REGENERATION IN PLANARIA DOROTOCEPHALA

by

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Introduction

The word "regeneration" has come to mean, in general usage, not only the replacement of a lost part, but also the development of a new, whole organism, or even a part of an organism. There are certain normal changes that occur in animals that are not the result of injury to the organism, and these have many points in common with the process of regeneration. They are generally spoken of as processes of physiological regeneration. The annual moulting of the feathers of birds, the periodic loss and growth of the horns of stags, the breaking down of cells in different parts of the body after they have been active for a time are examples of physiological regeneration. This group of phenomena must also be included under the term "regeneration" since it is not sharply separated from that including those cases of regeneration after injury, or loss of a part, and both processes appear to involve the same factors.

I must confess an interest in the properties of regeneration ever since my college days in General Zoology when we experimented with planaria and its powers of regeneration.

Acknowledgments

This work was done under the direction of Dr. H. H. Lane whose advice and criticism have been invaluable and who has loaned me valuable material and literature. I am also indebted to the late Dr. George T. McNair for suggestions and encouragement, and to my sister Sarah for help with the checking of the data and the plotting of the graphs.

Object of this Work

The object of this problem was to study the nature of regeneration and consider factors influencing the rate of regeneration as observed in the fresh-water flatworm, planaria.

The work was begun in early October of 1930 and being unfamiliar with the local field around Lawrence, Kansas, material was furnished by the laboratory instead of being collected in nearby ponds. Points distinctly in favor of the choosing of planaria were hardiness, adaptability to laboratory conditions, resistance to the shock of an operation, and rapidity of regeneration. On the other hand, the smallness of size, irritability, quickness of movement and food caused some difficulties in the handling and caring for the worms.

The effort was made to select one hundred planaria of equal medium size, but their activity was so great and

and their length changed so quickly that the results showed considerable variation. Great care was taken to make exact transverse cuts at the level decided upon, but here again activity interfered and there was a considerable percentage of errors in cutting. For operation each flatworm was placed on a paraffin block and a quick even cut was made with a sharp, thin scalpel, as soon as the worm became as quiet as possible. The anterior parts were measured and placed in tap water in a syracuse watch glass and properly labeled. Likewise the posterior parts were measured, labeled and placed in watch glasses filled with tap water. A control series was kept for each experiment. Measurements were made every second or third day and data charted. The worms were fed just enough raw liver to keep them in good condition without much growth.

Literature

Although a few cases of regeneration were spoken of by Aristotle and by Pliny, the phenomena of regeneration first attracted general attention through the remarkable observations and experiments of the Abbe Trembley, made in 1740. His interest was drawn to certain fresh-water polyps, Hydras, that were new to him, and in order to find out if the organisms were plants of animals he tried the effect of cutting them into pieces; for it was generally known that pieces of a plant made a new plant, but if an animal were cut into pieces, the pieces died. Trembley found that if the polyp were cut in two, it produced two polyps. Logically, he should have concluded that the new form was a plant, but from other observations, as to its method of feeding and of movement, Trembley concluded that the polyp was an animal, and that the property of developing a new organism from a part must belong to animals as well as to plants.

Trembley's first experiments were made in 1740, and the remarkable results were communicated by letter to several other naturalists. It came about that before Trembley's Memoirs had appeared, in 1744, his results were generally known, and several other observers had repeated his experiments, and extended them to other forms, and had even published an account of their own experiments, recognizing Trembley, however, as the first discoverer. Thus Reaumur, in 1742, described a number of other forms in which regeneration takes place; and Bonnet, in 1745, also described some experiments that he had made during the four preceding years. Widespread interest was aroused by these results, and many different kinds of animals were experimented with to test their power of regeneration. Most important of these new discoveries were those of Spallanzani, who published a short preliminary statement of his results, in 1768, in his "Prodromo".

Trembley found that when a hydra is cut in two, the time required for the development of the new individuals is less during warm than during cold weather. He also found that if a hydra is cut into three or four parts, each part produces a new individual. If these new hydras are fed, they grow to full size, and are then again cut into pieces, each piece will produce a new individual, or polyp. The new animals were kept in some cases for two years, and behaved in all respects as do ordinary polyps. Trembley also found that if the anterior, or head-end, with its tentacles is cut off, it also will make a new animal. If a hydra is cut lengthwise into two parts, the edges roll in and meet, and in an hour, or less, the characteristic form may be again assumed. New arms may appear later on the new individual. If a hydra is split lengthwise into four pieces, each piece will also produce a new polyp. If the head-end only of a hydra is split in two, each half becomes a new head, and a two-headed hydra results. If each of the new heads is split again, a four-headed hydra is produced, and if each of the four heads is once more split in two, and eight-headed hydra is formed. Each head behaves as a separate individual, and all remain united on the same stalk. If the foot-end of a hydra is split, a form with two feet is produced.

Réaumur repeated Trembley's experiment of cutting a hydra into pieces, and obtained the same results. He found also that certain fresh-water worms, as well as the terrestrial earthworm, regenerated when cut into pieces. Reaumur pointed out that regeneration is more likely to occur in fragile forms which are more exposed to injury. At the instigation of Réaumur, Guettard and Gérard de Villars examined the starfish, and some marine polyps, and they concluded that these forms also could regenerate.

Bonnet's experiments were made on several kinds of fresh-water worms, one of which was the annelid lumbriculus. His first experiments, in 1741, showed that when the worm is cut in two pieces a new tail develops at the posterior end of the anterior piece, and a new head at the anterior end of the posterior piece. He found that if a worm is cut into three, four, eight, ten, or even fourteen pieces, each piece produces a new worm; a new head appearing on the anterior end of each piece, and a new tail on the posterior end. The growth of the new head is limited in all cases to the formation of a few segments, but the new tail continues to grow longer, new segments being intercalated just in front of the end-piece that contains the anal opening. In summer the regeneration of a new part takes place in two or three days; in winter in ten to twelve days, this difference not being due to the time of year, but to the temperature. Bonnet found that if a newly regenerated head is cut off, a new one regenerates and if the second one is removed, a third new one develops, and in one case this occurred eight times, the ninth time only a bud-like outgrowth was formed. He thought that the capacity of a part to regenerate is in proportion to the number of times that the animal is liable to be injured under natural conditions. Bonnet found that short pieces from the anterior or posterior end of the body failed to regenerate, and usually died in a few days. Occasionally two new heads appeared at the anterior end of a piece and sometimes two tails at the posterior end.

Spallanzani made many experiments on a number of different animals, but unfortunately the complete account of his work was never published and we have only the abstract given in his "Prodromo" (1768). He made a large number of experiments with earthworms of several kinds, and found that a worm cut in two pieces may produce two new worms; or, at least, that the anterior piece produces a new tail which increases in length and may ultimately represent the posterior part of the body; the posterior piece, however, produces only a short head at its anterior end, but never makes good the rest of the part that was lost. A short piece of the anterior end fails to regenerate. Spallanzani also found that if much of the anterior end is cut off, the development of a new head by the posterior piece is delayed, and in some species, does not take place at all. If a new head is cut off, another is regenerated, and this occurred, in one case, five times. If, after a new head has developed, a portion only is cut off, the part removed is replaced, and if a portion of this new part is cut off, it is also regenerated. If a worm is split longitudinally into two pieces, the pieces die.

Spallanzani found that a tadpole can regenerate its tail; and if a part of the new tail is cut off, the remaining part will regenerate as much as is lost. Older tadpoles regenerate more slowly than younger ones. If a tadpole is not fed, it ceases to grow larger, but it will still regenerate its tail if the tail is cut off. Salamanders also regenerate a new tail, producing even new vertebrae. If a leg is cut off, it is regenerated; if all four legs are cut off, either at the same time or in succession, they are renewed. If the leg is cut off near the body, an imperfectly regenerated part is formed. Curiously enough, it was found that if the fingers or toes are cut off, they regenerate very slowly. If the fingers of one side and the whole leg of the opposite side are cut off at the same time, the leg may be regenerated as soon as are the fingers of the other side. If an animal is kept without food for two months after a leg has been cut off, the new leg will regenerate as rapidly as in another salamander that has been fed during this time. If the animal is kept longer without food, it will decrease in size, but nevertheless the new leg continues to grow larger. In one experiment, all four legs and the tail were cut off six times during the three summer months and were regenerated. Spallanzani calculated that 647 new bones must have been made in the new parts. The regeneration of the new limbs was as quickly carried out the last time as the first. Spallanzani also found that the upper and lower jaws of salamanders can regenerate.

These justly celebrated experiments of Trembley, Reáumur, Bonnet and Spallanzani furnished the basis of all later work in regeneration. Many new facts it is true have been discovered, and in many cases we have penetrated further into the conditions that influence the regeneration, but many of the important facts in regard to regeneration were made known by the work of these four naturalists. The twentieth century has seen a great deal of work and experimentation in the field of regeneration accomplished by such men as Morgan, Child, Stockard, Allen, Ellis, Zeleny, and many others.

The object of Allen's study was to find whether the greatest speed of regeneration occurs before or after or coincident with the completion of tissue differentiation. If the same correlation holds that was noted by Minot ('08) in ordinary growth, we should expect to find the rate of growth decreasing after the major tissues have been well developed and still more so after differentiation is essentially complete. Allen studied two distinct types, the mature, and the immature aniaml in securing information on the problem. The Oliogochaete worm, Limnodrilus claparedianus was the mature animal used and the other experiment was with the immature tadpole, Amblystoma. In the tadpoles which had their tails cut off just posterior to the anal opening, it was found that by the end of the first hour the epidermis had stretched over and very nearly covered the cut surface. In six hours it showed rapid proliferation with cells closely and irregularly massed and three or four deep. In two days the older regenerated epidermis was indistinguishable from the neighboring primary epidermis, though still proliferating at the tip. This general condition remained unchanged to the tenth day. Allen found that, when adult tissue is removed the resutlant regenerative growth reaches its greatest speed just preceding, or at least not later than, the time when the major somatic tissues become typically differentiated; and that when immature tissue is removed the resultant regenerative growth rate shows no relation of any sort to the differentiation of the more generalized somatic tissues. There is, however, a marked correlation with such a highly specialized tissue as muscle, the differentiation of which is coincident with the period of maximum growth rate.

Stockard in his studies of "Tissue Growth" says that the rate of regeneration from a peripheral cut on the Cassiopea disk is faster the nearer the disk center the cut is made. In the brittle stars Ophiocoma riisei and Ophiocoma echinata new arms regenerate faster as the old arms are cut off nearer their base of attachment to the body disk. The nearer the distal end a portion of arm is amputated, the slower will a new part regenerate. These experiments and those of several other workers all show that the rate of regeneration in diverse species of animals varies with the level of the cut, being faster as the cut surface is nearer the body center. The rate of regeneration does not bear the same definite relation to the extent of injury in all animal species. The Medusa, Casseopea, regenerates each oral arm at a rate which is independent of the degree of injury when replacing either one, two, four or six of its arms. If, however, eight arms are amputated, each arm is regenerated at a rate which is significantly greater than the regeneration rates in Medusae injured to any less extent. In the brittle-star, Ophiocoma Riisei, there is no relation between the rate of regeneration of the individual arms and the degree of injury. The rate of regeneration for individual arms in Ophiocoma echinata is faster when only a single arm is regenerating and successively slower when two, three, four or five arms are being replaced. The rate of regeneration is slower the greater the extent of injury.

The facts show that the rate of regeneration does not increase with an increase in the extent of injury in all animals but may actually respond in an opposite manner, or the rate of regeneration may even be independent of the extent of the injury. The unfed disk of Cassiopea decreases in size during regeneration in direct relation to the number of regenerating arms. Thus while the disks which are regenerating eight new arms grow then at the most rapid rate, these disks are also decreasing in size most rapidly. In _Ophiocoma riisei_ when all of the individuals are growing, those regenerating a larger number of arms increase in size slower than the specimens regenerating fewer arms. _Ophiocoma echinata_ regenerates each arm faster when only a few arms are cut and such individuals increase in size at about the same rate as do those which are regenerating each arm more slowly, although more arms are being replaced. Regenerating tissue possesses an excessive capacity for the absorption of nutriment and may do so even to the detriment of the old body tissue Stockard found.

In determining the relation of the amount of tail regenerated to the amount removed, Ellis used tadpoles of _Rama clamitans._ The rate of regeneration varies not only directly but proportionally with the distance the cut is removed from the tip of the tail. This proportional relation was between the length of the part of the tail removed and that regenerated at the end of twelve days. After a certain percentage of the amount removed had been regenerated, regeneration ceased entirely. The same percentage of the part removed was regenerated by all tadpoles maintained under uniform conditions and of the same age. Whether the amount removed was large or small, that is the amount of regeneration at the time regeneration ceased was proportional to the amount removed. The time elapsing between the operation and the cessation of regeneration varied with the amount removed.

The level of injury first producing death was 20 mm. cephalad from the tip of the tail when its tail length was 26 mm. The rate of regeneration varies as the level of injury until the 20 mm. level is reached. The first slow period of regeneration increases in length the higher the level of injury. Thus the time elapsing between the operation and the cessation of regeneration, that is the period of regeneration, varies with the level of injury, the higher the injury the longer the period - though the period is relatively longer for the series operated upon at the lower levels.

Zeleny found that an analysis of the progress of the regeneration in tadpoles brings out the fact that two distinct periods are to be recognized in rate of regeneration in its relation to level of the cut. During the first two to four days after the operation regeneration is confined to cell migration from the old tissues without cell division. During this period in the frog tadpoles there is no essential difference in length regenerated at the different levels and the specific rate is therefore much greater after shorter than

than after longer removals. In the second period with the initiation of rapid cell multiplication the rate of regeneration is greater the deeper the level and furthermore is directly proportional to the length removed. As soon as the bulk of material produced by cell division is considerably greater than that which was produced by cell migration there is an approach to constancy in specific length regenerated. This holds for all except the shortest removals. After the shortest removals the total regeneration is so small in amount that a large part of it is made up of the original migrated material. Therefore from these levels the specific regenerated lengths are greater than from the deeper levels even at a late period of regeneration. A further complication is introduced by the fact that regeneration is not complete. Only a certain per cent of the removed length is replaced and the end of the process is reached sooner after the shorter than after the longer removals. From the deepest level regeneration is still proceeding when it has stopped from the medium and shallowest ones. When the process is completed in all cases, the specific length is therefore slightly greater after both the longest and the shortest removals than after medium ones.

Morgan considers external and internal factors which influence regeneration in animals. There is a constant interchange of material and of energy that takes place between an animal and its surroundings, and this interchange may be influenced by such physical conditions as temperature, light, gravity and the like, or by such chemical conditions as the composition of the atmosphere or of the water surrounding the organism. We can study the process of regeneration either by keeping the regenerating organism under the same conditions that it is subject to in its natural environment, or else we can change the surrounding physical or chemical conditions. In this way we can determine how far the regeneration is affected by external changes and how far it is independent of them. If a change in the external conditions produces a definite change in the regeneration, then the new condition is called an external factor of regeneration. It has been shown by other writers that the rate at which regeneration takes place can be influenced by temperature. In general it may be stated that the limits of temperature under which normal growth takes place represent also the limits of temperature for regeneration. The limits of temperature within which regeneration takes place in Planaria torva have been determined. The worm was cut in two transversely through the pharynx, and the time required at different temperatures to produce a new head on the posterior piece was recorded. The lowest temperature at which regeneration was found to take place was 3 degrees C. Of six individuals kept at this temperature, only one regenerated at all, and in this one the eyes and brain were still incomplete after six months. The optimum temperature, or at least that at which regeneration takes place most rapidly, was found to be 29.7 degrees C.; a new head developed in 4.6 days at this temperature. At 31.5 degrees C. regeneration was slower, requiring 8.5 days to make a new head. At 32.degrees C. incomplete regeneration sometimes took place, but death occurred in about six days. At 33 degrees C. regeneration was very slight, and the animals died within three days. At 34 degrees C., and above this point, no regeneration took place, and death soon occurred.

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

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

Experiments have been made to test the effect of light on regeneration and it is certain that in many cases light has no effect on the process, either as to the quantity or the quality of the result. In one form, a tubularian hydroid, Eudendrium racemosum, it has been shown by Loeb, that the regeneration of the hydranth takes place only when the animal is exposed to light. When a colony of Eudendrium is brought into the laboratory and placed in an aquarium, new hydranths are regenerated in a few days. Loeb also determined the effect of chemical changes in the environment. Organisms that live in water may be affected by the quantity and the kinds of the salts contained in the water, and also by the dissolved gases. He placed pieces of the stem of tubularia in sea water of different degrees of concentration. After eight days the pieces, that had meanwhile produced hydranths, were measured. It was found that the maximum growth in length takes place, not in normal sea water, but in a much diluted solution. Temperature, and food, and substances in solution act alike on fixed and free forms and they are, it appears, both influenced in the same way by these reagents. The most significant fact that has been discovered in connection with the influence of external factors on regeneration is that the same factors that influence the normal growth of the organism also affect in the same way the regeneration.

Trembley, Spallanzani, and Bonnet knew that, in general, at the end of a piece of an animal from which a head had been cut off a new head developed, and from the posterior cut-surface of a piece a new posterior part regenerated. Allman was the first to give the name "polarity" to this phenomenon. In several animals regeneration takes place more readily from one end than from the other of the same cut, and this difference seems to be connected with the kind of new part that is to be regenerated, and not with the actual power of regeneration of the region itself. In the earthworm when two segments are cut off, two come back, and this holds good up to five segments. Beyond this, no matter how many are removed, only five at the most come back. A relation of some sort obtains between the old and the new parts, a sort of completing principle exists as a factor in the result, but when so much has been cut off that the old part cannot complete itself in the new material that is formed, then other factors must determine how many segments will be produced. In planarians we find a similar phenomenon. If much of the anterior end is cut off, only a head is formed at the anterior cut-surface of the posterior piece, and the intermediate region is absent. As soon as enough new material has been formed for the anterior end to appear, it begins to develop, and since it cannot develop below a certain minimal size, or rather since the tendency to produce a head approaching the maximum size is stronger than the tendency to produce as much as possible of the missing anterior end, all the new material goes into the new head.

In the planarian the possibility of subsequently replacing the missing region behind the head exists, and the intermediate part is later produced, the head being carried farther forward. The same is true of the new posterior end of the earthworm, in which a growing region is established at a very early stage, in front of the tip of the tail, but no such growing region is present at the anterior end in the earthworm. These differences appear to be connected with the general phenomena of growth in these forms. In the planarian interstitial growth can take place in any part of the body, hence the possibility of producing a missing region is present in all parts of the worm.

Regeneration in Planaria Dorotocephala

Among the flatworms, the fresh-water planarians show remarkable powers of regeneration. If the anterior end is cut off at any level, a new head is produced (figure 1,c). The new worm is at first too short, that is, the new head is too near the pharynx, but changes take place in the region behind the new head that lead to the development of new material in this part. The new head is, in consequence, carried farther and farther forward until the typical relations of the parts have been formed, when the growth in the region behind the head comes to an end (Fig. 1,c'). Similar changes take place when the posterior end is cut off, as shown in Figure 1 B and B'. The new part contains the new pharynx that is proportionately too near the head, but the pharynx is carried farther backwards by the formation of new material in front of it, until it has reached its typical distance from the head. In these planarians the results are somewhat complicated, owing to the old part changing its form, especially if the piece is not fed; but

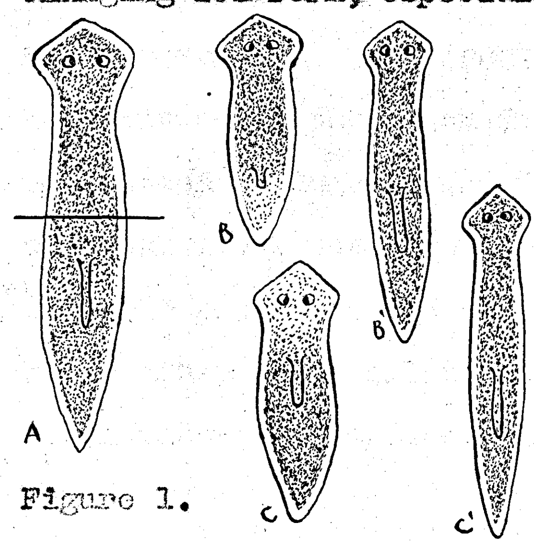


Figure 1

A. Normal worm

B, B' Regeneration of anterior half.

C, C' Regeneration of posterior half.

Figure 1.

the main facts are given above, and a more complete account of the changes that occur will be given later.

Not only does regeneration take place in an antero-posterior direction, but in many animals also at the side. If a planarian is cut in two lengthwise, the growth of the new part is at the expense of the old tissues which is a phenomenon of the greatest importance. In the course of five or six days after the operation, there develops new material along the cut-side of each piece, and a new pharynx appears at the border between the old and the new parts. However, this paper will consider regeneration that takes place in an antero-posterior direction only, with due consideration of the level of the cut.

Experimentation with the planaria was begun early in October but the first results were not used, as the technique of preparing and handling the worms was at first not well in hand. The methods used for operation on the flatworms were as simple as could be devised.

The main supply of planaria were kept in a large flat glass dish in plenty of tap water which was replaced fresh every day. No direct effort was made to control the temperature of the water during the experiments which varied between 66 degrees and 84 degrees F. Food in the form of fresh liver was given once a week. The liver was placed in small pieces in the water and left for a period of two hours after which all traces of the meat were removed and fresh water was replaced in the dish. The operated series were also fed in the same manner but not on days when measurements were taken. The operated series were kept in syracuse watch glasses filled with tap water. The rim of the glass was vaselined to keep evaporation from taking place. A control series was kept for each experiment in the same manner as the operated series; only measurements were taken once a week instead of every second or third day as in the case of the operated series.

For operation each flatworm was placed on a paraffin block and a quick even cut was made with a sharp, thin scalpel as soon as the worm became as quiet as possible. The worms for operation were selected for their medium size and removed from the supply dish by means of a large-mouthed pipette. After the operation the cut pieces were placed by means of a camels hair brush in the labeled watch glasses. Measurements were made by removing the worm by means of the camels hair brush from the watch glass to a glass slide. This was placed on a dissecting scope and a metric rule was placed under the slide. Measurements were made by the use of a hand doublet when the worm was fully extended.

Throughout the experimentation an effort was made to consider all the factors which might influence the amount of regeneration. These were generally controlled directly, but if this were impossible, they were reduced to a constant by the introduction of a control series of uninjured planaria whose reaction to the factors of the experiment were noted. The factors of greatest importance here considered are:

1. Environment. The worms were kept in clean glass containers.

2. Food. Only fresh liver was given to the worms.

3. Temperature. The temperature was not controlled directly but the average temperature of the water in the dishes was taken as a standard temperature for the experiments.

4. Light. At no time did any of the worms receive direct sunlight. Neither were they kept in the dark, but rather in diffused day light.

5. Age. There was no definite way of obtaining the absolute age of any of the planaria. It was assumed that planaria of the same species collected from the same part of a given pond and of practically the same body measurements were about the same age. There must of course be some exceptions to this calculation of age, but the uniformity of results and data show that there was probably little error from this source.

6. Individual Variation. The effect of individual variation was eliminated as far as possible by the use of large numbers of planaria.

Experiment I

November 2, 1930 ten individual planaria with an average of 14.8 mm. in length were operated upon. The average amount of tail removed was 7.7 mm. The extremes of the operation were 7.5 mm. in one case and 8 mm. in two cases. The set was composed of ten individuals in a control series with an average body length of 15.5 mm.

The level of the cut was above the pharynx and regeneration took place more rapidly than when the level of the cut was lower. Compare Figure 1, Experiment I, with Figure 1, Experiment II, which is for a cut of lower level. The amount regenerated was much greater in the cut of deeper level. The decrease in the rate of regeneration is practically the same in the two curves shown and corresponds to ordinary growth decrease.

Data for Experiment I

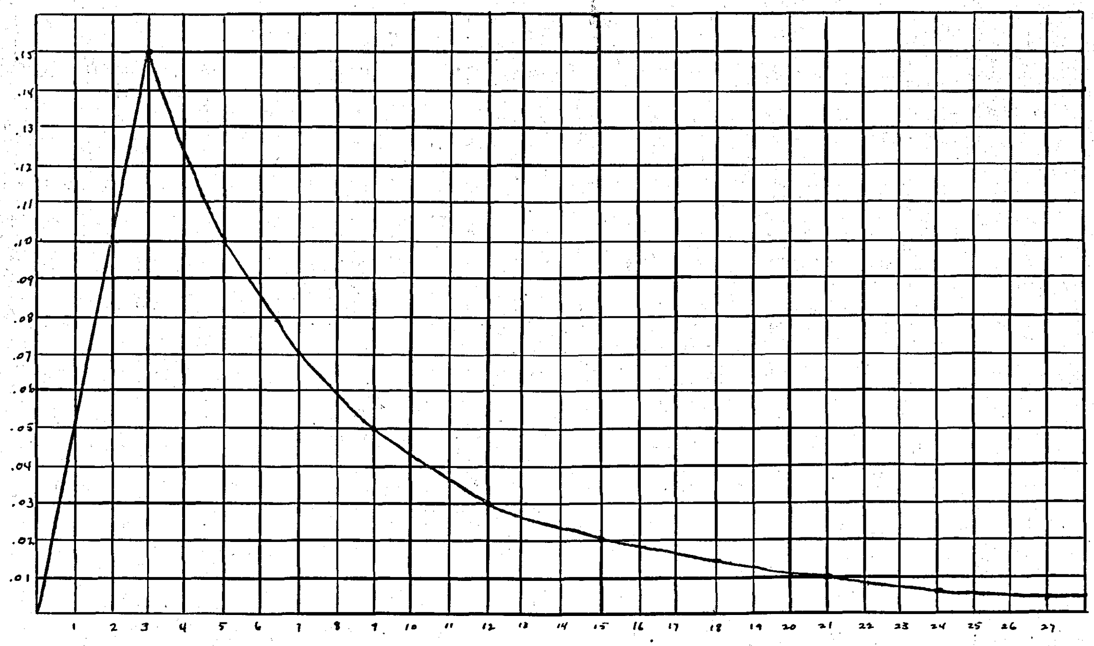
Figure 1 - Regeneration of New Tails.

	November									
Dates of Measurements	2-5	7	9	11	14	17	20	23	26	29
Amounts of Regeneration in mm.	8-21	8.41	8.55	8.65	8.74	8.8	8.848	8.878	8.896	8.912
Days in Period between Measurements	3	2	2	2	3	3	3	3	3	3
Gain during Periods in mm.	.44	.20	.14	.10	.09	.06	.048	..03	.018	.016
Rate during Periods	.15	.10	.07	.05	.03	.02	.016	.01	.006	.005
Measurements of Control Series in mm.	15.5			15.3			15.2			15.

The first horizontal row of figures gives the dates upon which each measurement was made; below each date is the average amount of tail in millimeters regenerated by the operated series at that time. The third row of figures gives the number of days that have elapsed since the last measurement. The fourth row gives the gain in length during these periods. The firth row contains the rate in millimeters per day, at which the tail grew during each period. The meqsurements of body length are the averages for the control series and are given in the columns headed by the dates upon which the measurement was made.

Data for Experiment I

Figure 1 - Regeneration of New Tails



Curve of rates of regeneration for Experiment I. The days elapsing since the operation are placed upon the base line. The rates at which the tail is increasing in length are measured above the base line at the end of the corresponding periods. The curve indicates a rapid increase at first with a gradual decrease in amount of regeneration until the normal is reached.

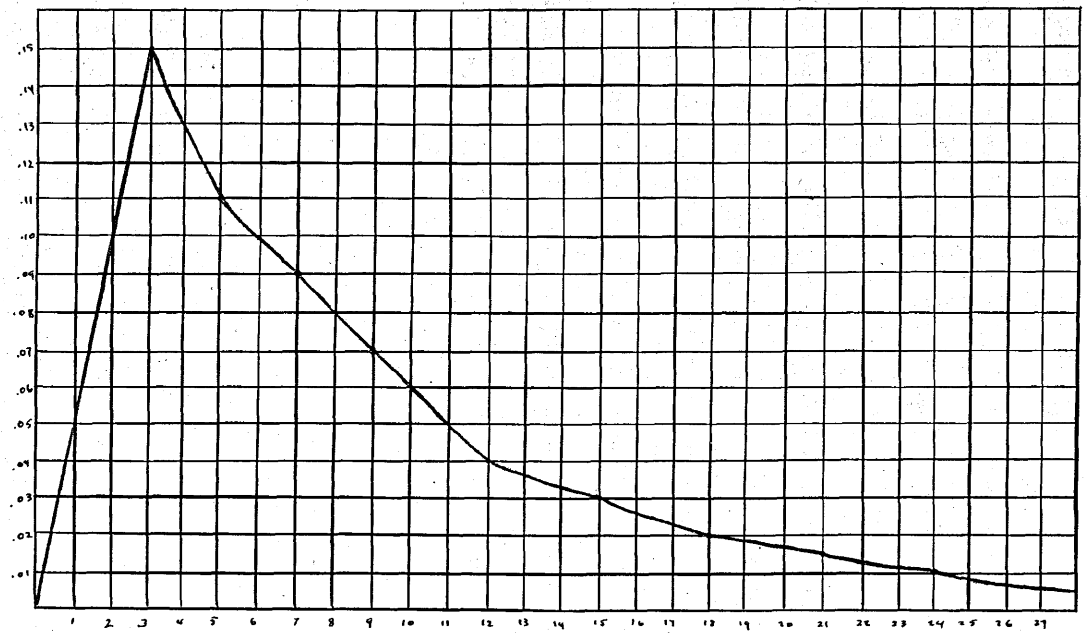
Data for Experiment I

Figure 2 - Regeneration of New Heads.

	November									
Dates of Measurements	2-5	7	9	11	14	17	20	23	26	29
Amounts of Regeneration in mm.	7.54	7,76	7.93	8.07	8.19	8.28	8.34	8.39	8.42	8.44
Days in Period between Measurements	3	2	2	2	3	3	3	3	3	3
Gain during Periods in mm.	.44	.22	.17	.14	.12	.09	.06	.05	.03	.02
Rate during Periods	.15	.11	.09	.07	.04	.03	.02	.016	.01	.006
Measurements of Control Series in mm.	14.8			14.5			14.4			14.2

Data for Experiment I

Figure 2 - Regeneration of New Heads



Experiment II

February 17, 1931, ten individuals 6 - 11 mm. in length were operated upon. The average amount of tail removed was 5.83 mm., the extremes of the operation were 4 mm. in one case and 7 mm. in two cases. The set was composed of ten individuals in a control series. The average body length of the operated series was 8.91 mm., and of the control series 8.94 mm. During the last of this experiment the animals were subjected to two irregularities of treatment. They were left without food for fourteen days at one time and allowed to live in foul water for four days at a later period. The set was discontinued March 17, twenty-seven days after operation. Figure 1 gives the average of the results of the measurements made on this set.

Data for Experiment II

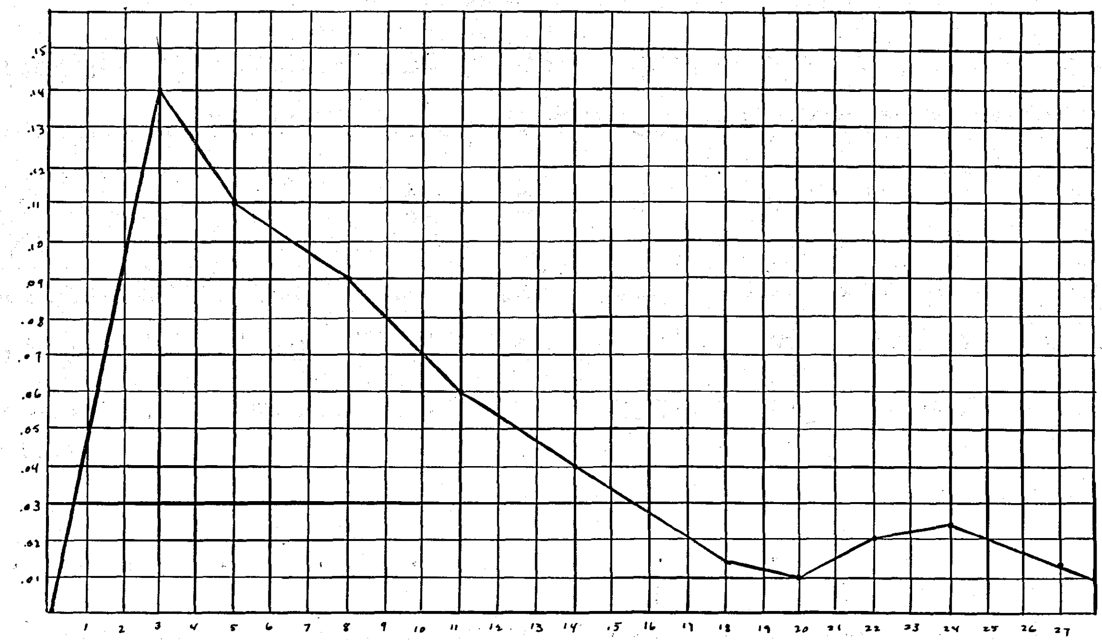
Figure 1 - Regeneration of New Tails

	February				March					
Dates of Measurements	17-21	23	25	28	3	7	9	11	13	16
Amounts of Regeneration in mm.	3.43	3.65	3.83	4.00	4.13	4.19	4.21	4.25	4.30	4.35
Days in Period between Measurements	4	2	2	3	3	4	2	2	2	3
Gain during Periods in mm.	.43	.22	.18	.17	.13	.06	.02	.04	.05	.05
Rate during Periods	.11	.11	.09	.06	.04	.015	.01	.02	.025	.016
Measurements of Control Series in mm.	8.18			8.14			8.1			8.06

The first horizontal row of figures gives the dates upon which each measurement was made; below each date is the average amount of tail in millimeters regenerated by the operated series at that time. The third row of figures gives the number of days that have elapsed since the last measurement. The fourth row gives the gain in length during these periods. The fifth row contains the rate in millimeters per day, at which the tail grew during each period. The measurements of body length are the averages for the control series and are given in the columns headed by the dates upon which the measurement was made.

Data for Experiment II

Figure 1 - Regeneration of New Tails



Curve of rates of regeneration for experiment II. The days elapsing since the operation are placed upon the base line. The rates at which the tail is increasing in length are measured above the base line at the end of the corresponding periods. The curve indicates the decrease in amount of regeneration with a slight increase toward the close of the period.

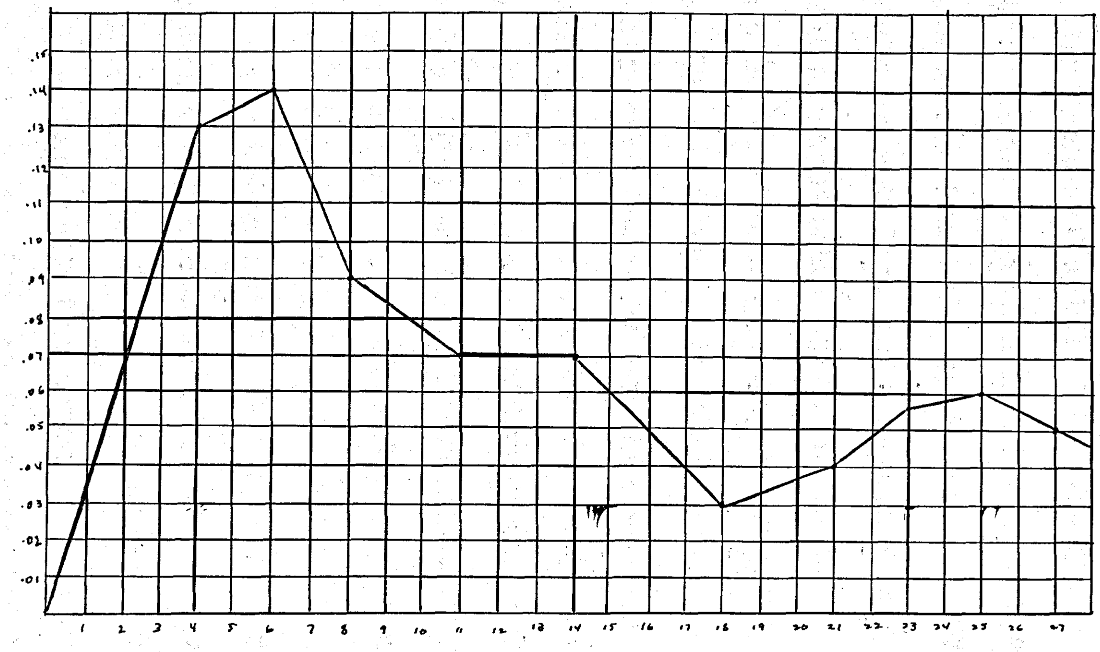
Data for Experiment II

Figure 2 - Regeneration of New Heads

	February				March					
Dates of Measurements	17-21	23	25	28	3	7	10	12	14	17
Amounts of Regeneration in mm.	6.53	6.80	6.97	7.17	7.37	7.49	7.61	7.72	7.84	7.94
Days in Period between Measurements	4	2	2	3	3	4	3	2	2	3
Gain during periods in mm.	.53	.27	.17	.20	.20	.12	.12	.11	.12	.10
Rate during Periods	.13	.14	.09	.07	.07	.03	.04	.055	.06	.05
Measurements of Control Series in mm.	8.94			8.8			8.76			8.74

Data for Experiment II

Figure 2 - Regeneration of New Heads



Curve of rates of regeneration for experiment II. The days elapsing since the operation are placed upon the base line. The rates at which the head is increasing in length are measured above the base line at the end of the corresponding periods. The curve indicates the decrease in amount of regeneration. There is a lack of uniformity of gradual rate of change of regeneration as shown by the irregularities in the curve which is due to the death of two animals in the operated series.

A glance at the data is sufficient to reveal a general similarity between the changes in the rate of regeneration in planaria and the changes in the rate of growth throughout the life of the animal. The rate of regeneration and of ordinary growth decreases from a maximum attained soon after the process begins. The decrease is rapid at first and then becomes gradually slower and slower. Minot ('08) conducted an elaborate series of experiments upon the rate of growth in rabbits, guinea-pigs and man, and expressed his results graphically by constructing curves showing the per cent of increment per day throughout the life of the animal. These curves show that the rate of growth undergoes four different kinds of change, which correspond in general with the four intervals of change in the rate of regeneration. It was found that immediately following the removal of a part of the posterior half the rate of increase in length is slow but rises rapidly to a maximum which it reaches on the third day. From this point there is a rapid decrease for about ten days and then a progressively slower decrease until the process is complete. The curve expressing these rate changes is shown in Figures 1 and 2. It agrees in general features with the curve of the ordinary growth of rabbits and guinea-pigs as it is described by Minot.

In connection with the factors controlling the process of regeneration the changes in rate show that the full force of the tendency to regenerate is attained within a short period after the operation, but that the retarding factors appear early and increase in intensity rapidly at first and then more slowly until they have completely overcome the positive growth tendencies.

There are two possible explanations for the low rate in the initial interval of regeneration.

1. It may be that the shock given the worms by the operation makes them too weak to begin regeneration at once.

2. It may be that some time is consumed immediately after the operation in the formation of an embryonic tissue which serves as a basis for subsequent regeneration.

In connection with the description of the experiments a partial analysis of the results has been attempted and much of the ground gone over need not be traversed again here, but the more general bearings of the facts may now be discussed. The problems of special interest are those connected with the rate of growth at different levels.

The question whether the difference in rate can be explained as due to the amount of food available at each level has been sufficiently examined. Ample evidence was found showing that the differences in rate of growth are not due to differences in the available food supply. It would be erroneous to conclude from this that the available food supply has no influence on any of the phenomena of regeneration, for it has been shown that the size of the new tail, for example, is affected by the amount of food, in the

the same way as the rest of the body, and it has also been shown that when starvation has gone beyond a certain point, even the formation of new parts may be delayed, or stopped before the animal perishes from hunger. But despite these effects, the experiments show that the rate of formation of new parts as seen in the regeneration of new heads and in the growth in the length of the tails of planaria takes place at the same rate, whether the animal is fed or starved, provided there still remains enough food for the formation of new material. The meaning of this relation seems to be that the greater power of assimilation of a young part makes possible for this part to draw the necessary nourishment from the protoplasm although the amount of nourishment present in the protoplasm is below that which is necessary to maintain in "statu quo" the differentiated tissues. The most important consideration in this case is that the material of the new part is derived directly from that of the old, so that the difference is one of condition only, and is therefore, a reversible process. In other words, because a tissue has become differentiated it has not lost the potentiality of becoming young again, provided it gives up its differentiation. This consideration has a bearing on the problem of the difference of rate at different levels.

Let us return now to the main problem of the factors involved in the growth of the new part in the posterior regeneration of the tail of planaria. As a result of removal of the posterior end there is a proliferation of new materials, and this as we have seen, appears to take place at about the same time for all levels, despite the fact that the rate of regeneration is different for all levels. The exposure itself may appear to give the stimulus that calls forth the proliferation, but it seems improbable that this is the immediate cause, since the greater part of the proliferation takes place after the closure of the skin over the wound. It seems more probable that the real stimulus is to be sought for in the loss of the connection with the old part, in other words, to the loss of the normal pressure relations essential for the normal equilibrium. The terminal part is quickly formed in the proliferated materials. Between the terminal part and the old part there is also laid down a growing zone that is a normal structure for the posterior end. The growing region has the same potentialities for all levels and it continues to grow until some retarding influence delays and then prevents its further growth.

We have also seen that the retarding influence is connected with the completion of the normal form, hence it is in the nature of a formative influence. We may compare the retardation of a regenerating part to the retardation seen in the growth of the whole organism. The growth of many animals slowly decreases as the typical form or size is approached.

In the case of the posterior growth under consideration, the clue to the solution of the manner of growth is to be found, I think, in the relation of the new part to the parts lying proximad to them. But what is the nature of this relation that determines the formation of the successive parts? The old part has a certain differentiation as well as the potentiality of forming the whole of the distal or other regions. The relation in question must depend in some way upon differentiation, but differentiation in itself cannot be assumed to be a formative factor, since we know of no such influence extending from cell to cell. If, however, the differentiation is an expression of certain pressure relations that have determined the differentiation and which since they still remain, determine the pressure relations of the neighboring parts and determine the kind of new differentiation that will take place, the new part thus formed will, in turn, influence the differentiation of the next part that develops and the process will continue until the completion of the typical form has been accomplished.

The new growth will come to an end when the last formed part has developed - when differentiation is of such a kind that the resulting pressures thereby established no longer act as a stimulus on the growing region to produce another new part. In the formation of a new tail the pressure relation is a gradually decreasing quantity and along with this decrease there goes a decrease in the stimulus to further growth that ultimately comes to an end. This analysis shows why there should be a gradual slowing down of the regeneration as the normal form is approached. It is apparent that this retardation will be the same whether it occurs near the end of an old part, or, as a new part approaches completion for, on the hypothesis, the conditions will be the same in each. The hypothesis gives at least a formal explanation of the facts and I can find no other that will. The most problematical part of the hypothesis is, I think, the assumption regarding the nature of the influence of the formed part upon the unformed part. I have assumed this to be a pressure relation of some kind. Possibly some other condition may be found that expresses this relation more correctly, but the remainder of the argument may stand even if it be found that the nature of the influence is different from that which I have assumed. My assumption has, however, the advantage that it puts into the same category the influences that determined the formation of a terminal part, and the subsequent growth of a posterior end, namely a condition of pressure or tension. My pressure hypothesis has also the advantage, I think, that it involves only a known quantity. It appeals on the whole to phenomena that are known to occur in living things; for response to pressure, or stereotropism, in adult animals and plants is well known. That growth is influenced by pressure is also known. Less familiar perhaps is the assumption that differentiation is itself a response to a pressure relation rather than due only to the kind of material contained in a cell, although the latter also may be a factor that enters at times into the result.

Results and Conclusions

1. The rate of regeneration and of ordinary growth decreases from a maximum attained soon after the process begins.

2. The decrease is rapid at first and then becomes gradually slower and slower until the process is complete.

3. The retarding factors appear early and increase in intensity rapidly at first and then more slowly until they have completely overcome the positive growth tendencies.

4. Differences in rate of growth are not due to differences in the available food supply.

5. Regenerating tissue possesses an excessive capacity for the absorption of nutriment and may do so even to the detriment of the old body tissue.

6. Tissues in regions undergoing regeneration show very distinct indications of differentiation.

7. The growing region has the same potentialities for all levels and it continues to grow until some retarding influences delays and prevents further growth.

8. The retarding influence is connected with the completion of the normal form.

9. The new growth will come to an end when the last formed part has developed, when differentiation is of such a kind that the resulting pressures thereby established

no longer act as a stimulus on the growing region to produce another new part.

10. This is a pressure relation.

Bibliography.

Of the literature available, only that which was found distinctly useful is listed here.

Allen, W. E.

1911 - "A Study of the Relation of Tissue Differentiation to Rate of Growth during Regeneration". Biol. Bull. 21:187-206

Bardeen, Charles Russell

"The Inhibitive Action of the Roentgen Rays on Regeneration in Planarians". J. Exp. Zool. Vol. I pp. 191-195.

Child, C. M.

1904 - "Some Experimental Modifications of Form Regulation in Leptoplana". J. Exp. Zool. Vol. I No. 1.

"Anterior and Lateral Regeneration". J. Exp. Zool. Vol. I, No. 4.

1906 - "The Relation Between Regulation and Fission in Planaria". Biol. Bull. Vol. XI, No. 3.

"The Relation Between Functional Regulation and Form Regulation". J. Exp. Zool. Vol. III No. 4.

1909 - "The Regulatory Change of Shape in Planaria Dorotocephala". Biol. Bull. 16:277-296

Durbin, M. L.

1909 - "An Analysis of the Rate of Regeneration Throughout the Regenerative Process". J. Exp. Zool. Vol. III pp 397-420.

Ellis, M. M.

1909 - "The Relation of the Amount of Tail Regenerated to the Amount Removed in Tadpoles of Rana Clamitans". J. Exp. Zool. Vol. VII 421-456.

Morgan, T. H.

1901 - "The Physiology of Regeneration". J. Exp. Zool. Vol. III 457-500.

"The Dynamic Factor in Regeneration" Biol. Bull. 16:265-276.

"Regeneration" MacMillan, N. Y. 1901.

Morgulis, S.

1907 - "Observations and Experiments in Lumbriculus". J. Exp. Zool. Vol. IV. 549-574.

"Regulation of the Water Content in Regeneration" J. Exp. Zool. Vol. X. 321-348.

1909 - Contributions to the Physiology of Regeneration Experiments on Podarke Obscura." J. Exp. Zool. 7:595-642.

Scott, G. G.

1907 - "Further Notes on the Regeneration of the Fins of Fundulus heteroclitus". Biol. Bull. 12:385-400.

1909 - "Regeneration in Fundulus and its Relation to the Size of the Fish". Biol. Bull. 17:343-353.

Stockard, C. R.

1909 - "Studies of Tissue Growth - Functional Activity, Form Regulation, Level of the Cut and Degree of Injury as Factors in Determing the Rate of Regeneration. The Reaction of Regenerating Tissue in the Old Body". J. Exp. Zool. Vol. 6:433-471.

Zeleny, C.

1916 - "Studies on the Factors Controlling the Rate of Regeneration". Ill. Biol. Monograph Vol. III, No. 1.

"The Effect of Successive Removal upon the Rate of Regeneration". Proceedings of Nat. Academy of Science. Vol. II p. 487. August, 1916.

"A Comparison of the Rates of Regeneration from Old and from New Tissue". Ibid. Vol. II p.484.

"The Effect of Degree of Injury, Level of Cut and Time Within the Regenerative Cycle upon the Rate of Regeneration." Ibid. Vol. III pp. 211-217. March, 1917.