

THE PHYSIOLOGY OF REGENERATION

BY

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WITH SEVEN FIGURES

Lest the title mislead someone expecting to find in the following pages an account of the processes of assimilation and of respiration, that presumably take place during regeneration, I ought to state that I shall deal only with the physiology of the growth process as shown in the regeneration of a new part. Morphogenesis does not express my meaning in all respects, for I am not concerned so much with changes in form as with the rate of growth and of differentiation. If I have taken a liberty in using the term physiology to cover these kinds of changes, my excuse must be that we are dealing with phenomena that lie on the borderland, where physiology and morphology overlap, and appear to merge into each other.

It is generally, if tacitly, assumed that when undifferentiated cells are supplied with food materials growth must follow, but I shall try to show, on the contrary, that whether or not growth takes place depends not so much on the available food supply as on a formative influence that regulates both the kind and the amount of growth. The nature of this formative influence is the most difficult and problematical factor with which we shall have to deal.

RATE OF REGENERATION IN STARVED AND WELL-FED SALAMANDERS

If the rate of regeneration is in any way connected with the food supply, the fact ought to become at once apparent by comparing the process in well-fed and in starved individuals. I have carried out such an experiment with the salamander, *Diemyctylus viridescens*. The results show that we must be careful to distinguish, in the use of the word *rate*, between a simple increase in size, and

the rapidity of the process of differentiation (or development in the narrower sense) of the new part.

Zeleny¹ has recently shown that the increase in size of regenerating arms of the brittle-star and of the legs of the crayfish is determined, in part, by the number of the appendages removed. The more parts removed, the faster each regenerates. Zeleny discusses amongst other factors, the possible relation of this result to the food supply, and points out that the larger the number of appendages removed the greater will be the temporary surplus of food, for the amount necessary to nourish the entire leg may be greater than that used at first in the growth of the small new part. Whilst pointing out the possibility of this interpretation Zeleny carefully refrains from committing himself to it as the only explanation of his results.

It seemed to me that this question might be tested in the following way. If the rate is determined by the food supply, then if two sets of individuals are selected, and one set starved and the other fed, the latter should be in a better condition for regeneration than the former. If the same number of parts is removed in each, the well-fed individuals should regenerate faster than the starved ones.

If fewer parts are removed from the well-fed individuals than from the starved ones, nevertheless the well-fed individuals should regenerate faster, for, the greater amount of food given ought to much more than outweigh the surplus in the starved ones due to the absence of more parts.

The experiment was carried out with salamanders collected in the autumn. They were in excellent condition when caught, although not so large as they soon became when fed on pieces of beef. The individuals were kept for several weeks, without much food, before the experiments began. In some individuals one leg was removed, in others two, in others three, and in still others one, two, or three legs and also the tail, which was cut off near the base. Duplicate sets were prepared, each containing several of these different kinds of individuals. One lot was kept without food and the other fed about every other day on small pieces of raw beef.

¹Zeleny, C. A Study of the Regeneration of the Arms of the Brittle-Star. *Biol. Bull.*, vi, 1903. Compensatory Regulation. *Jour. Exp. Zool.*, ii, 1905.

As the new limbs developed they were carefully compared and in some cases measured. It was soon seen that no constant difference could be detected in the two sets, or between different kinds of individuals of the same set, if the regeneration of the new legs is measured by their rate of differentiation. Therefore, food does not seem to be the main factor in the result. But another fact also came to light. The new legs of the well-fed individuals were larger than those of the starved ones. This difference is correlated with the great difference in size between the two sets, for the well-fed animals grew to a large size, while the starved ones dwindled almost to a skeleton. By actual weight after eleven weeks one well-fed individual weighed 3.48 gms. and a starved one .67; the latter having, therefore, only one-fifth the weight of the former. This difference in weight is not due to the storage of fat; but all of the organs of the body, heart, liver, pancreas, intestine, skin, muscles, etc., are larger in well-fed animals. The new limbs also partake of the general condition of well being, so far as size is concerned; in other words, they developed in proportion to the size of the old part. Measurements of the new limbs show that those of the well-fed individuals have outstripped those of the starved individuals. The difference in diameter was especially marked, while the length of the new limb seemed to show less difference.

It has been stated that no difference in the rate of differentiation was found, but owing to the very considerable individual variation, small differences, if they exist, might have been easily overlooked, and while this must be freely granted, the main result was quite definite that no appreciable difference was seen, while the difference in size was quite apparent. There is another consideration in this connection. If the difference in size of the new parts, in relation to the number of parts removed, depends on the surplus of food, the detection of the difference might largely depend on the size of the part removed. In a form like the salamander, where the legs are relatively very small in proportion to the rest of the body, the difference in the amount of surplus food would be so small that we would not expect to detect any difference in the relative sizes, even if it exists, when one or when three legs are cut off. In fact, I could detect no such difference in these forms,

when individuals lacking one, two or three legs were compared with each other; neither in the starved nor in the well-fed sets. When the whole of the tail is removed the loss becomes proportionately greater but still I failed to note any differences. It is to be remembered that Zeleny's results show only an increase in size of the new part, and not in rate of differentiation, and my own results show to some extent the same thing; at least, this difference was found between the well-fed and starved sets, if not between the individuals with one or with more parts removed. In the latter case measurable results might depend, as stated above, on the relation between the relative size of the body and of the parts removed.

Zeleny's important discovery, regarding the relation between the size of the new parts and the number of parts removed, bears a close resemblance to another curious fact in regard to the rate of regeneration. If the distal end of the tail is removed it regenerates more slowly than when more of the tail is cut off. Thus the more the material removed the greater the rate of regeneration of the new part. Stated in this form the two results appear to be identical. This question may now be considered.

RATE OF REGENERATION OF THE TAIL OF DIEMYCTYLUS AT DIFFERENT LEVELS

If the tail of one individual is cut off near the base and of another near the outer end, a great difference in the rate of growth of the new tail becomes apparent. *The nearer the cut to the outer end the slower the rate of regeneration.*¹ In general it may be said that the rate of development of the new tail is directly proportional to the distance of the cut surface from the distal end of the tail. A few actual measurements will bear out this statement.

¹Spallanzani observed that it takes as long for the toe of a salamander to regenerate as it does for an entire leg. King found in the starfish that regeneration is more rapid from the base of the arm than from its tip. The results are in accord with the fact just stated for the tail of the salamander.

INCREASE IN LENGTH OF REGENERATING TAIL

		Jan. 29. Cut Off at Base			Cut Off Near Tip	
March	3	3½	4	4½	1	2
	10	3	3½	4	¾	2
	17	4	5½	6	1½	3½
	24	4	6	6½	2	4
	31	4½	6	7	2	4
April	7	4½	6	7	2	4
	14	5	6	7	2	4
	28	6	7	7	—	4½

INCREASE IN LENGTH OF REGENERATING TAIL

		Feb. 3. Cut Off at Base			Cut Off Near Tip		
March	3	3	3	4	1	1	1½
	10	4	3½	5	1½	1	1½
	17	5½	6	7	2	1½	3
	24	6	—	8	2½	2	—
	31	7	—	8	2½	2¼	—
April	7	7½	—	8	—	2¼	—
	14	7	—	7½	—	2¼	—
	28	7½	—	7½	—	3	—

What is the meaning of this result? Is it due to the larger amount of food-material available when more of the old tail is removed? This possibility was tested by starving one lot of animals. The results are not in accord with the assumption. For example: In the second half of the preceding table in the last two columns of the series "cut off at base" and in the last column of the series "cut off near tip," the records of individuals are given that were not fed after March 6. No decrease in the rate of regeneration is to be found. In fact these individuals as long as they lived did even better than the others. The salamanders were in a well-fed condition at the beginning of the experiment and the materials derived from their own bodies sufficed, during the time of the experiment, to give sufficient materials for maximum regenerative growth. In the experiment in which the legs as well as the tail were cut off, it was apparent that the new tails in the starved set were not as large, as in the well-fed set. In the starved animal the old tail also was very emaciated and much smaller in the ver-

tical and transverse diameters than was the tail of the well-fed individuals. The difference in length was not so apparent, which is probably due to the loss in the bones being less than that in the other tissues.

The normal tail of *Diemyctylus* is much bigger at its base than nearer its distal end, so that the cross-section of the base is larger than that of the tip. Can this difference account for the difference in the rate of regeneration from the two levels? At the beginning of the new growth this relation may account for the difference in size of the new part, because the amount of material proliferated from a large surface may be greater than that from a small one. This, in itself, would not account for the results, if, at each level, the new part, from beginning to end, were as broad as the old part, but such is not the case. The new part has more the shape of a somewhat flattened cone, with a broader base in one case than in the other, but tapering quickly to its apex. Therefore, if more material were proliferated from a broader base the cone would be longer *i. e.*, it would be in proportion to the base. On the other hand the period of proliferation is short, and the basal parts soon differentiate into their organs. Subsequent growth takes place near the tip. Hence after the first period is passed, the new tail must, in both cases, continue to grow in length through its own activity, and its increase in length must henceforth be due to this activity and not to proliferation from the base. It may appear that the difference in rate is due to some initial difference in the material at different levels of the old tail. If it were simply a question of material, *per se*, we should expect the new growth from a basal surface to be as rapid during the later stages of formation of the new tail as at first, since the material for both came from the same level, but this is not the case. Hence, I conclude, that the cause of the difference observed is not due to a difference in the old materials that go to produce the new part. The analysis leads here to the same conclusion as in other cases of posterior growth to be described, in all of which the result appears to be due to some retarding influence that appears as the growth approaches its natural terminus. The retardation is the same for the growth at the end of a new part (that arises from the base) and for the new growth

that begins from the old part near its end. A discussion of this point will be left until other cases have been considered.

THE RATE OF REGENERATION OF THE EARTHWORM AT
DIFFERENT LEVELS

In order to study the rate of posterior regeneration in the earthworm, *Allolobophora foetida*, at different levels the worms were cut in two at the following places; (1), near the posterior end, removing 20 to 25 segments; (2) near the middle, *i. e.*, at about the 50th segment; and (3), just behind the girdle at about the 33d segment. It is not advantageous to cut further forward, for, as I have shown elsewhere, the power of regenerating a posterior end ceases rather suddenly about the level of the 15th segment. The following tables give the results of three experiments of this kind. Table I is for a set of worms 55 days old (September 30 to November 24). Table II is for the same set 35 days old (September 30 to November 4). Table III is another set 57 days old (December 28 to February 23).

TABLE I					
September 30 to November 24					
WORMS CUT NEAR POSTERIOR END, AT MIDDLE AND BACK OF GIRDLE.				ANTERIOR END INTACT	
<i>Posterior End</i>		<i>Middle</i>		<i>Behind Girdle</i>	
<i>Old</i>	<i>New</i>	<i>Old</i>	<i>New</i>	<i>Old</i>	<i>New</i>
83	7	49	34	35	45
84	7	53	27	33	54
67	12	49	38	37	37
73	12			32	0
64	17			31	25
81	6			32	0
65	16			34	30+

If we consider the data given in these tables we find in the first series, Table I, that from the *posterior level* the number of new segments regenerated is small, the maximum number being 17. In this case there were 64 old segments, showing that the regenerating end was not very near the posterior end, since about 36 segments must have been removed, or what is more probable some of the posterior segments pinched off after the operation. In the other cases where

25 were absent six or seven new segments regenerated. From the *middle region* the number of new segments is 27, 34, 38; a much larger number than in the last case. From the region behind the girdle the number of new segments is 25, 30, 37, 45, 54; which is on the whole a still larger number than the last, although the difference is not very great. The highest number, 54, is higher than the greatest number for the middle region which is 38.

In Table II only one worm is recorded for the posterior level, and this has only 8+ new segments. In the middle region the numbers for the clearest cases are 26+, 34+, 34+. In the region behind the girdle the only worm that regenerated normally had 52 new segments. The results in this table are few but they agree with those of the last one.

TABLE II
September 30 to November 4.
WORMS CUT AS IN TABLE I. INTACT ANTERIOR ENDS

<i>Posterior End</i>		<i>Middle</i>		<i>Behind Girdle</i>	
<i>Old</i>	<i>New</i>	<i>Old</i>	<i>New</i>	<i>Old</i>	<i>New</i>
77	8+	50	26+	38	52+
		55	34+		
		47	34+		
		51	13+		
		53	18+		

In the series recorded in Table III, the number of new segments for the posterior region is variable, owing in part to the fact that the levels from which the regeneration occurs are somewhat different, as shown by counting the old segments. There is one case with a very large number, viz: 24 (with 72 old segments) which gives almost the complete number, but in the other cases where the cut was made at about the 80th segment, only from 5 to 11 new segments regenerated. From the middle region the number of new segments is greater than for the last level, giving a maximum of 51, but most of the other cases produced about 30 to 40. From the region of the girdle the numbers are still larger, with a maximum of 61, the others varying from 31 to 40, or thereabouts. In this table also the data show the same relation between the rates at anterior and posterior levels as do the other two.

TABLE III

December 28 to February 23

TWENTY-FIVE WORMS IN EACH SET WERE CUT (A) IN FRONT OF GIRDLE; (B) BEHIND GIRDLE;
(C) AT MIDDLE; (D) NEAR POSTERIOR END

<i>Posterior End (D)</i>		<i>At Middle (C)</i>		<i>Behind Girdle (B)</i>		<i>In Front of Girdle (A)</i>	
<i>Old</i>	<i>New</i>	<i>Old</i>	<i>New</i>	<i>Old</i>	<i>New</i>	<i>Old</i>	<i>New</i>
72	13	about 50	42	about 34	61	19	0
76	8	about 50	35		37	19	0
74	10	about 50	33		31	20	0
75	11	about 50	34		19 (abn)	21	22 (abn)
78	8	about 50	38		36-40 (abn)	23	17-20 (abn)
88	5	about 50	42		55 (abn)	26	23
71	17	about 50	11		Abn.	25	50
68	4	about 50	51		40+ (abn)		
72	24	about 50	40				
		about 50	24				
		about 50	30				

There is also another series, Table IV (56 days old), including only two levels. From the middle level of the worm the maximum number of new segments is 58 (with only 41 old segments present), while the other individuals have between 23 and 43. From the region in front of the girdle (22 to 26 old segments) the numbers are very variable; 40 new segments being the maximum. Comparison of these results with those in the other tables shows again that in the middle and anterior regions the number of new segments is large, and much greater in number than when the worm is cut in two nearer the posterior end.

TABLE IV

January 6 to March 3

<i>ANTERIOR END INTACT; CUT AT MIDDLE OF WORM</i>		<i>A FEW ANTERIOR SEGMENTS CUT OFF ALSO CUT AT MIDDLE OF WORM.</i>		<i>INTACT ANTERIOR END, CUT IN FRONT OF GIRDLE</i>	
<i>Old</i>	<i>New</i>	<i>Old</i>	<i>New</i>	<i>Old</i>	<i>New</i>
56	39	about 50	30	22	0
55	43	about 50	45	24	0
41	58	about 50	35	24	12+
47	44	about 50	18	24	3+
	23	about 50	50	24	40 (about)
	32	about 50	45	26	30 (about)
	38	about 50	26		
	30	about 50	42		
		about 50	48		
	30	about 50	30		
	30	about 50	48	48	
	24	about 50	23		
		about 35	55		

What is the cause of this difference in the rate of regeneration at different levels? The amount of food available at different levels might appear to furnish the most probable explanation of such differences. For instance, if the food is digested in the anterior part of the body, let us say in the stomach and the anterior part of the stomach-intestine, there will be the same amount present in the worms cut at the three levels; but since on the hypothesis the posterior end uses up more food than it digests, the surplus for regenerative purposes will be greater the less there remains of this posterior region. Hence at the level of the girdle, the regeneration will be more rapid than at the middle, and at the middle more rapid than at the posterior end. I tried to test this possible interpretation in the following way. The head ends of some of the worms were cut off, and also at the same time the posterior ends at the same three levels as before. For two or three weeks, or more, the worms were unable to obtain food, hence if the same difference in the rate of regeneration at the three levels were to occur the assumption that the difference is due to a food relation is disproven. Such was found to be the case, as the following data show. The series were made at the same time as were those given in the preceding tables with which, therefore, they are to be compared. Thus Table V should be compared with Table I; Table VI with Table II; and Table VII with Table IV.

TABLE V
September 30 to November 24
WORMS CUT NEAR POSTERIOR END, AT MIDDLE AND BEHIND GIRDLE; ALSO A FEW ANTERIOR
SEGMENTS REMOVED

<i>Posterior End</i>		<i>Middle</i>		<i>Behind Girdle</i>	
<i>Old</i>	<i>New</i>	<i>Old</i>	<i>New</i>	<i>Old</i>	<i>New</i>
69	9	60	39	34	29
84	7	64	29	37	40
83	9	54	42	35	48
76	13	46	38	34	33+
87	5			33	35 (about)
64	17			34	49
79	16			34	35

As shown in this table there is a marked difference in rate of regeneration between the posterior and middle levels, but not between the middle and the girdle levels; in fact, rather in favor of

the latter, but since the same difference in rate is found between the girdle level and the tail level as is found in intact worms, the results show that the difference in rate is not due to a difference in the food supply.

In the next series, Table VI, the only survivors were those cut at the level of the girdle (except one worm cut at the middle which did not regenerate at all).

TABLE VI
September 30 to November 24
WORMS CUT BEHIND GIRDLE AND A FEW ANTERIOR SEGMENTS CUT OFF

<i>Old</i>	<i>New</i>
35	58+
35	29
—	0
—	abnormal
—	very short

Owing to the difficulty in counting the segments in these worms the results are unsatisfactory; but so far as they go the results show, when compared with those of Table II, that the rate is about the same in both, yet while confirmatory the number of cases is too small to be of much value. In the middle columns of Table IV there are recorded other cases of worms cut in two at the middle, and at the same time some of the head-segments were also removed. The number of posterior segments that regenerated is about the same as in the corresponding set for the same level with intact anterior ends as recorded in the first columns of Table IV. These results, taken in connection with those given above, seem to show that whether the worms are with or without food for a considerable period of the time the number of the segments produced is about the same. Suppose we reverse the argument and assume that, since in the starved worms the materials for regeneration must be supplied by the reserve materials in the worms themselves, then the longer the piece the greater will be the sum total of the reserve supply and hence we should expect more regeneration; but the facts contradict this assumption, for the longer the worms the slower the regeneration. Here again we find that the results cannot be explained as due to the food factor. If we assumed that the anterior end is the storehouse for the reserve, and that the

posterior end merely uses up the food, and, therefore, the longer the piece the less material available for posterior regeneration, we might appear to offer a formal explanation of the results, but there are no facts in favor of this assumption, and the experimental results that are next to be described negative such a conclusion.

In order to examine the relation between the rate of regeneration and the size of the piece the following experiment was made. The worms were cut near the middle and at about the 20th segment from the posterior end in one series and in the other series at the middle and in front of the girdle. The pieces between these levels were used in both cases. In the former only a very few posterior segments regenerated (only one or two), and in the latter cases only 6, 7 and 12. The results are shown in Table VII. This is a distinct difference, to be sure, although the number of segments in both cases falls below those of the check series. The small number of cases is no doubt partly responsible for the few new segments at the middle region, as shown by other experiments to be described, but that this is not the whole question is also shown by the next series.

TABLE VII
January 6 to March 3

ABOUT 20 POSTERIOR SEGMENTS CUT OFF; ALSO CUT AT MIDDLE OF WORM.	New Segments	CUT IN TWO AT MIDDLE AND IN FRONT OF GIRDLE; NEW SEGMENTS FROM MIDDLE	LEVEL	New Segments
	0			6
	1			12
	1			7
	0			
	0			
	1			
	0			
	0			
	2			
	1			
	1			
	0			
	0			

In order to obtain a series of still shorter pieces, the worms were cut into five pieces, each in the following way. Each worm was

first cut in two in the middle (about the 50th segment). The anterior end was then cut into three pieces, by one cut behind the girdle and by another halfway between the anterior end and the girdle. The posterior end was also cut into three pieces; thus, 20 segments were cut from the posterior end and thrown away, the remaining part was then cut in two. All of the pieces of one kind were kept together, and were killed and examined after two months (January 6 to March 3). The check set of longer pieces will be found in Table IV.

TABLE VIII

January 6 to March 3

WORMS CUT INTO SIX PIECES (TAIL PIECE THROWN AWAY). FOR DETAILS, SEE TEXT

E		D		C		B		A	
<i>Old</i>	<i>New</i>	<i>Old</i>	<i>New</i>	<i>Old</i>	<i>New</i>	<i>Old</i>	<i>New</i>	<i>Old</i>	<i>New</i>
13	15	12	18	14	14	26	15 (about)	19	knob
17	4	11	18	13	17			23	18
16	5	12	17	15	14			22	o
15	2	21	6	8	14			19	o
15	o	19	20	15	8				
17	o	16	14	15	knob				
17	o	16	35	14	14				
17	o	11	15	14	18 (about)				
		17	o	—	o				
		21	5	—	o				
		12	8	—	o				

For convenience the pieces will be called A, B, C, D, E; the first being nearer the anterior end, etc. If we examine the piece in the reverse order, beginning with E, we find relatively few new posterior segments in these pieces, although in one case the surprising number of 15 segments have developed. It seems not unlikely that in this case a piece was misplaced. In the D series the number of segments is very variable, ranging from 5 to 35, the latter number giving nearly the entire number lacking at the posterior end. In the C series, whose posterior end is at the middle of the worm, the number of segments is about the same as in the last case, except there is no such extreme case as that mentioned. In the B series only one piece was alive (of the 25 cut off) with about the same number of new segments as in the last cases. In the A series only one

piece had regenerated (from the posterior end of the 23d segment) with 18 poorly developed segments.

With the exception of the E pieces this series does not show any very marked difference for the different levels. There is a possible source of error in the pieces from the posterior region, for I have sometimes found these breaking up into smaller pieces, or pinching off pieces from the posterior end. The small number of the old segments in some of these pieces, where the expectation is about 15, may be due to this; but granting the possibility of such occurrences the main results cannot be due to this factor as will be seen by examining the number of old segments present in the pieces.

If we compare this table with the longer pieces of Table IV, comparing, for example, those cut at the middle of the worm in the two cases, we find that fewer segments regenerate as a rule in these very short pieces. It will be recalled that in none of these pieces does a head develop at the anterior end; on the contrary, most of the more posterior pieces develop a heteromorphic tail on the anterior end. How far this heteromorphic development may affect the result is not clear, but that the small number of posterior segments cannot be due to this factor is shown by comparison with somewhat longer pieces that may also develop heteromorphic tails. The great mortality of these short pieces indicates that they are not under very favorable conditions, although the death rate is especially high at first, and while later the small pieces appear to be in a healthy condition and may live for several months, yet after two or three months they all die of starvation. One point, however, is fairly clear that although the small pieces lack the power to produce new parts at the maximum rate for a given level, yet the retardation is far from being proportionate to their small size. From this fact we may safely conclude that the amount of the food supply in the piece is not the main factor in its rate of regeneration, although when it has decreased below a certain point regeneration may stop or be much retarded for want of materials. These negative conclusions in regard to the rate of growth are useful in so far as they clear the way for the discussion of the main problem of what factors regulate the rate of growth, for, if it is not due to

food the road is clear to search in some other direction for the meaning of the facts. Before attacking this fundamental question I should like briefly to bring into relation with the foregoing results certain other facts that I have already published.¹

I have shown that if 10 to 12 posterior segments are cut from the posterior end of a worm, and then the next 10 to 12 segments are cut off, the piece lying between these cuts does not as a rule produce any new posterior segments, even after three and a half months. Whole worms, however, that lack the last 10 or 12 segments regenerate a few segments in this time. In another experiment, long pieces from the middle of the worm, having the anterior end also removed, were compared with similar pieces with the head end intact. The rate of posterior regeneration was the same for both. Again, some worms with only a few posterior segments removed were compared with similar ones which lacked also the head end. The regeneration at the posterior end was the same in both after a month showing that the results do not depend on the question of the taking in of food. These and a few other experiments are in harmony with the results described above.

So far the results have been judged by the number of the new segments produced; in other words, by the *differentiation* of the new part. If, however, we test the results by the size of the new part certain differences are apparent. The most general result is seen at once when well-fed worms are compared with starving pieces.

The new part in the well-fed individual is larger, and this is especially noticeable in the diameter of the piece, that often approaches that of the old part. The individual segments are also larger in the well-fed worms, so that for an equivalent number of segments the new part is longer. In the starved worms the new segments are often very small, especially when the old part is a very short piece. In striking contrast to this difference in size, which is so apparent that it is not necessary to take measurements to discover it, the number of segments is, as we have seen, approximately the same in a well-fed and in a starved individual. These

¹Morgan, T. H. Regeneration in *Allolobophora foetida*. Archiv f. Entw. Mech., 1897.

results show that while the size of the new part is dependent on the food supply, the growth and differentiation of the new part is to a large extent independent of this factor. The difference in the size of the new part in the two cases shows, nevertheless, that the new part is affected by the conditions of the food supply, and it is probable that the smaller number of posterior segments regenerated by very short pieces is the result of the lack of food for further growth, hence the pieces from different regions show approximately the same number of segments, but the difference in rate of regeneration of larger pieces at different levels cannot be accounted for in this way as the experiments first described have shown. So long as there is enough food material in the blood or other fluids of the body to allow growth to take place at all it goes on at a rate determined by the peculiarities of each level, and largely independent of the food supply.

If my conclusions from the data are correct, and the difference in the rate of development of the new parts at different levels is not, in the main, due to differences in the food supply, to what is the difference due? The answer that first suggests itself is that the difference must be due to differences in the materials of the worm at different levels. If by differences at different levels we mean the differentiation of the parts, or the kind of material that exists at each level, then the conclusion may express a part of the truth, but if we mean that each level possesses limitations in its powers of growth, not possessed by other levels the answer can be shown to be inadequate. For example, if the rate of regeneration from the middle of the worm were determined by certain peculiarities of its material, we should expect the same rate to continue until the entire missing part was replaced; and if we were then to cut off the tip of the new tail after it had completed itself it ought to regenerate as rapidly as does the new part from the middle of the worm, because its material has come from that level. While I have not actually carried out this experiment there can be no doubt that the newly regenerated tail would show at its tip the same retardation shown by the unregenerated tail. Furthermore, if this view were correct how could we explain the termination of the growth process when the normal number of segments has been

replaced? Why should not the new part continue indefinitely to grow? If we can find the explanation for the cessation of growth at the proper terminus we can probably find also an explanation for the difference in the rate at different levels, for, as can be shown, the two things appear to be one and the same. In other words, as the new part grows longer its materials change, and this change is of such a kind that it leads to the cessation of growth. Hence starting under different conditions at different levels the same end result will be reached in all cases, and when the terminus is reached the growth should slowly decline as we find in fact that it does. It is this idea that will be developed later.

There is another point that must be mentioned here. The difference in rate is not so much due to an initial difference in the appearance of new material at different levels, as to the relatively greater slowness of the growth of a terminal part after it has been once started. This seems to mean that the stimulus for the formation of the new part is so much greater than the difference in the rate for each level, that the latter becomes entirely overshadowed in the formation of the first new tissue. Moreover, the proliferation of new material as the result of an injury may depend on a different set of factors than the subsequent rate of growth of a new part after the original stimulus that led to the proliferation has disappeared.

In order to compare the rate of regeneration at the beginning and toward the end of its period of growth some of the same lot of worms that gave the records of Table IV were kept alive for another month (January 6 to April 6) in order to see how much further the regeneration of new segments would continue. The results are given in the following Table IX.

TABLE IX

CUT AT MIDDLE		CUT BEHIND GIRDLE		CUT NEAR POSTERIOR END	
<i>Old</i>	<i>New</i>	<i>Old</i>	<i>New</i>	<i>Old</i>	<i>New</i>
48	52	33	13 (abn)	75	10
59	32	32	72	75	8
41	53	36	60	63	17
52	36	34	52		
51	40	34	55		
51	46	33	45		
53	42				

The number of new segments from the middle region is about the same as it was a month earlier; the full number having been nearly reached, although not quite in a few cases. More new segments are found in the worms cut just behind the girdle; the number approaching completion here also. In the cases where about 25 segments had been cut from the posterior end, the number removed had not yet been made good, and the same holds for the single case where about 37 old posterior segments had been removed. The results show a much smaller proportionate increase during the third month than during the two preceding ones. In other words, as the new part approaches completion its rate of regeneration declines.

REGENERATION OF THE CAUDAL FIN OF *FUNDULUS*

Experiments on the regeneration of the caudal fin of *Fundulus* were carried out during the summer of 1905, while occupying the Table of Columbia University at the Marine Laboratory at Woods Hole, and continued throughout the present winter at the New York Aquarium, where, thanks to the admirable arrangements and to the opportunity for scientific work extended by the Director, Mr. Chas. Townsend, it has been possible to carry on the work under the most favorable conditions.

In two former papers¹ dealing with the regeneration of the fins of *Fundulus* and of some other species of fish I have described the differences in rate of regeneration from the basal and distal parts of an oblique cut made through the tail. This difference I referred correctly, as further results have shown, to the retardation of the growth on the more distal parts. This retardation, however, did not seem to me to belong to the same category of facts as the retardation from a more distal squarely-cut surface, and it was primarily in order to account, if possible, for this difference that I again undertook a re-examination of the facts. The new results have cleared up this point satisfactorily, and I have gone further and been able to obtain data that bear on the more fundamental question of the rate of growth from different levels.

¹Morgan, T. H. Regeneration in Teleosts. *Archiv f. Ent. mech.*, x, 1900. Further Experiments on the Regeneration of the Tail of Fishes. *Archiv f. Ent. mech.*, xiv, 1902.

This problem of the rate of growth at different levels would seem to be a comparatively simple one, but I have found it somewhat baffling, owing to the great individual differences in the rate of growth in different fish. Two methods of study have been followed. In the one, the tails were removed from a number of fish at two or three different levels, and the rate of regeneration measured from time to time in the living fish, or a few fish were killed at intervals and then measured. In the other case the records of the rate of growth of the same fish were kept which gives more accurate data.

The results may be briefly summarized in the following statements. The rate of growth is, *at first*, nearly the same whether the cut is made near the base or near the outer end of the tail. This period covers that of the first proliferation of new material. Very soon, the new parts grow more rapidly from the basal than from the more distal cut surface. In general, the nearer the new part approaches its completion, the slower its regeneration, so that the new part from a distal cut surface very soon grows with extreme slowness, while that from the basal cut continues for a longer time to grow, but it, too, as it gets longer, shows an ever increasing retardation in its growth.

In regard to obliquely cut surfaces, I can confirm my former statements, namely, that regeneration from the more distal part of the oblique surface is much retarded after the first period of proliferation is over. Moreover, this retardation is far greater than that seen in a cross-cut surface at the same distance from the end of the tail, and consequently is not due to the same factor. I can now give some further experiments that throw a great deal of light on this retarding factor in oblique regeneration.

If the tail of a fish is cut off in such a way (Fig. 1) that two cross-cut surfaces *a* and *b* are exposed at different levels, it will be found that on *a*, regeneration progresses much more slowly (after its first beginning) than on *b*. The rate of growth on *b* is as fast or faster than that on a cross-cut surface of the whole tail at this level, while that on *a* is very much slower, and seems almost to come to an end after a time unless the new part from *b* catches up to the

new part on *a*, after which the two new parts continue to grow forward together until the tail is completed.

In the reverse case the cut surface *a* is made the broader of the two, as shown in Fig. 2. Under these circumstances the retardation is smaller on *a*, while that on *b* is the same as in the former case, which is the same as that of an entire cross cut for this level. The results show that the retardation of the distal surface is in proportion to its height, while the growth on the basal cut surface is the same as the regeneration for an entire cross-cut surface at this level. The results are the same whether the distal partial surface is at the top or at the bottom of the tail.

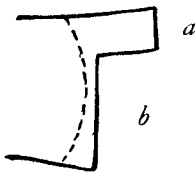


FIG. 1

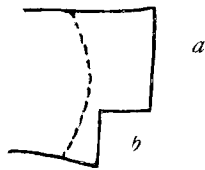


FIG. 2

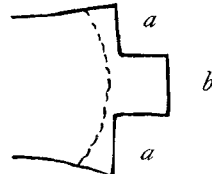


FIG. 3

It might be supposed that the retardation on the outer partial cut *a* is due to this part lying at one side of the tail, where it might be supposed to receive less nourishment. I tested and disproved this possibility in the following way. The tail was cut off as shown in Fig. 3. Here the middle portion has a small and independent cut surface. Regeneration takes place from all three surfaces, but the growth from the outer one *a* was markedly less than that from the other two. After a time the new upper and the lower parts, from the upper and the lower basal cut surfaces, catch up to the middle part, and after this has occurred the three parts, completely united, grow forward as a single organ to complete the length of the tail. In order to prevent this latter result I have cut away after a time the new parts above and below, leaving the middle part intact, and in some cases this was done two or three times before the upper and lower parts had caught up to the middle part. In this way I hoped to discover whether the middle part would finally grow out to its full length, or whether it would not go

beyond a size proportionate to the width of the piece from which it arose. It was found that the new middle part continued to grow, but with extreme slowness. Whether it would finally reach its full length I do not know, but it is not improbable that it would do so.

These results explain, in a way, the regeneration from an oblique surface. If we consider any part of the outer oblique surface by itself alone, it is in the same condition as the outer part in the preceding cases of partial cut surfaces, and the retardation of the growth in both would seem to be due to the same factors, whatever these may be. The outer part of an oblique surface cannot complete itself, except very slowly if at all, until the new tissue from the more proximal parts of the same cut surface has caught up, and set it free, so to speak. This is, in fact, what occurs. The nature of the retarding influence will be discussed after some further facts have been considered.

The details of the experiments in which the rate of regeneration at different levels was examined may now be given.

The first experiment was with *Fundulus majalis*. The operation was carried out on July 7, and the first measurements were made July 26. The same lot of living fish gave all of the measurements. The tails were cut near the outer or distal end, near the base, and obliquely between these two levels. From the basal cut surface the new parts measured $1\frac{7}{8}$, 2, 2, $2\frac{1}{4}$, $2\frac{1}{2}$, $2\frac{1}{2}$, 3, 3. From the distal cut surface the new parts measured 2, 2, $1\frac{7}{8}$, $1\frac{3}{4}$, $1\frac{7}{8}$, 2. Already at this time some of the basal cuts had regenerated faster than the distal ones. The oblique cut surfaces were measured near the top and near the base where the length was greatest. The two measurements for each fish are bracketed, the distal one standing above. They are $\left\{ \begin{array}{l} 1 \\ 3 \end{array} \right\}$ $\left\{ \begin{array}{l} 1 \\ 2 \end{array} \right\}$ $\left\{ \begin{array}{l} 1 \\ 2 \end{array} \right\}$ $\left\{ \begin{array}{l} 1 \\ 2\frac{1}{2} \end{array} \right\}$ The basal measurements correspond with those of the same level given above. The distal measurements are about half as much as the distal measurement given above. Five days later, August 1, the following measurements were taken.

Basal, $3\frac{1}{2}$, 4, 3, 3, $3\frac{1}{2}$, $4\frac{1}{2}$, $3\frac{1}{2}$, 4, 4, $4\frac{1}{2}$

Distal, 4, $3\frac{2}{3}$, $3\frac{1}{2}$, 3, $3\frac{1}{4}$, $3\frac{1}{2}$

Oblique, $\left\{ \begin{array}{l} 2\frac{1}{2} \\ 4 \end{array} \right\}$ $\left\{ \begin{array}{l} 2\frac{1}{2} \\ 4\frac{1}{4} \end{array} \right\}$ $\left\{ \begin{array}{l} 1\frac{3}{4} \\ 4 \end{array} \right\}$ $\left\{ \begin{array}{l} 1 \\ 3\frac{2}{3} \end{array} \right\}$

There seems to be little or no difference at this time between the basal and the distal cut surfaces. The oblique surfaces are much shorter at the distal part than at the basal, as in the last case.

Eight days later the following measurements were taken:

Basal, 7, 5, 6, 7, 5, 6, 6, 7, 6

Distal, 6, 5, 5, 2, 4, 4

Oblique, $\begin{Bmatrix} 3 \\ 6 \end{Bmatrix} \begin{Bmatrix} 3 \\ 6 \end{Bmatrix} \begin{Bmatrix} 2 \\ 5 \end{Bmatrix} \begin{Bmatrix} 2 \\ 5 \end{Bmatrix} \begin{Bmatrix} 2 \\ 4 \end{Bmatrix}$

The new parts from the basal surfaces are noticeably ahead in most cases of those from the distal surfaces. The oblique cuts show the same relation as before.

Eight days later, August 17, the results were as follows:

Basal, 7, 8, 8, 7, 6, 5, 8, 6, 6

Distal, 5, 7, 4, 5, $4\frac{1}{2}$, 4

Oblique, $\begin{Bmatrix} 2 \\ 6 \end{Bmatrix} \begin{Bmatrix} 3 \\ 7 \end{Bmatrix} \begin{Bmatrix} 4 \\ 8 \end{Bmatrix} \begin{Bmatrix} 3 \\ 6 \end{Bmatrix} \begin{Bmatrix} 3 \\ 7 \end{Bmatrix}$

The relation is the same here as before. It is noticeable that even at this time the distal cut surfaces had not regained their full length. Eleven days later the measurements were about the same, no appreciable increase in length being noted. As the fish were not in as good condition as at first the last results are no doubt due to this. Other results, to be described later, where the fish were under better conditions, show that the new growth continues after this time.

The next series of experiments were made with *Fundulus heteroclitus*. The tails were cut off on July 7 and the first measurements made July 20.

Basal, 2 +, 1, $1\frac{1}{2}$, to $1\frac{3}{4}$, $1\frac{1}{4}$ to $1\frac{1}{2}$, 2, 1

Distal, $1\frac{1}{4}$, $1\frac{3}{4}$, 2, $1\frac{1}{2}$, $1\frac{1}{2}$ to $1\frac{3}{4}$, $1\frac{1}{2}$, $1\frac{1}{2}$, $1\frac{1}{2}$, $1\frac{1}{2}$

Oblique, $\begin{Bmatrix} 1\frac{1}{2} \\ 2 \end{Bmatrix} \begin{Bmatrix} 1\frac{1}{2} \\ 3 \end{Bmatrix} \begin{Bmatrix} 1 \\ 2+ \end{Bmatrix} \begin{Bmatrix} 1 \\ 3 \end{Bmatrix} \begin{Bmatrix} 1 \\ 2 \end{Bmatrix} \begin{Bmatrix} 1 \\ 2\frac{1}{2} \end{Bmatrix} \begin{Bmatrix} 1 \\ 2 \end{Bmatrix} \begin{Bmatrix} 1 \\ 2\frac{1}{2} \end{Bmatrix}$
 $\begin{Bmatrix} 1 \\ 2\frac{1}{2} \end{Bmatrix} \begin{Bmatrix} 1 \\ 3 \end{Bmatrix} \begin{Bmatrix} 1 \\ 2\frac{1}{2} \end{Bmatrix} \begin{Bmatrix} 1 \\ 2 \end{Bmatrix} \begin{Bmatrix} 1 \\ 2\frac{1}{2} \end{Bmatrix}$

At this time, 13 days after the operation, there is nothing to show that there is any difference between basal and distal rates of growth. In the measurements from the oblique surface the basal growth is noticeably greater than the distal, and even also greater than the basal growth from the basal cut surface. Whether this is a

real difference, or one due to unintentional cutting nearer to the base in oblique cuts, is difficult to decide, but the latter view is the more probable. The following experiments were carried out on *Fundulus heteroclitus* in the New York Aquarium between December 8, 1905, and January 30, 1906. The water in which the fish lived was warmed to about 69° F. Measurements of the amount of old material cut off were made. They are given below:

Basal, 10, 10, 9½, 9, 9, 9, 9, 8

Distal, 5½, 5½, 5, 5, 5, 5, 4½

Large fish were used in this experiment. The distance from the rounded end of the scales covering the base of the tail to the end of the tail measured, as shown above, about 10. Hence the basal cut passes just at the edge of the scales. It was found better not to cut closer than this level to the base; for further forward there is soon reached a region from which regeneration is abnormal and often delayed. The first measurements (of the tails of a few fish that were killed and put into formalin) after 21 days (December 29) were as follows:

Basal, 3½, 3, 3, 3

Distal, 3½, 3½, 2½, 2½

The rate at the two levels seems to be nearly the same at this time. The next measurements were made after 38 days (January 15).

Basal 5½, 5½, 5, 4

Distal, 4, 4, 3½, 3½, 3½

The new part from the base has now outstripped that from the distal level, although the latter has not yet reached its full growth.

The third and last set of measurements were made after 58 days, January 30.

Basal, 6, 6, 5½, 5, 5

Distal, 3½, 3

By this time the basal surfaces have regenerated nearly twice as fast as have the distal ones, although the latter still fall short of their full length, which they would have attained had they regenerated as fast as the basal cut surfaces.

In the last series the new tails had not regained their full length after 58 days. In order to obtain later stages for comparison a

new series was started February 16 and continued until May 11, a period of 84 days. The results are given in the following tables. For comparison I give a few measurements of the lengths of the old pieces cut off February 16. The pieces cut off from the distal end measured in 10 cases 3, 3, $3\frac{1}{4}$, $3\frac{1}{2}$, $3\frac{1}{2}$, $3\frac{1}{2}$, $3\frac{1}{2}$, $3\frac{1}{2}$, 4, $4\frac{1}{2}$; and from the basal end 7, 7, $7\frac{1}{4}$, $7\frac{1}{2}$, 8, 8, 8, 9. Subsequent examination showed that some of the distal cuts had been unintentionally made nearer the middle part of the tail (those measuring 4 mm. above, for instance) and these have been included in the tables in the column marked "Middle."

		<i>Basal</i>				<i>Middle</i>		<i>Distal</i>						
March	3	1½	2	2½	2½	1¾	2	1½	1½	1½			
	17	2½	3½	4		3	3½	1½	1½	2			
	31	4½	4½					2	2	2¼			
April	16	4¼	4½	4½		3	4	1	1¼	2	2	2¼	2¼
	31	3¾	3⅞	4		2¾		1½	2	2	2½	2¾	
May	11	4	3	3¾	3⅞	3		1	2½	2¼	2		

The results show that even after 84 days the new parts from the distal end had not reached their full growth, neither had the new part from the basal cut, although it was longer than the former, and enough had been produced to have completed the new parts of the distal cut had it regenerated as fast as the basal. There is for both levels a marked decrease in rate in the later stages which is the greater the more distal the cut surface.

The slow rate of regeneration from distal cut surfaces, shown in the preceding results, led me to examine this question further. A terminal piece was cut from the tip of the tail much shorter than in any of the preceding cases in order to see whether the same delay would manifest itself in this case, or whether the initial growth would replace at once so small a part. On March 3 a very narrow piece measuring 2 mm. was cut from the tip of the tail. On April 6 the new part measured $\frac{3}{4}$ mm.; on April 14, $1\frac{1}{2}$; on April 28, 1 mm. In a control, in which as much as 7 mm. was cut off, the new part measured 2 mm. on April 6, and 3 mm. on April 28. In both cases the fish had been kept in dishes in the laboratory and were under rather poor conditions, lacking food and sufficient air. Nevertheless the regeneration went on at about the same rate as when the conditions are better.

In another case, 2 mm. was cut off from the distal end of the tail on February 17. On March 1 the new part measured 1 mm.; on March 17 it measured $\frac{2}{3}$ mm.; on March 24, $1\frac{1}{4}$ mm.; on April 6, $1\frac{1}{2}$, on April 31, 2 mm. Thus it took two and a half months to reproduce 2 mm. at the distal end, an amount that could be produced in two weeks from a basal cut. In another case $1\frac{1}{2}$ mm. was cut off on February 17. On March 17 the new part measured about 1 mm.; on March 24 it still measured 1 mm.; and on April 31, 1 mm. Thus at the end of this time the part cut off had not been completely restored.

In the same series as the one of December 8 to January 30, other kinds of operations were also carried out. In several cases cross-cut surfaces were made at two levels, as shown in Figs. 4 and 5. The rate of regeneration from the two levels is shown in the following figures, with the numbers attached. The first series is from December 8 to December 29.

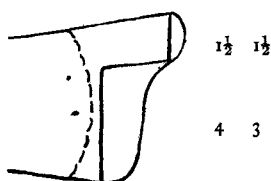


FIG. 4

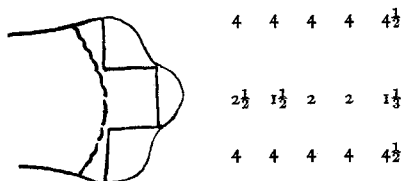


FIG. 5

It will be seen if these results are compared with those of the preceding tables, in which entire cross-cut surfaces are involved, that the regeneration from the distal partial surface in these cases is greatly inhibited, being not more than one-third as much as from an entire surface at the same level. The statement holds both when the partial surface is at the edge and when it is in the middle of the tail. The next series (December 8 to January 5) gives further cases of this sort.

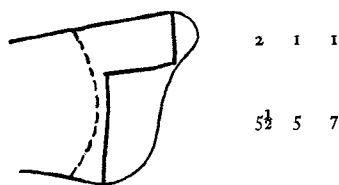


FIG. 4a

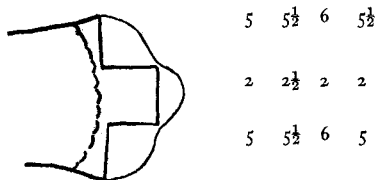


FIG. 5a

During the interval of 38 days between these two series the new part from the outer cut surface has grown very little, but that from the inner cut surface has grown as fast, and apparently a little faster, than from the entire cut surface. Here also the greater regeneration from the base is probably due to unintentionally cutting the partial basal surface nearer the base of the tail as examination showed. The third series (January 30) gave the following measurements:

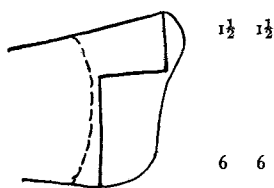


FIG. 4b

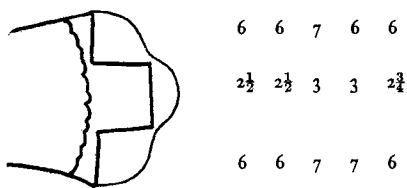


FIG. 5b

The new part from the outer surface is longer in some cases, and this is undoubtedly due to the new growth from the basal surfaces having reached and passed the level of the distal cut. Other series show that in this way the tail may finally complete itself.

Three other series of experiments gave essentially the same results, and, therefore, need not be cited. One point of some additional interest is furnished by these other series. The rate of regeneration from the outer partial cut surface is greater the broader, *i. e.*, the higher dorso-ventrally, the cut surface. This result shows that the retardation is directly connected with the height of the cut surface, and only secondarily with its distance from the base of the tail.

REGENERATION OF THE TAIL OF THE GOLD FISH *CARASSIUS-AURATUS*

This fish has a forked tail which introduces some new factors into the problem. The most important fact connected with the regeneration of a forked tail is that the new part becomes forked very early, before, in fact, the new part has grown out to the level of the old notch. This shows that the distal end of the new tail

is very early laid down, although the later growth must take place very near the outer edge of the new tail; the tail retaining its forked form during the whole of the subsequent growth. The results for three cases in which the tails were cut squarely off, one near the base, the other two nearer the distal end, are shown in Fig. 6. The distance of the first from the notch is 8 divisions (= 4 mm.), and of the latter 2 divisions (= 1 mm.), and of a third 4 (= 2 mm.) The measurements give the rate in growth in the middle and at the upper and lower parts where the lobes grow out.

The results show that the growth is rapid at first, especially when we consider that the healing over of the cut surface and that the arrangement of the new materials take place during this time. The notch soon appears in the new tail owing to less rapid growth in this region. It will be noticed that the middle region is nearer its definitive end than the upper and lower parts of the tail and this may account for its retardation. It also seems, as far as I could judge, that the notch appears sooner when the regeneration is from a distal than from a more basal cut surface, and this is in accord with the idea just expressed. It is not improbable, however, that the appearance of the notch is due to other factors. The upper and the lower parts continue to grow longer after the middle part has reached or nearly reached its goal. At least this seems to be the case, although the middle part may also continue to grow, but so slowly that its progress was not observed in the somewhat rough measurements that I have made.

In another experiment with gold fish, measurements were not taken but sketches of each fish were made at intervals, and as the results illustrate some other points, they may be briefly mentioned here. In one set the exceedingly slow growth of the middle part was noted, the notch had not appeared after 45 days, although the middle had nearly reached its outer level. The upper and the lower parts had grown faster, but had not reached their limit at this time. By January 13 the notch was present, although the level of the old notch had not been reached. Its appearance is due to the greater growth above and below. In another set the notch appeared soon because the cut was made nearer to the old notch. The upper and the lower parts had grown past the level

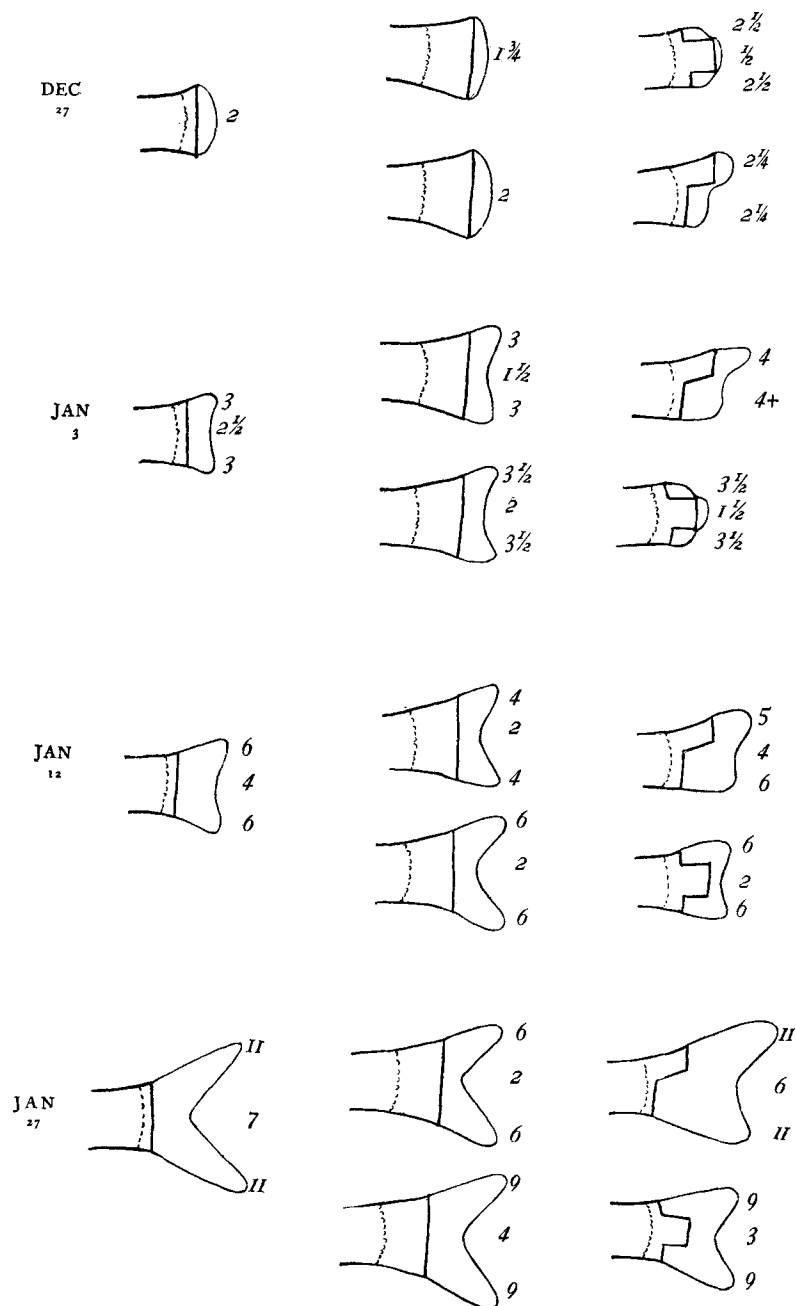


FIG. 6

of the old notch. In another case a small outer and a large inner surface was left. The new growth on the latter was removed once. The development was delayed, and even on February 15 it had not reached its full length. At this time the notch was present and the upper and the lower parts were of the same length.

AN EXAMINATION OF THE RELATION BETWEEN THE INCREASE IN
SIZE IN NORMAL AND IN REGENERATING SALAMANDERS

The rate of growth at different stages of embryonic and larval development and its cessation in many species when a certain size or condition has been reached furnishes some of the most perplexing and important problems of morphology and physiology. It has proven difficult in the case of normal development to attack this problem except by measuring the different rates of growth at different stages of development, but this offers little opportunity to test experimentally any conclusions that the measurements may lead one to draw. It would seem that a chance to study the problem experimentally is afforded in the remarkable power to regenerate shown by many animals. The surprising fact has never, I believe, been sufficiently appreciated, that regeneration means a sudden and rapid renewal of the growth process, which takes place not only in those animals that have unlimited powers of normal growth, but also in those whose normal growth is limited within rather narrow boundaries. The fact that an animal that has ceased to grow larger will replace a lost part shows that its growth has come to an end not because of the loss of the power to grow, but because of some retardation of normal growth that has taken place.

It is commonly supposed in the case of an animal that has reached adult size that an equilibrium has become established between the amount of food that can be digested and the body weight. Suppose, if this is true, that a part of the body of appreciable weight is suddenly removed without affecting the surface of absorption of the digestive tract, will the animal quickly regain its lost weight before the missing part has regenerated? In the case of an animal that has not reached its upper limit of adult size, and

in the case of one having no well defined upper limit the problem is more complicated, but if the size at any one time is due to an equilibrium between the food digested and the body weight, we might anticipate on the theory that an animal lacking a part of the body would increase more rapidly in proportion to its weight than an intact animal. The salamander that I have used to test this point belongs to the latter class. The experiment was as follows: The tails of a few salamanders were cut off at the base, the animals being weighed both before and after the operation. They were then weighed at intervals of a week during the period of regeneration of a new tail. Control, intact animals were also kept under the same conditions and their increase in weight also recorded. The tailless animals have had their body weight suddenly decreased by the loss of the tail, while the organs of digestion remain as before, hence, since the tailless animals have less body weight to keep up, they might be expected to increase in proportion to their weight faster than the check series. If both sets increase in weight, as the results show to be the case, the body weight of both would be expected to increase at the same rate, but the amount of material that goes to nourish the tail in the tailed animals might go as additional increase to the body of the tailless ones. Hence, as I have said, we might expect at most only a greater increase in the tailless set, in proportion to the amount removed in the tail which is about one-seventh to one-tenth of the total weight. Unless the weights show a very constant although small difference it would not be possible to detect the supposed influence. Unfortunately, the outcome has shown that the increase is too variable to furnish very definite information on this question.

There is a further possible complication in the results. The presence of a regenerating stump may in itself react on the rest of the body, and cause the general growth to take place faster, in somewhat the same way that the presence of young in the uterus of a guinea pig, itself not fully grown, does not interfere with the normal growth of the parent,¹ but on the contrary, the powers of

¹Minot, C. S. Seriescence and Rejuvenation. *Jour. Physiol.*, xii, 1891.

assimilation seem to be increased, since under the conditions the mother digests enough material not only for her own normal growth but also for that of her rapidly growing progeny as well. In the case of the guinea pigs the result is complicated by the amount of food taken by the mother, which may be greater during pregnancy since the amount does not appear to have been regulated; but in the case of the salamanders the amount given was carefully controlled.

The salamanders were kept in flat dishes containing half an inch to an inch of water, and were fed on small pieces of raw beef about every other day. As they take food from the forceps the amount that each takes can be regulated, which would not be the case if the food were simply left in the dishes. As the animals grow larger they can swallow larger pieces, hence for some time their weight can be steadily increased by increasing the size of the piece of beef given to each.

The animals were not young, so that their increase in size is not to be ascribed to the increase from youth to age. The life history of this species insures the maturity of all the individuals, since Gage¹ has shown that they pass two years on land before undergoing the final changes that transform them into the aquatic *Diemyctylus*. The susceptibility of the animals to the amount of food is shown not only in their rate of increase, but also by the rapid loss in weight when food is scarce or absent, although they may be kept alive for several months deprived of food. Hence in weighing the animals care must be taken to weigh them at corresponding times from the last feeding. Failure in this respect is at once shown in the weight, as a few cases in the tables indicate. As a rule the last feeding was 24 hours before the time of weighing. The average of the five heaviest animals that I obtained by a steady and abundant feeding for three months was 3.14 grams and the minimum average weight for five starved individuals kept in a similar dish was 1.00. In the well-fed set there were considerable individual differences. Thus the largest individual in the same set weighed 3.25 and the smallest 1.62 grams or about half. I isolated one of

Gage, S. H. Life History of the Vermilion Spotted Newt. *Am. Natural.*, xxv, 1891.

the smallest individuals in the hope of determining the cause of this difference and found that it took less food than the others. No doubt its smaller size is directly connected with this condition.

The most important fact connected with the increase in size of *Diemyctylus* is that the increase is not due to the storage of fat, as the weight of a man who had reached adult size might be increased by over feeding, but to an actual increase in size of nearly all of the organs of the body. The increase is, therefore, to be ascribed to growth, not the storage of reserve material. The starved animal may live for a long time at the expense of its formed tissues. This possibility indicates that an animal of average size, kept without food, may still be able to supply a regenerating part with materials for growth—the interesting point being that one part is rapidly growing at the expense of the rest of the body. The simplest interpretation of this result is, I think, that there must be present in the blood at all times a certain, although perhaps variable, amount of nourishing materials, and that a newly regenerating part has the power to take from the blood the materials that it needs for growth, even when the amount present in the blood has fallen so low that the rest of the tissues cannot maintain themselves, but break down to supply the blood with a certain amount of nutriment. If this idea expresses approximately the relation that exists, it follows that while the new part requires a certain amount of food in order to continue growing, it can take advantage of a condition that the older or differentiated tissues cannot make use of; in fact, when the latter slowly lose ground. There is apparently a similarity in this respect between an embryo and the newly regenerating part. Since in regeneration the new part is formed directly out of the old tissues we may assume that this property of young parts is something connected with their lack of differentiation, which is lost when differentiation takes place, and is regained again when the differentiation is lost.

In the first experiment the tails of six salamanders (three males and three females) were cut off near the base. In another set (also of three males and three females) the animals were intact. The former weighed 1.46 grams a piece (average weight) and the tails cut off weighed about 0.16 gram. Owing to a loss of blood, etc.,

the tailless animals were weighed again the next day, and their weight (1.28 grams) was found to be in this case about the same or a little greater, due perhaps to the absorption of water by the cut surfaces. The control set were at the beginning a little heavier, weighing 1.85 grams. The following table gives the average individual weight of these two sets of animals from December 12 to February 3. On January 5 one of the tailless animals died, and on January 10 another.

TABLE SHOWING THE INCREASE IN WEIGHT OF TAILLESS AND INTACT SALAMANDERS
TABLE A

	Tailless	Rate of Increase	Control Intact	Rate of Increase	Tailless	Rate of Increase
Dec. 12.....	1.46 (intact)		1.85			
12.....	1.22 (tailless)					
13.....	1.28	.06				
18.....	1.53	.25	1.74	— .11		
27.....	1.57	.04	1.65	— .09		
Jan. 5.....	1.88	.31	1.98	.33	{ 2.28 (intact)	
13.....	1.95	.07	2.06	.08	{ 2.08 (tailless)	
20.....	1.96	.01	2.13	.07	{ Feb. 4	
27.....	2.13	.17	2.36	.23	{ 2.01	
Feb. 3.....	2.08	— .05	2.23	— .13		
10.....	2.18	.10	2.30	.07	2.12	.11
17.....	2.22	.04	2.34	.04	2.33	.21
24.....	2.36	.14	2.45	.11	2.34	.01
March 3.....	2.34	— .02	2.56	.11	2.41	.07
10.....	2.45	.11	2.43	— .13	2.51	.10
17.....	2.38	— .07	2.41	— .02	2.45	— .06
24.....	2.64	.26	2.78	.37	2.73	.28
31.....	2.66	.02	2.66	— .12	2.80	.07
April 7.....	2.83	.17	2.78	.12	2.84	.04

From this table it appears that both sets steadily increased in weight, but the increase was greater in the tailless set. The difference in rate during the first two weeks, before the new tail has appreciably developed is much greater in the tailless set. There is a gain of .35 for the tailless animals and a loss of .20 in the intact set. If the next week is taken into account when both sets made great gains the balance remains nearly the same. Their

differences may be due to uncontrolled factors—and in part I suspect this must be so, for there is no assignable cause for the decrease in the intact series—but if the difference is directly connected with the absence of the tail in one set it is obvious that the entire loss was made good, which is a greater increase than we should anticipate since the expectation would only be a proportionately greater increase in the tailless set.

The tables also show that after four months the tailless set had doubled the weight while the intact ones had increased only one and a half times.

The greater increase in weight in the tailless set might be supposed to be due to the stimulus of the growing part on the body as a whole, as said above; or the difference may be a purely fortuitous one, the tailless animals happening to be a faster growing set. By January 27 the new tails were about one-half to one-third of an inch long, and their increase in length continuously adds new weight to the tailless set. In order to see if the same result would follow if some of the intact individuals had their tails removed, they were separated in two lots; one lot kept intact and the other curtailed. The last two columns of the table give the rate of growth of the tailless set. The average weight before the operation was 2.28 grams; afterward 2.08 and the following day 2.01. The data are controlled by those of the middle columns. It will be noticed that the control, February 3, weighed a little less than the animals whose tails were to be cut off. During the two weeks following the operation the tailless salamanders gained .32 gram while the intact ones gained only .11 gram. If the third week is also taken into account the tailless animals gained .33 gram, and the intact ones .22 gram, which gives still a difference in favor of the former. When the experiment closed the tailless animals had gained .83 gram and the intact .55 gram.

Still not convinced that the difference in these two cases might not be due to uncontrolled conditions, I started two new sets on March 2. The results are given in the next two tables. The animals used had been kept all winter in the laboratory, and were under-fed, but for ten days before the operation they had been well-fed. Two of the animals in this set had had their legs cut off

some time before and were regenerating new ones, which introduces, perhaps, a disturbing factor in the result.

TABLES SHOWING THE INCREASE IN WEIGHT OF TAILLESS AND INTACT SALAMANDERS

TABLE B

	TAILLESS		CONTROL	
		Rate of inc.		Rate of inc.
March 2..	Intact			
	1.46			
	tailless			
	1.32		2.05	
4..	1.30	—	—	—
10..	1.45	.15	2.41	.36
17..	1.46	.01	2.12	.29
24..	1.67	.21	2.22	.10
31..	1.96	.29	2.33	.11
	one sick		one died	
April 7..	1.93	.03	2.57	.24
	one died			
14..	1.93	.00	2.70	.13

TABLE C

	TAILLESS		CONTROL	
		Rate of inc.		Rate of inc.
March 2..	Intact		1.24	
	1.68	—	—	—
	tailless	.01	1.40	.16
	1.48	.00	1.53	.13
3..	1.56	.20	1.63	.20
10..	1.57	.35	1.67	.04
17..	1.57	.04	1.67	.00
24..	1.76	.02	1.71	.04
31..	1.91			
April 7..	1.96			
14..	1.98			

The left hand table (B) shows that during the first two weeks the tailless set again gained faster, but there is a strange rise and fall in the control set that probably makes the result of little value. If we take another week into account when the disturbance may have had time to subside the rate of increase of the tailless set is still double that of the intact ones.

In the right hand table (C) the control animals increased in weight at first much faster than the tailless, but later the tailless ones gained much more rapidly than the control.

Our examination of the tables shows that it would be hazardous to ascribe the greater initial gain (in three of the former cases) in the tailless animals to the loss of the tail, although this may be the case. The difference when it occurs seems too great to be due to a *proportionately* greater increase as the result of the loss of a part; and if not due to variable factors, *i. e.*, accidental, it may mean that the changes taking place at the cut surface incite the digestive tract to greater activity or the cells of the body to greater assimilation.

In the first series (Table A) there is not only an initial greater

gain in the tailless set but an actually greater increase in weight throughout the series. This might be attributed to the influence of the regenerating tail on the growth of the rest of the body, but as the difference is not found in the other three series, and in one set, in fact, the intact animals grew faster, we must conclude that there is no clear evidence in favor of the view that a regenerating part has in its later stages at least an influence on the digestive or assimilative changes that take place in other parts of the body. The great powers of growth in a regenerating part may be local in their influence and not transferable to other parts. The question is, however, worthy of further examination.

CONCLUSIONS

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 rate of growth from partial as compared with entire cut surfaces, and the rate of growth on different parts of the same oblique surface.

The question whether the differences 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 part, for example, is affected by the amount of food, in 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 the limb of *Diemyctylus*, and in the growth in length of the tail of the earthworm and of the salamander 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 it possible for this part to draw the necessary nourishment from the blood, although the amount present in the blood is below that which is necessary to maintain *in statu quo* the differentiated tissues. These slowly decrease in size and in the number of their cells, while the new part is increasing in size and in the number of its cells. The ultimate physiological-chemical basis on which this difference between differentiated and undifferentiated materials rests is entirely unknown at present. 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, as will be apparent later on.

It has been shown in the fish that the rate of growth is retarded on a partial surface, provided the surface does not connect at one or at both ends with the rest of the tail. For instance if the tail is cut off, as shown in Fig. 7A, the outer free cut surface *a* regenerates more slowly than does an entire cross-cut at the same level; but if the partial surface is continuous with the rest of the tail at the same level as in Fig. 7A at *b*, it regenerates at the same rate as does the entire cut surface at this level. Results similar to the last are found when a square piece is cut out of the middle of the tail, as in Fig. 7B. The proximal, partial cross-cut surface *b*, continuous both above and below with the rest of the tail, regenerates as fast (or faster than) an entire cross-cut at the same level. It is to be remembered that the longitudinally exposed edge connecting the cross-cut surface *a* and *b*, does not proliferate in a vertical direction except in so far as to cover the exposed surface and to complete the structure as far as the next fin ray.¹ Additions to the

¹The explanation of this seems to be that fin rays cannot develop new ones except from the cut ends of the old ones. If one were split lengthwise it would probably complete itself, but not produce new ones.

new part from the basal cut surface are not made from this source, and the rate of growth of the basal part is not, therefore, increased in this way, but the growth from the base seems to be faster, nevertheless, along the line of the longitudinal cut surface, as seen for example in Figs. 7A and B, than at the opposite edge where the new part is free. In some way the presence of the new material along the longitudinal edge accelerates in its vicinity the growth of the new part from the base.

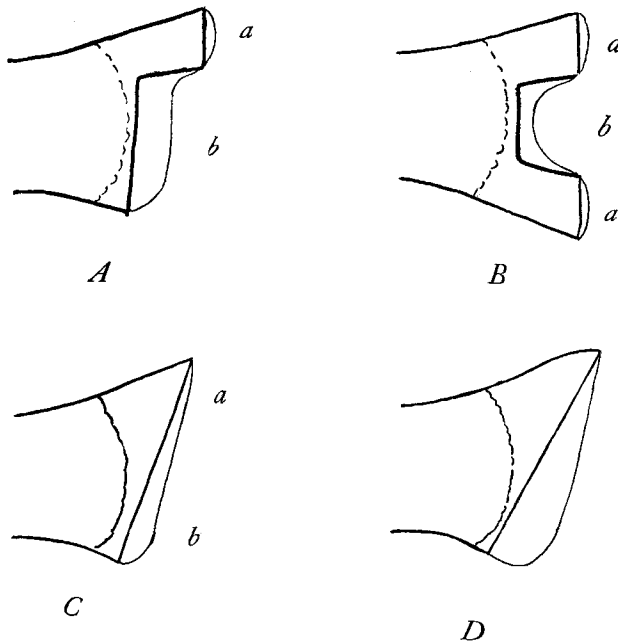


FIG. 7

What factor retards the development of a free, partial cut surface? The retardation is not due to the level, for it occurs at all levels alike. It is in proportion to the height of the free cut surface, hence the retardation must be in some way connected with the height of the base from which the new part arises. One might be inclined to interpret this result as due to a proportionate devel-

opment, by which I mean that the regulation of the growth is such that its rate is proportional to the base from which it arises. But even if this were the case, it would give no causal explanation of the results, unless it be assumed that proportionate development is in itself a vitalistic explanation and a causal one in that sense. But the facts do not seem to bear out this interpretation, for, while the growth on a free partial surface is undoubtedly delayed, there are some indications that it might continue slowly toward the natural terminus of its development. At least in the gold fish I have obtained evidence of this sort in two cases, where the lower lobe grew out slowly to its original length. In *Fundulus* the evidence is not so clear, but mainly on account of insufficient material.

Now the factor that seems to be responsible for the retardation in the growth from a free partial surface appears to be one involving the pressure relations in the new part. Observations show that the new part from a partial surface is rounded at the sides (as is also the new part from an entire surface), and this condition at the sides appears to be responsible for the retardation of the rest of the new part, for, since the rounding is nearly the same for a new part from a partial and from an entire surface, the retarding influence will be the greater the shorter the base. In contrast with this retardation from a *free* partial surface we have seen that when a partial surface is not free but continuous at one or at both ends with the rest of the tail, as at *b* in Fig. 7A, there is no retardation. In this case the side of the new part, that is continuous with the old part, is not rounded, but even with the rest of the new growth, hence the rate of growth is not retarded on this side, while on the free side the relation is the same as when the growth takes place from an entire cut surface. In fact, the growth of the new part on the side in connection with the old tail often seems to be accelerated, especially along the longitudinal cut surface that is also proliferating a little new material, and this condition is in accord with my interpretation.

The new part from an oblique surface also shows a retardation of growth on the more distal parts of the cut surface, and the difference between the rate of growth on the more distal and the more basal parts is in proportion to the degree of obliqueness of the

cut surface, as seen in Figs. 7C and D. The retardation in this case seems to call for the same explanation as that observed on a partial surface, for, if we consider by itself alone any part of the more distal surface it may be treated in the same way as a partial cut surface, because, despite the fact the parts lying next to it at a lower level grow faster than the part in question, yet owing to the fact that the part below is still behind that of the lower end of the postulated part, there will be a retarding influence on the growth of the latter. The pull or tension that exists will, on the theory, hold back the parts just above it. If we imagine this same influence existing throughout the whole of the new growth on an oblique surface, we can get an insight into the factor at work, and see how this case falls under the same head as that of the growth from a partial surface. Our analysis and experiments with oblique cut surfaces lead, therefore, to the conclusion that the slower growth over the more distal part of the oblique surface is not due to its more distal position, for comparison with cross-cuts at the same level disprove this interpretation, but the result is due to another factor. This factor is a formative one in the sense that the failure of the maximum potential of growth over the more distal part of an oblique surface is due directly to the new growth below it not having reached the same level, and owing to this difference there arises a pull or tension on the part that retards its maximum possible rate.

Before taking up again for further analysis the principal question of the retardation of growth at different levels, I should like to clear the way still further by referring briefly to the kind of regeneration from the anterior cut surface of the earthworm (or of *Lumbriculus*) and from the oral and basal ends of a piece of the stem of *Tubularia*. Both of these cases may appear to stand in contradiction to the conclusions so far reached in regard to posterior growth in *Diemycytus*, *Fundulus* and *Lumbricus*. In reality, as I hope to show, there is no contradiction between the interpretation of the two classes of facts.

When one segment is cut from the anterior end of the earthworm, it is replaced by one; when two are cut off, two regenerate; when three are removed, three regenerate, and so on up to five, although for five sometimes only four come back. When more

than five are cut off only five (or sometimes only four) regenerate. This rule holds only for the anterior end. As the region of the gizzard is approached (about the 15th segment) the head, if it develops at all, is abnormal; and behind this level there regenerates a heteromorphic tail from the anterior end. In *Lumbriculus*, when from one to seven anterior segments are removed, the same number is regenerated; when more than seven, six or seven come back; and this rule holds for the greater part of the length of the worm, since there is no such regional limitation in this species for head formation as in the earthworm.

In the regeneration of the anterior parts in these two species there is to be observed no such difference in rate from different levels as seen in posterior regeneration, and this is the relation referred to above that may seem to be in contradicton to the conclusions reached in the case of posterior growth. But it is to be recalled that we are dealing here only with the part that is first laid down, and not with subsequent growth after the terminal organs have been formed. The comparison should properly be made in the two cases only between the terminal organs. The formation of the head segments that are all laid down at the same time is comparable with the formation of the terminal posterior segments that are formed in all cases of posterior growth. I have no observations in the earthworm showing that there is any difference in the time of formation of the posterior terminal segment from cuts at different levels, and in the salamander I could detect no such difference. If such exists the difference is slight and in this respect the conditions are similar to the formation of a head at different levels, which also seems to take place at the same time, although the possibility of slight differences that were not detected must be granted for both cases. In both head and terminal segment the centripetal influences seem to be the predominating ones.

These considerations show that, in principle, there is no conflict between anterior and posterior regeneration. The difference found in the latter case is due to later growth in the posterior end, and no such growth takes place in the anterior end.

It has been shown in *Tubularia* that the time required for the formation of a new hydranth depends on the distance of the cut

surface from the old hydranth.¹ The nearer the cut surface to the oral end the quicker the regeneration. The same law holds also for the development of the aboral hydranth from the aboral end of a piece. In both of these cases we are again dealing with the development of a terminal organ in the formation of which the centripetal influences predominate. Therefore, the difference in the rate of appearance of the hydranths at different levels involves only the appearance of a terminal organ, which is, as I have tried to show, a different problem from that of the growth of an organ as it approaches the terminus of its growth. This difference in the formation of the hydranth of *Tubularia* is due, I believe, to the amount of stem differentiation at different levels. The gradation of this differentiation is from the oral to the aboral end of a piece of the stem. Whether after the hydranth has emerged, the stalk grows faster from a cut surface nearer the base remains to be examined.

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 salamander, fish and earthworm. 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. 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 parts; in other words, to the loss of the normal pressure relations essential for the normal equilibrium. I base this inference mainly on the results of grafting experiments in hydra where, when dissimilar regions are united, each part completes itself at the line of graft, although the actual cut surfaces are completely united, and the subsequent changes may not take place for a week or more after the operation, when the effects of the injury as such must have long since passed away.

The terminal part is quickly formed in the proliferated materials. Between the terminal part and the old part there is also laid

¹This has been shown by Driesch, Stevens and Morgan.

down a growing zone that is a normal structure for the posterior end. I have already given my reasons for supposing that the growing region has the same potentialities for all levels,¹ and that 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. I have also compared 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 segments or parts to the parts lying proximal to them. At first this is the old part, and the first segment develops in relation to this part, the next one develops in relation to the first new one, and so on for the whole series. But what, it may be asked, 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 new 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, whose 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 forma-

¹An exception for tail formation must be made for the most anterior end, and for head formation in the region behind the gizzard of the earthworm.

tion 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, and 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 terminate 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.

I have expressed elsewhere the idea that polarity is an expression of the gradation of differentiated materials. We may now push the analysis further and refer the polarity to a gradation in the pressure relations, since these are the dynamical expression of the gradation of the materials, as shown in their differentiation. These differences can be traced to the egg where the differences in the pressure relations of the cells give rise to the later differentiation.