

Grafts of White and Green Hydranths

A few experiments were performed with green and white hydras to discover if possible whether a hydranth that was grafted in the side of another hydra kept its individuality or whether the tissues of the two hydras fused. In one of the two successful grafts in which a short green head was grafted into a white hydra at about the middle region, the graft retained its individuality and was of approximately the same size and in about the same position at the end of two weeks as at the time of graft (Fig. 52). As the hydra did not live until the graft pinched off, the final result was undecided, but it seemed probable that it would pinch off at the line of union of graft and stock. The second graft was more interesting for the reason that the green grafted head increased in size until of equal length with the head of the stock and then migrated down toward the foot end of the white hydra (Fig. 46). The point to be noted is that in increase in size, the new material came not from the larger white hydra but was formed by the green hydranth. The graft not only kept its individuality but also completed itself by regenerating new material. A third experiment of somewhat different kind shows the same principle. A white and green head were grafted together by their aboral ends, the green one being somewhat shorter than the white one (Fig. 53). Both hydranths kept their individuality and the green one regenerated new tissue so as to become of equal length with the white one (Fig. 54). In this condition, the graft died, evidently just before separation would have taken place.

From these experiments we conclude that the grafted hydranth, although intimately associated with the stock, keeps its individuality. This conclusion agrees with that of Miss King who, in similar experiments used hydras of different shades of green as stock and graft.

Group D

Graft of Green Foot in White Hydra

In four out of five cases in which the lower half of a hydra was grafted by its cut oral surface into the middle region of a normal hydra, the grafted foot was absorbed. The history of the other case was as follows: On February 22, a green hydra was cut in two, and the posterior half was grafted by its oral surface into the middle of the body of a white hydra. On February 25, the graft had moved down somewhat toward the foot of the stock (Fig. 55). On February 27 a new head was evidently being formed at the union of graft and stock, of graft material (Fig. 56). On February 28 the graft had quite completed itself, having formed several tentacles, but was still attached near the foot region to the stock (Fig. 47). On February 29, the green hydranth including part of the grafted foot and the regenerated head separated off from the white hydra, leaving, however, a small amount of green foot tissue attached to the stock (Fig. 57).

From these experiments I should conclude that if the graft asserts itself sufficiently not to be absorbed by the stock, it maintains its individuality, forms its own tentacles and separates off as a complete hydra.

Group E

Reversed Polarity in Green and White Grafts

Cases of heteromorphosis in hydra produced by grafting have been reported by Wetzel, Peebles, and King. A heteromorphic foot has been produced by Wetzel by removing the foot ends of the two hydras, grafting the two aboral ends together and cutting off one head close to the tentacles. A normal hydra was produced with a foot at the former oral end. Peebles performed the reverse experiment, cutting off the heads of two hydras, grafting them together by their oral surfaces and subsequently cutting off one foot close to the line of union. Heteromorphic heads were produced in five cases on the exposed aboral surface. King succeeded in obtaining heteromorphic structures by cutting off both ends of a head-to-head or a foot-to-foot graft close to the line of union, leaving a ring of tissue with two aboral or two oral ends exposed. In most cases, she found that normal hydras resulted, one end having reversed its polarity so that a head was produced from the exposed aboral surface or a foot from the exposed oral surface. It has never been definitely shown whether these heteromorphic structures are really formed from material whose polarity has been reversed through the influence of the complementary structure or whether the material has rearranged itself so that polarity is not really reversed.

That such a rearrangement is possible is shown by an experiment in which I cut a hydra longitudinally and reversed the two halves so that each free end consisted of half foot and half head with tentacles (Fig. 58). Two days later it was evident that the foot material of each end was migrating from its position near the tentacles to the middle of the body (Fig. 59). That this structure was produced by a migration of the foot material and not merely by a split along the line of graft, separating the original half head and half foot, is shown by the fact that the free foot was not of equal length with the hydranth, but was only a small projection from the surface. The final result of this graft was the separation of the two original half-hydras into two complete hydras (Figs. 60, 61). In this case, then, there has been a migration and rearrangement of material. Another instance of migration is the experiment described in Group C, where the grafted hydranth moved down along the stock (Fig. 46) from the middle to the foot region. Many similar cases of migration have been described by King and Rand and Hefferan.

Is it not possible that in the case of the supposed heteromorphic heads, the aboral material of the graft has wandered in, leaving exposed the oral material of either stock or graft, so that the head really develops not from an aboral layer of tissue but from the oral layer?

The answer to this question has been definitely determined, I think, by the following experiments: A white and a green hydra were both cut a little beneath the tentacles and the two cut oral surfaces grafted together. A few hours later, after the graft had become secure, the green portion was cut off leaving a circle of green tissue with aboral end exposed and attached by its oral end to the oral surface of the headless white hydra (Fig. 62). In three cases, tentacles appeared at the free aboral end and these tentacles were formed of green material, the oral material of the white stock having no part in their composition (Fig. 63). In a few other cases, one or two of the tentacles were formed of white material, the rest of green (Fig. 64). Whether this result was due to a somewhat oblique cut, so that the circle of green was minimal in one place, or whether the material rearranged itself, some of the green migrating posteriorly and the white anteriorly was not determined. In the former cases, where all the tentacles were green, there was no migration of the white oral material. These experiments did not show, however, that there might not have been a migration of material in the green tissue itself, the oral material going anteriorly and the aboral posteriorly. In one experiment, it was conclusively shown that such migration did *not* take place. In this case tentacles formed not only on the exposed green surface, but also at the union of graft and stock (Fig. 48). Moreover, both sets of tentacles were composed entirely of green material. One set must therefore have come from the oral material which still remained at the junction of graft and stock, and the other set from the aboral material which lay at the exposed surface. This was an undoubted case of heteromorphosis in the strictest sense of the word, and throws some light on the meaning of reversal of polarity in connection with heteromorphic structures. We find in this experiment, that although polarity has been reversed in so far as normal foot-producing material becomes head-forming material under the influence of the larger piece, the original polarity which determined that the head-forming material should be head-forming has not been lost. It would seem that the original polarity of the reversed piece is not altered, but that on account of the relation of the graft to the stock, a secondary polarity has been assumed. This secondary polarity is that of the stock which asserts itself in the new material which now becomes a part of its own body. If this secondary polarity be conceived as preponderating over the primary polarity of the graft, this original polarity would be entirely submerged, and this is probably the case in the former experiments where only heteromorphic tentacles were formed. On the other hand if the primary polarity preponderates over the secondary we should have tentacles at the junction of graft and stock and no heteromorphic structure. Many cases of this result have been reported by Peebles and King.

In three cases I succeeded in obtaining heteromorphic feet by grafting the two cut aboral ends of a white and a green hydra and then cutting off the head of the green end leaving an exposed oral surface of green tissue (Fig. 65). This graft resulted in the formation of a normal hydra consisting of a head end of white material and a foot end of green material (Fig. 66). The foot end consisted of a true foot as evidenced by the presence of the characteristic sticky secretion which made the foot adhere to the substratum. These were evidently cases of heteromorphosis, the polarity of the stock so influencing the graft as to preponderate over its original polarity, thus calling forth a foot from its exposed oral surface. The production of heteromorphic feet in these experiments confirms the like experiment of Wetzel and is opposed to the results of Peebles who obtained no heteromorphic foot in the fifteen cases tried. No hook-like processes such as described by Wetzel as preceding the formation of a heteromorphic foot appeared in my experiments.

CONCLUSIONS

As a result of the experiments recorded in this paper, the following conclusions may be drawn:

1. The fate of a graft in Hydra viridis depends on several factors. Rand states that "the fate of the graft depends upon its degree of specialization." King states that "the fate of a graft depends, not upon its degree of specialization, but primarily on its size and to some extent on its position in the stock." From my experiments, I should conclude that the fate of a graft depends (1) primarily on its specialization. If the tissue grafted is a small

amount of body wall tissue, or pure tentacle tissue, or pure hypostome tissue (tissue anterior to the tentacles) or a small amount of foot tissue, it is absorbed. If it is tissue lying at the base of the tentacle, whether it includes a tentacle or not, it becomes part of a new hydranth which under its stimulation grows out from the stock. The fate of a graft depends (2) on the size of the piece grafted. A large piece of body wall tissue with the oral end exposed in a lateral graft gives rise to a new hydranth, a small piece is absorbed. A large piece from the foot may produce a new hydranth when grafted laterally, a small piece is absorbed. The fate of a graft depends (3) on the position it occupies in the stock. If in the circlet of tentacles, a new hydranth is not produced by the graft of a tentacle with peristome tissue at its base, but in any other region of the body a new hydranth may be produced. In the foot region this hydranth is very minute; in the middle region it is of normal size. The fate of a graft depends (4) on its polarity. If a band of tissue is grafted by its *aboral* end to the oral surface of a half-hydra, leaving the oral surface of the graft exposed, a normal hydra is produced, the tentacles growing on the exposed oral surface. If a band of tissue is reversed and grafted by its *oral* end to the oral surface of a half-hydra, leaving exposed the aboral surface of the graft, unless sufficiently small, tentacles grow out at the line of union of the two components, and not at the free end.

2. No matter how specialized a tissue has become, it can, when grafted, be made over into a different kind of tissue and be incorporated into the body of the stock. For example, the material that had become differentiated into tentacle tissue can lose its differentiation and become body wall tissue and function as such. Likewise, differentiated tissue of the stock can, under the influence of special grafts, be made over into other kinds of differentiated tissue. For example, the foot tissue can, under the influence of a grafted tentacle with basal tissue attached, be transformed into the body wall and tentacle tissue of a new hydranth.

3. A grafted hydranth and a grafted foot, when not absorbed, keep their individuality and do not become one with the stock.

4. A new hydranth can be stimulated to grow out from a hydra by (1) the graft of the peristome tissue at the base of the tentacle, with

or without the tentacle itself, and by (2) the graft of the material of a regenerating hydranth and by (3) the graft of the material of a bud. Neither a wound nor the graft of any other kind of tissue will stimulate the stock to send out a new hydranth.

5. A reversal of polarity may take place in a graft, resulting in the production of a heteromorphic structure, if the polarity of the stock preponderates over that of the graft.

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Plate I

Figs. 1–5 Graft of tentacle with peristome tissue in middle of stock.

Figs. 6–8 Absorption of new hydranth.

Figs. 9–13 Graft of tentacle with peristome tissue in foot of stock.

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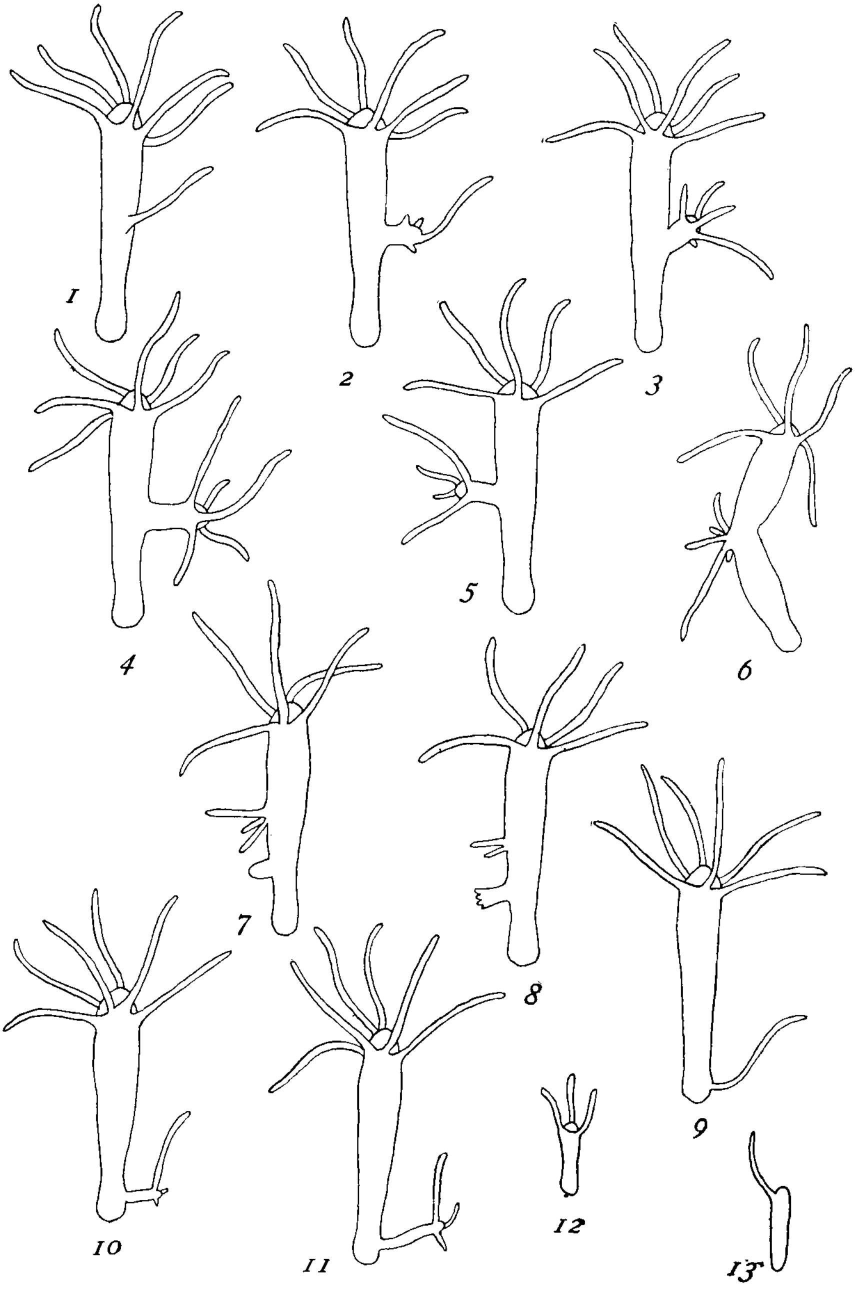

Plate II

Figs. 14–15 Abnormal hydra produced by foot-region graft.

Figs. 16–17 Graft of tentacle with peristome tissue in circlet of tentacles.

Fig. 18 Graft of tentacle with peristome tissue below circlet and above middle.

Figs. 19–21 Fusion of old and new hydranths.

Fig. 22 Grafted tentacle included in old circlet.

Fig. 23 Graft of tentacle with peristome tissue between foot and middle.

Figs. 24—26 Transformation of grafted tentacle into new hydranth.

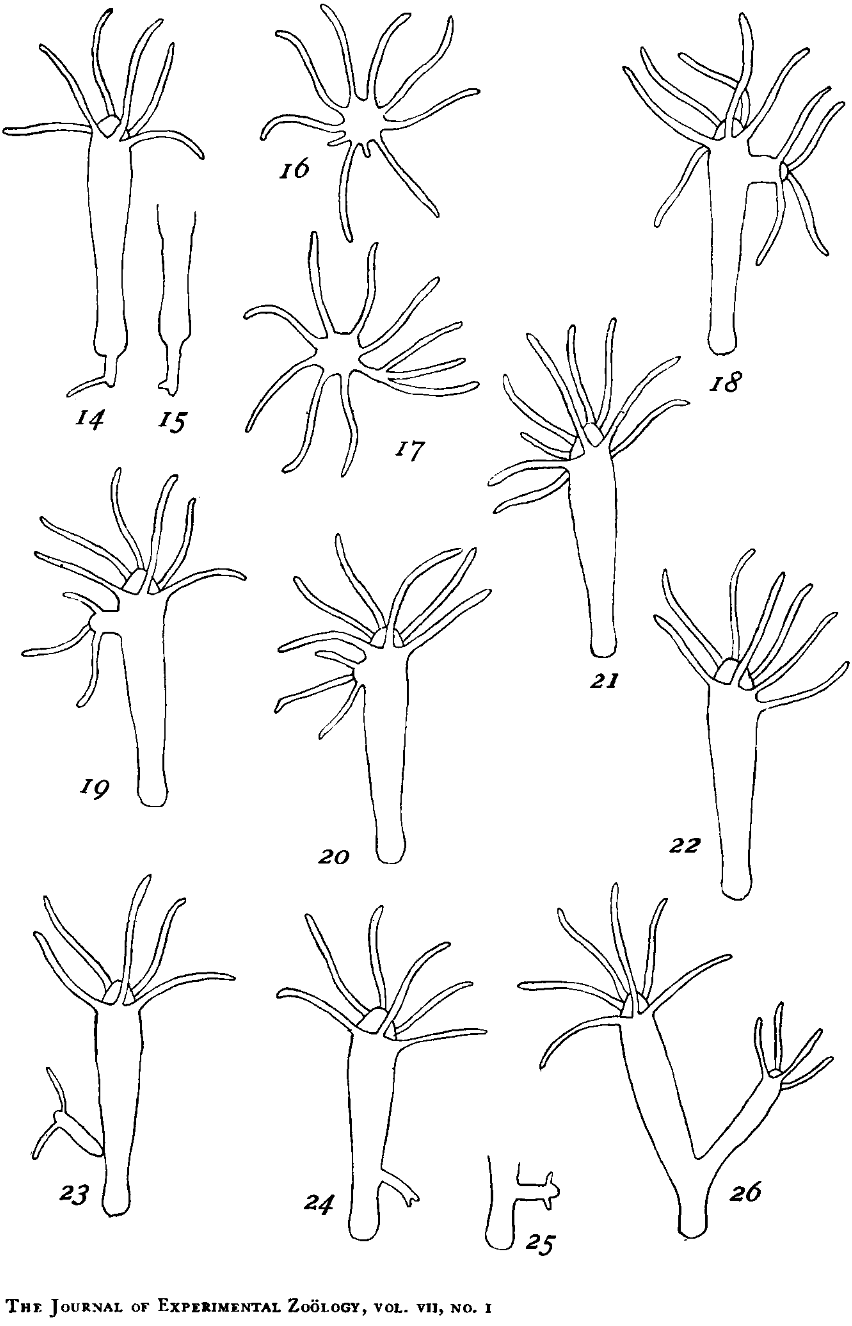
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Plate III

Figs. 27–29 Graft of two tentacles with peristome tissue in opposite sides of stock.

Figs. 30–31 Absorption of one new hydranth, and growth of other.

Figs. 32–33 Migration and fusion of two new hydranths.

Fig. 34 Absorption of one grafted tentacle.

Figs. 35–37 Graft of peristome tissue without tentacle in middle region.

Figs. 38–39 Graft of peristome tissue in foot.

PLATE III

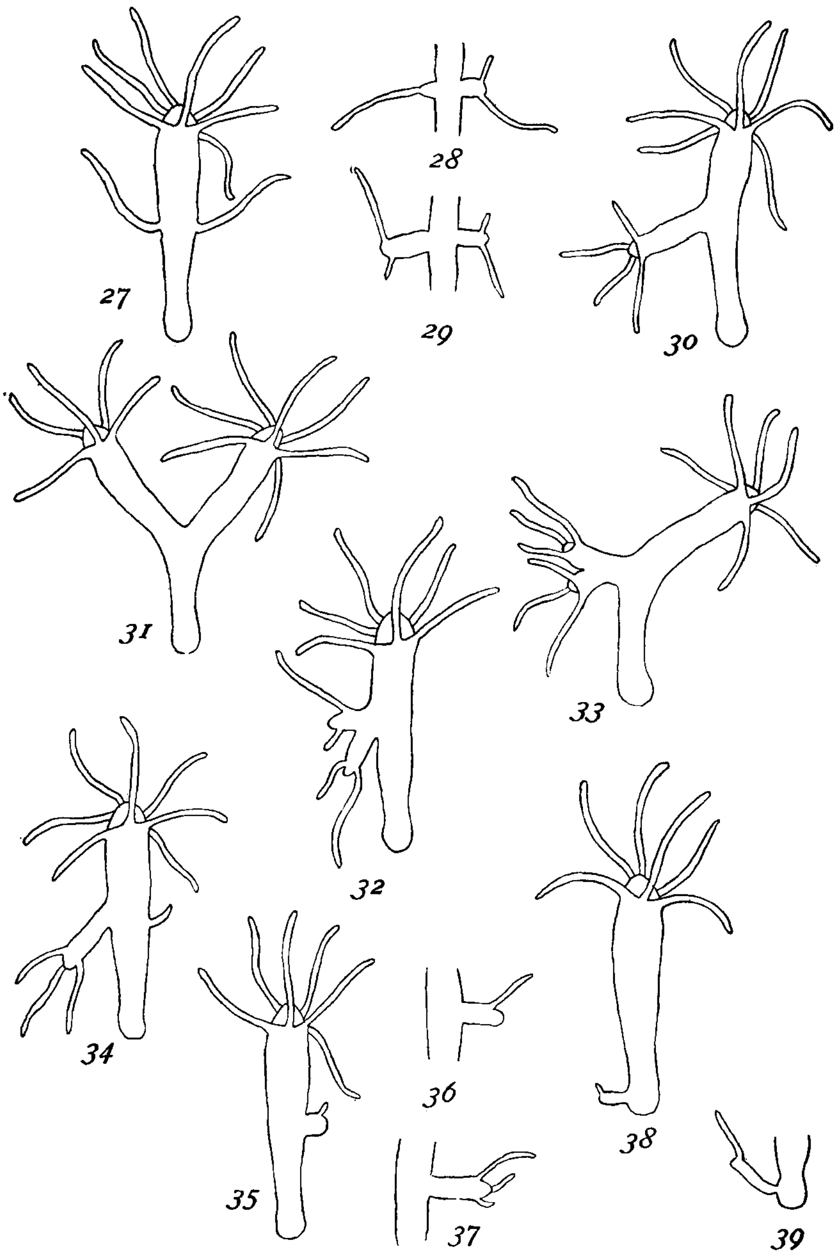

Plate IV

Figs. 40–41. Graft of regenerating tissue.

Figs. 49–50 Small hydra produced by graft of white tentacle in foot of green hydra.

Fig. 51 Graft of green tentacle without peristome in white hydra.

Fig. 52 Graft of green hydranth in white hydra.

Figs. 53–54 Graft of green and white heads.

Figs. 55–57 Graft of green foot in white hydra.

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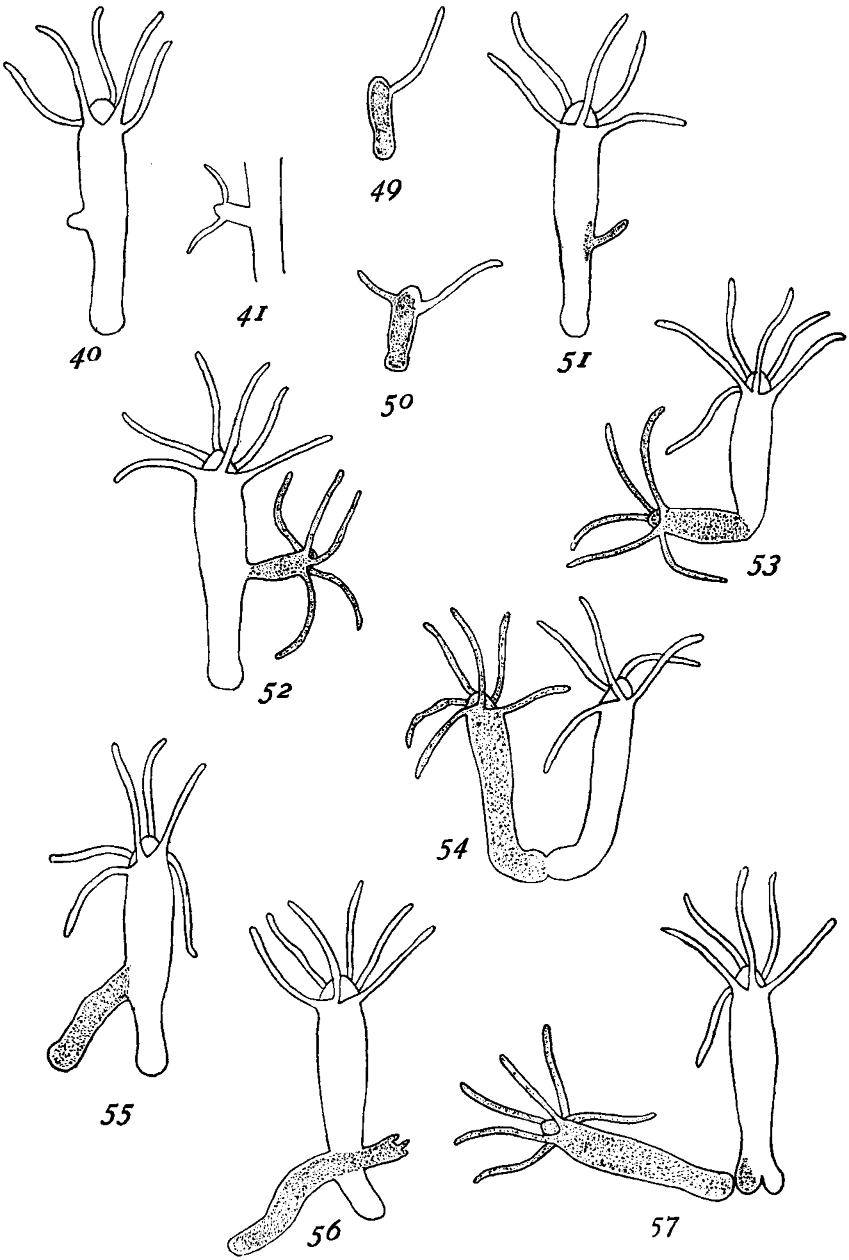

Plate V

Fig. 42 Graft of white tentacle with peristome in middle of green hydra.
Fig. 43 Graft of white tentacle with peristome in foot of green hydra.
Fig. 44 Graft of green tentacle without peristome in white hydra.
Fig. 45 Graft of green body tissue in white hydra.
Fig. 46 Graft of green hydranth in white hydra.
Fig. 47 Graft of green foot in white hydra.
Fig. 48 Heteromorphosis in reversed ring of green tissue grafted on white stock.

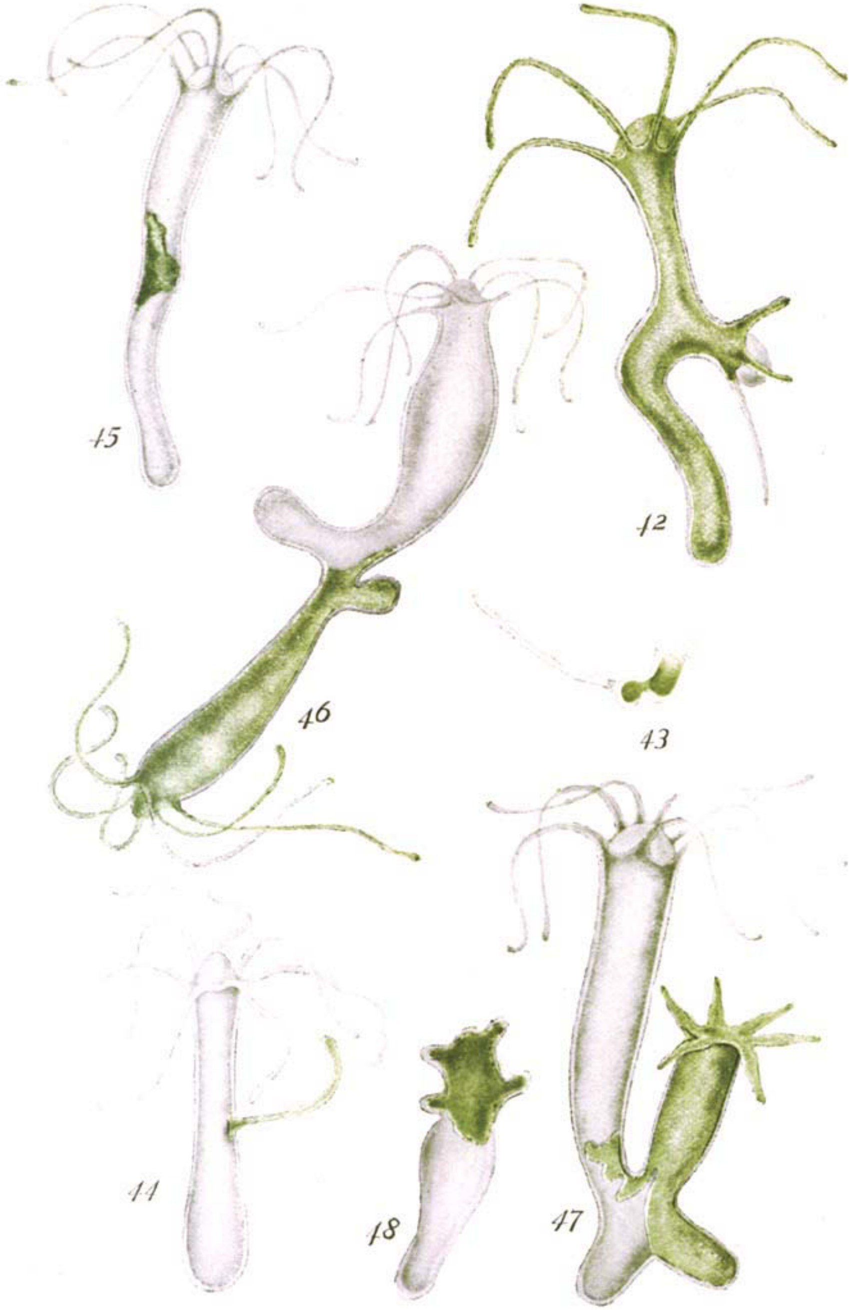

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Plate VI

Figs. 58–61 Graft of reversed halves of a longitudinally split hydra.

Figs. 62–64 Heteromorphic heads in green and white graft.

Figs. 65–66 Heteromorphic foot in green and white graft.

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