ILLINOIS BIOLOGICAL MONOGRAPHS

Vol. III August, 1916 No. 1

STUDIES ON THE FACTORS CONTROLLING THE RATE OF REGENERATION

BY

CHARLES ZELENY

PRICE $1.25

PUBLISHED BY THE UNIVERSITY OF ILLINOIS
UNDER THE AUSPICES OF THE GRADUATE SCHOOL
URBANA, ILLINOIS

UNIVERSITY OF ILLINOIS STUDIES PUBLISHED

ILLINOIS BIOLOGICAL MONOGRAPHS

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CHARLES ZELENY

Contributions from the
Zoological Laboratory of the University of Illinois, No. 73

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INTRODUCTION.

The present studies of the factors controlling rate of regeneration are a continuation of previous work on the subject. An advance in knowledge concerning certain of the factors has made possible an extension of the experimental analysis of others. The present studies are therefore closely related. In fact in several cases a single series of individuals has been of value in connection with more than a single factor. The definite determinations of the effect of level of the cut and of the change in rate during the regeneration cycle have been of particular value.

The precautions taken to meet the demands of the experiments are not discussed in detail because they have already been given in previous papers. The frog tadpoles (when they can be used) are in all respects more suitable than salamander larvae. When collected late in the fall they can be kept at a fairly constant size and the results obtained under these conditions are not complicated with growth phenomena. They have proved to be remarkably uniform in several series. The salamander larvae on the other hand vary in rate of regeneration from day to day. The factors involved in this fluctuation were not discovered and could not be remedied but may be related in some way to the fact that these animals require living active food and the feeding reactions are therefore more complicated than in frog tadpoles and more subject to disturbance.

In regard to certain factors, such as the degree of injury, in which expected differences in rate are slight the writer has felt that he might be biased in making the measurements and in a number of cases this work was therefore delegated to a person who had no preconceptions concerning the result.

In making averages elimination of individual cases is avoided except for a few very aberrant values. Such exceptional values are in every case however included in the tables. In many cases where only slight differences are to be expected several different kinds of comparisons are made so as to bring out the correct relation as completely as possible.

As in the past all data obtained by the writer on the particular factors in question are given. The practice of selective elimination would be dangerous because of the large value of factors not at present under experimental control.

Discussions of the results of other workers are included in the previous papers and need not be repeated here. The principal need at present seems to be an extension of knowledge of these factors by multiplying the number of series of carefully controlled experiments. While it would be interesting to know why a particular series differs from others with respect to a certain factor it is not always possible to discuss the matter profitably in the absence of evidence as to all the factors concerned.

Particular emphasis must be laid on the fact that in connection with some at least of the factors it has been possible to make out very definite quantitative relations. These have been checked up in a number of cases by agreement between separate series of experiments. The success in this direction has made it very probable that with a more accurate control of external conditions there will be a considerable further advance in our knowledge of the factors controlling rate of regeneration.

PART I

THE RATE OF REGENERATION FROM NEW TISSUE COMPARED WITH THAT FROM OLD TISSUE

In comparing first and second regenerations from the same level one of the difficulties that presents itself is the impossibility of making the second cut exactly in the path of the first. This is true not only because of the error in manipulation but also because the old and the new tissues become intermingled and do not retain a distinct dividing line. At the cut surface there is old tissue alone, old and new tissue, or new tissue alone according as the second cut comes inside of the first level, exactly at the level, or outside of it.

The experiments about to be described were devised with a view to the testing of the relative rates from old and from new tissue. Other factors being eliminated, are new cells which are recently produced in a regenerating part able to carry on a repetition of the process more expeditiously than old cells which have not been directly concerned in such a process?

There has been no selective elimination of data. As in former papers of a similar character all the data obtained by the author on the topic at hand are included.

Experiment I Series 3628-3675

Tadpoles of *Rana clamitans* with an average length of 33.4 mm. were used. They were fed just enough to keep them in good condition without much growth. All were collected at one time in a single pool and during the course of the experiment factors apart from the one under investigation were made as nearly alike as possible. This elimination of outside factors was facilitated by subdividing the tadpoles into sets of two each, the two individuals of a set being exactly alike except for the factor under consideration and one being used for regeneration from old tissue and the other for regeneration from new tissue.

Within each set the tail of tadpole 1 was removed at B (Fig. 1) and the tail of tadpole 2 at A. The distance between A and B was 2 or 3 mm. After 21 days of regeneration the second operation on both tadpoles came between A and B and therefore in old tissue in tadpole 1 and in new tissue in tadpole 2. This procedure, insuring

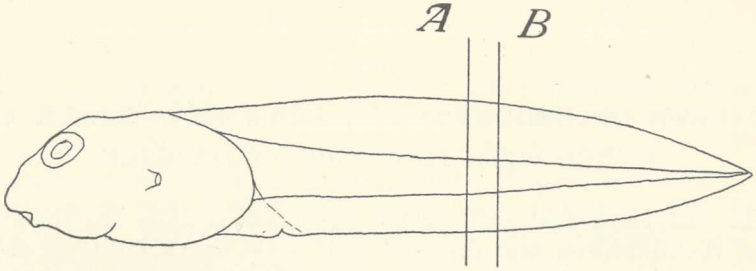

Figure 1. Outline of tadpole of *Rana clamitans*. Individuals used for regeneration from old tissue have the original removal level at B and the second level at A. Individuals used for regeneration from new tissue have the original removal level at A and the second level at B. Regenerations from the second levels are compared.

approximately the same level in the two cases, is necessary because level of the cut has a great influence upon rate of regeneration. Eleven pairs of individuals were used in the comparison. The precautions taken to eliminate possible error are treated fully elsewhere for similar cases (Zeleny 1909a, 1909b).

The data are given in Table 1. The removed tail lengths are the lengths of the original removed portions of the tail plus or minus the

EXPLANATION OF TABLE I.

Note 1. The removed length is the length of the original removed portion of the tail plus or minus the distance of the new cut surface from the dividing line between the old and new tissue.

Note 2. The lengths as given are the living lengths. Measurements were made on material killed in Gilson's mercuro-nitric mixture and preserved in 85% alcohol. Sets I and IX were measured both when alive and after killing and preserving. From them the shrinkage coefficient was obtained and this made possible the reduction of all the data to the living basis.

Note 3. The specific amount regenerated in any case is the amount regenerated per unit of removed length.

Note 4. The average includes only the sets in which both individuals are present.

TABLE I.

Series 3628-3675

Set	Old or New tissue at cut surface	Catalog number	Total length mm.	Tail length mm.	Removed length mm.	Regeneration Time 6 days		Regeneration Time 8 days	
						Regenerated length mm.	Specific length regenerated	Regenerated length mm.	Specific length regenerated
I	old	3628	38.0	24.1	13.2	2.2	0.17	3.5	0.27
	new	3629	39.2	24.6	12.8	2.3	0.18	3.1	0.24
II	old	3633	35.7	23.2	12.3	2.0	0.16	3.4	0.28
	new	3632	33.8	22.1	10.2	1.8	0.18	—	—
III	old	3636	35.8	23.1	12.8	2.0	0.16	3.25	0.25
	new	3637	38.4	25.0	11.9	2.4	0.20	3.5	0.29
IV	old	3641	32.9	20.8	11.3	1.7	0.15	3.4	0.30
	new	3640	31.4	20.4	9.3	2.2	0.24	—	—
V	old	3645	37.5	23.8	11.5	2.2	0.19	—	—
	new	3644	42.8	29.2	15.1	2.3	0.15	—	—
VI	old	3649	37.0	25.6	11.2	2.3	0.21	3.1	0.28
	new	3648	35.9	23.3	9.9	2.1	0.21	3.1	0.31
VII	old	3652	31.3	20.8	—	—	—	—	—
	new	3653	29.0	19.2	9.2	2.4	0.26	3.6	0.39
VIII	old	3656	31.8	21.1	13.2	2.4	0.18	—	—
	new	3657	33.0	22.0	11.7	2.7	0.23	4.9	0.42
IX	old	3660	26.5	17.0	9.6	2.5	0.26	3.5	0.36
	new	3661	29.4	19.0	8.7	2.0	0.23	3.0	0.34
X	old	3668	31.1	20.8	9.2	1.6	0.17	2.3	0.25
	new	3669	32.4	21.8	8.9	1.8	0.20	2.5	0.28
XI	old	3672	24.4	15.8	8.6	2.7	0.31	3.5	0.41
	new	3673	28.5	18.1	8.7	1.9	0.22	3.5	0.40
Average of old			32.9	21.5	11.3	2.16	0.196	3.19	0.303
Average of new			34.0	22.2	10.7	2.15	0.204	3.12	0.310
Old—ahead						0.01	—	0.07	—
New—ahead						—	0.008	—	0.007
Old—Times ahead						4	3½	3	3
New—Times ahead						6	6½	3	3

distances of the new cut surface from the dividing line between the old and the new tissue. The regenerated lengths as given are the living lengths. Measurements were made on material killed in Gilson's mercuro-nitric fluid and preserved in 85% alcohol. Sets I and IX were measured both when alive and after killing and preserving. From them the shrinkage coefficient was obtained and this made possible the reduction of all the data to the living basis. The averages include only the sets in which both individuals are present. The specific amount of regeneration is the amount regenerated per unit of removed length. It has been shown that within wide limits this is a constant if the only variable in the experiment is the amount removed. This statement holds for all levels in the present experiment.

The table shows that the average amount regenerated at the end of six days is 2.16 mm. from the old tissue levels and 2.15 mm. from the new tissue levels. The new tissue levels however represent the shorter amount removed, 10.7 mm. as opposed to 11.3 for the old tissue levels. This gives an average specific rate of 0.204 for the new levels and 0.196 for the old levels. The difference is probably not significant. The individual specific amounts in pairs, putting the old tissue first and the new tissue second in each case, are 0.17 and 0.18, 0.16 and 0.18, 0.16 and 0.20, 0.15 and 0.24, 0.19 and 0.15, 0.21 and 0.21, 0.18 and 0.23, 0.26 and 0.23, 0.17 and 0.20, and 0.31 and 0.22. The old tissue is ahead three times, the new six times and there is a tie in one case.

At the end of eight days the result is similar. There is a slight advantage in favor of the new tissue level but this cannot be considered as significant. The average amount regenerated is 3.19 mm. from old tissue levels and 3.12 mm. from new tissue levels. The specific amount regenerated is 0.303 for the old and 0.310 for the new level. The individual amounts by pairs putting the old tissue level first as before are 0.27 and 0.24, 0.25 and 0.29, 0.28 and 0.31, 0.36 and 0.34, 0.25 and 0.28, and 0.41 and 0.40. Each level is ahead of the other in three of the six cases.

Experiment II Series 3676-3765

Tadpoles of *Rana clamitans* with an average length of forty mm. were used. The experiment was designed for a study of the effect of successive removal on the rate of regeneration but incidentally furnishes valuable data for the present problem. In removing the regenerated portion, the cut in most cases did not come exactly at the border. In some cases it was too near the base of the tail and therefore the cells at the cut surface were old unregenerated cells. In other cases

it was too near the tip of the tail and the cells at the cut surface were newly regenerated ones.

The operations were at different levels in different individuals but the determination of the specific amounts of regeneration according to the method given in the explanation of Experiment I eliminates these differences within wide limits. It does not hold when the level of the cut is very near the tip or near the base of the tail. In the present experiment the specific amount is a fair constant for all removed lengths of over 4 mm. The individuals with a removed length of less than 4 mm. are therefore treated separately. Likewise it does not hold for the first few days of regeneration during which regeneration is confined to active migration of cells over the cut surface without any new formation by cell division. Separate comparisons are made at 4, 6, 8, 10, 12½, 18 and 56 days of regeneration. The data are given in Tables 2 to 17.

Taking first the cases with a removed length of over 4 mm. there is at four days a specific amount of 0.043 for old tissue and of 0.045 for new tissue. At six days the amounts are respectively 0.135 and 0.143, at eight days 0.216 and 0.224, at ten days 0.292 and 0.293, at twelve and a half days 0.331 and 0.337, at eighteen days 0.352 and 0.348, and at fifty-six days 0.345 and 0.346. The two are approximately equal though in six out of the seven cases the new tissue is ahead. The average difference in favor of the new tissue is 0.003.

For removed amounts of less than 4 mm. the data are unsatisfactory because there are only three individuals with regeneration from new tissues. The data are however of value in comparison with the others. The specific amounts at the different days, again putting the old tissue first in each case, are 0.119 and 0.160 for four days, 0.317 and 0.327 for six days, 0.444 and 0.467 for eight days, 0.506 and 0.520 for ten days, 0.517 and 0.517 for twelve and a half days, 0.501 and 0.507 for eighteen days, and 0.475 and 0.325 for fifty-six days. In the last the absorption of the tail had begun before the measurement was made and the comparison is therefore not valid for our purposes. In the first, 0.119 for old and 0.160 for new at four days, the great difference between individual cases on each side makes a comparison of doubtful validity. There are other data however which make it probable that the initial migration of the cells takes place more rapidly from new than from old tissue. For the other levels there is on the average a slight difference (0.011) in favor of the regeneration from new tissue. With but a single exception, which is a tie, the new tissue is ahead of the old. The differences favoring the new tissue are greater than those for the larger removals. This again may be due to the fact that a larger percent-

age of the regenerated material is derived from the old by migration and a smaller percentage by cell division. The data unfortunately are based on such a small number of individuals, especially in the case of new tissue levels, that too much stress should not be laid on the differences.

TABLE 2

Series 3676-3765 Over 4 millimeters removed Regeneration: 4 days

Old tissue				New tissue			
Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated	Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated
3720	4.7	0.42	0.09	3756	4.8	0.36	0.07
3684	5.5	0.15	0.03	3751	6.7	0.45	0.07
3715	7.9	0.24	0.03	3697	7.3	0.48	0.07
3757	8.0	0.42	0.05	3721	8.5	0.57	0.07
3694	8.7	0.45	0.05	3733	8.5	0.36	0.04
3685	9.3	0.60	0.06	3734	8.5	0.48	0.06
3686	14.5	0.60	0.04	3739	9.4	0.36	0.04
3753	16.8	0.42	0.03	3722	12.5	0.60	0.05
3723	18.4	0.30	0.02	3716	12.7	0.39	0.03
3699	21.0	0.54	0.03	3698	12.9	0.50	0.04
				3759	15.5	0.30	0.02
				3705	17.6	0.72	0.04
				3717	17.6	0.42	0.02
				3687	19.7	0.54	0.03
Average			0.043	Average			0.045

Note 1. No. 3734 is left out in making up the averages because its specific amount from six days of regeneration on is very much in excess of that of any of the others. A probable explanation is that the end of the tail in this individual had been removed and regeneration had just started when the present operations were begun. If this is true it belongs to a longer removed length than indicated and the specific rate is wrong. Besides a highly exceptional individual even if not explained should be left out in determining the average value.

TABLE 3

Series 3676-3765 Over 4 millimeters removed Regeneration: 6 days

Old tissue				New tissue			
Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated	Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated
3720	4.7	0.84	0.18	3756	4.8	1.0	0.21
3648	5.5	0.7	0.13	3751	6.7	1.1	0.16
3715	7.9	0.9	0.11	3697	7.3	1.2	0.16
3757	8.0	1.2	0.15	3721	8.5	1.3	0.15
3694	8.7	1.5	0.17	3733	8.5	1.0	0.12
3685	9.3	1.2	0.13	3734	8.5	2.1	0.25
3686	14.5	2.1	0.14	3739	9.4	1.0	0.11
3753	16.8	2.0	0.12	3722	12.5	1.6	0.13
3723	18.4	2.3	0.12	3716	12.7	1.7	0.13
3699	21.0	2.2	0.10	3698	12.9	1.5	0.12
				3759	15.5	2.0	0.13
				3705	17.6	2.0	0.11
				3717	17.6	2.6	0.15
				3687	19.7	2.6	0.18
Average			0.135	Average			0.143

TABLE 4

Series 3676-3765 Over 4 millimeters removed Regeneration: 8 days

Old tissue				New tissue			
Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated	Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated
3720	4.7	1.1	0.23	3756	4.8	1.3	0.27
3684	5.5	1.2	0.22	3751	6.7	1.7	0.25
3715	7.9	1.7	0.22	3697	7.3	1.7	0.23
3757	8.0	2.1	0.26	3721	8.5	1.9	0.22
3694	8.7	2.2	0.25	3733	8.5	1.9	0.22
3685	9.3	1.9	0.20	3734	8.5	3.1	0.36
3686	14.5	3.4	0.23	3739	9.4	1.8	0.19
3753	16.8	2.5	0.15	3722	12.5	2.6	0.21
3723	18.4	3.7	0.20	3716	12.7	2.4	0.19
3699	21.0	4.3	0.20	3698	12.9	3.3	0.26
				3759	15.5	3.0	0.19
				3705	17.6	3.6	0.20
				3717	17.6	3.6	0.20
				3687	19.7	5.6	0.28
Average			0.216	Average			0.224

TABLE 5

Series 3676-3765 Over 4 millimeters removed Regeneration: 10 days

Old tissue				New tissue			
Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated	Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated
3720	4.7	1.3	0.28	3756	4.8	1.7	0.35
3684	5.5	1.4	0.25	3751	6.7	2.1	0.31
3715	7.9	2.3	0.29	3697	7.3	2.2	0.30
3757	8.0	2.8	0.35	3721	8.5	2.3	0.27
3694	8.7	3.2	0.37	3733	8.5	2.4	0.28
3685	9.3	2.3	0.25	3734	8.5	4.5	0.53
3686	14.5	4.8	0.33	3739	9.4	2.4	0.26
3753	16.8	3.8	0.23	3722	12.5	3.6	0.29
3723	18.4	5.3	0.29	3716	12.7	3.4	0.27
3699	21.0	5.9	0.28	3698	12.9	4.3	0.33
				3759	15.5	4.2	0.27
				3705	17.6	4.8	0.28
				3717	17.6	5.2	0.30
				3687	19.7	6.0	0.30
Average			**0.292**	**Average**			**0.293**

TABLE 6

Series 3676-3765 Over 4 millimeters removed Regeneration: 12-13 days

Old tissue				New tissue			
Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated	Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated
3720	4.7	1.3	0.28	3756	4.8	1.8	0.37
3684	5.5	1.4	0.25	3751	6.7	2.4	0.36
3715	7.9	2.6	0.33	3697	7.3	2.4	0.33
3757	8.0	3.1	0.39	3721	8.5	2.6	0.31
3694	8.7	3.4	0.39	3733	8.5	2.6	0.31
3685	9.3	2.8	0.30	3734	8.5	5.7	0.67
3686	14.5	5.3	0.37	3739	9.4	3.0	0.32
3753	16.8	5.2	0.31	3722	12.5	3.9	0.31
3723	18.4	6.5	0.35	3716	12.7	4.2	0.33
3699	21.0	7.1	0.34	3698	12.9	5.0	0.39
				3759	15.5	4.8	0.31
				3705	17.6	6.4	0.36
				3717	17.6	6.0	0.34
				3687	19.7	6.6	0.34
Average			**0.331**	**Average**			**0.337**

TABLE 7

Series 3676-3765 Over 4 millimeters removed Regeneration: 17-18-19 days

Old tissue				New tissue			
Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated	Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated
3720	4.7	1.3	0.28	3756	4.8	1.6	0.33
3684	5.5	1.5	0.27	3751	6.7	2.5	0.37
3715	7.9	2.6	0.33	3697	7.3	2.3	0.32
3757	8.0	3.2	0.40	3721	8.5	2.3	0.27
3694	8.7	3.4	0.39	3733	8.5	2.6	0.31
3685	9.3	3.0	0.32	3734	8.5	6.4	0.75
3686	14.5	5.2	0.36	3739	9.4	2.9	0.31
3753	16.8	6.4	0.38	3722	12.5	3.5	0.28
3723	18.4	8.1	0.43	3716	12.7	5.1	0.40
3699	21.0	7.5	0.36	3698	12.9	5.4	0.42
				3759	15.5	6.7	0.43
				3705	17.6	6.2	0.35
				3717	17.6	6.7	0.38
				3687	19.7	7.0	0.36
Average			**0.352**	**Average**			**0.348**

TABLE 8

Series 3676-3765 Over 4 millimeters removed Regeneration: 55-56-57 days

Old tissue				New tissue			
Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated	Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated
3720	4.7	1.3	0.28	3756	4.8	—	——
3684	5.5	1.4	0.25	3751	6.7	2.3	0.34
3715	7.9	2.8	0.35	3697	7.3	2.1	0.29
3757	8.0	3.1	0.39	3721	8.5	2.2	0.26
3694	8.7	—	——	3733	8.5	2.8	0.33
3685	9.3	2.6	0.28	3734	8.5	6.6	0.78
3686	14.5	—	——	3739	9.4	—	——
3753	16.8	7.1	0.42	3722	12.5	4.2	0.34
3723	18.4	8.3	0.45	3716	12.7	4.4	0.35
3699	21.0	7.2	0.34	3698	12.9	5.4	0.42
				3759	15.5	6.6	0.43
				3705	17.6	6.0	0.34
				3717	17.6	6.4	0.36
				3687	19.7	—	——
Average			**0.345**	**Average**			**0.346**

TABLE 9

Series 3676-3765 Over 4 millimeters removed Summary Tables 2 to 8

Table number	Days of regeneration	Old tissue Specific length of regeneration	New tissue Specific length of regeneration	Old ahead	New ahead
2	4	0.043	0.045		0.002
3	6	0.135	0.143		0.008
4	8	0.216	0.224		0.008
5	10	0.292	0.293		0.001
6	12, 13	0.331	0.337		0.006
7	17, 18, 19	0.352	0.348	0.004	
8	55, 56, 57	0.345	0.346		0.001
Average					0.003

TABLE 10

Series 3676-3765 Less than 4 millimeters removed Regeneration: 4 days

Old tissue				New tissue			
Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated	Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated
3676	1.3	0.27	0.27	3696	2.1	0.48	0.23
3682	1.6	0.18	0.11	3749	2.8	0.30	0.11
3730	1.6	0.39	0.24	3750	3.5	0.48	0.14
3754	1.6	0.06	0.04				
3718	2.1	0.06	0.03				
3731	2.7	0.15	0.06				
3713	2.8	0.36	0.13				
3719	3.1	0.36	0.12				
3701	3.2	0.42	0.13				
Average			0.119	Average			0.160

TABLE 11

Series 3676-3765 Less than 4 millimeters removed Regeneration: 6 days

Old tissue				New tissue			
Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated	Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated
3676	1.3	0.6	0.46	3696	2.1	0.85	0.40
3682	1.6	0.6	0.37	3749	2.8	0.6	0.21
3730	1.6	0.75	0.47	3750	3.5	1.3	0.37
3754	1.6	0.55	0.34				
3718	2.1	0.45	0.21				
3731	2.7	0.5	0.19				
3713	2.8	0.8	0.29				
3719	3.1	0.84	0.27				
3701	3.2	0.8	0.25				
Average			0.317	Average			0.327

TABLE 12

Series 3676-3765 Less than 4 millimeters removed Regeneration: 8 days

Old tissue				New tissue			
Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated	Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated
3676	1.3	0.9	0.69	3696	2.1	1.0	0.48
3682	1.6	0.9	0.56	3749	2.8	1.2	0.43
3730	1.6	0.9	0.56	3750	3.5	1.7	0.49
3754	1.6	0.9	0.56				
3718	2.1	0.7	0.33				
3731	2.7	0.8	0.29				
3713	2.8	0.9	0.32				
3719	3.1	1.1	0.35				
3701	3.2	1.1	0.34				
Average			0.444	Average			0.467

TABLE 13

Series 3676-3765 Less than 4 millimeters removed Regeneration: 10 days

Old tissue				New tissue			
Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated	Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated
3676	1.3	0.9	0.69	3696	2.1	1.1	0.52
3682	1.6	1.0	0.62	3749	2.8	1.4	0.50
3730	1.6	0.9	0.56	3750	3.5	1.9	0.54
3754	1.6	1.1	0.69				
3718	2.1	1.0	0.48				
3731	2.7	1.0	0.37				
3713	2.8	0.9	0.32				
3719	3.1	1.4	0.45				
3701	3.2	1.2	0.37				
Average			**0.506**	**Average**			**0.520**

TABLE 14

Series 3676-3765 Less than 4 millimeters removed Regeneration: 12-13 days

Old tissue				New tissue			
Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated	Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated
3676	1.3	0.9	0.69	3696	2.1	1.0	0.48
3682	1.6	1.0	0.62	3749	2.8	1.4	0.50
3730	1.6	0.9	0.56	3750	3.5	2.0	0.57
3754	1.6	1.2	0.75				
3718	2.1	1.0	0.48				
3731	2.7	1.0	0.37				
3713	2.8	0.9	0.32				
3719	3.1	1.4	0.45				
3701	3.2	1.3	0.41				
Average			**0.517**	**Average**			**0.517**

TABLE 15

Series 3676-3765 Less than 4 millimeters removed Regeneration: 17-18-19 days

Old tissue				New tissue			
Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated	Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated
3676	1.3	0.9	0.69	3696	2.1	1.0	0.48
3682	1.6	1.0	0.62	3749	2.8	1.3	0.47
3730	1.6	0.9	0.56	3750	3.5	2.0	0.57
3754	1.6	1.2	0.75				
3718	2.1	0.5	0.24				
3731	2.7	—	——				
3713	2.8	0.9	0.32				
3719	3.1	1.3	0.42				
3701	3.2	1.3	0.41				
Average			0.501	Average			0.507

TABLE 16

Series 3676-3765 Less than 4 millimeters removed Regeneration: 55-56-57 days

Old tissue				New tissue			
Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated	Catalog number	Length removed mm.	Length regenerated mm.	Specific length regenerated
3676	1.3	0.7	0.54	3696	2.1	0.7	0.33
3682	1.6	1.1	0.69	3749	2.8	0.9	0.32
3730	1.6	0.7	0.44	3750	3.5	—	——
3754	1.6	1.1	0.69				
3718	2.1	—	——				
3731	2.7	—	——				
3713	2.8	0.5	0.18				
3719	3.1	—	——				
3701	3.2	1.0	0.31				
Average			0.475	Average			0.325

TABLE 17

Series 3676-3765 Less than 4 millimeters removed Summary Tables 10 to 16

Table number	Days of regeneration	Old tissue Specific length of regeneration	New tissue Specific length of regeneration	Old ahead	New ahead
10	4	0.119	0.160		0.041
11	6	0.317	0.327		0.010
12	8	0.444	0.467		0.023
13	10	0.506	0.520		0.014
14	12, 13	0.517	0.517	0.000	0.000
15	17, 18, 19	0.501	0.507		0.006
16	55, 56, 57	0.475	0.325	0.150	
Average					0.011

Note 1. Because of the great variability in the data the average for the four-day period is not of much value and is therefore not included in the grand average.

Note 2. The absorption of the regenerated portion of the tail was proceeding so rapidly by the fifty-fifth day of regeneration that this average should not be included in the grand average.

Experiment III Series 3557-3624

This experiment was planned for a study of the effect of repeated removal and regeneration upon the rate of metamorphosis but it yields data of value for the present problem. Tadpoles of *Rana clamitans* with an average length of about 40 mm. were used. In some cases the cuts were made inside of the first level and therefore in old tissue and in other cases outside of the first level and therefore in new tissue.

The data include third, fourth and fifth successive regenerations. The time of regeneration is 37 days for the third and 36 days for the fourth and for the fifth regenerations. The length of time is more than sufficient for the completion of the process of regeneration in so far as it is completed. The data therefore do not serve for the rate but for the completeness of regeneration from old as compared with new levels. Approximately one-half of the original tail length was removed but measurements were not made of individual removed lengths, so that

specific rates of regeneration can not be calculated. However the removed lengths were so nearly alike as to make the regenerated lengths of value in direct comparison.

The data are given in Table 18. The average length of the third regeneration is 7.9 mm. for both the old and the new tissue basis. For the fourth regeneration the value from old tissue is 5.3 mm. and from new tissue 5.5 mm. The corresponding values for the fifth regeneration are 6.6 mm. and 5.9 mm. Averaging the individual cases for all three regenerations the old tissue average is 6.5 mm. and the new tissue average 6.6 mm., an advantage in favor of the latter of 0.1 mm. This difference can not be considered as significant, especially since for the individual regenerations the two levels give equal regenerated lengths for the third, the new is slightly ahead at the fourth and the old is ahead at the fifth.

On the whole the data for Experiment III agree with those for Experiments I and II. There is no striking difference between completeness of regeneration from old and from new tissue levels, though a small difference favoring the latter persists in practically all the comparisons.

TABLE 18

Rana clamitans Series 3557-3624

Regenerated tail length from new tissue compared with that from old tissue during the third, fourth and fifth regenerations

	Third regeneration		Fourth regeneration		Fifth regeneration		Third, fourth and fifth regenerations combined	
	37 Days		36 Days		36 Days			
	Old tissue	New tissue	Old tissue	New tissue	Old tissue	New tissue	Old tissue	New tissue
	2.0	5.7	4.4	4.9	4.7	5.2		
	6.8	6.6	4.5	5.4	5.5	5.7		
	6.9	8.0	4.8	5.5	6.2	5.9		
	7.5	8.3	4.9	6.1	6.5	6.8		
	7.9	9.3	5.0		7.1			
	9.0	9.7	5.1		7.2			
	9.4		5.8		7.9			
			6.1		8.0			
			7.3					
Average in mm.	7.9	7.9	5.3	5.5	6.6	5.9	6.5	6.6
Difference in mm.	0.0	0.0		+0.2	+0.7			+0.1

Discussion

While the knowledge of the relative rates of regeneration for old and new tissue is essential for accurate determination of other factors its main interest is in its bearing on the question of the character of control of the process of regeneration. Evidence from a great many directions points toward the conclusion that regeneration is not wholly a direct response of the injured cells at the cut surface nor of those in the immediate neighborhood of the cut surface. It is more and more evident that conditions in parts of the body remote from the injured region are involved. If rate of regeneration were determined wholly by the character of the cells at the cut surface we would expect that cells in process of active proliferation, such as those that are starting to build up a new tail, would respond much more promptly than those which have become more highly differentiated and hence more stable. Regenerating cells ought to furnish a much better basis than old ones. We find however that there is no striking difference in the two cases. Regeneration proceeds at approximately the same rate whether old or new cells have furnished the basis for the new material. It is true that the data show on the average a slight advantage in favor of the new tissue, especially during the early periods, but this advantage is small and it is doubtful whether it can be considered as significant. There is some evidence that the earliest stages of regeneration, those due to cell migration exclusively, are more rapid from new than from old tissue. If this evidence is reliable an explanation is found for the slight advantage in favor of the new tissue at later periods.

Summary

1. A comparison of the rate of regeneration in tadpoles of *Rana clamitans* in cases where there are newly regenerated cells at the cut surface with those in which only old cells are present shows, on the whole, little difference between the two.

2. The slight difference favors the new cells but may not be significant.

3. In Experiment I the specific length of regeneration at the end of 6 days was 0.196 from old tissue and 0.204 from new tissue.

4. In the same experiment at the end of 8 days the specific length from the old was 0.303 and from the new 0.310.

5. In Experiment II the general result was similar to that in Experiment I. The amounts of regeneration in the two cases are very nearly equal and the slight difference is in favor of the new tissue.

6. Experiment III shows that as regards completeness of regen-

eration there is again essential similarity between the old tissue and the new tissue levels.

7. The result strengthens the view that the rate of regeneration is controlled in large part by factors not inherent in the character or condition of the cells near the cut surface.

8. In the case of the earliest stages, those in which there is cell migration but no cell division, there is some evidence that the rate of regeneration may be greater from new than from old tissue.

PART II

THE EFFECT OF SUCCESSIVE REMOVAL UPON THE RATE AND COMPLETENESS OF REGENERATION

One of the most interesting facts in connection with regeneration is the ability to replace a part after repeated removal. The present set of experiments was made in continuation of previous studies of the effect of successive removal upon the rate of regeneration (Zeleny 1907, 1908, 1909). The earlier studies show that as a rule the rate of regeneration following a first removal is no greater than that following second and later removals if the effect of age is eliminated. Where a difference exists it seems to be in favor of the later regenerations.

The matter is of very great interest in connection with general problems of development and particularly in connection with the question as to the existence or non-existence of a necessary limit to the amount of living substance that a single individual may produce during its life cycle. Does the production of a group of tissues use up a part of a certain store of developmental energy or of developmental factors possessed by the individual or is this store inexhaustible or perchance even increased by exercise of the function? These questions warrant more extended study especially in view of the additional analysis that has been made of other factors controlling the rate of regeneration. The paper includes all the unpublished data that have been obtained on the problem at hand. In general these data support the conclusions previously reached. The descriptions of the individual experiments will first be given and they will be followed by a discussion of the general results.

Experiment I Rana clamitans Series 3628-3675

Material and Method The tadpoles were collected on December 9, 1911. At the time of the operation on December 20 the average total length was 33.0 mm. and the average tail length 21.6 mm. Forty-eight individuals were divided into twelve sets of four each. The four individuals of a set are called *a, b, c,* and *d.* Approximately one-half in length of the tail was removed by a transverse cut in *c* and *d.* After 21 days the regenerated portion of the tail was removed. In individual *c* the second cut came inside of the border line between old and new

tissue and in individual *d* it came outside of that line. Of the two individuals available for second regeneration in each set, the one with the cut nearer to the tip of the tail was chosen as individual *c* and the other as individual *d*. In this way the second regeneration levels were equalized. A first removal of a half of the tail was made in individuals *a* and *b* at the same time that the second removal was made in *c* and *d*. A direct comparison of the rate of the second regeneration with that of the first was thus made possible without the complication due to internal factors such as difference in age, or external factors such as temperature and food.

Measurements of regenerated lengths were made at the end of six and of eight days, other experiments having shown that the period of most rapid growth comes at about this time.

Elsewhere there is a comparison of the rate of regeneration from new tissue with that from old tissue. Here the chief concern is the comparison of the rate of the second regenerations, including both old tissue and new tissue levels, with first regenerations.

Data The results of the experiment are given in Table 19 for six-day regenerations and in Table 20 for eight-day regenerations. At the end of six days the average length of first regenerations is 2.01 mm. and of second regenerations 2.18 mm. The first exceeds the second in two cases, the second exceeds the first in eight and one is tied. The corresponding average specific amounts are 0.194 and 0.205. In five cases the first exceeds the second and in six the second exceeds the first.

At eight days the average length of the first regenerations is 3.06 mm. and of the second 3.42 mm. The first exceeds the second in three sets and the second exceeds the first in seven sets. The corresponding average specific amounts are 0.298 and 0.323. In four the first exceeds the second regeneration and in six the second exceeds the first.

Comparing the first regenerations on the one hand with second regenerations from old tissue and on the other hand with second regenerations from new tissue it is found, including only complete sets, that at the end of six days the average first regeneration length is 2.01 mm. while that of the second from new tissue is 2.15 mm. and from old tissue 2.16 mm. The corresponding average specific amounts are 0.194 for first regenerations and 0.196 for second regenerations from old tissue and 0.204 for second regenerations from new tissue.

At eight days the first regeneration lengths average 3.06 mm. while second regenerations from old tissue average 3.19 and those from new tissue 3.12. The corresponding specific lengths are 0.298 for first regenerations and 0.303 for second from old tissue and 0.310 for second from new tissue.

TABLE 19

Rana clamitans Series 3676-3765

Comparison of first and second regenerations Age factor eliminated

Six Days

Series	Regeneration		Total length	Tail length	Length removed	Length regenerated	Specific length regenerated	Average length regenerated	Average specific length regenerated
I	1	individual a	35.5	23.1	11.5	1.9	0.17		
		individual b	34.5	21.9	9.4	1.8	0.19	1.85	0.180
	2	c from old tissue	38.0	24.1	13.2	2.2	0.17		
		d from new tissue	39.2	24.6	12.8	2.3	0.18	2.25	0.175
II	1	a	34.5	23.0	—	—	—		
		b	33.9	22.2	9.6	1.7	0.18	1.70	0.180
	2	c old	35.7	23.2	12.3	2.0	0.16		
		d new	33.8	22.1	10.2	1.8	0.18	1.90	0.170
III	1	a	36.2	23.3	9.7	1.7	0.18		
		b	34.1	22.5	10.9	1.9	0.17	1.80	0.175
	2	c old	35.8	23.1	12.8	2.0	0.16		
		d new	38.4	25.0	11.9	2.4	0.20	2.20	0.180
IV	1	a	33.1	21.2	12.6	2.2	0.17		
		b	32.4	20.8	10.2	1.7	0.17	1.95	0.170
	2	c old	32.9	20.8	11.3	1.7	0.15		
		d new	31.4	20.4	9.3	2.2	0.24	1.95	0.195
V	1	a	40.8	27.3	11.9	2.1	0.18		
		b	39.4	26.4	12.7	2.2	0.17	2.15	0.175
	2	c old	37.5	23.8	11.5	2.2	0.19		
		d new	42.8	29.2	15.1	2.3	0.15	2.25	0.170
VI	1	a	37.0	24.5	10.2	1.9	0.19		
		b	35.7	24.6	12.9	2.2	0.17	2.05	0.180
	2	c old	37.0	25.6	11.2	2.3	0.21		
		d new	35.9	23.3	9.9	2.1	0.21	2.20	0.210
VII	1	a	31.2	20.1	11.9	2.1	0.18		
		b	28.0	18.6	8.4	2.0	0.24	2.05	0.210
	2	c old	31.3	20.8	—	—	—		
		d new	29.0	19.2	9.2	2.4	0.26	2.40	0.260

TABLE 19 (Continued)

Series	Regeneration		Total length	Tail length	Length removed	Length regenerated	Specific length regenerated	Average length regenerated	Average specific length regenerated
	1	a	28.7	18.5	10.0	2.1	0.21		
		b	32.0	21.5	8.5	2.2	0.26	2.17	0.235
VIII									
	2	c old	31.8	21.1	13.2	2.4	0.18		
		d new	33.0	22.0	11.7	2.7	0.23	2.55	0.205
	1	a	29.8	19.1	10.1	2.3	0.23		
		b	26.9	17.0	10.7	2.3	0.22	2.30	0.225
IX									
	2	c old	26.5	17.0	9.6	2.5	0.26		
		d new	29.4	19.0	8.7	2.0	0.23	2.25	0.245
	1	a	32.1	21.5	12.0	2.3	0.19		
		b	32.4	21.5	10.1	2.0	0.20	2.15	0.195
X									
	2	c old	32.7	22.4	—	—	—		
		d new	30.0	19.8	—	—	—	—	—
	1	a	30.9	20.9	10.2	2.0	0.20		
		b	30.1	20.2	9.4	1.8	0.20	1.90	0.200
XI									
	2	c old	31.1	20.8	9.2	1.6	0.17		
		d new	32.4	21.8	8.9	1.8	0.20	1.70	0.185
	1	a	28.0	18.0	11.0	2.2	0.20		
		b	26.3	16.4	10.4	2.1	0.20	2.15	0.200
XII									
	2	c old	24.4	15.4	8.6	2.7	0.31		
		d new	28.5	18.1	8.7	1.9	0.22	2.30	0.265
	1		**32.7**	**21.4**	**10.6**			**2.01**	**0.194**
Average									
	2		**33.4**	**21.8**	**10.9**			**2.18**	**0.205**

The data as a whole show an advantage in favor of the second regeneration as compared with the first. This is seen not only when the direct regenerated lengths are taken but also when the specific amounts are used. Elsewhere it is shown that the specific amount of regeneration is independent of the level of the cut and therefore a constant within the limits of removal as used in this experiment. The specific amount

determinations are therefore more accurate for our purposes than the direct values of length regenerated.

The first regeneration is slightly below the second not only in case the latter is from new cells but also in case it is from old cells. The difference between first and second regenerations therefore can not be due entirely to the presence in the former of cells which are already undergoing regeneration.

TABLE 20

Rana clamitans Series 3628-3675

First and second regenerations compared Age factor eliminated

Eight days

Series	Regeneration		Length removed	Length regenerated	Specific length regenerated	Average length regenerated	Average specific length regenerated
	1	individual a	11.5	3.1	0.26		
		individual b	9.4	2.5	0.27	0.28	0.265
I							
	2	c from old tissue	13.2	3.5	0.27		
		d from new tissue	12.8	3.1	0.24	3.30	0.255
	1	a	—	—	—		
		b	9.6	2.1	0.22	2.10	0.220
II							
	2	c old	12.3	3.4	0.28		
		d new	10.2	—	—	3.40	0.280
	1	a	9.7	2.7	0.28		
		b	10.9	3.4	0.31	3.05	0.295
III							
	2	c old	12.8	3.25	0.25		
		d new	11.9	3.5	0.29	3.37	0.270
	1	a	12.6	3.25	0.26		
		b	10.2	3.25	0.32	3.25	0.290
IV							
	2	c old	11.3	3.4	0.30		
		d new	9.3	—	—	3.40	0.300
	1	a	11.9	—	—		
		b	12.7	4.0	0.31	4.00	0.310
V							
	2	c old	11.5	—	—		
		d new	15.1	—	—	—	—

TABLE 20 (Continued)

Series	Regeneration		Length removed	Length regenerated	Specific length regenerated	Average length regenerated	Average specific length regenerated
VI	1	a	10.2	3.6	0.35		
		b	12.9	3.6	0.28	3.60	0.315
	2	c old	11.2	3.1	0.28		
		d new	9.9	3.1	0.31	3.10	0.295
VII	1	a	11.9	3.8	0.32		
		b	8.4	3.25	0.39	3.52	0.355
	2	c old	—	—	—		
		d new	9.2	3.6	0.39	3.60	0.390
VIII	1	a	10.0	3.25	0.32		
		b	8.5	3.4	0.40	3.32	0.360
	2	c old	13.2	—	—		
		d new	11.7	4.9	0.42	4.90	0.420
IX	1	a	10.1	3.2	0.32		
		b	10.7	3.4	0.32	3.30	0.320
	2	c old	9.6	3.5	0.36		
		d new	8.7	3.0	0.34	3.25	0.350
X	1	a	12.1	3.6	0.30		
		b	10.1	2.3	0.23	2.95	0.265
	2	c old	—	—	—		
		d new	—	—	—	—	—
XI	1	a	10.2	3.5	0.34		
		b	9.4	2.5	0.27	3.00	0.305
	2	c old	9.2	2.3	0.25		
		d new	8.9	2.5	0.28	2.40	0.265
XII	1	a	11.0	—	—		
		b	10.4	2.7	0.26	2.70	0.260
	2	c old	8.6	3.5	0.41		
		d new	8.7	3.5	0.40	3.50	0.405
Average	1		10.6			3.06	0.298
	2		10.9			3.42	0.323

EXPERIMENT II RANA CLAMITANS SERIES 3676-3765

Material and Method Ninety tadpoles with an average total length of about 40 mm. and an average tail length of 27 mm. were used in the experiment. The plan consisted in the removal of a portion of the tail in a part, S, of the individuals, the remaining part, F, being left uninjured at the time. After S had been regenerating a new tail for twenty-two days both S and F were operated upon. In S the regenerating tails were removed by a cut which came at the border line between the old and the new tissues. In F an operation was made similar to the original one on S and leaving the same amount of old tail in both S and F. The procedure is similar to that shown in Figure 1. S and F were now allowed to regenerate and a direct comparison is possible between a second regeneration in S and a first regeneration in F.

Measurements were made of regenerated lengths at 4, 6, 8, 10, 12½, 18 and 56 days. The operations were made at six levels corresponding approximately to the removal respectively of $^1/_{18}$, $^1/_{10}$, ⅙, ⅓, ½ and ⅔ of the tail. Four of these levels, $^1/_{10}$, ⅓, ½ and ⅔, had at least five individuals each for each regeneration. The other two levels, $^1/_{18}$ and ⅙, had less than five individuals per regeneration but are included in the tables though their averages are not as reliable as those of the others.

The method as described agrees in principle with that pursued in Experiment I. It has a decided advantage over a direct comparison within a single individual because it eliminates the age factor as well as the effects of change in external conditions such as temperature and food.

Data The results of the experiment are given in Tables 21 to 30 and in Figures 2 and 3. The data show on the whole a tendency for the second regeneration to remain in advance of the first for eight or ten days after the operation. The first regeneration then catches up and even slightly surpasses the other; this is apparent both when the regenerated lengths are taken directly and when they are corrected for difference in level of the cut and put in terms of specific regenerated length or the length regenerated per unit of removed length.

In making the comparisons certain general features must be borne in mind. The maximum rate of regeneration is reached on or near the seventh day, earlier for the smaller removals and later for the larger removals. The whole regeneration, in so far as it is completed, is finished in nearly all cases at 12½ days, again somewhat earlier for the smaller and somewhat later for the larger removals. In the tadpoles used in the present experiment about four-tenths in length of the removed tail is replaced before regeneration stops. This was found to be

generally true of tadpoles of this size in *Rana clamitans*. The percent regenerated is somewhat greater for the smallest removals than for the others. After the maximum is reached there is a tendency toward decrease of the regenerated region though this is hard to determine with accuracy because the boundary between old and new tissue becomes more and more obscure as time goes on. For this reason the data for 56 days of regeneration are not as reliable as the others.

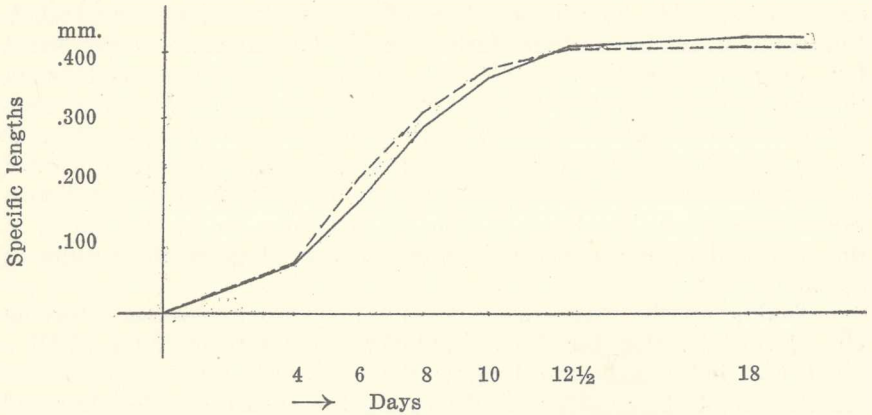

Figure 2. Specific regenerated lengths during the regenerative period for both first and second regenerations. Tadpole tail of *Rana clamitans*. Series 3676-3765.

Broken line = second regeneration.
Unbroken line = first regeneration.

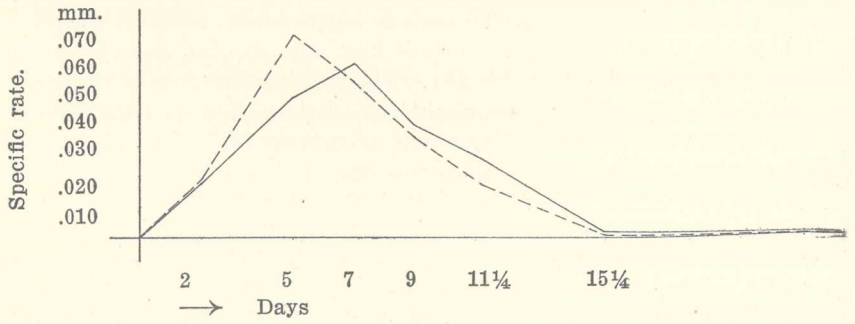

Figure 3. Change in specific rate of regeneration during the regenerative period for both first and second regenerations. Tadpole tail of *Rana clamitans*. Series 3676-3765.

Unbroken line = first regeneration.
Broken line = second regeneration.

At the four-day period the amount of regeneration is so small that there is a large probable error and these data should be used with caution. For the $^1/_{18}$ and $^1/_6$ removals the number of individuals is so small that the data for these levels do not compare in accuracy with the others and they will therefore be passed over for the present.

The data are presented in Tables 21 to 30. Tables 21 to 26 give respectively the regenerations for the six different levels beginning with the shortest removal. Table 27 collects all the data of amounts regenerated and Table 28 all the data of specific amounts regenerated. Figure 2 gives in graphic form the specific amounts regenerated for each regeneration. Table 29 gives the differences between the first and second regenerations for each of the different levels at each of the seven times of measurement. It includes the differences in specific length as well as those in absolute length. The specific lengths furnish the better basis for comparison and will be used in the following discussion unless otherwise stated. Table 30 compares the specific rates in the first and second regenerations and Figure 3 gives the results in graphic form.

Taking up the regeneration from the different levels and leaving out of consideration for the present the two levels with too small a number of individuals, the data for the $^1/_{10}$ level as given in Table 4 are the first to be considered. There are five individuals for first and seven for second regenerations. The second regeneration is ahead in specific length from the fourth to the tenth day. At 12½ days the two are tied and at 56 days the first is ahead. Regeneration is completed in 12½ days and beyond this time there is a decrease in regenerated material. The decrease is greater in the second than in the first regeneration, hence the ascendency of the latter at 56 days. During the whole period of active regeneration the second regeneration remains ahead.

There are eight individuals for the first regeneration and eleven for the second at the ⅓ level (Table 24). The specific amounts of regeneration are strikingly similar throughout the whole period of regeneration. The two departures from equality are an advantage of 0.01 for the second regeneration at 8 days and a disadvantage of 0.02 at 18 days. These departures are in the direction of the general rule observed at other levels that the second regeneration tends to be ahead at the earlier periods and the first at later periods, the advantage in the later case being due to the earlier completion of regeneration and absorption of regenerated material in the second regenerations than in the first ones. In this instance the first regeneration does not gain an advantage until after the second has reached its maximum.

At the ½ level there are 5 individuals for the first regeneration and 8 for the second (Table 25). The second is ahead until the eighth day.

Beginning with the tenth day the first is ahead. In general the advantage of the first increases as time goes on. The growth of new tissue does not terminate until the eighteenth day or after.

At the ⅔ level there are five individuals for the first and ten for the second regeneration (Table 26). The second is ahead of the first until the tenth day, after which the first is in the lead. Regeneration is not stopped until the eighteenth day or later.

At all four of these levels the specific length of the second regeneration tends to be ahead until the tenth day (Table 28 and Figure 2). The maximum rate of regeneration is reached before this time and somewhat earlier by the second than by the first regeneration, hence the relative gain by the latter after the tenth day (Table 30 and Figure 3). The stopping of regeneration also comes earlier for the second than for the first regeneration as does the beginning of absorption of regenerated material.

The data in Experiment I concern the amount of regeneration at six and at eight days. At the corresponding times in Experiment II the second regeneration is ahead of the first. There is a full agreement between the two experiments in this regard.

The more rapid rate of the second regeneration at the start may at first sight seem to be due to the presence of at least some cells which have been actively engaged in previous regenerations. If the second cut comes outside of the boundary between old and new cells the latter cover the whole new cut surface. Even if the cut seems to be exactly at the original cut level there will be some new cells at the regenerating surface. These cells which are already regenerating may be expected to adjust themselves more readily to the new conditions than old ones which have not been engaged in such a process. In another place the relative rates from old and from new tissue are described and a slight early difference favoring the new tissue is made out. While this slight initial advantage may be explained in this way it is probably confined to the period of cell migration and is not a factor in the period of cell division which begins on the second day or later. It is evident that on the whole the control of rate is not a matter inherent in the cells in the neighborhood of the cut surface. Indications point rather to a more central control of the process.

TABLE 21

Rana clamitans Series 3676-3765
Comparison of first and second regenerations Age factor eliminated
One-eighteenth of tail removed

	Catalog number	Removed length mm.	Length regenerated in mm. 4 Days	6 Days	8 Days	10 Days	12½ Days	18 Days	56 Days
First regeneration	3706	1.4	0.24	0.54	0.9	1.0	1.0	0.9	0.7
	3742	1.7	0.30	0.40	0.7	0.8	0.9	0.9	0.7
	Average	1.5							
Second regeneration	3676	1.3	0.27	0.60	0.9	1.0	1.0	1.0	0.7
	3682	1.6	0.18	0.60	0.9	1.0	1.0	1.0	1.1
	3730	1.6	0.39	0.75	0.9	0.9	0.9	0.9	0.7
	3754	1.6	0.06	0.55	0.9	1.1	1.2	1.2	1.1
	Average	1.5							
Av. length—First regen.			0.27	0.47	0.8	0.9	0.9	0.9	0.7
Av. length—Second regen.			0.22	0.62	0.9	1.0	1.0	1.0	0.9
Increase or decrease			—0.05	+0.15	+0.1	+0.1	+0.1	+0.1	+0.2
Specific lg.—First regen.			0.17	0.30	0.53	0.58	0.61	0.60	0.45
Specific lg.—Second regen.			0.15	0.42	0.60	0.67	0.67	0.67	0.60
Increase or decrease			—0.02	+0.12	+0.07	+0.09	+0.06	+0.07	+0.15

TABLE 22

Rana clamitans Series 3676-3765
Comparison of first and second regenerations Age factor eliminated
One-tenth of tail removed

	Catalog number	Removed length mm.	Length regenerated in mm. 4 Days	6 Days	8 Days	10 Days	12½ Days	18 Days	56 Days
First regeneration	3688	2.5	0.12	0.3	0.3	0.7	0.9	0.9	0.7
	3707	3.2	0.24	0.8	1.1	1.4	1.4	1.3	0.7
	3724	2.6	0.06	0.5	0.8	1.1	1.4	1.4	1.2
	3743	2.5	0.03	0.1	0.4	0.8	1.0	1.0	1.7
	3760	3.1	0.30	0.6	0.9	1.1	1.2	1.1	1.1
	Average	2.6							
Second regeneration	3677	2.0	0.30	0.6	0.9	0.9	0.9	0.9	0.7
	3696	2.1	0.48	0.8	1.0	1.1	1.0	1.0	0.7
	3713	2.8	0.36	0.8	0.9	0.9	0.9	0.9	0.5
	3719	3.1	0.36	0.8	1.1	1.4	1.4	1.3	—
	3749	2.8	0.30	0.6	1.2	1.4	1.4	1.3	0.9
	3750	3.5	0.48	1.3	1.7	1.9	2.0	2.0	—
	3701	3.2	0.42	0.8	1.1	1.2	1.3	1.3	—
	Average	2.8							
Av. length—First regen.			0.15	0.5	0.7	1.0	1.2	1.1	1.1
Av. length—Second regen.			0.39	0.8	1.1	1.3	1.3	1.2	0.8
Increase or decrease			+0.24	+0.3	+0.4	+0.3	+0.1	+0.1	—0.3
Specific lg.—First regen.			0.06	0.18	0.27	0.38	0.46	0.42	0.42
Specific lg.—Second regen.			0.14	0.30	0.39	0.46	0.46	0.43	0.29
Increase or decrease			+0.08	+0.12	+0.12	+0.08	0.00	+0.01	—0.13

TABLE 23

Rana clamitans Series 3676-3765

Comparison of first and second regeneration Age factor eliminated

One-sixth of tail removed

	Catalog number	Removed length mm.	Length regenerated in mm. 4 Days	6 Days	8 Days	10 Days	12½ Days	18 Days	56 Days
First regeneration	3708	5.3	0.54	1.2	1.9	2.1	2.3	2.3	1.8
	3726	4.3	0.42	0.9	1.3	1.4	1.4	1.4	1.4
	3762	4.1	0.57	1.0	1.2	1.5	1.7	1.8	1.4
	Average	4.6							
Second regeneration	3678	5.0	0.20	0.5	0.9	1.0	1.1	1.1	—
	3684	5.5	0.15	0.7	1.2	1.4	1.4	1.5	1.4
	3702	4.7	0.42	0.8	1.1	1.3	1.3	1.3	1.3
	3720	4.6	0.06	0.3	1.3	1.8	2.3	1.9	2.0
	3756	4.8	0.36	1.0	1.3	1.7	1.8	1.6	—
	Average	4.9							
Av. length—First regen.			0.51	1.0	1.5	1.7	1.8	1.8	1.5
Av. length—Second regen.			0.24	0.7	1.2	1.4	1.6	1.5	1.6
Increase or decrease			—0.27	—0.3	—0.3	—0.3	—0.2	—0.3	+0.1
Specific lg.—First regen.			0.11	0.22	0.33	0.37	0.39	0.39	0.34
Specific lg.—Second regen.			0.05	0.14	0.24	0.29	0.33	0.31	0.33
Increase or decrease			—0.06	—0.08	—0.09	—0.08	—0.06	—0.08	—0.01

TABLE 24

Rana clamitans Series 3676-3765
Comparison of first and second regenerations Age factor eliminated
One-third of tail removed

	Catalog number	Removed length mm.	Length regenerated in mm. 4 Days	6 Days	8 Days	10 Days	12½ Days	18 Days	56 Days
First regeneration	3690	9.7	0.48	1.0	1.7	2.4	2.6	2.7	2.2
	3709	8.8	0.48	1.3	2.0	2.6	3.2	3.4	3.3
	3727	8.3	0.48	1.1	1.6	2.0	2.2	2.2	2.2
	3745	10.0	0.54	1.8	2.4	3.8	4.4	4.8	4.2
	3744	6.0	0.36	1.0	1.3	1.7	1.8	1.7	—
	3761	6.6	0.39	1.0	1.5	1.9	2.2	2.3	1.8
	3763	8.5	0.57	1.1	1.8	2.4	2.9	3.1	—
	3689	6.3	0.30	0.7	1.2	1.5	1.6	1.7	1.4
	Average	8.2							
Second regeneration	3679	8.4	0.30	0.7	1.4	1.9	1.9	2.1	—
	3685	9.3	0.60	1.2	1.9	2.3	2.8	3.0	2.6
	3697	7.3	0.48	1.2	1.7	2.2	2.4	2.3	2.1
	3703	9.3	0.45	1.3	2.0	2.5	2.6	2.5	2.5
	3715	7.9	0.24	0.9	1.7	2.3	2.6	2.6	2.8
	3721	8.7	0.57	1.3	1.9	2.3	2.6	2.3	2.2
	3733	8.5	0.36	1.0	1.9	2.4	2.6	2.6	2.8
	3734	8.5	0.48	2.1	3.1	4.5	5.7	6.4	6.6
	3739	9.6	0.36	1.0	1.8	2.4	3.0	2.9	—
	3751	6.7	0.45	1.1	1.7	2.1	2.4	2.5	2.3
	3757	8.0	0.42	1.2	2.1	2.8	3.1	3.2	3.1
	Average	8.4							
Av. length—First regen.			0.45	1.1	1.7	2.3	2.6	2.7	2.5
Av. length—Second regen.			0.42	1.1	1.8	2.3	2.6	2.6	2.5
Increase or decrease			—0.03	0.0	+0.1	0.0	0.0	—0.1	0.0
Specific lg.—First regen.			0.05	0.13	0.21	0.28	0.31	0.33	0.30
Specific lg.—Second regen.			0.05	0.13	0.22	0.28	0.31	0.31	0.30
Increase or decrease			0.00	0.00	+0.01	0.00	0.00	—0.02	0.00

TABLE 25

Rana clamitans Series 3676-3765
Comparison of first and second regenerations Age factor eliminated
One-half of tail removed

	Catalog number	Removed length mm.	Length regenerated in mm. 4 Days	6 Days	8 Days	10 Days	12½ Days	18 Days	56 Days
First regeneration	3710	12.3	0.42	1.8	2.9	3.7	3.9	3.9	3.9
	3728	12.8	0.60	1.7	2.8	3.9	4.8	5.4	5.8
	3746	13.3	0.54	1.7	2.4	4.1	5.7	7.0	6.8
	3764	14.6	0.42	1.3	2.5	4.2	5.3	6.8	6.5
	3765	12.2	0.30	1.5	2.3	3.2	3.9	4.5	4.5
	Average	13.0							
Second regeneration	3686	14.5	0.60	2.1	3.4	4.8	5.3	5.2	—
	3698	14.9	0.50	1.5	3.3	4.3	5.0	5.4	5.4
	3704	14.5	0.45	2.2	3.3	4.4	5.2	5.5	5.4
	3716	12.7	0.39	1.7	2.4	3.4	4.2	5.1	4.4
	3722	12.5	0.60	1.6	2.6	3.6	3.9	3.5	4.2
	3740	13.9	0.30	1.1	2.1	3.0	4.6	5.6	6.8
	3752	12.2	0.54	1.7	2.5	3.4	4.1	4.0	—
	3758	11.0	0.60	1.5	2.2	2.9	3.6	4.1	4.9
	Average	13.1							
Av. length—First regen.			0.46	1.6	2.6	3.8	4.7	5.5	5.5
Av. length—Second regen.			0.50	1.7	2.7	3.7	4.4	4.8	5.2
Increase or decrease			+0.04	+0.1	+0.1	—0.1	—0.3	—0.7	—0.3
Specific lg.—First regen.			0.03	0.12	0.20	0.29	0.36	0.42	0.42
Specific lg.—Second regen.			0.04	0.13	0.21	0.28	0.34	0.37	0.40
Increase or decrease			+0.01	+0.01	+0.01	—0.01	—0.02	—0.05	—0.02

TABLE 26

Comparison of first and second regenerations Age factor eliminated
Rana clamitans Series 3676-3765
Two-thirds of tail removed

	Catalog number	Removed length mm.	Length regenerated in mm. 4 Days	6 Days	8 Days	10 Days	12½ Days	18 Days	56 Days
First regeneration	3692	16.8	0.51	1.1	2.2	3.2	4.3	5.0	5.2
	3693	17.2	0.48	1.8	3.3	5.0	6.5	7.3	6.6
	3711	17.0	0.54	1.8	3.6	5.6	7.0	7.7	8.3
	3729	16.1	0.48	1.9	3.3	4.6	5.5	6.7	6.4
	3749	16.2	0.54	1.2	2.7	4.2	5.6	7.1	7.8
	Average	16.7							
Second regeneration	3680	16.0	0.60	1.9	3.0	4.2	5.2	6.4	6.6
	3681	21.2	0.84	3.0	4.0	5.6	6.3	7.3	7.2
	3687	19.7	0.54	3.6	5.6	6.0	6.6	7.0	—
	3699	21.0	0.54	2.2	4.3	5.9	7.1	7.5	7.2
	3705	17.6	0.72	2.0	3.6	4.8	6.4	6.2	6.0
	3717	17.6	0.42	2.6	3.6	5.2	6.0	6.7	6.4
	3723	18.4	0.30	2.3	3.7	5.3	6.5	8.1	8.3
	3735	16.5	0.48	2.0	3.4	5.5	6.5	7.8	8.0
	3741	16.0	0.30	1.9	3.0	4.4	5.8	6.9	7.0
	3753	16.8	0.42	2.0	2.5	3.8	5.2	6.4	7.1
	Average	18.1							
Av. length—First regen.			0.51	1.56	3.02	4.52	5.78	6.76	6.86
Av. length—Second regen.			0.52	2.35	3.67	5.07	6.16	7.03	7.09
Increase or decrease			+0.01	+0.79	+0.65	+0.55	+0.38	+0.27	+0.23
Specific lg.—First regen.			0.03	0.09	0.18	0.27	0.35	0.40	0.41
Specific lg.—Second regen.			0.03	0.13	0.20	0.28	0.34	0.39	0.39
Increase or decrease			0.00	+0.04	+0.02	+0.01	—0.01	—0.01	—0.02

TABLE 27

Rana clamitans Series 3676-3765
Comparison of first and second regenerations Age factor eliminated
Average lengths regenerated in mm.

Approx. fraction of tail removed	Regeneration	Number of individuals	Average length removed in mm.	Average length regenerated in mm.						
				4 Days	6 Days	8 Days	10 Days	12½ Days	18 Days	56 Days
1/18	1	2	1.5	0.3	0.5	0.8	0.9	0.9	0.9	0.7
	2	4	1.5	0.2	0.6	0.9	1.0	1.0	1.0	.0.9
1/10	1	5	2.6	0.1	0.5	0.7	1.0	1.2	1.1	1.1
	2	7	2.8	0.4	0.8	1.1	1.3	1.3	1.2	0.8
1/6	1	3	4.6	0.5	1.0	1.5	1.7	1.8	1.8	1.5
	2	5	4.9	0.2	0.7	1.2	1.4	1.6	1.5	1.6
1/3	1	8	8.2	0.4	1.1	1.7	2.3	2.6	2.7	2.5
	2	10	8.4	0.4	1.1	1.8	2.3	2.6	2.6	2.5
1/2	1	5	13.0	0.5	1.6	2.6	3.8	4.7	5.5	5.5
	2	8	13.1	0.5	1.7	2.7	3.7	4.4	4.8	5.2
2/3	1	5	16.7	0.5	1.6	3.0	4.5	5.8	6.8	6.9
	2	10	18.1	0.5	2.3	3.7	5.1	6.2	7.0	7.1

TABLE 28

Rana clamitans Series 3676-3765

Comparison of first and second regenerations Age factor eliminated
Specific lengths regenerated

Approx. fraction of tail removed	Regeneration	Number of individuals	Average length removed in mm.	Specific length regenerated in mm. 4 Days	6 Days	8 Days	10 Days	12½ Days	18 Days	56 Days
1/18	1	2	1.5	0.17	0.30	0.53	0.58	0.61	0.60	0.45
	2	4	1.5	0.15	0.42	0.60	0.67	0.67	0.67	0.60
1/10	1	5	2.6	0.06	0.18	0.27	0.38	0.46	0.42	0.42
	2	7	2.8	0.14	0.30	0.39	0.46	0.46	0.43	0.29
1/6	1	3	4.6	0.11	0.22	0.33	0.37	0.39	0.39	0.34
	2	5	4.9	0.05	0.14	0.24	0.29	0.33	0.31	0.33
1/3	1	8	8.2	0.05	0.13	0.21	0.28	0.31	0.33	0.30
	2	10	8.4	0.05	0.13	0.22	0.28	0.31	0.31	0.30
1/2	1	5	13.0	0.03	0.12	0.20	0.29	0.36	0.42	0.42
	2	8	13.1	0.04	0.13	0.21	0.28	0.34	0.37	0.40
2/3	1	5	16.7	0.03	0.09	0.18	0.27	0.35	0.40	0.41
	2	10	18.1	0.03	0.13	0.20	0.28	0.34	0.39	0.39
All levels—Average—First				0.075	0.173	0.287	0.362	0.413	0.427	0.390
All levels—Average—Second				0.077	0.208	0.310	0.377	0.408	0.413	0.385
First ahead				—	—	—	—	0.005	0.014	0.005
Second ahead				0.002	0.035	0.023	0.015	—	—	—

TABLE 29

Rana clamitans Series 3676-3765

Comparison of first and second regenerations Age factor eliminated

Difference between first and second regenerations

Approx. fraction of tail removed	Average length removed in mm.	Number of individuals			Increase--second over first regeneration						
		First regen.	Second regen.		4 Days	6 Days	8 Days	10 Days	12½ Days	18 Days	56 Days
1/18	1.5	2	4	Length in mm.	—0.1	+0.1	+0.1	+0.1	+0.1	+0.1	+0.2
				Specific length	—0.02	+0.12	+0.07	+0.09	+0.08	+0.07	+0.15
1/10	2.7	5	7	Length in mm.	+0.3	+0.3	+0.4	+0.3	+0.1	+0.1	—0.3
				Specific length	+0.08	+0.12	+0.12	+0.08	0.00	+0.01	—0.13
1/6	4.8	3	5	Length in mm.	—0.3	—0.3	—0.3	—0.3	—0.2	—0.3	+0.1
				Specific length	—0.06	—0.08	—0.09	—0.08	—0.06	—0.08	—0.01
1/3	8.3	8	10	Length in mm.	0.0	0.0	+0.1	0.0	0.0	—0.1	0.0
				Specific length	0.00	0.00	+0.01	0.00	0.00	—0.02	0.00
1/2	13.1	5	8	Length in mm	0.0	+0.1	+0.1	—0.1	—0.3	—0.7	—0.3
				Specific length	+0.01	+0.01	+0.01	—0.01	—0.02	—0.05	—0.02
2/3	17.6	5	10	Length in mm.	0.0	+0.7	+0.7	+0.6	+0.4	+0.2	+0.2
				Specific length	0.00	+0.04	+0.02	+0.01	—0.01	—0.01	—0.02

TABLE 30

Rana clamitans Series 3676-3765

Specific rates of first and second regenerations during each of the time periods

Approx. fraction of tail removed	Regeneration	Number of individuals	Average length removed in mm.	Specific rate of regeneration						
				0-4 Days	4-6 Days	6-8 Days	8-10 Days	10-12½ Days	12½-18 Days	18-56 Days
1/18	1	2	1.5	0.042	0.065	0.115	0.025	0.015	—0.002	—0.004
	2	4	1.5	0.037	0.135	0.090	0.035	0.000	0.000	—0.002
1/10	1	5	2.6	0.015	0.040	0.045	0.055	0.040	—0.007	—0.001
	2	7	2.8	0.035	0.080	0.045	0.035	0.000	—0.005	—0.004
1/6	1	3	4.6	0.027	0.055	0.055	0.020	0.010	0.000	—0.001
	2	5	4.9	0.012	0.045	0.050	0.025	0.025	—0.004	0.001
1/3	1	8	8.2	0.012	0.040	0.040	0.035	0.015	0.004	—0.001
	2	10	8.4	0.012	0.040	0.045	0.030	0.015	0.000	—0.000
1/2	1	5	13.0	0.007	0.045	0.040	0.045	0.035	0.011	0.000
	2	8	13.1	0.010	0.045	0.040	0.035	0.030	0.005	0.001
2/3	1	5	16.7	0.007	0.030	0.045	0.045	0.040	0.009	+0.000
	2	10	18.1	0.007	0.050	0.035	0.040	0.030	0.009	0.000
All levels—Average—First				0.018	0.046	0.057	0.037	0.026	0.002	—0.001
All levels—Average—Second				0.019	0.066	0.051	0.033	0.017	0.001	—0.001
First ahead				—	—	0.006	0.004	0.009	0.001	—
Second ahead				0.001	0.020	—	—	—	—	—

EXPERIMENT III AMBLYSTOMA PUNCTATUM SERIES 3962-3999

Material and Method Eggs of Amblystoma punctatum in the cleavage stages were collected on March 18, 1913, and hatched in the laboratory on April 9. The first operations were made on April 23, at which time also five controls were killed and preserved. These when measured gave an average total length of 13.1 mm. and an average tail length of 5.3 mm. Ninety individuals were used for the regeneration study. In thirty individuals two-thirds in length of the tail was removed on April 23. The regenerated portion in these was removed on May 10 and at the same time in a second thirty individuals two-thirds of the tail was removed. On May 21 the first thirty were operated on for the third time, the second thirty for the second time, and the third thirty for the first time. To insure as accurate a comparison as possible the ninety individuals though they were approximately of equal size were divided into thirty groups of three each, a selection being made so that the three members of a group were as much alike as possible. In each group one of the three members was used for the first regeneration, one for the second and the third for the third regeneration. This procedure gave a possibility of comparing the first, second and third regenerations without error due to difference in size, age, or in external conditions.

Three individuals from each thirty were killed two days after the last operations, four in four days, five in six days, five in eight days, six in ten days and seven in fourteen days.

At the end of the experiment, control individuals gave an average total length of 31.5 mm. and an average tail length of 10.5 mm.

Data The data are given in Tables 31 and 32. The specific amounts of regeneration were not determined because the removed lengths were alike and hence the comparison of absolute lengths gives the same results as a comparison of specific amounts.

The average regenerated lengths at each of the six different times will be taken up first. At two days the average regenerated lengths for the first, second and third regenerations are respectively 0.22, 0.25 and 0.26 mm. At four days the corresponding amounts are 0.66, 0.75 and 1.00. At six days they are 1.36, 1.40 and 1.36, but the low value of the third regeneration is due to a single exceptional individual. At eight days the figures are 2.18, 2.68 and 2.68. At ten days they are 3.55, 3.82 and 4.20 and at fourteen days 5.34, 6.12 and 6.08. In all cases, except the one at six days explained above, both second and third regenerations are ahead of the first. The third regeneration is greater than the second at two, four and ten days, is equal to the second at eight days and less than the second at six and fourteen days. Since the low

average for the third regeneration at six days is due to a single exceptional individual it is more proper to put the third ahead of the second at this time.

A comparison of the three regenerations by individual cases is shown in Table 32. At each of the six times taken the number of cases showing a more rapid regeneration is greater for the third regeneration than for the first and also greater for the second than for the first. The third is ahead of the second at two times (more properly three times) and equal to the third at four times (more properly three).

When all the individual cases are taken together both third and second regenerations are again distinctly ahead of the first as shown by the totals in Table 32. The third is ahead of the second in twelve cases (more properly thirteen) and the second ahead of the first in eight cases (more properly seven).

Each of the three comparisons shows that both second and third regenerations are more rapid than first regenerations. The third regeneration shows a slight advantage over the second instance in all three of the comparisons. In this instance the difference can not be due to the presence of newly regenerated cells in the one case and not in the other.

TABLE 31

Amblystoma punctatum Series 3967-3998
Comparison of lengths of first, second and third regenerations
Age factor eliminated

Regeneration time in days	Catalog number	Regenerated lengths in mm.		
		First regeneration	Second regeneration	Third regeneration
2	3967	0.2	0.25	0.3
	3968	0.25	0.3	0.27
	3969	0.2	0.2	0.2
	Average	0.22	0.25	0.26
4	3970	0.75	0.9	1.0
	3971	0.7	—	1.6
	3972	0.5	0.75	0.8
	3973	0.7	0.6	0.6
	Average	0.66	0.75	1.00

TABLE 31 (Continued)
Amblystoma punctatum Series 3967-3998
Comparison of lengths of first, second and third regenerations
Age factor eliminated

Regeneration time in days	Catalog number	Regenerated lengths in mm.		
		First regeneration	Second regeneration	Third regeneration
6	3974	1.2	1.2	—
	3975	1.4	1.5	1.5
	3976	1.3	1.4	1.6
	3977	1.5	1.2	0.6
	3978	1.4	1.7	1.7
	Average	1.36	1.40	1.36
8	3980	1.7	2.4	2.7
	3981	1.9	—	2.9
	3982	2.3	2.6	3.0
	3984	2.4	2.8	2.1
	3985	2.6	2.9	2.7
	Average	2.18	2.68	2.68
10	3986	4.1	3.8	4.7
	3987	3.6	3.7	—
	3988	3.5	—	3.9
	3989	2.6	3.9	3.5
	3990	3.2	4.25	4.6
	3991	4.3	3.5	4.35
	Average	3.55	3.82	4.20
14	3992	5.5	5.5	5.7
	3993	5.0	5.75	5.7
	3994	—	6.7	6.9
	3995	5.0	5.7	6.9
	3997	6.9	6.7	5.2
	3998	4.3	6.35	—
	Average	5.34	6.12	6.08

TABLE 32

Amblystoma punctatum Series 3967-3998 Age factor eliminated
Comparison of lengths of first, second and third regenerations
Comparison of individual cases

Comparisons	Two days	Four days	Six days	Eight days	Ten days	Fourteen days	Totals
3rd regen. > 1st	2	3	3	4	5	3	20
3rd regen. = 1st	1	0	0	0	0	0	1
3rd regen. < 1st	0	1	1	1	0	1	4
2nd regen. > 1st	2	2	3	4	3	3	17
2nd regen. = 1st	1	0	1	0	0	1	3
2nd regen. < 1st	0	1	1	0	2	1	5
3rd regen. > 2nd	1	2	1	2	3	3	12
3rd regen. = 2nd	1	1	2	0	0	0	4
3rd regen. < 2nd	1	0	1	2	1	3	8

EXPERIMENT IV AMBLYSTOMA PUNCTATUM SERIES 3962-3999

The series used for Experiment III furnishes another set of data for the effect of successive removal. When the third operation was made the removed regenerated tails of the first thirty individuals represented an eleven-day second regeneration and those of the second thirty-individuals an eleven-day first regeneration. A direct comparison is thus possible between the first and the second regenerations. It is not possible to make a cut exactly at the border line between old and new tissue and therefore the measurement of the removed regenerating tail is not as accurate a determination as is the direct measurement of a regenerating unremoved tail.

The data are shown in Table 33. Twenty-five individuals are available for each regeneration. The average of the first regenerations is 4.55 ±0.11 and of the second regenerations 4.50 ±0.10. The first regeneration is ahead of the second in ten cases, the second is ahead of the first in twelve cases and three cases are equal. The first comparison shows a slight difference in favor of the first regeneration but this is so much less than the probable error that it can not be considered as significant. The second comparison shows a slight advantage in favor of the second regeneration. On the whole the data indicate essential equality between the first and the second regenerations at eleven days.

TABLE 33

Amblystoma punctatum Series 3962-3999 Age factor eliminated
Comparison of first and second regenerations
Eleven days

Catalog number	First regen. mm.	Second regen. mm.	First ahead of second	Second ahead of first	First and second equal
3967	4.0	4.4		0.4	
3968	3.7	3.5	0.2		
3969	4.9	5.1		0.2	
3970	4.5	4.7		0.2	
3972	4.7	4.7			*
3973	3.9	4.3		0.4	
3975	4.5	5.7		1.2	
3976	4.9	4.9			*
3977	3.8	3.7	0.1		
3978	4.1	4.5		0.4	
3980	4.9	5.0		0.1	
3981	3.5	4.4		1.1	
3982	5.0	4.7	0.3		
3984	5.1	4.0	1.1		
3985	5.8	4.3	1.5		
3986	3.8	4.1		0.3	
3989	4.8	5.5		0.7	
3990	5.5	4.3	1.2		
3991	4.1	4.6		0.5	
3992	4.6	4.1	0.5		
3993	4.5	4.5			*
3994	4.9	5.0		0.1	
3995	4.5	4.0	0.5		
3997	5.1	4.2	0.9		
3998	4.8	4.3	0.5		
	4.55±0.11	4.50±0.10	ten times	twelve times	three times

EXPERIMENT V AMBLYSTOMA PUNCTATUM SERIES 6042-6100F

This series was devised for a study of the effect of repeated removal of the tail upon the rate of metamorphosis. The removed tails were preserved and they give some data on the comparison of successive regenerations. The interest of the results lies in the fact that the successive regenerations are compared within single individuals. Thus the effect of the age factor is not eliminated. Environmental differences such as those of temperature may also be factors.

The eggs were hatched on March 25 to 29, 1915. Approximately one-half in length of the tail was removed in each of the indi-

viduals on April 5. The new tissue was removed on April 17 and again on May 1, May 10 and May 19, making five removals in all. The second removal gives the first regeneration, the third the second, and so on. The regenerated lengths were therefore determined by measurement of removed parts. This does not give as accurate a determination as does direct measurement without removal because the cut can not in ordinary practice be made exactly at the border line between old and new tissue.

The data are given in Table 34. The first regeneration covers a twelve-day period, the second fourteen days and the third and fourth each nine days.

The third and fourth regenerations are the only ones that have the same time interval. Ten individuals are available for this comparison. The average for the third regeneration for these ten is 1.30 mm. and of the fourth regeneration 1.17 mm. When all individuals are taken without regard to representation of both regenerations the average for the third regeneration is 1.28 and for the fourth 1.17. In seven of the ten former cases the third is ahead of the fourth regeneration, in two they are tied and in one the fourth is ahead of the third. The data therefore show an advantage of the third over the fourth regeneration.

The first regeneration ran twelve days and the second fourteen days. The maximum rate of regeneration comes on or near the ninth day and the rate has declined to a low point by the fourteenth day. However it is not possible to make the necessary correction because of lack of data on the rate curve for this particular set of larvae. Some facts may however be obtained by a comparison. Sixteen individuals for each of the two regenerations are available for comparison. The average for the first regeneration in these is 2.06 mm. and for the second 2.01 mm. In seven the first is ahead of the second, in seven the second is ahead of the first, and two are tied. When all individuals are taken without regard to representation of both regenerations the average for the first regeneration is 1.99 ±0.03 mm. for a twelve-day period and for the second regeneration 2.01 for a fourteen-day period. The difference between the two values is not significant, but when the longer time interval taken by the second regeneration is considered the conclusion is reached that the first regeneration is more rapid than the second.

The data thus indicate a progressive decrease in rate from the first to the fourth regenerations. This result taken in connection with the results obtained from the experiments in which the age factor is eliminated makes it highly probable that the decrease in rate of regeneration observed here is due to increase in age and not to the effect of successive removal.

TABLE 34

Amblystoma punctatum Series 6042-6100 F Age factor eliminated
Successive regenerations in single individuals

Catalog number	First regeneration mm. Twelve days	Second regeneration mm. Fourteen days	Third regeneration mm. Nine days	Fourth regeneration mm. Nine days
6042	2.0	2.4	1.6	
6043	1.8	2.5	1.5	1.4
6044	2.2			
6046	2.2	2.2	1.3	1.3
6047	2.2	2.1	1.4	1.3
6048	2.0	2.2		
6049	2.3	1.8	1.5	1.0
6050	1.8			
6052	2.0			
6053	1.9			
6055	1.9			
6056	1.7			
6057	2.0			
6058	2.0			
6059	1.9			
6061	1.5			
6062	2.3			
6065	1.5			
6067	1.6			
6068	1.6			
6071	2.0			
6072	1.9			
6076	2.1			
6077	2.0			
6079	2.0			
6080	1.8			
6081	1.9			
6082	1.9	2.0	1.0	1.1
6083	2.0	2.1	1.3	1.1
6084	1.9			
6085	2.0	2.0	1.5	1.5
6086	2.2			
6087	1.8	1.0	0.8	
6088	2.1	2.4	1.4	1.2
6090	2.5			
6093	2.3	2.2	1.0	0.8
6094	2.1			

TABLE 34 (Continued)

Catalog number	First regeneration mm. Twelve days	Second regeneration mm. Fourteen days	Third regeneration mm. Nine days	Fourth regeneration mm. Nine days
6096	2.1			
6097	2.5	1.6		
6098	1.8	2.1		
6099	2.2			
6100D	2.0			
6100E	1.8	1.6		
6100F	2.2	2.0	1.1	1.0
Average	**1.99±0.03**	**2.01**	**1.28**	**1.17**
Rate per day	**0.166**	**0.144**	**0.142**	**0.130**

EXPERIMENT VI BUFO AMERICANUS SERIES 6283-6323

This series was designed for the study of the effect of successive removal of the tail upon the rate of metamorphosis. The lengths of the removed regenerating tails however are of some value in a comparison of successive regenerations though here as in Experiment V age and external factors are not eliminated.

The eggs were laid on April 20-21, 1915. The tadpoles were collected on April 27 and the first removals were made on April 28. The first metamorphosis was completed on June 11. The average total length at the time of the first removal was 10.9 mm. and the average tail length 6.4 mm. The average removed length was 3.8 mm., which is approximately 60 per cent of the tail length. The second removal was made on May 7 and gives a nine-day period for the first regeneration. The third removal of May 17 gives a ten-day period for the second regeneration. The fourth removal on May 26 gives a nine-day period for the third regeneration. As in the case of Experiment V the cuts could not in practice be made to come exactly at the border line between old and new tissue and the accuracy of the measurements is therefore not as great as in those cases in which the lengths were taken directly from the animal without removal of the tail.

The data are shown in Table 35. The first, second and third regeneration lengths are given for sixty individuals. The first and third regenerations have the same time interval and are therefore directly comparable. The average for the first regeneration is 1.94 ±0.02 mm.

and for the third 1.80 ±0.03 mm., a difference in favor of the first regeneration of 0.14 ±0.05 mm. This represents a regeneration of 0.51 mm. per unit of removed length in the first regeneration and 0.47 mm. per unit in the third regeneration. A comparison of individual cases shows that the first regeneration is ahead of the third in 36 individuals, the third is ahead of the first in 18 individuals and 5 are tied. The difference between the two regenerations is thus probably significant. As in Experiment V the decrease is probably due to the age factor.

The second regeneration has a time interval of ten days, one day more than the first and third regenerations. In the absence of knowledge concerning the rate curve for toad tadpoles of this age no correction can be applied. The rates per day for the three regenerations are however given in the table.

TABLE 35

Bufo americanus Series 6283-6323 Age factor not eliminated

Successive regenerations of tail

Catalog number	First regeneration Nine days Length in mm.	Second regeneration Ten days Length in mm.	Third regeneration Nine days Length in mm.
6283 a	2.1	2.0	1.9
b	1.5	2.1	1.6
c	1.7	2.1	1.7
6285 a	2.3	1.9	2.0
b	2.3	2.1	2.0
c	2.0	2.4	2.4
6287 a	1.9	2.1	2.1
b	2.1	2.0	1.9
c	1.8	2.3	1.8
6289 a	1.9	2.3	2.2
b	2.1	2.0	1.7
c	2.2	2.1	2.0
6291 a	2.1	2.2	2.0
b	1.9	1.9	1.8
c	2.0	1.9	1.4
6295 a	2.0	2.0	2.0
b	2.1	2.1	1.8
c	1.8	1.9	1.9
6297 a	1.9	2.0	2.0
b	1.9	2.1	1.8
c	1.9	2.0	1.8
6299 a	1.8	2.0	1.5
b	2.0	2.0	1.7

TABLE 35 (Continued)

Catalog number	First regeneration Nine days Length in mm.	Second regeneration Ten days Length in mm.	Third regeneration Nine days Length in mm.
c	1.8	1.9	1.3
6301 a	2.0	1.5	1.4
b	1.8	1.9	1.2
c	1.7	1.8	1.4
6303 a	1.9	1.9	1.3
b	1.9	1.9	1.5
c	1.8	2.0	1.6
6305 a	1.9	2.0	1.5
b	1.7	1.8	2.0
c	2.1	2.2	1.9
6307 a	2.1	1.8	2.0
b	1.9	2.0	2.1
c	1.8	1.9	1.8
6309 a	2.0	1.9	1.9
b	1.9	1.9	2.0
c	2.0	2.0	1.7
6311 a	1.7	2.4	1.8
b	1.8	1.9	2.1
c	2.0	2.1	2.0
6313 a	2.0	2.3	1.7
b	1.8	1.7	2.0
c	1.7	1.7	1.5
6315 a	1.9	2.4	2.0
b	2.0	2.1	1.6
c	..	1.9	1.9
6317 a	1.9	2.0	1.3
b	1.8	2.2	2.0
c	1.9	2.1	1.8
6319 a	2.2	2.0	2.0
b	2.1	2.0	2.0
c	1.9	1.9	2.0
6321 a	2.3	2.0	1.8
b	1.7	2.0	1.9
c	2.0	2.3	1.3
6323 a	2.1	2.1	1.7
b	1.8	2.3	2.0
c	1.9	2.2	2.0
Average	1.94±0.02	2.02±0.02	1.80±0.03
Rate per day	0.216	0.202	0.200

EXPERIMENT VII RANA CLAMITANS SERIES 3557-3624

This experiment deals with the relative completeness of regeneration after successive removals within single individuals. Age and external factors are not eliminated. A more complete description of the experiment is given under "Completeness of Regeneration." The tail length averaged approximately 17.0 mm. About one-half of the tail was removed at the first operation. At succeeding operations the cut came as near as possible to the border line between old and new tissue. The first removals came on October 23, 1911, the second on November 28, the third January 3, the fourth February 9, the fifth March 16 and the sixth April 4. At the time of the last removal the hind legs were just starting to grow.

The data are given in Table 36. The first regeneration interval is 36 days, the second 36, the third 37, the fourth 36 and the fifth 39 days. Each one of these is more than sufficient for the completion of the regenerative process. The individuals are divided into three sets, A, B, and C. A, with seven individuals, has no record for the first regeneration; the second regeneration is 9.8 mm., the third 9.3, the fourth 8.5 and the fifth 8.6. B, also with seven individuals, has no record for the first regeneration; the second is 9.1 mm., the third 8.9, the fourth 7.2 and the fifth 7.8. C, with nineteen individuals, has a first regeneration average of 8.6 mm., a second of 8.0, a third of 7.5, a fourth of 5.5 and a fifth of 6.4. In all the cases there is a decrease in the amount regenerated with successive removal except for the fifth regeneration, which has in each case an increase over the fourth. It is probable that

TABLE 36

Rana clamitans Series 3564-3624 Age factor not eliminated
Completed successive regenerations compared

Set	Catalog number	Number of individuals	First regeneration 36 Days	Second regeneration 36 Days	Third regeneration 37 Days	Fourth regeneration 36 Days	Fifth regeneration 39 Days
A	3564 to 3570	7	..	9.8	9.3	8.5	8.6
B	3578 to 3584	7	..	9.1	8.9	7.2	7.8
C	3586 to 3624	19	8.6	8.0	7.5	5.5	6.4

the decrease is due to increase in age. The increase from the fourth to the fifth regeneration may be due to some special characteristic of the stage immediately preceding metamorphosis or it may merely indicate the existence of some uncontrolled external factor such as food or temperature.

Discussion

The evidence shows clearly that when the age factor is eliminated there is no decrease in rate of regeneration with successive removal. On the contrary the second regeneration is more rapid than the first up to the period of maximum rate. The second regeneration however passes its maximum sooner than does the first and after the tenth day the latter therefore catches up to the former in total amount regenerated. There is no striking difference between the second and the third regenerations but in each comparison the third has a slight advantage.

When the successive regenerations in single individuals are compared, the rate decreases with successive removal. This decrease is undoubtedly due to the age factor.

The possibility has suggested itself that the second regeneration starts out at a more rapid rate than the first because the cells at the cut surface were undergoing regenerative changes at the time of the new operation and can therefore start the process much faster than can the old cells at the first surface of regeneration. Following a first removal there is a considerable degree of reorganization of the cells at the cut surface, accompanied by active migration. During this period, which in *Rana clamitans* lasts two or three days, there is little or no mitotic cell division. Then follows a division period which reaches its maximum at seven to ten days. Its decline is associated with the oncoming of tissue differentiation (Sutherland 1915, Metcalf 1915).

A special study has been made of the relative rates of second regenerations from old cells following a cut inside of the first removal level and from new cells following a cut outside of the first level. This comparison shows only a very slight difference in favor of the new cells and this is largely confined to the early stages, the period of cell migration.

The period of increase in rate is the period of active cell multiplication and the decline in rate is associated with cell differentiation. The second regeneration therefore reaches the period of differentiation slightly in advance of the first regeneration.

Apart from the slowing due to age there is no indication of a limitation of the amount of new material that may be produced by regeneration. The actual limitation comes not from the using up of

regenerative or developmental energy or of determiners by repeated regeneration but from changes in the non-regenerating part associated with age. In another place there is a discussion of the possibility that there may be an effect upon the rate of developmental processes in the organism as a whole due to continued regeneration of a part. This is studied particularly in connection with the effect of regeneration upon rate of metamorphosis in Amphibia.

Regeneration studies in general and those on successive regeneration in particular make it improbable that there is a definite number of cell generations between the fertilized egg and the end product, the differentiated cells. The possibility that certain cells may remain in an early cell generation can not be wholly excluded as an explanation of at least a part of first regeneration phenomena. Under suitable stimulation such cells may be postulated to take up development where it had left off. The definite descriptions of de-differentiations of cells as well as other facts of regeneration argue against this conclusion. The view that there can be no such definite number of cell generations is strengthened by the facts of successive regeneration. It does not seem probable that embryonic cells of an early cell generation can be held in reserve through repeated regenerations.

The explanation of regeneration by the theory of duplicate sets of determiners meets difficulties in undiminished successive regenerations. The greater the number of repeated regenerations the greater the difficulties of explanation on this basis. Of course the difficulty does not hold for the hypothesis that every cell or nearly every cell contains a full set of determiners.

The earlier appearance of the maximum rate in the second than in the first regeneration may be due to the more rapid progress of the cells in the early cell migration period alone or it may be due to the acceleration of the whole developmental cycle.

Summary

1. The age factor was eliminated in Experiments I to IV. Experiments I and II deal with tadpoles of Rana clamitans and Experiments III and IV with larvae of Amblystoma punctatum.

2. In Experiment I approximately one-half of the tail was removed. At six days the average first regeneration length is 2.01 mm. and the average second regeneration length 2.18 mm. In five cases the first exceeds the second and in six the second exceeds the first. The corresponding specific lengths are 0.194 and 0.205. The first regeneration exceeds the second in two sets, the second exceeds the first in eight and one is tied. The second regeneration has the advantage in all the comparisons.

3. At eight days in Experiment I the average first regeneration length is 3.06 mm., and the second 3.42 mm. The first exceeds the second in three sets and the second exceeds the first in seven. The corresponding average specific lengths are 0.298 and 0.323. In four sets the first regeneration exceeds the second and in six the second exceeds the first. The second regeneration has the advantage in all the comparisons.

4. The advantage of the second regeneration over the first in Experiment I holds true of second regenerations from both old tissue and new tissue levels.

5. In Experiment II observations were made at the 1/10, 1/3, 1/2 and 2/3 levels in a sufficient number of individuals to yield valid data. Regeneration measurements were made at each of these levels 4, 6, 8, 10, 12½, 18 and 56 days after the operations. The second regeneration at all of them tends to be ahead of the first until the tenth day, after which the first regeneration catches up. The maximum rate for both regenerations is reached before this time and earlier for the second than for the first regeneration.

6. In Experiment III two-thirds of the tail was removed. A comparison of the first, second and third regenerations was made at 2, 4, 6, 8, 10 and 14 days.. At two days the first, second and third regenerations average respectively 0.22, 0.25 and 0.26 mm. The corresponding values at four days are 0.66, 0.75 and 1.00; at six days 1.36, 1.40 and 1.46; at eight days 2.18, 2.68 and 2.68; at ten days 3.55, 3.82 and 4.20; at fourteen days 5.34, 6.12 and 6.08. The advantage is in favor of the second and third regenerations as opposed to the first and of the third as opposed to the second. Individual comparisons at each of the different times as well as in the experiment as a whole show the same results.

7. The removed tails in the preliminary procedure of Experiment III furnish the data of Experiment IV and allow a comparison of the first and second regenerations at eleven days. The procedure is however subject to greater error than that of Experiments I to III. Twenty-five individuals for each regeneration give an average of 4.55 ±0.11 mm. for the first regeneration and 4.50 ±0.10 mm. for the second regeneration. The first regeneration is ahead of the second in ten cases, the second ahead of the first in twelve cases and three are equal. The two regenerations must be considered as essentially equal.

8. In Experiments V and VI the age factor is not eliminated. Successive regenerations in single individuals are compared. In Experiment V one-half of the tail in Amblystoma larvae was removed. In Experiment VI 60 per cent of the tail of toad tadpoles was removed.

The time intervals vary somewhat in each set but it is evident in both cases that there is a decrease in rate of regeneration from the first to the third and fourth regenerations. This decrease is undoubtedly due to increase in age and not to successive removal.

9. In Experiment VII a comparison of the completeness of regeneration in single individuals of *Rana clamitans* shows a progressive decrease in amount regenerated from the first to the fourth regeneration and an increase from the fourth to the fifth. In this experiment also the age factor is not eliminated and the decrease is probably due to increase in age.

PART III

THE EFFECT OF LEVEL OF THE CUT UPON THE RATE AND COMPLETENESS OF REGENERATION

The present study gives a description of some experiments made to define more accurately than has been done the exact relation between the level of the cut and rate of regeneration and especially the relation of this factor to the other factors affecting rate and completeness of regeneration. The factor is one of great interest because if it is true that the ratio between length regenerated per unit time and length removed is a constant it follows that no matter how much material is removed regeneration is always completed in the same time. It is therefore of great interest to determine the extent to which this statement is true, to analyze the elements of the level factor and to determine its relation to other factors.

Experiment I Rana clamitans Series 3676-3765

The tadpoles were collected on December 9, 1911, and first removals were made in two-thirds of the individuals on December 22. A second removal was made in these individuals on January 8, and at the same time a first removal in the other one-third. Measurements were made four, six, eight, ten, twelve and a half, eighteen and fifty-six days after the operations of January 8. The first and second regenerations are treated separately and the second regenerations are taken up first because they have a larger number of individuals and therefore give the more uniform results.

Second Regenerations

The different amounts removed approximate 6, 10, 18, 31, 49 and 67 per cent of the tail length. There are four individuals at the lowest removal, averaging 1.5 mm., seven at the next, averaging 2.8 mm., five at the third with an average of 4.9 mm., ten at the fourth with 8.4 mm., eight at the fifth with 13.1 mm., and ten at the sixth with 18.1 mm. The data are given in tables 37 to 40 and in graphic form in figures 4 to 17.

The regenerated lengths at ten days will be taken up first because at this time the period of maximum rate has been passed and its full effect is represented. Differentiation of the tissues has begun but there

is still a considerable production of new cells by mitotic division except in the individuals with the two shortest removals in which the process is completed. The regenerated lengths for the six levels beginning with the shortest removal are respectively 1.0, 1.3, 1.4, 2.3, 3.7 and 5.1 mm. The data are given in the last two columns of table 37. There is very distinctly an increase in regenerated length with increase in removed length. Dividing the regenerated length by the removed length at each level, the fractions obtained are

$$\frac{1.0}{1.5}, \quad \frac{1.3}{2.8}, \quad \frac{1.4}{4.9}, \quad \frac{2.3}{8.4}, \quad \frac{3.7}{13.1}, \quad \text{and} \quad \frac{5.1}{18.1},$$

which give the specific regenerated lengths or lengths regenerated per unit of removed lengths. These values are 0.67, 0.46, 0.29, 0.28, 0.28 and 0.28. They show a remarkable constancy for removed lengths of 4.9 mm. and over. The relations between removed lengths and regenerated lengths are further shown in figure 4 which gives the removed lengths along the horizontal axis and the regenerated lengths parallel to the vertical axis. The plotted line of correlation between the two values is straight except for the two lowest removed lengths. The specific lengths are given in Figure 5 in which the removal lengths again are along the horizontal axis and the lengths regenerated per unit of removed length parallel to the vertical axis. The line of correlation is straight and parallel to the horizontal axis for the four highest removals. For these therefore the regenerated length is directly proportional to the removed length or in other words within these limits the same percentage of the removed length is regenerated in each within the given time of ten days.

The two lowest removed lengths give a higher specific rate than the others. They regenerate a higher percentage of the removed length within the given time.

The ten day period is chosen as the first example because it is the first one to receive the full benefit of the periods of maximum rate of regeneration, the periods during which rapid multiplication of cells takes place. The other periods give results which agree in general features after the first few days with those at ten days but depart from them in certain respects.

The remaining periods will now be taken up in turn beginning with the shortest.

During the first four days after the operation the rate of regeneration is slow, the new tissue being derived largely from migration of cells over the cut surface. Measurements of regeneration at this time are especially subject to error because of the small amount regenerated

and because of irregularity in the outer edge of the regenerating tissue. The regenerated lengths at four days are respectively 0.22, 0.39, 0.24, 0.42, 0.50 and 0.52 mm. These data are given in table 37 and are rep-

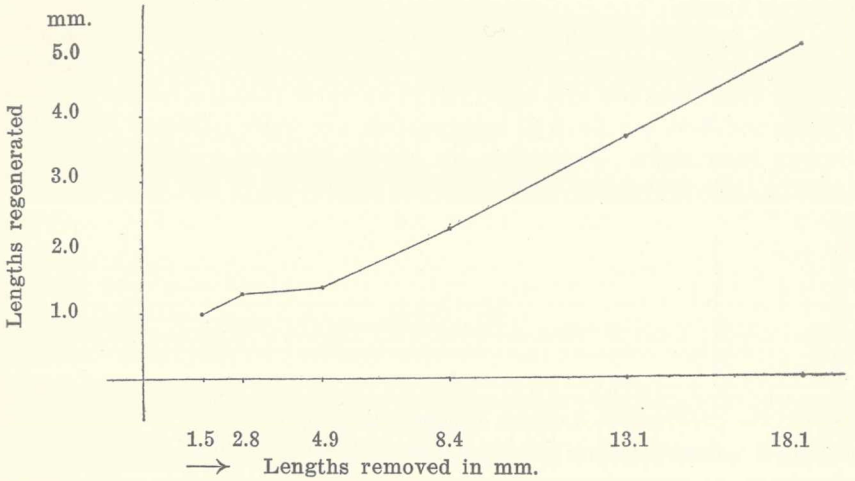

Figure 4 *Rana clamitans* Second regenerations Ten days

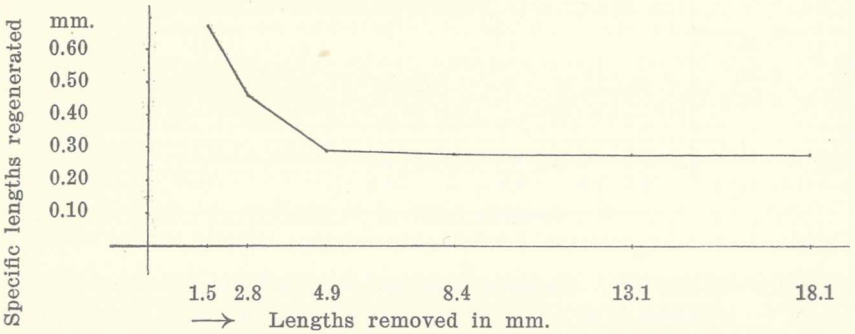

Figure 5 *Rana clamitans* Second regenerations Specific lengths Ten days

resented graphically in figure 6. Dividing the regenerated lengths by the removed lengths the fractions obtained are

$$\frac{0.22}{1.50}, \quad \frac{0.39}{2.80}, \quad \frac{0.24}{4.90}, \quad \frac{0.42}{8.40}, \quad \frac{0.50}{13.10} \quad \text{and} \quad \frac{0.52}{18.10}$$

giving specific lengths of 0.15, 0.14, 0.05, 0.05, 0.04 and 0.03. These relations are represented graphically in figure 7. There is on the whole a slight increase in regenerated length with increase in removed length but this increase is not proportional to the amount removed so that the proportion regenerated decreases with increase in removed length. The

approach to equality in regeneration at this time is probably due to the fact that the new tissue is largely made up of migrating cells and there is not a striking difference in the extent of the migration at the different levels.

The specific length of material regenerated after the smallest removals is greater than that regenerated after the larger removals not only at four days but also later. It is probable that the factors involved during the first few days of regeneration are quite different from those during later days. Following the injury there is a disintegration of injured cells associated with an active migration of the epidermal cells

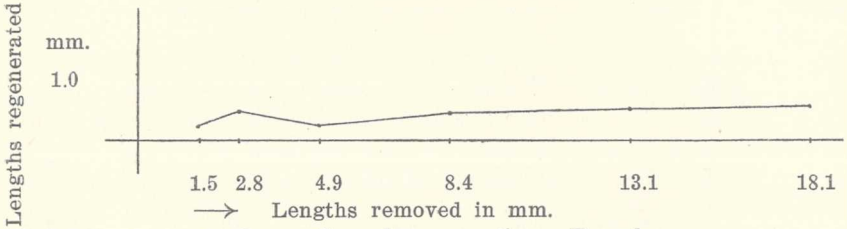

Figure 6 *Rana clamitans* Second regenerations Four days

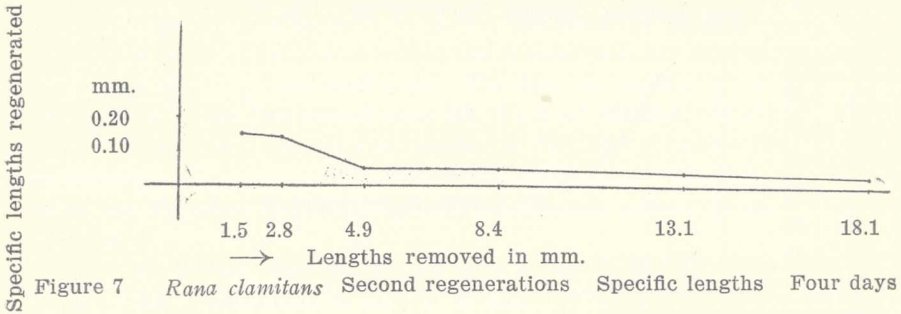

Figure 7 *Rana clamitans* Second regenerations Specific lengths Four days

over the cut surface. There is practically no mitotic cell division. The rapid multiplication of cells comes later. These processes of cell migration apparently are not essentially different at the different levels. They are local responses of the cells at the cut surface. With the appearance of rapid cell multiplication there is a marked difference at different levels though the shortest removals still show a greater specific length than the others probably because in their case the migrated cells make up a large percent of the total material of the new part.

Between the end of the fourth and the end of the sixth day after the operation mitotic cell division becomes very rapid and the rate of regeneration for second regenerations reaches its maximum at a majority of the levels on the sixth day. At six days the regeneration for the six levels is respectively 0.62, 0.80, 0.70, 1.1, 1.7 and 2.3 mm., as shown in

Table 37. A graphic representation is given in Figure 8. There is a gradual increase with increase in removed length. The fractions obtained by dividing by the removed lengths are:

$$\frac{0.62}{1.5}, \quad \frac{0.80}{2.8}, \quad \frac{0.70}{4.9}, \quad \frac{1.1}{8.4}, \quad \frac{1.7}{13.1} \quad \text{and} \quad \frac{2.3}{18.1}$$

They give specific lengths of 0.42, 0.30, 0.14, 0.13, 0.13 and 0.13. The smaller removals still have the larger specific lengths but with removals of 4.9 mm. and more there is an approach to constancy. The relations are shown graphically in Figure 9.

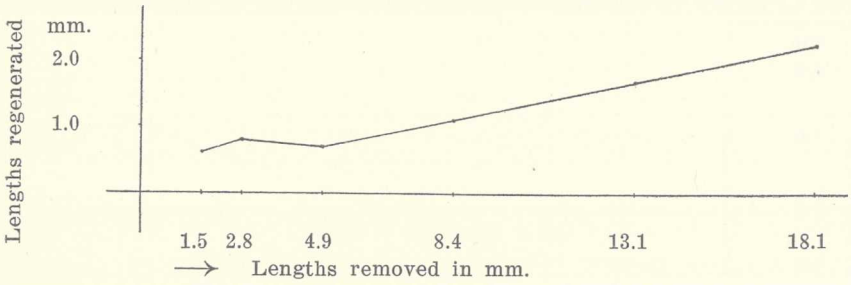

Figure 8 *Rana clamitans* Second regenerations Six days

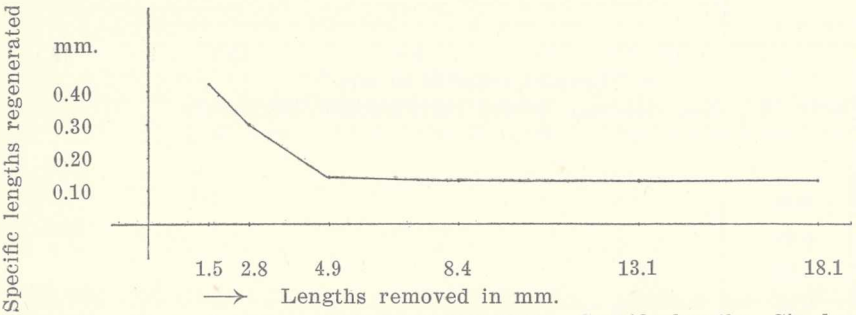

Figure 9 *Rana clamitans* Second regenerations Specific lengths Six days

The rate of regeneration between the sixth and the eighth day for second regenerations is not quite as high as for the preceding period, but mitotic divisions are still very numerous and differentiation of the cells is just beginning. At eight days the regenerated lengths are respectively 0.9, 1.1, 1.2, 1.8, 2.7 and 3.7 mm. as shown in table 37. The increase in regeneration with increase in removed length is evident. The relations are shown in Figure 10. Dividing by the removed lengths the fractions obtained are

$$\frac{0.9}{1.5}, \quad \frac{1.1}{2.8}, \quad \frac{1.2}{4.9}, \quad \frac{1.8}{8.4}, \quad \frac{2.7}{13.1} \quad \text{and} \quad \frac{3.7}{18.1}$$

giving the specific regenerations 0.60, 0.39, 0.24, 0.22, 0.21, 0.20. There is a graphic representation in Figure 11. As before, the two shortest removals give the highest specific rates but beyond these there is an approach to constancy though there is still a slight decrease with increase in removal.

The ten day values have already been given.

Between ten and twelve and a half days after the operation there is no further growth in the case of the two shortest removals. In the two medium removals the process is completed at twelve and a half days. In the two longest removals there is still a small amount of

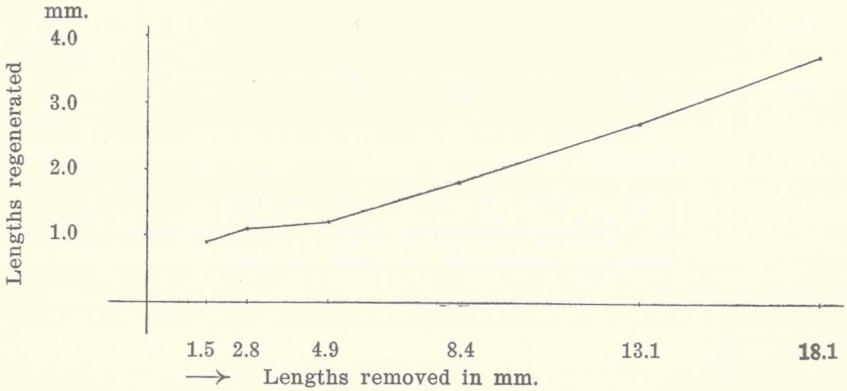

Figure 10 *Rana clamitans* Second regenerations Eight days

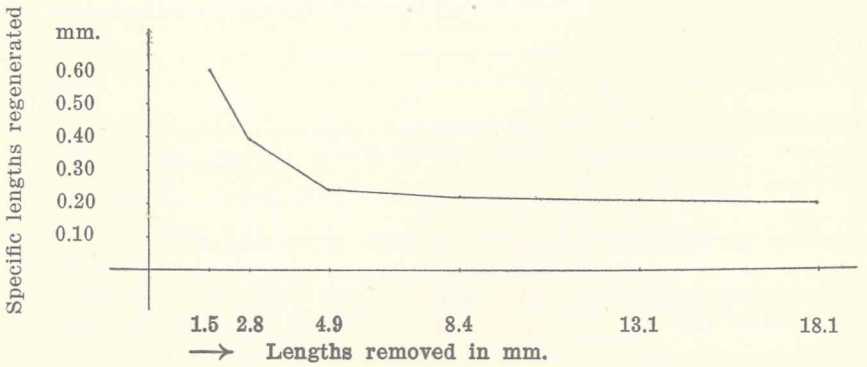

Figure 11 *Rana clamitans* Second regenerations Specific lengths Eight days

proliferation after this time. At twelve and a half days the regenerated lengths are 1.0, 1.3, 1.6, 2.6, 4.4 and 6.2 mm. as shown in Table 38. The increase with increase in removed length is continuous. This is shown

in graphic form in figure 12. Dividing by the removed lengths the fractions obtained are

$$\frac{1.0}{1.5}, \quad \frac{1.3}{2.8}, \quad \frac{1.6}{4.9}, \quad \frac{2.6}{8.4}, \quad \frac{4.4}{13.1} \quad \text{and} \quad \frac{6.2}{18.1}$$

giving specific lengths of 0.67, 0.46, 0.33, 0.31, 0.34 and 0.34. The graph for specific lengths is shown in figure 13. There is still a fair

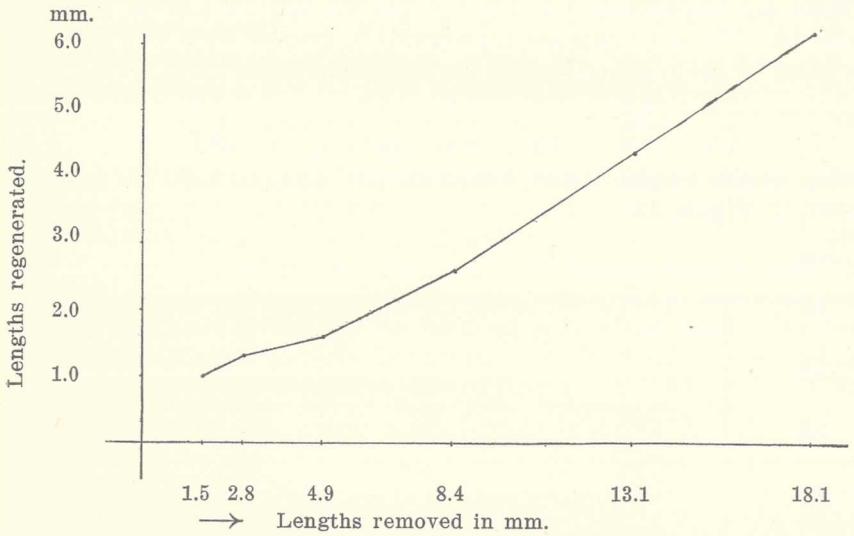

Figure 12 *Rana clamitans* Second regenerations Twelve and a half days

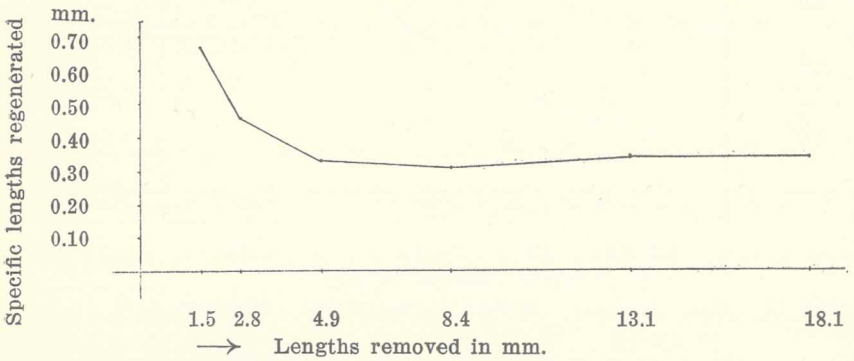

Figure 13 *Rana clamitans* Second regenerations Specific lengths Twelve and a half days

approach to constancy with removals of 4.9 mm. and above. The relative increase in the case of the higher removals is due to the fact that regeneration is continuing in them after it has stopped in the others.

Therefore the data after this time are values for the completeness of regeneration rather than for the rate.

Between twelve and a half and eighteen days after the operation there is no further regeneration in the tails with the four shortest removals. Two of them even exhibit a decrease in size. The two longest removals show only a slight increase. At eighteen days the regenerated lengths are respectively 1.0, 1.2, 1.5, 2.6, 4.8 and 7.0 mm. as given in Table 38. The same data are represented in graphic form in Figure 14. Dividing by the removed lengths the fractions obtained are

$$\frac{1.0}{1.5}, \quad \frac{1.2}{2.8}, \quad \frac{1.5}{4.9}, \quad \frac{2.6}{8.4}, \quad \frac{4.8}{13.1}, \quad \text{and} \quad \frac{7.0}{18.1}$$

giving specific lengths of 0.67, 0.43, 0.31, 0.31, 0.37 and 0.39. The graph is shown in Figure 15.

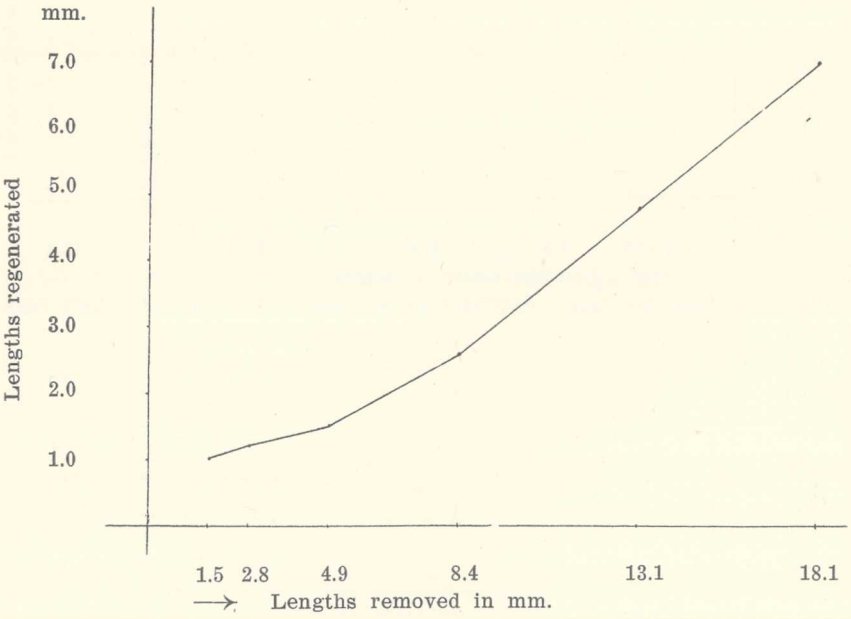

Figure 14 *Rana clamitans* Second regenerations Eighteen days

At eighteen days there is very little regeneration at any of the levels and at some of them, especially the shorter removals, a considerable absorption of regenerated material. Regeneration may therefore be considered as completed at this time. However the measurements for 56 days are given in order to show the changes. The regenerated lengths at that time are 0.9, 0.7, 1.6, 2.5, 5.2 and 7.1 mm. These data

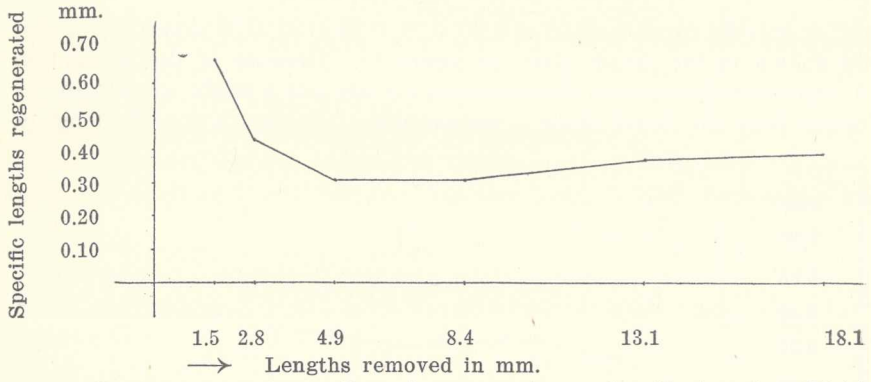

Figure 15 *Rana clamitans* Second regenerations Specific lengths Eighteen days

are given in table 38 and are represented in graphic form in figure 16. Dividing by the average removed lengths, which differ somewhat from the previous ones because of the death of certain individuals, the fractions obtained are

$$\frac{0.9}{1.5}, \quad \frac{0.7}{2.6}, \quad \frac{1.6}{4.9}, \quad \frac{2.5}{8.2}, \quad \frac{5.2}{13.2} \quad \text{and} \quad \frac{7.1}{17.9}$$

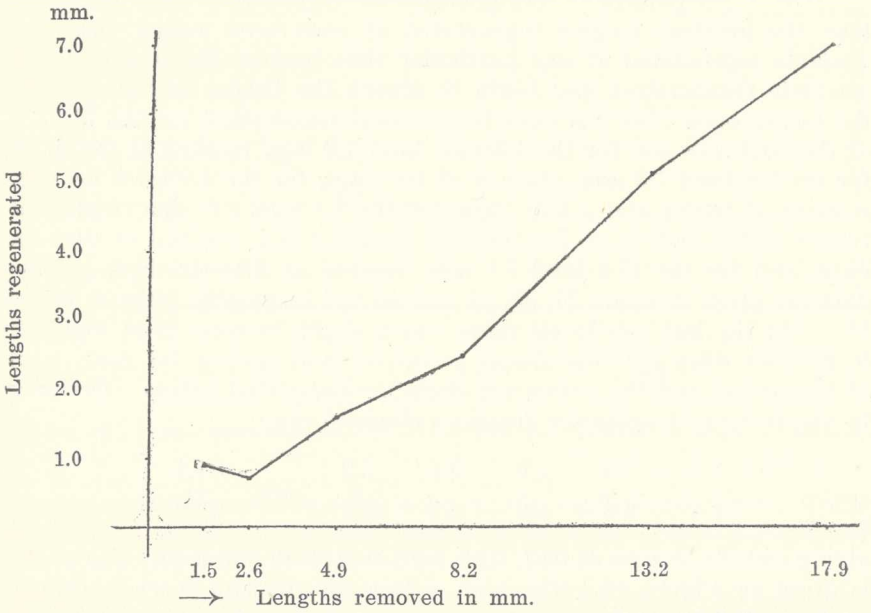

Figure 16 *Rana clamitans* Second regenerations Fifty-six days

giving specific regenerations of 0.60, 0.27, 0.33, 0.31, 0.39 and 0.40. These are shown in the graph given in figure 17. Because of the absorption of regenerated tissue in the shorter removals and a slight growth in the longer ones the latter show a comparative increase in specific lengths.

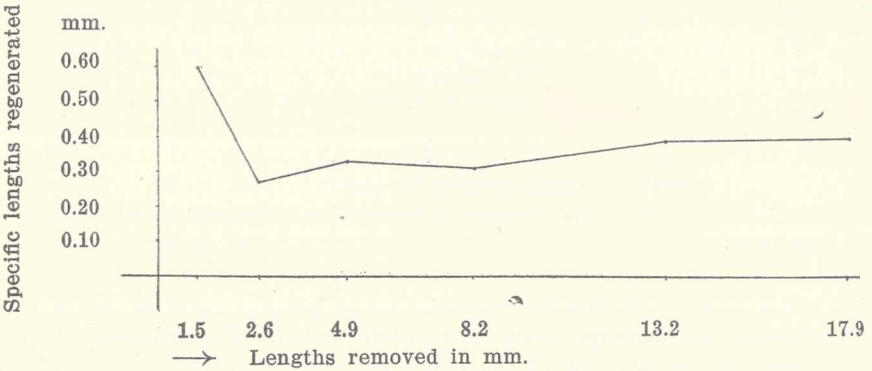

Figure 17 *Rana clamitans* Second regenerations Specific lengths Fifty-six days

For a comparison of completeness of regeneration it is better to take the greatest lengths regenerated at each level rather than the amounts regenerated at any particular time because the shorter levels complete regeneration and begin to absorb the tissues sooner than do the longer ones. On this basis the greatest regenerated lengths at each of the six levels are, for the 1.5 mm. level 1.0 mm. reached at ten days, for the 2.8 level 1.3 mm. reached at ten days, for the 4.9 level 1.6 mm. reached at twelve and a half days, for the 8.4 level 2.6 mm. reached at twelve and a half days, for the 13.2 level 5.2 mm. reached at fifty-six days, and for the 17.9 level 7.1 mm. reached at fifty-six days. These data are given in tables 37, 38, 39 and 40 and in graphic form in figure 18. At the last two levels there was a slight increase from eighteen to fifty-six days but this almost certainly came during the early part of the period and the values are therefore completed values. Dividing by the removed lengths the fractions obtained are

$$\frac{1.0}{1.5}, \quad \frac{1.3}{2.8}, \quad \frac{1.6}{4.9}, \quad \frac{2.6}{8.4}, \quad \frac{5.2}{13.2} \quad \text{and} \quad \frac{7.1}{17.9}$$

giving specific lengths of 0.67, 0.46, 0.33, 0.31, 0.39 and 0.40. The graph is given in Figure 19. The high values for the two short levels are probably due to the fact that the cells migrating to the cut surface form

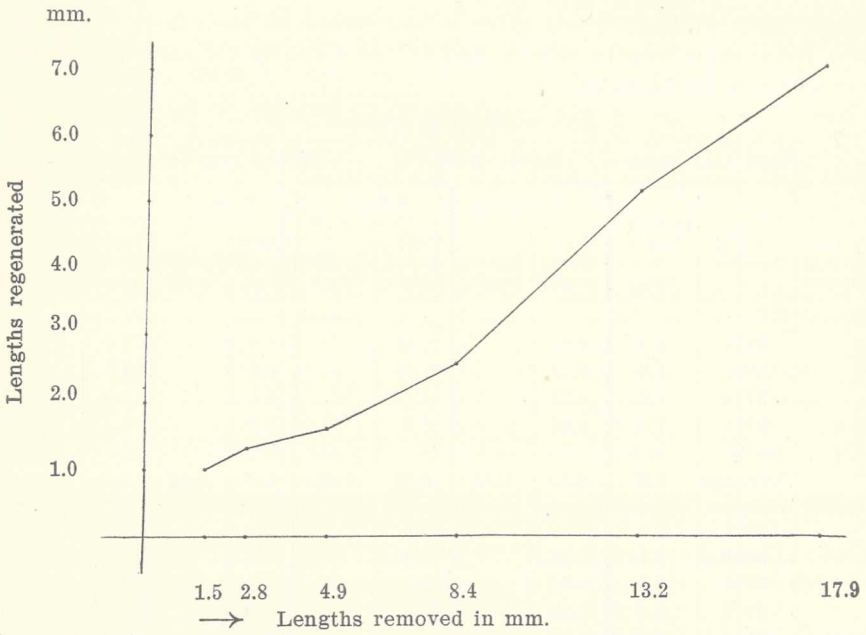

Figure 18 *Rana clamitans* Second regenerations Completeness

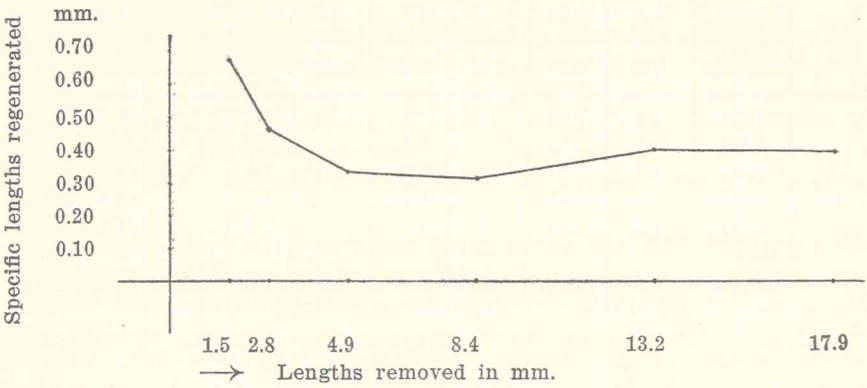

Figure 19 *Rana clamitans* Second regenerations Specific lengths Completeness

a large proportion of the total mass of the regenerated organ. Since apparently the length of this mass of cells is very much alike at all levels as indicated by the facts of the four day regenerations, the specific lengths for these short removals are greater than for the others. The high values of the two longest removals are due to a continuation of

regeneration at these levels after it has ceased at the others. At ten days the specific lengths regenerated are very nearly the same at all the levels except the first two.

TABLE 37

Rana clamitans Series 3676-3765 Second regenerations

Percent removed Average	Catalog number	Removed length in mm.	4 Days		6 Days		8 Days		10 Days	
			Regenerated length mm.	Specific length	Regenerated length mm.	Specific length	Regenerated length mm.	Specific length	Regenerated length mm.	Specific length
	3676	1.3	0.27		0.60		0.9		1.0	
	3682	1.6	0.18		0.60		0.9		1.0	
	3730	1.6	0.39		0.75		0.9		0.9	
6	3754	1.6	0.06		0.55		0.9		1.1	
	Average	**1.5**	**0.22**	**0.15**	**0.62**	**0.42**	**0.9**	**0.60**	**1.0**	**0.67**
	3677	2.0	0.30		0.6		0.9		0.9	
	3696	2.1	0.48		0.8		1.0		1.1	
	3701	3.2	0.42		0.8		1.1		1.2	
	3713	2.8	0.36		0.8		0.9		0.9	
10	3719	3.1	0.30		0.8		1.1		1.4	
	3749	2.8	0.48		0.6		1.2		1.4	
	3750	3.5	0.42		1.3		1.7		1.9	
	Average	**2.8**	**0.39**	**0.14**	**0.80**	**0.30**	**1.1**	**0.39**	**1.3**	**0.46**
	3678	5.0	0.20		0.5		0.9		1.0	
	3684	5.5	0.15		0.7		1.2		1.4	
	3702	4.7	0.42		0.8		1.1		1.3	
18	3720	4.6	0.06		0.3		1.3		1.8	
	3756	4.8	0.36		1.0		1.3		1.7	
	Average	**4.9**	**0.24**	**0.05**	**0.70**	**0.14**	**1.2**	**0.24**	**1.4**	**0.29**

TABLE 37 (Continued)

Rana clamitans Series 3676-3765 Second regenerations

Percent removed Average	Catalog number	Removed length in mm.	4 Days		6 Days		8 Days		10 Days	
			Regenerated length mm.	Specific length	Regenerated length mm.	Specific length	Regenerated length mm.	Specific length	Regenerated length mm.	Specific length
	3679	8.4	0.30		0.7		1.4		1.9	
	3685	9.3	0.60		1.2		1.9		2.3	
	3697	7.3	0.48		1.2		1.7		2.2	
	3703	9.3	0.45		1.3		2.0		2.5	
	3715	7.9	0.24		0.9		1.7		2.3	
31	3721	8.7	0.57		1.3		1.9		2.3	
	3733	8.5	0.36		1.0		1.9		2.4	
	3739	9.6	0.36		1.0		1.8		2.4	
	3751	6.7	0.45		1.1		1.7		2.1	
	3757	8.0	0.42		1.2		2.1		2.8	
	Average	8.4	0.42	0.05	1.1	0.13	1.8	0.22	2.3	0.28
	3686	14.5	0.60		2.1		3.4		4.8	
	3698	14.9	0.50		1.5		3.3		4.3	
	3704	14.5	0.45		2.2		3.3		4.4	
	3716	12.7	0.39		1.7		2.4		3.4	
	3722	12.5	0.60		1.6		2.6		3.6	
49	3740	13.9	0.30		1.1		2.1		3.0	
	3752	11.2	0.54		1.7		2.5		3.4	
	3758	11.0	0.60		1.5		2.2		2.9	
	Average	13.1	0.50	0.04	1.7	0.13	2.7	0.21	3.7	0.28
	3680	16.0	0.60		1.9		3.0		4.2	
	3681	21.2	0.84		3.0		4.0		5.6	
	3687	19.7	0.54		3.6		5.6		6.0	
	3699	21.0	0.54		2.2		4.3		5.9	
	3705	17.6	0.72		2.0		3.6		4.8	
	3717	17.6	0.42		2.6		3.6		5.2	
67	3723	18.4	0.30		2.3		3.7		5.3	
	3735	16.5	0.48		2.0		3.4		5.5	
	3741	16.0	0.30		1.9		3.0		5.4	
	3753	16.8	0.42		2.0		2.5		3.8	
	Average	18.1	0.52	0.03	2.3	0.13	3.7	0.20	5.1	0.28

TABLE 38

Rana clamitans Series 3676-3765 Second regenerations

Percent removed Average	Catalog number	Removed length in mm.	12½ Days		18 Days		56 Days		Highest values	
			Regenerated length mm.	Specific length	Regenerated length mm.	Specific length	Regenerated length mm.	Specific length	Regenerated length mm.	Specific length
	3676	1.3	1.0		1.0		0.7		1.0	
	3682	1.6	1.0		1.0		1.1		1.1	
	3730	1.6	0.9		0.9		0.7		0.9	
6	3754	1.6	1.2		1.2		1.1		1.2	
	Average	**1.5**	**1.0**	**0.67**	**1.0**	**0.67**	**0.9**	**0.60**	**1.0**	**0.67**
	3677	2.0	0.9		0.9		0.7		0.9	
	3696	2.1	1.0		1.0		0.7		1.1	
	3701	3.2	0.9		0.9		0.5		1.2	
	3713	2.8	1.4		1.3				1.4	
10	3719	3.1	1.4		1.3		0.9		1.4	
	3749	2.8	2.0		2.0				2.0	
	3750	3.5	1.3		1.3				1.9	
	Average	**2.8**	**1.3**	**0.46**	**1.2**	**0.43**	**0.7**	**0.27**	**1.3**	**0.46**
	3678	5.0	1.1		1.1				1.1	
	3684	5.5	1.4		1.5		1.4		1.5	
	3702	4.7	1.3		1.3		1.3		1.3	
18	3720	4.6	2.3		1.9		2.0		2.3	
	3756	4.8	1.8		1.6				1.8	
	Average	**4.9**	**1.6**	**0.33**	**1.5**	**0.31**	**1.6**	**0.33**	**1.6**	**0.33**
	3679	8.4	1.9		2.1				2.1	
	3685	9.3	2.8		3.0		2.6		3.0	
	3697	7.3	2.4		2.3		2.1		2.4	
	3703	9.3	2.6		2.5		2.5		2.5	
	3715	7.9	2.6		2.6		2.8		2.8	
	3721	8.7	2.6		2.3		2.2		2.6	
31	3733	8.5	2.6		2.6		2.8		2.8	
	3739	9.6	3.0		2.9				3.0	
	3751	6.7	2.4		2.5		2.3		2.5	
	3757	8.0	3.1		3.2		3.1		3.2	
	Average	**8.4**	**2.6**	**0.31**	**2.6**	**0.31**	**2.5**	**0.31**	**2.6**	**0.31**

TABLE 38 (Continued)

Percent removed Average	Catalog number	Removed length in mm.	12½ Days		18 Days		56 Days		Highest values	
			Regenerated length mm.	Specific length	Regenerated length mm.	Specific length	Regenerated length mm.	Specific length	Regenerated length mm.	Specific length
	3686	14.5	5.3		5.2				5.3	
	3698	14.9	5.0		5.4		5.4		5.4	
	3704	14.5	5.2		5.5		5.4		5.5	
	3716	12.7	4.2		5.1		4.4		5.1	
	3722	12.5	3.9		3.5		4.2		4.2	
49	3740	13.9	4.6		5.6		6.8		6.8	
	3752	11.2	4.1		4.0				4.1	
	3758	11.0	3.6		4.1		4.9		4.9	
	Average	13.1	4.4	0.34	4.8	0.37	5.2	0.39	5.2	0.39
	3680	16.0	5.2		6.4		6.6		6.6	
	3681	21.2	6.3		7.3		7.2		7.3	
	3687	19.7	6.6		7.0				7.0	
	3699	21.0	7.1		7.5		7.2		7.5	
	3705	17.6	6.4		6.2		6.0		6.4	
67	3717	17.6	6.0		6.7		6.4		6.7	
	3723	18.4	6.5		8.1		8.3		8.3	
	3735	16.5	6.5		7.8		8.0		8.0	
	3741	16.0	5.8		6.9		7.0		7.0	
	3753	16.8	5.2		6.4		7.1		7.1	
	Average	18.1	6.2	0.34	7.0	0.39	7.1	0.40	7.1	0.40

TABLE 39

Rana clamitans Series 3676-3765 Summary Second regenerations
Lengths regenerated at different levels at different times

Percent of tail length removed	Length removed in mm.	Number of individuals	Days after operation						
			4	6	8	10	12½	18	56
6	1.5	4	0.22	0.6	0.9	1.0	1.0	1.0	0.9
10	2.8	7	0.39	0.8	1.1	1.3	1.3	1.2	0.7
18	4.9	5	0.24	0.7	1.2	1.4	1.6	1.5	1.6
31	8.4	10	0.42	1.1	1.8	2.3	2.6	2.6	2.5
49	13.1	8	0.50	1.7	2.7	3.7	4.4	4.8	5.2
67	18.1	10	0.52	2.3	3.7	5.1	6.2	7.0	7.1

TABLE 40

Rana clamitans Series 3676-3765 Summary Second regenerations
Lengths regenerated at different levels at different times

Percent of tail length removed	Length removed in mm.	Number of individuals	Days after operation						
			4	6	8	10	12½	18	56
6	1.5	4	0.15	0.42	0.60	0.67	0.67	0.67	0.60
10	2.8	7	0.14	0.30	0.39	0.46	0.46	0.43	0.27
18	4.9	5	0.05	0.14	0.24	0.29	0.33	0.31	0.33
31	8.4	10	0.05	0.13	0.22	0.28	0.31	0.31	0.31
49	13.1	8	0.04	0.13	0.21	0.28	0.34	0.37	0.39
67	18.1	10	0.03	0.13	0.20	0.28	0.34	0.39	0.40

First Regenerations

The data for first regenerations are from a different set of individuals than those for second regenerations. The two kinds of operations were made on the same day. The general results obtained from the first regenerations are in full agreement with those obtained from the second regenerations but there is greater variability because of the smaller number of individuals. The average per cents of the tail length removed are respectively 6, 10, 17, 30, 48 and 62 for the six levels. The first of these has two individuals averaging 1.5 mm. of removed tail, the second five individuals with an average of 2.6 mm., the third three individuals with an average of 4.6, the fourth eight with an average of 8.2, the fifth five with an average of 13.0 and the sixth five with an average of 16.7. The data for these experiments are given in Tables 41, 42, 43 and 44 and in Figures 20 to 35.

The progress of a first regeneration is similar to that of a second except that the maximum is reached later in the case of first regenerations. In the present series the maximum specific rate for first regenerations comes between the sixth and the eighth day after the operation. A comparison of the two regenerations is made in the section on the effect of successive removal. The change in rate during the process of regeneration is also discussed in a separate section.

The lengths regenerated during the first four days are respectively 0.27, 0.15, 0.51, 0.45, 0.46 and 0.51 for the six levels. There is no regular increase with removed length. The data are given in Table 41 and in Figure 20. The specific lengths regenerated are 0.17, 0.06, 0.11, 0.05, 0.03 and 0.03. They are shown in Table 41 and in Figure 21. As in the case of the second regeneration the shortest removals have the largest proportional amounts. This first period being the period of cell

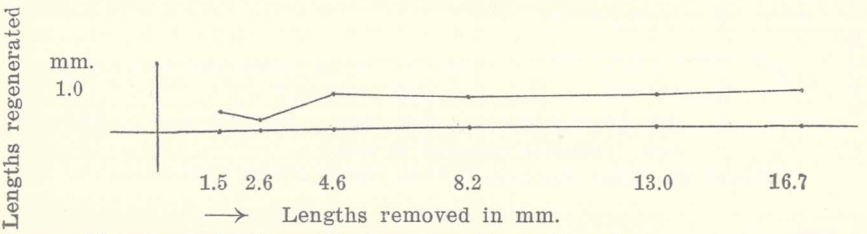

Figure 20 *Rana clamitans* First regenerations Four days

migration with very little cell division it is probable that the length of the material furnished in this way, as measured along the main axis of the individual, is independent of the level of the cut. The area of the cut surface of course is greater at the more proximal than at the more distal levels so that the actual total mass of regenerated material is greater at the deeper levels.

At six days the rate of first regenerations is rapidly increasing, the maximum rate coming between six and eight days. The lengths regenerated at six days are respectively 0.47, 0.5, 1.0, 1.1, 1.6 and 1.6 mm.

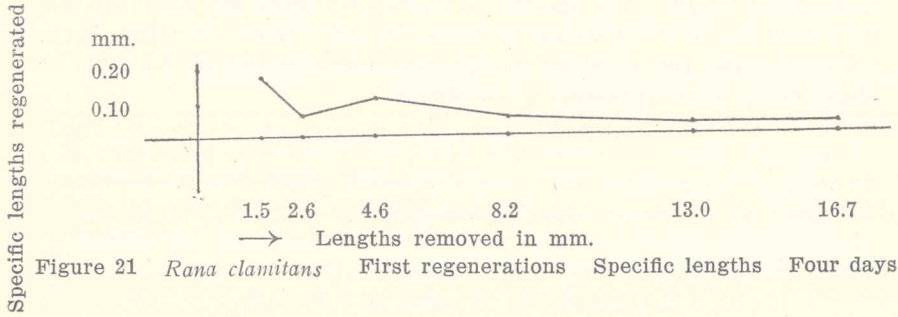

Figure 21 *Rana clamitans* First regenerations Specific lengths Four days

They are shown in Table 41 and in Figure 22. There is in general an increase with increase in removed length but the first is not proportional to the second. The specific lengths are 0.30, 0.18, 0.22, 0.13, 0.12 and 0.09 as shown in Table 41 and Figure 23. The shorter removals still have proportionately the greater regenerations.

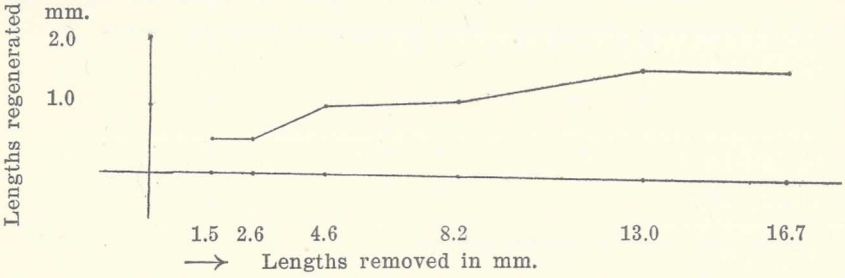

Figure 22 *Rana clamitans.* First regenerations Six days

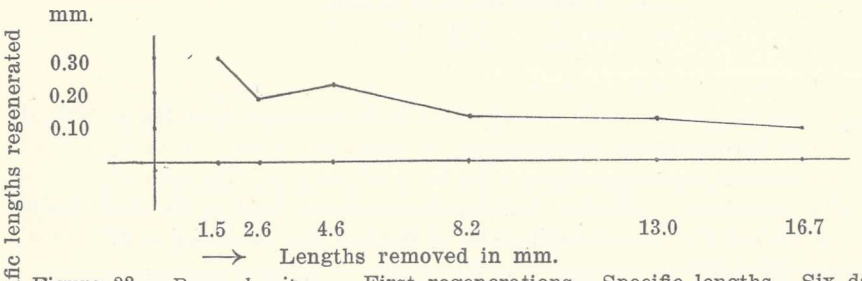

Figure 23 *Rana clamitans* First regenerations Specific lengths Six days

The maximum rate of regeneration is reached between the sixth and the eighth day. The regenerated lengths at eight days are 0.8, 0.7, 1.5, 1.7, 2.6 and 3.0 mm. The data are shown in Table 41 and Figure 24. With one exception there is increase in regenerated length with increase in removed length. The specific regenerated lengths are 0.53, 0.27, 0.33, 0.21, 0.20 and 0.18 as shown in Table 41 and Figure 25. The shortest removals have the greatest specific regenerations but at 8.2 mm. and above there is an approach to constancy.

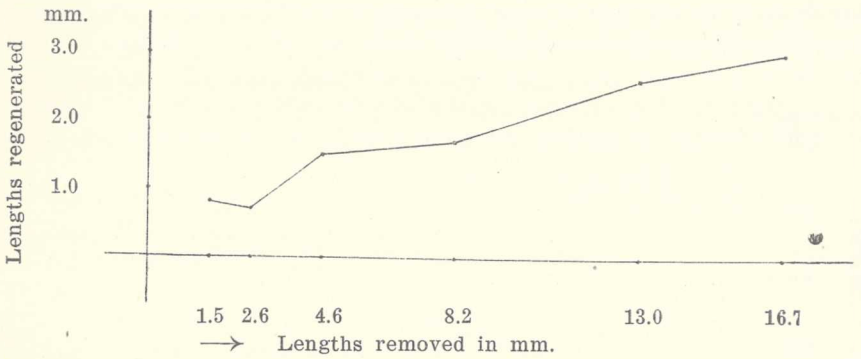

Figure 24 *Rana clamitans* First regenerations Eight days

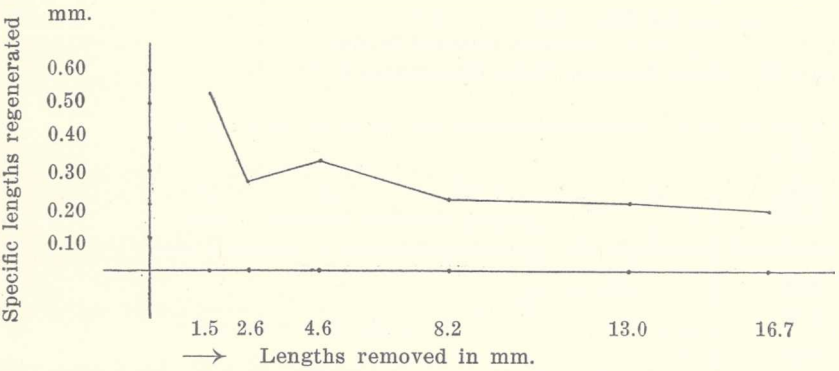

Figure 25 *Rana clamitans.* First regenerations Specific lengths Eight days

Between the eighth and the tenth day there is a rapid decrease in rate associated with tissue differentiation. The regenerated lengths at ten days are 0.9, 1.0, 1.7, 2.3, 3.8 and 4.5 mm. as shown in Table 41 and Figure 26. There is an uninterrupted increase in regeneration with increase in removed length. The specific lengths are 0.58, 0.38, 0.37, 0.28, 0.29 and 0.27 as shown in Table 41 and Figure 27.

Between ten and twelve and a half days regeneration is slow, reaching its end for the two shortest removals at the latter day. The regenerated lengths at twelve and a half days are 0.9, 1.2, 1.8, 2.6, 4.7 and

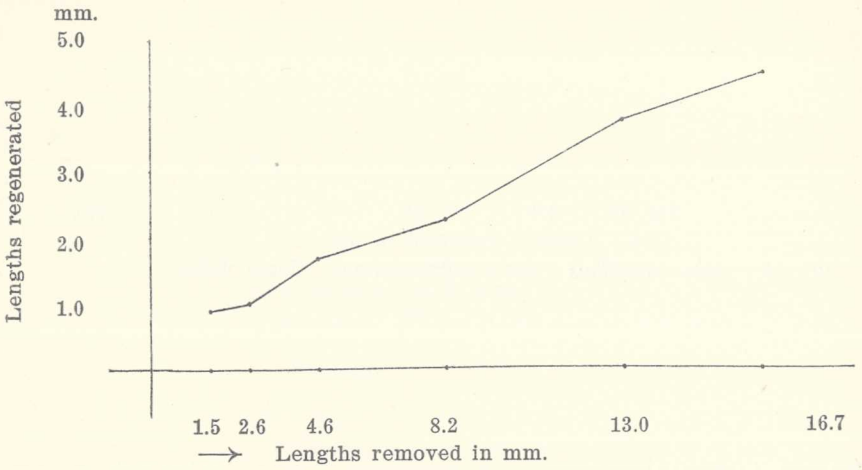

Figure 26 *Rana clamitans.* First regenerations Ten days

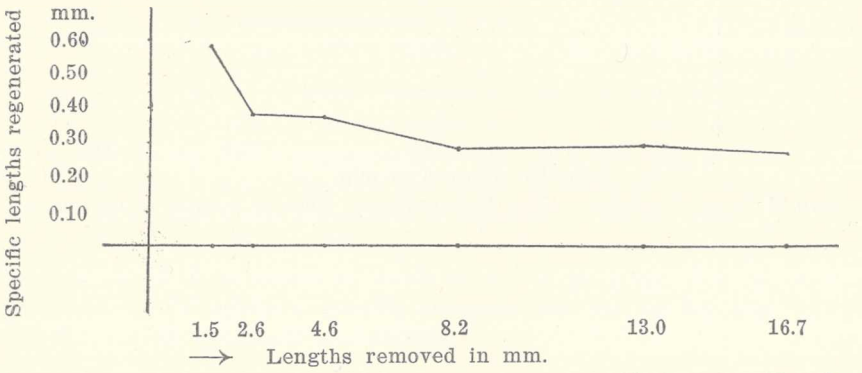

Figure 27 *Rana clamitans* First regenerations Specific lengths Ten days

5.8 mm. as shown in Table 42 and Figure 28. There is a steady increase with increase in removed length. The specific lengths are 0.61, 0.46, 0.39, 0.31, 0.36 and 0.35 as shown in Table 42 and Figure 29. There is an approach to constancy in the four largest removals.

Between twelve and a half and eighteen days the regenerated material is decreasing in the case of the two shortest removals, has made no progress in the third and in the three longest removals the increase is very slight. The data therefore are of value more particularly in connection with the problem of the relative completeness of regeneration from the different levels. The regenerated lengths at eighteen days are

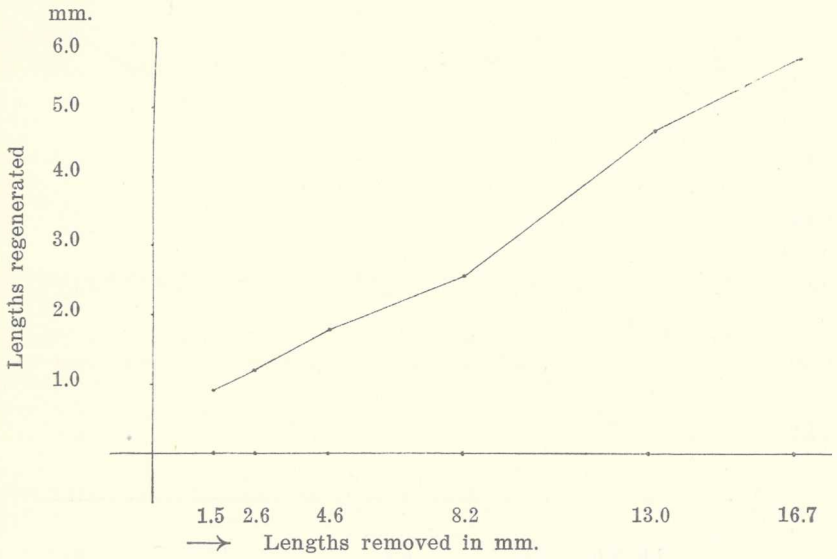

Figure 28 *Rana clamitans* First regenerations Twelve and a half days

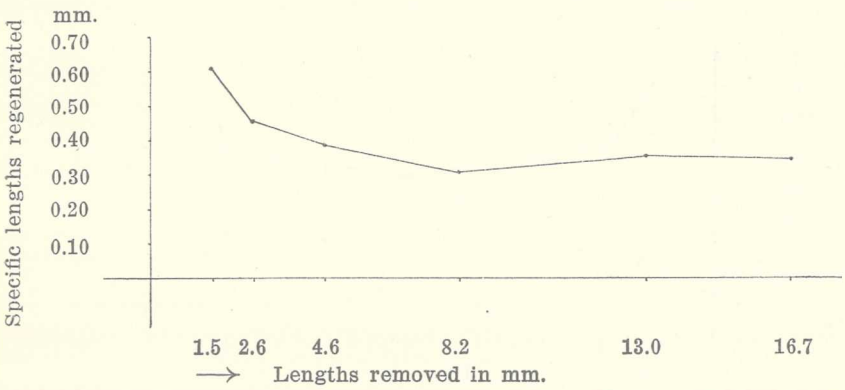

Figure 29 *Rana clamitans* First regenerations Specific lengths Twelve and a half days

0.9, 1.1, 1.8, 2.7, 5.5 and 6.8 mm. as shown in Table 42 and Figure 30. There is a regular increase with increase in removed length. The specific lengths are 0.60, 0.42, 0.39, 0.33, 0.42 and 0.40 as shown in Table 42 and Figure 31. Though there are irregularities the specific lengths approach constancy at all levels except the most distal one.

Between eighteen and fifty-six days there is practically no increase

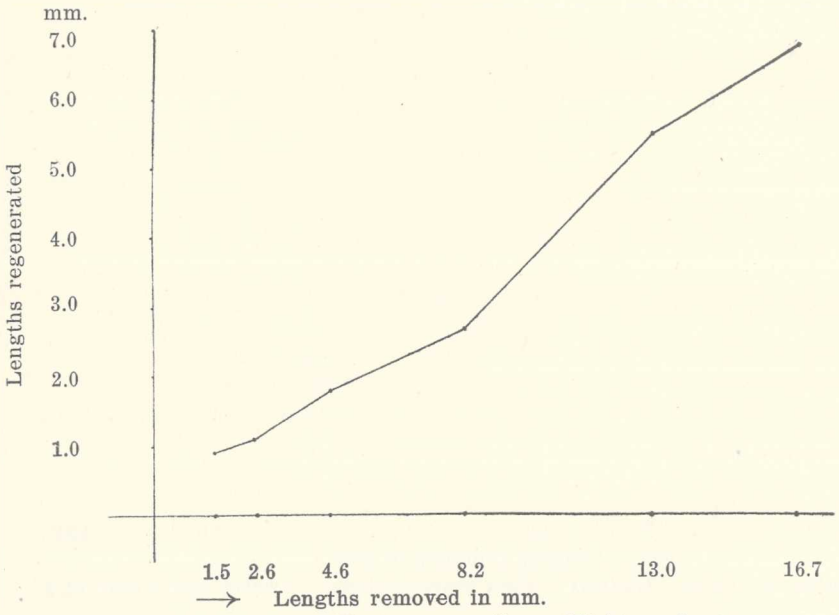

Figure 30 *Rana clamitans.* First regenerations Eighteen days

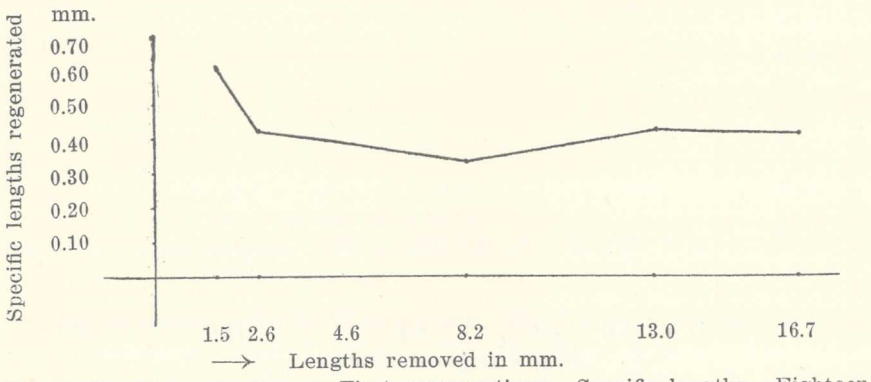

Figure 31 *Rana clamitans* First regenerations Specific lengths Eighteen days

in regenerated length and some absorption of material especially with the shorter removals. The regenerated lengths at fifty-six days are 0.7, 1.1, 1.5, 2.5, 5.5 and 6.9 mm. as shown in Table 42 and Figure 32. There is a regular increase in regeneration from the shortest to the longest removal. The specific lengths are 0.45, 0.42, 0.34, 0.30, 0.42 and 0.41 as shown in Table 42 and Figure 33. These data are of value only for

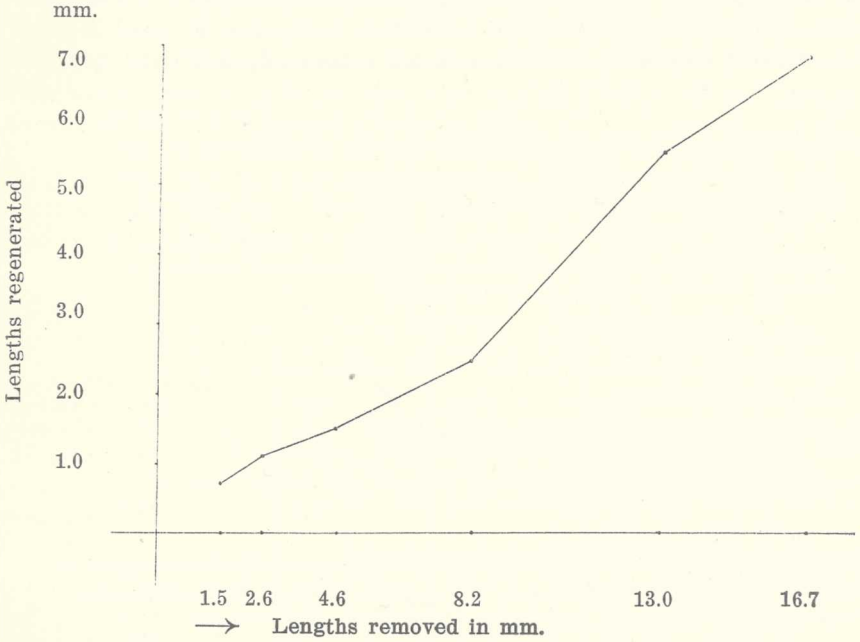

Figure 32 *Rana clamitans* First regenerations Fifty-six days

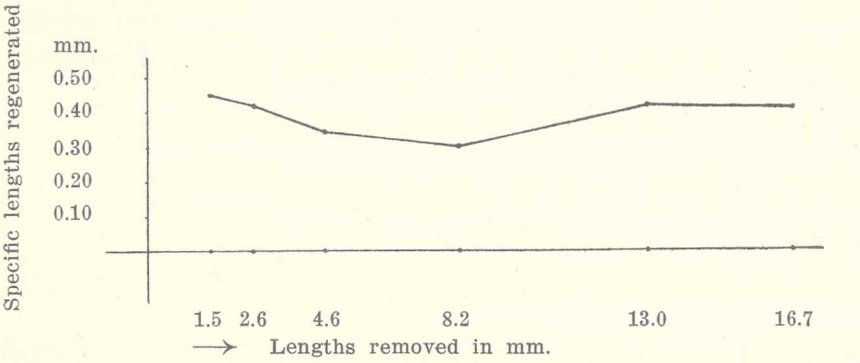

Figure 33 *Rana clamitans* First regenerations Specific lengths Fifty-six days

a comparison of completeness of regeneration but for such a comparison it is better in some ways to compare the regenerations at the time when absorption has not begun. The greatest average regenerated length attained for the 1.5 mm. level is 0.9 mm. at ten days, for the 2.6 level 1.2 at twelve and a half days, for the 4.6 level 1.8 at twelve and a half days, for the 8.2 level 2.7 at eighteen days, for the 13.0 level 5.5 at eighteen days and for the 16.7 level 6.9 at fifty-six days. There is an uninterrupted increase from the shortest to the longest removal in complete amount regenerated. This is shown graphically in Figure 34. The

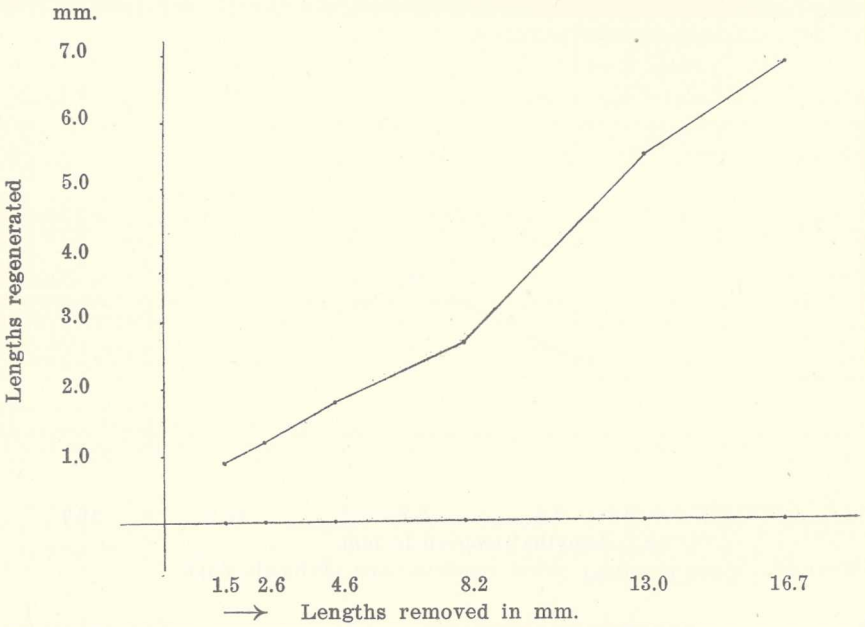

Figure 34 *Rana clamitans* First regenerations Completeness

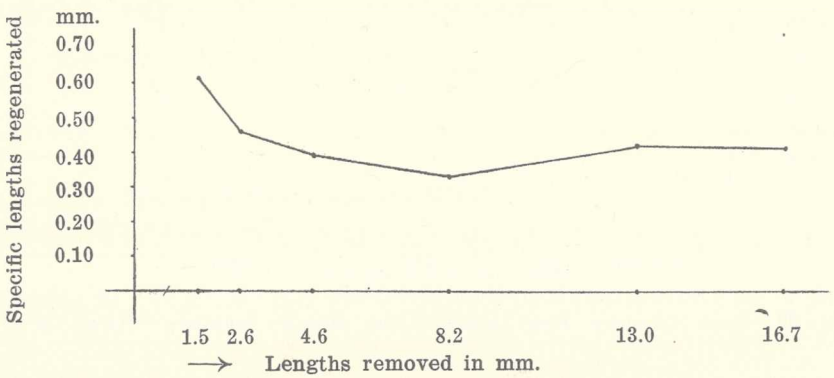

Figure 35 *Rana clamitans* First regenerations Specific lengths Completeness

completed regenerations are less than the removed lengths. The specific completed regenerated lengths obtained as before by dividing by the removed lengths are 0.61, 0.46, 0.39, 0.33, 0.42 and 0.41, as shown in table 42 and figure 35. The greater specific lengths from the shortest removals are probably due as in the case of the second regenerations to the fact that a greater proportion of their substance is made up of cells that have migrated over the cut surface during the first stages of regeneration. This migrated material is not essentially different in axial length at the different levels. The largest removals have a greater specific length than the medium ones because regeneration continues at the former levels after it has ceased at the latter.

On the whole there is no essential difference between the results obtained from first regenerations and those obtained from second regenerations. The latter give the more regular data because the averages are taken from a larger number of individuals.

TABLE 41

Rana clamitans Series 3676-3765 First regenerations

Percent removed Average	Catalog number	Removed length in mm.	4 Days		6 Days		8 Days		10 Days	
			Regenerated length mm.	Specific length	Regenerated length mm.	Specific length	Regenerated length mm.	Specific length	Regenerated length mm.	Specific length
	3706	1.4	0.24		0.54		0.9		1.0	
6	3742	1.7	0.30		0.40		0.7		0.8	
	Average	**1.5**	**0.27**	**0.17**	**0.47**	**0.30**	**0.8**	**0.53**	**0.9**	**0.58**
	3688	2.5	0.12		0.3		0.3		0.7	
	3707	3.2	0.24		0.8		1.1		1.4	
	3724	2.6	0.06		0.5		0.8		1.1	
10	3743	2.5	0.03		0.1		0.4		0.8	
	3760	3.1	0.30		0.6		0.9		1.1	
	Average	**2.6**	**0.15**	**0.06**	**0.5**	**0.18**	**0.7**	**0.27**	**1.0**	**0.38**

TABLE 41 (Continued)

Percent removed Average	Catalog number	Removed length in mm.	4 Days		6 Days		8 Days		10 Days	
			Regenerated length mm.	Specific length	Regenerated length mm.	Specific length	Regenerated length mm.	Specific length	Regenerated length mm.	Specific length
	3708	5.3	0.54		1.2		1.9		2.1	
	3726	4.3	0.42		0.9		1.3		1.4	
17	3762	4.1	0.57		1.0		1.2		1.5	
	Average	4.6	0.51	0.11	1.0	0.22	1.5	0.33	1.7	0.37
	3690	9.7	0.48		1.0		1.7		2.4	
	3709	8.8	0.48		1.3		2.0		2.6	
	3727	8.3	0.48		1.1		1.6		2.0	
	3745	10.0	0.54		1.8		2.4		3.8	
	3744	6.0	0.36		1.0		1.3		1.7	
30	3761	6.6	0.39		1.0		1.5		1.9	
	3763	8.5	0.57		1.1		1.8		2.4	
	3689	6.3	0.30		0.7		1.2		1.5	
	Average	8.2	0.45	0.05	1.1	0.13	1.7	0.21	2.3	0.28
	3710	12.3	0.42		1.8		2.9		3.7	
	3728	12.8	0.60		1.7		2.8		3.9	
	3746	13.3	0.54		1.7		2.4		4.1	
48	3764	14.6	0.42		1.3		2.5		4.2	
	3765	12.2	0.30		1.5		2.3		3.2	
	Average	13.0	0.46	0.03	1.6	0.12	2.6	0.20	3.8	0.29
	3692	16.8	0.51		1.1		2.2		3.2	
	3693	17.2	0.48		1.8		3.3		5.0	
	3711	17.0	0.54		1.8		3.6		5.6	
62	3729	16.1	0.48		1.9		3.3		4.6	
	3749	16.2	0.54		1.2		2.7		4.2	
	Average	16.7	0.51	0.03	1.6	0.09	3.0	0.18	4.5	0.27

TABLE 42

Rana clamitans Series 3676-3765 First regenerations

Percent removed Average	Catalog number	Removed length in mm.	12½ Days		18 Days		56 Days	
			Regenerated length mm.	Specific length	Regenerated length mm.	Specific length	Regenerated length mm.	Specific length
	3706	1.4	1.0		0.9		0.7	
	3742	1.7	0.9		0.9		0.7	
6								
	Average	**1.5**	**0.9**	**0.61**	**0.9**	**0.60**	**0.7**	**0.45**
	3688	2.5	0.9		0.9		0.7	
	3707	3.2	1.4		1.3		0.7	
	3724	2.6	1.4		1.4		1.2	
10	3743	2.5	1.0		1.0		1.7	
	3760	3.1	1.2		1.1		1.1	
	Average	**2.6**	**1.2**	**0.46**	**1.1**	**0.42**	**1.1**	**0.42**
	3708	5.3	2.3		2.3		1.8	
	3726	4.3	1.4		1.4		1.4	
17	3762	4.1	1.7		1.8		1.4	
	Average	**4.6**	**1.8**	**0.39**	**1.8**	**0.39**	**1.5**	**0.34**
	3690	9.7	2.6		2.7		2.2	
	3709	8.8	3.2		3.4		3.3	
	3727	8.3	2.2		2.2		2.2	
	3745	10.0	4.4		4.8		4.2	
	3744	6.0	1.8		1.7			
30	3761	6.6	2.2		2.3		1.8	
	3763	8.5	2.9		3.1			
	3689	6.3	1.6		1.7		1.4	
	Average	**8.2**	**2.6**	**0.31**	**2.7**	**0.33**	**2.5**	**0.30**
	3710	12.3	3.9		3.9		3.9	
	3728	12.8	4.8		5.4		5.8	
	3746	13.3	5.7		7.0		6.8	
48	3764	14.6	5.3		6.8		6.5	
	3765	12.2	3.9		4.5		4.5	
	Average	**13.0**	**4.7**	**0.36**	**5.5**	**0.42**	**5.5**	**0.42**
	3692	16.8	4.3		5.0		5.2	
	3693	17.2	6.5		7.3		6.6	
	3711	17.0	7.0		7.7		8.3	
62	3729	16.1	5.5		6.7		6.4	
	3749	16.2	5.6		7.1		7.8	
	Average	**16.7**	**5.8**	**0.35**	**6.8**	**0.40**	**6.9**	**0.41**

TABLE 43

Rana clamitans Series 3676-3765 Summary First regenerations
Lengths regenerated at different levels at different times

Percent of tail length removed	Length removed in mm.	Number of individuals	Days after operation						
			4	6	8	10	12½	18	56
6	1.5	2	0.27	0.5	0.8	0.9	0.9	0.9	0.7
10	2.6	5	0.15	0.5	0.7	1.0	1.2	1.1	1.1
17	4.6	3	0.51	1.0	1.5	1.7	1.8	1.8	1.5
30	8.2	8	0.45	1.1	1.7	2.3	2.6	2.7	2.5
48	13.0	5	0.46	1.6	2.6	3.8	4.7	5.5	5.5
62	16.7	5	0.51	1.6	3.0	4.5	5.8	6.8	6.9

TABLE 44

Rana clamitans Series 3676-3765 Summary First regenerations
Specific lengths regenerated at different levels at different times

Percent of tail length removed	Length removed in mm.	Number of individuals	Days after operation						
			4	6	8	10	12½	18	56
6	1.5	2	0.17	0.30	0.53	0.58	0.61	0.60	0.45
10	2.6	5	0.06	0.18	0.27	0.38	0.46	0.42	0.42
17	4.6	3	0.11	0.22	0.33	0.37	0.39	0.39	0.34
30	8.2	8	0.05	0.13	0.21	0.28	0.31	0.33	0.30
48	13.0	5	0.03	0.12	0.20	0.29	0.36	0.42	0.42
62	16.7	5	0.03	0.09	0.18	0.27	0.35	0.40	0.41

EXPERIMENT II AMBLYSTOMA PUNCTATUM SERIES 4600-5052

The eggs were hatched on March 29 to April 4, 1913. Operations on the tail were made on May 7 in numbers 4600-4752 and on May 10 in numbers 4800-5052. The removed lengths were approximately $^1/_{10}$, $^1/_5$, $^1/_3$, $^1/_2$ and $^3/_4$ of the tail length. Measurements of the regenerated tissue were made at 2, 4, 6, 8-9, 10-11, 13, 15-16 and 17-18 days after the operation. The data are given in Tables 45 to 54 and in Figures 36 to 51.

The salamander larvae are much more irregular in their regeneration as well as in ordinary growth than frog tadpoles. The measurements

in the present experiment were made on killed individuals so that only a single regeneration measurement is made in a single individual. This procedure also tends toward a greater variability in the data. The number of individuals in any particular measurement also is less than for the second regeneration of frog tadpoles.

Notwithstanding all these unwelcome factors the general features of regeneration are similar to those for the tadpole experiment. The regenerated length at any time is approximately proportional to the removed length. It is true even in the earliest measurements. As for frog tadpoles the shorter removals have proportionately a larger regeneration than the others at practically each time of measurement. The approach to equality in specific lengths is true only of the lengths of removal equal to one-fifth or more of the tail length.

At two days the regenerated lengths are respectively 0.10, 0.15, 0.15, 0.47 and 0.53 mm. for the five levels of removal. They give specific lengths of 0.07, 0.07, 0.04, 0.08 and 0.06 as shown Table 45 and Figures 36 and 37.

At four days the regenerated lengths are 0.12, 0.15, 0.30, 0.41 and 0.40 mm. and the specific lengths 0.11, 0.07, 0.07, 0.07 and 0.05 as shown in Table 46 and Figures 38 and 39.

At six days the regenerated lengths are 0.32, 0.47, 0.62, 0.70 and 1.02 mm. and the specific lengths 0.30, 0.20, 0.16, 0.12 and 0.11 as shown in Table 47 and Figures 40 and 41.

At eight to nine days the regenerated lengths are 0.40, 0.65, 0.80, 1.40 and 1.52 mm. and the specific lengths 0.44, 0.28, 0.23, 0.23 and 0.19 as shown in Table 48 and Figures 42 and 43.

At ten to eleven days the regenerated lengths are 0.50, 0.63, 1.54, 2.22 and 2.22 mm. and the specific lengths 0.62, 0.26, 0.43, 0.41 and 0.27 as shown in Table 49 and Figures 44 and 45.

At thirteen days the regenerated lengths are 0.78, 0.92, 1.74, 2.40 and 3.60 mm and the specific lengths 0.74, 0.43, 0.48, 0.44 and 0.48 as shown in Table 50 and Figures 46 and 47.

At fifteen to sixteen days the regenerated lengths are 0.80, 1.30, 1.37, 2.80 and 3.80 mm. and the specific lengths 0.67, 0.61, 0.40, 0.48 and 0.54 as shown in Table 51 and Figures 48 and 49.

At seventeen to eighteen days the regenerated lengths are 0.70, 1.40, 1.60, 3.80 and 4.67 mm. and the specific lengths 0.67, 0.62, 0.41, 0.66 and 0.57 as shown in Table 52 and Figures 50 and 51.

A summary of regenerated lengths is given in Table 53 and of specific regenerated lengths in Table 54.

Since the experiment was closed at eighteen days and since the measurements at different times were made on different individuals it is not possible to make as accurate a comparison of completeness of

regeneration as in the case of the frog tadpoles. For the three shortest removals regeneration is probably completed at this time but this is not true for the two longest ones. In this respect as in others there is an agreement with the former experiment. The percent of the removed tail that is regenerated is greater for all levels than in the frog tadpoles. It is probable also that if the longest removals had been allowed to complete their regenerations their specific regenerations as in the case of the frog tadpoles would have been shown to be greater than those for medium levels.

TABLE 45

Amblystoma punctatum. Series 4600-5052. Average tail length =10.9 mm.
Regeneration: 2 days

Percent of tail length removed Average	Catalog number	Removed length mm.	Regenerated length mm.	Specific length regenerated
14	5022	1.5	0.1	
	Average	**1.5**	**0.10**	**0.07**
	4641	2.3	0.2	
	4741	1.9	0.1	
20	4841	2.2	0.1	
	5050b	2.3	0.2	
	Average	**2.2**	**0.15**	**0.07**
	4811	3.9	0.3	
	4911	3.3	0.1	
32	5012	3.3	0.05	
	Average	**3.5**	**0.15**	**0.04**
	4601	6.0	0.3	
	4801	6.0	0.7	
53	5001	5.4	0.4	
	Average	**5.8**	**0.47**	**0.08**
	4631	9.5	0.7	
	4831	9.1	0.6	
81	5032	7.9	0.3	
	Average	**8.8**	**0.53**	**0.06**

The data from both experiments show that except for very short removals the length regenerated in a given time is approximately proportional to the length removed.

TABLE 46

Amblystoma punctatum. Series 4600-5052. Average tail length=10.9 mm.
Regeneration: 4 days

Percent of tail length removed Average	Catalog number	Removed length mm.	Regenerated length mm.	Specific length regenerated
	4622	0.9	0.1	
	4722	0.8	0.2	
	4822	1.5	0.1	
10	4922	0.8	0.1	
	5024	1.6	0.1	
	Average	**1.1**	**0.12**	**0.11**
	4742	2.4	0.1	
21	4842	2.3	0.2	
	Average	**2.3**	**0.15**	**0.07**
	4612	3.7	0.3	
	4712	3.6	0.2	
37	4812	4.5	0.4	
	5012	4.2	0.3	
	Average	**4.0**	**0.30**	**0.07**
	4602	6.0	0.2	
	4702	6.0	0.05	
	4802	6.2	0.7	
55	4902	6.0	0.5	
	5004	5.9	0.6	
	Average	**6.0**	**0.41**	**0.07**
	4632	9.3	0.4	
	4732	8.5	0.2	
	4832	10.9	0.6	
76	4932	5.9	0.4	
	5034	7.1	0.4	
	Average	**8.3**	**0.40**	**0.05**

TABLE 47

Amblystoma punctatum. Series 4600-5052. Average tail length=10.9 mm. Regeneration: 6 days

Percent of tail length removed Average	Catalog number	Removed length mm.	Regenerated length mm.	Specific length regenerated
	4623	1.0	0.3	
	4723	0.8	0.2	
10	4923	1.6	0.6	
	——	1.1	0.2	
	Average	**1.1**	**0.32**	**0.30**
	4643	2.1	0.7	
	4743	2.2	0.2	
21	4843	2.1	0.4	
	4943	2.8	0.6	
	Average	**2.3**	**0.47**	**0.20**
	4613	3.6	0.6	
	4713	2.9	0.6	
	4820b	4.4	0.8	
35	4913	4.5	0.6	
	5013	3.6	0.5	
	Average	**3.8**	**0.62**	**0.16**
	4603	6.1	0.4	
	4703	6.7	0.4	
54	4803	5.3	1.4	
	5003	5.6	0.6	
	Average	**5.9**	**0.70**	**0.12**
	4633	8.8	0.6	
	4733	8.2	0.9	
82	4833	10.6	1.8	
	5033	7.9	0.8	
	Average	**8.9**	**1.02**	**0.11**

TABLE 48

Amblystoma punctatum Series 4600-5052 Average tail length=10.9 mm.
Regeneration: 8-9 days (8 for 4800-5052, 9 for 4600-4752)

Percent of tail length removed Average	Catalog number	Removed length mm.	Regenerated length mm.	Specific length regenerated
	4724	0.7	0.2	
	4824	1.1	0.7	
8	5026	1.0	0.3	
	Average	**0.9**	**0.40**	0.44
	4644	2.3	0.6	
	4844	2.3	1.0	
21	4944	2.2	0.7	
	5045	2.3	0.3	
	Average	**2.3**	**0.65**	**0.28**
	4614	3.0	0.8	
	4624	3.0	0.4	
	4714	3.4	1.2	
31	4814	3.7	1.0	
	4914	3.2	0.6	
	5016	4.3	0.8	
	Average	**3.4**	**0.80**	**0.23**
	4604	6.0	1.8	
	4704	6.3	1.7	
	4804	5.9	1.4	
56	4904	5.4	0.9	
	5006	6.8	1.2	
	Average	**6.1**	**1.40**	**0.23**
	4634	8.3	2.1	
	4734	8.0	1.3	
	4834	9.0	1.6	
75	4934	7.5	1.1	
	Average	**8.2**	**1.52**	**0.19**

TABLE 49

Amblystoma punctatum Series 4600-5052 Average tail length=10.9 mm.
Regeneration: 10-11 days (10 for 4800-5052, 11 for 4600-4752)

Percent of tail length removed Average	Catalog number	Removed length mm.	Regenerated length mm.	Specific length regenerated
	4725	0.6	0.3	
	4825	0.8	0.6	
7	5027	1.0	0.6	
	Average	**0.8**	**0.50**	**0.62**
	4746	2.6	0.4	
	4845	2.6	1.0	
23	5046	2.2	0.5	
	Average	**2.5**	**0.63**	**0.26**
	4620b	3.0	1.9	
	4715	3.0	1.8	
	4815	4.3	2.0	
33	4920	3.6	1.2	
	5017	3.9	0.8	
	Average	**3.6**	**1.54**	**0.43**
	4605	5.2	2.8	
	4705	4.6	1.4	
	4805	6.3	2.8	
50	4910b	5.0	1.9	
	5007	6.0	2.2	
	Average	**5.4..**	**2.22**	**0.41**
	4735	7.5	2.5	
	4835	9.4	2.9	
74	4935	7.6	1.3	
	5037	8.1	2.2	
	Average	**8.1**	**2.22**	**0.27**

TABLE 50

Amblystoma punctatum. Series 4600-5052. Average tail length=10.9 mm. Regeneration: 13 days

Percent of tail length removed Average	Catalog number	Removed length mm.	Regenerated length mm.	Specific length regenerated
	4626	1.4	0.8	
	4726	1.0	0.6	
	4830b	1.0	0.7	
10	4926	1.0	0.9	
	5028	0.9	0.9	
	Average	**1.1**	**0.78**	**0.74**
	4646	1.9	1.4	
	4745	2.2	0.8	
19	4846	2.6	1.0	
	4946	1.9	0.5	
	Average	**2.1**	**0.92**	**0.43**
	4616	3.6	1.8	
	4716	3.2	2.0	
	4816	3.9	2.5	
32	4916	3.7	1.6	
	5018	3.7	0.8	
	Average	**3.6**	**1.74**	**0.48**
	4706	6.4	2.3	
	4806	5.8	2.9	
50	4910	5.0	2.5	
	5008	4.5	1.9	
	Average	**5.4**	**2.40**	**0.44**
	4636	8.1	3.4	
	4740b	7.7	4.7	
69	4936	7.4	3.5	
	5038	6.7	2.8	
	Average	**7.5**	**3.60**	**0.48**

TABLE 51

Amblystoma punctatum. Series 4600-5052. Average tail length=10.9 mm.
Regeneration: 15-16 days (15 for 4800-5052, 16 for 4600-47g2)

Percent of tail length removed Average	Catalog number	Removed length mm.	Regenerated length mm.	Specific length regenerated
11	4927	1.2	0.8	
	5029	1.2	0.8	
	Average	**1.2**	**0.80**	**0.67**
19	4647	1.9	1.5	
	4747	2.7	1.7	
	4847	2.4	1.0	
	4950b	1.9	1.2	
	5049	1.8	1.1	
	Average	**2.1**	**1.30**	**0.61**
32	4617	3.1	1.4	
	4717	3.5	1.3	
	4917	3.2	1.8	
	5019	4.1	1.0	
	Average	**3.5**	**1.37**	**0.40**
53	4607	5.7	2.7	
	4807	5.7	2.9	
	4817	5.2	2.6	
	5009	6.6	3.0	
	Average	**5.8**	**2.80**	**0.48**
64	4937	7.0	3.8	
	Average	**7.0**	**3.80**	**0.54**

TABLE 52

Amblystoma punctatum. Series 4600-5052. Average tail length=10.9 mm. Regeneration: 17-18 days (18 for 4800-5052, 17 for 4600-4752)

Percent of tail length removed Average	Catalog number	Removed length mm.	Regenerated length mm.	Specific length regenerated
	4828	1.0	0.8	
10	4929	1.1	0.6	
	Average	**1.0**	**0.70**	**0.67**
	4648	2.1	1.5	
	4749	2.6	2.1	
	4848	2.1	1.1	
20	4949	2.2	1.3	
	5050	2.2	1.0	
	Average	**2.2**	**1.40**	**0.62**
	4718	3.9	1.6	
36				
	Average	**3.9**	**1.60**	**0.41**
	4608	5.4	4.2	
	4708	6.6	4.1	
53	4808	5.4	3.1	
	Average	**5.8**	**3.80**	**0.66**
	4838	9.4	5.0	
	4939	6.4	4.5	
76	5040	9.0	4.5	
	Average	**8.3**	**4.67**	**0.57**

TABLE 53

Amblystoma punctatum Series 4600-5052 Summary Regenerated lengths (Tables 45 to 52)

Percent of tail length removed Average	Length removed mm. Average	Average length regenerated in mm.							
		2 Days	4 Days	6 Days	8-9 Days	10-11 Days	13 Days	15-16 Days	17-18 Days
10	1.1	0.10	0.12	0.32	0.40	0.50	0.78	0.80	0.70
21	2.2	0.15	0.15	0.47	0.65	0.63	0.92	1.30	1.40
34	3.7	0.15	0.30	0.62	0.80	1.54	1.74	1.37	1.60
53	5.8	0.47	0.41	0.70	1.40	2.22	2.40	2.80	3.80
74	8.1	0.53	0.40	0.94	1.52	2.22	3.60	3.80	4.70

TABLE 54

Amblystoma punctatum Series 4600-5052 Summary Specific lengths regenerated (Tables 45 to 52)

Percent of tail length removed Average	Length removed mm. Average	Average specific regenerated lengths							
		2 Days	4 Days	6 Days	8-9 Days	10-11 Days	13 Days	15-16 Days	17-18 Days
10	1.1	0.07	0.11	0.30	0.43	0.62	0.74	0.67	0.67
21	2.2	0.07	0.06	0.20	0.28	0.26	0.43	0.61	0.62
34	3.7	0.04	0.07	0.16	0.23	0.43	0.48	0.40	0.41
53	5.8	0.08	0.07	0.12	0.23	0.41	0.44	0.48	0.66
74	8.1	0.06	0.15	0.11	0.19	0.27	0.48	0.54	0.57

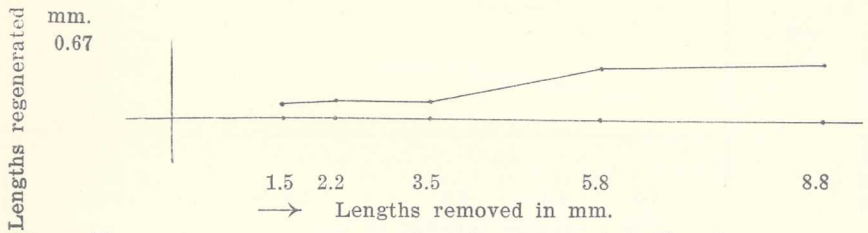

Figure 36 *Amblystoma punctatum* Lengths regenerated Two days

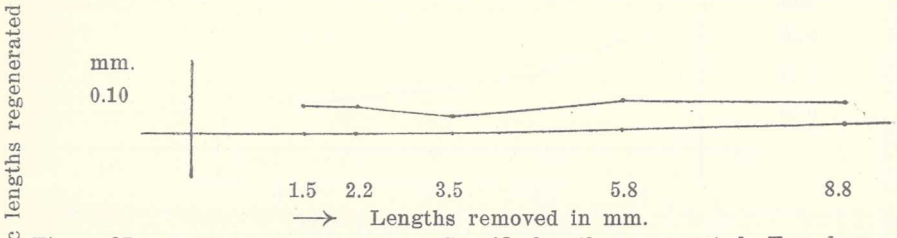

Figure 37 *Amblystoma punctatum* Specific lengths regenerated Two days

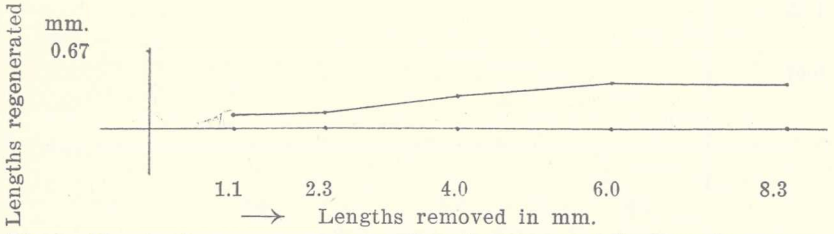

Figure 38 *Amblystoma punctatum* Lengths regenerated Four days

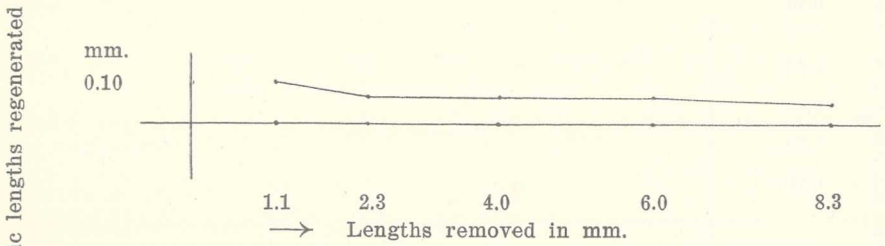

Figure 39 *Amblystoma punctatum* Specific lengths regenerated Four days

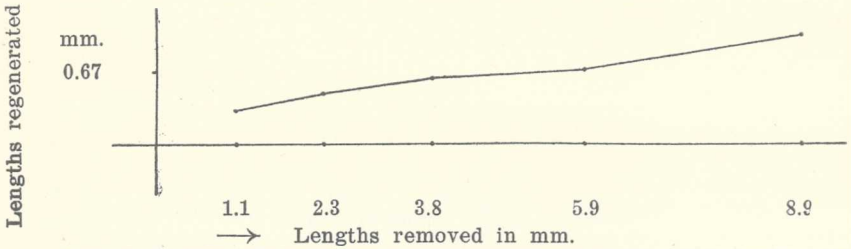

Figure 40 *Amblystoma punctatum* Lengths regenerated Six days

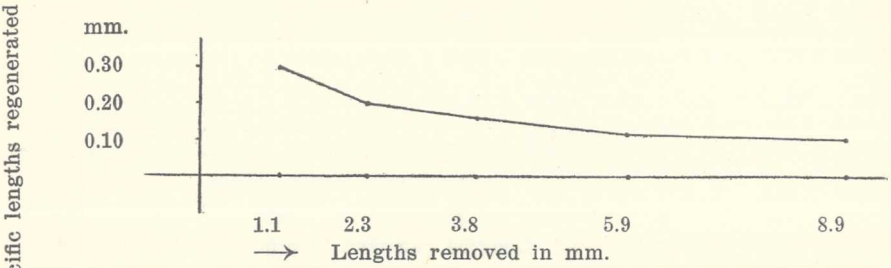

Figure 41 *Amblystoma punctatum* Specific lengths regenerated Six days

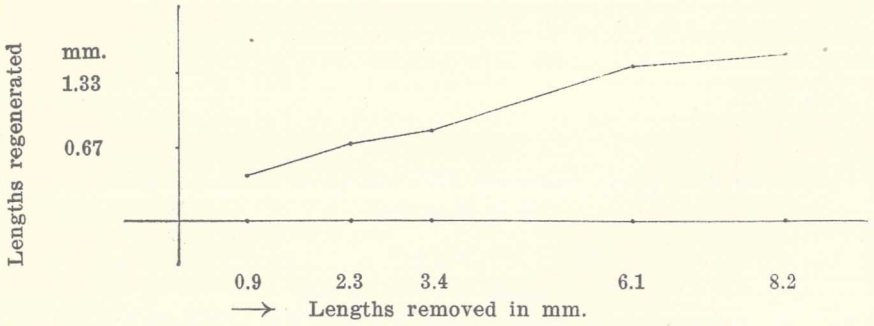

Figure 42 *Amblystoma punctatum* Lengths regenerated Eight to nine days

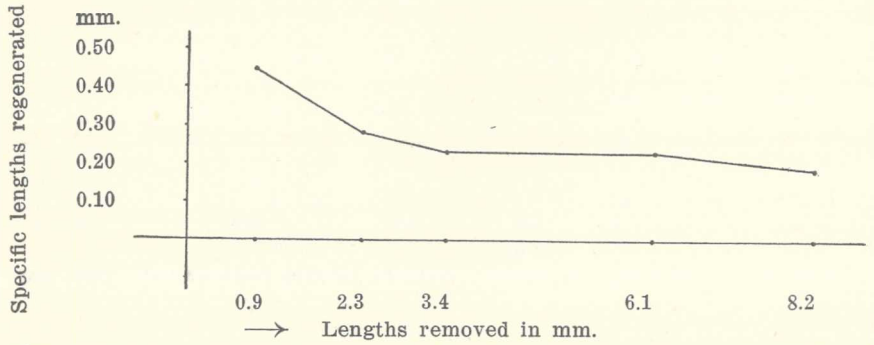

Figure 43 *Amblystoma punctatum* Specific lengths regenerated Eight to nine days

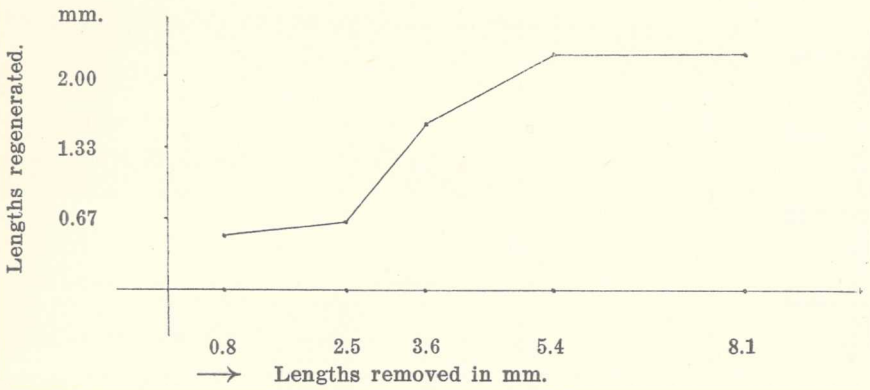

Figure 44 *Amblystoma punctatum* Lengths regenerated Ten to eleven days

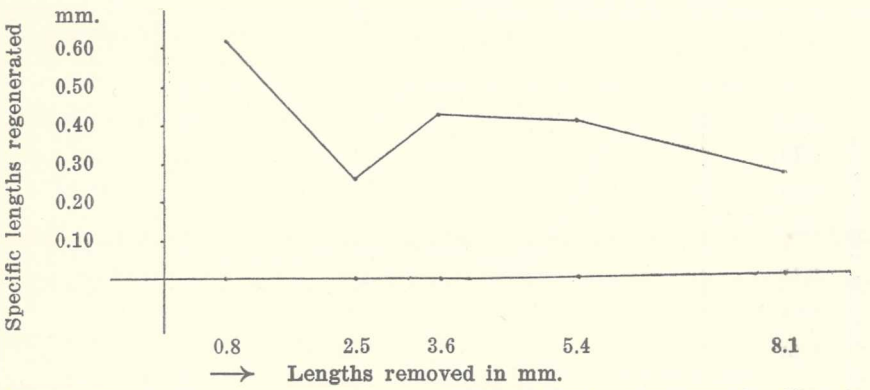

Figure 45 *Amblystoma punctatum* Specific lengths regenerated Ten to eleven days

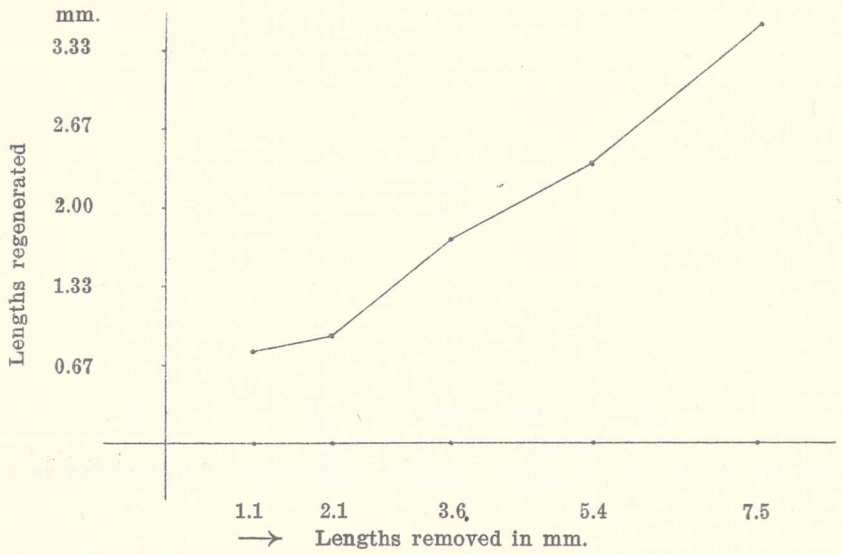

Figure 46 *Amblystoma punctatum* Lengths regenerated Thirteen days

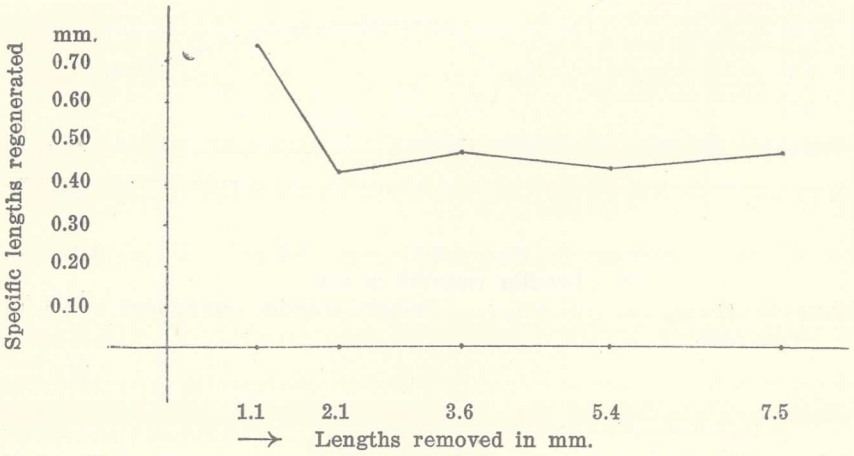

Figure 47 *Amblystoma punctatum* Specific lengths regenerated Thirteen days

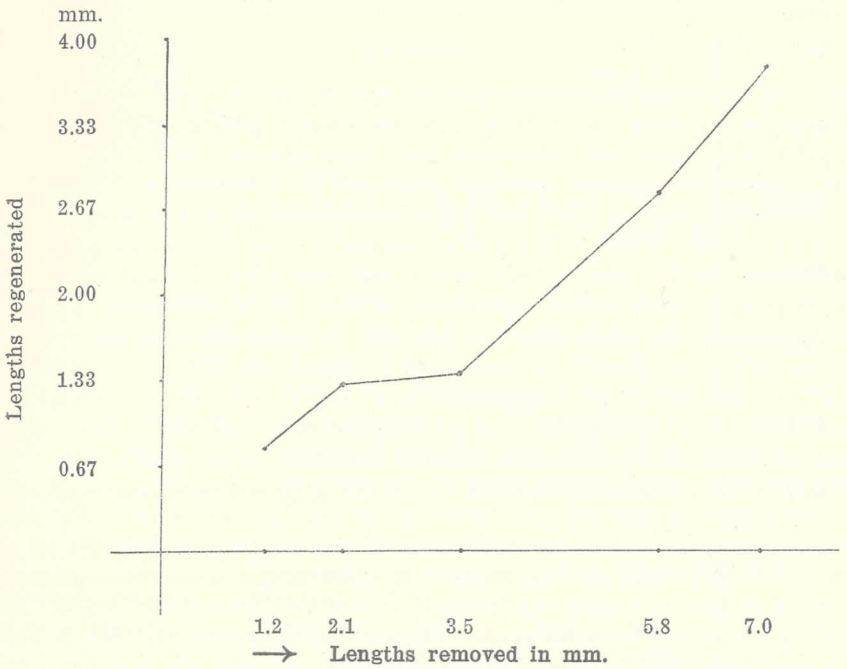

Figure 48 *Amblystoma punctatum* Lengths regenerated Fifteen to sixteen days

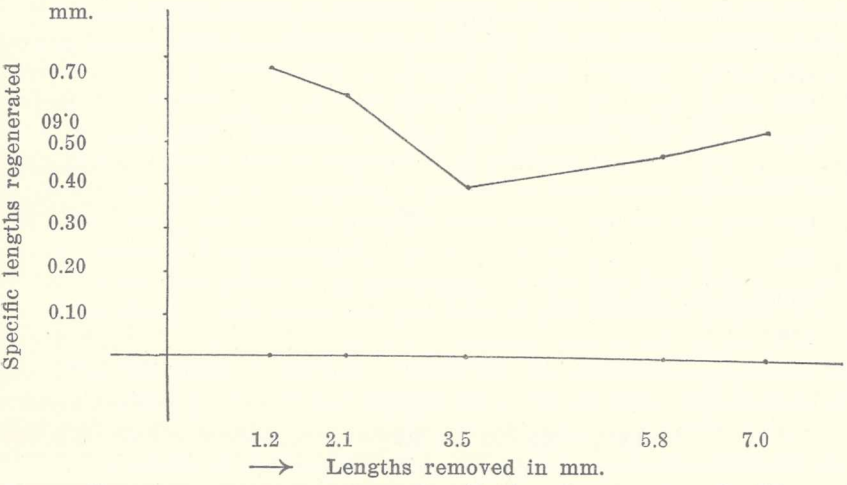

Figure 49 *Amblystoma punctatum* Specific lengths regenerated Fifteen to sixteen days

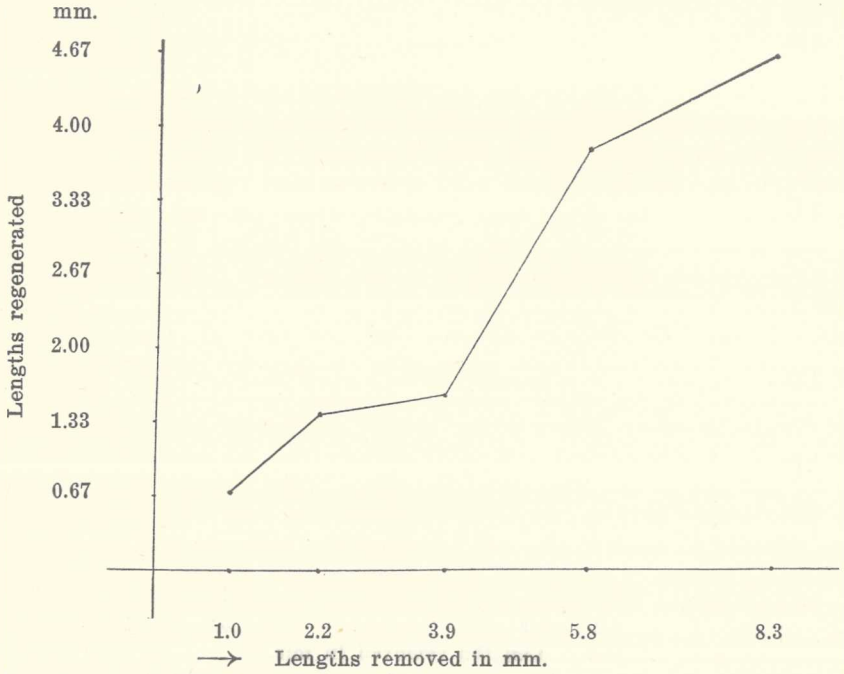

Figure 50 *Amblystoma punctatum* Lengths regenerated Seventeen to eighteen days

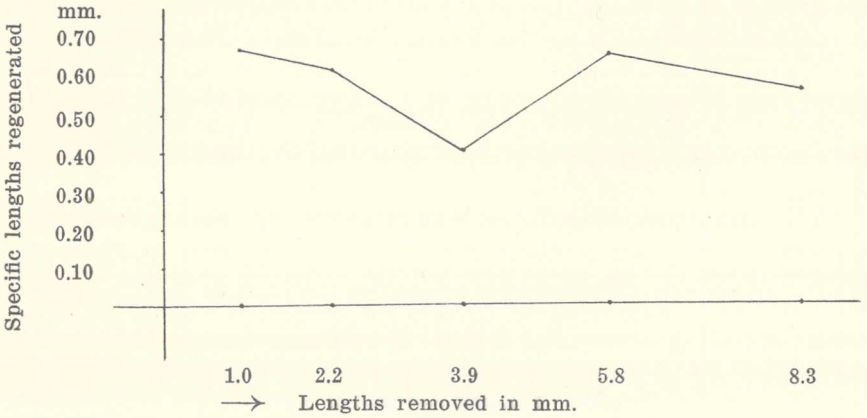

Figure 51 *Amblystoma punctatum* Specific lengths regenerated Seventeen to eighteen days

Discussion

That the level of the cut has an important influence upon the rate of regeneration has been made out by a number of investigators (Spallanzani 1768, King 1898, Morgan 1906, Stockard 1908, Ellis 1909, Morgulis 1909a, b, and others). Their work indicates that regenerations from deeper levels are on the whole more rapid that from more superficial ones. The data obtained from the present experiments confirm this conclusion and make possible a further analysis of the relation. They show that in the regeneration of the tail of amphibian larvae there is a striking relation between the level of the cut and the rate of regeneration. Within wide limits the length regenerated is directly proportional to the distance of the cut surface from the original tip of the tail. Within these limits therefore regeneration at any particular time after the operation has the same degree of completeness from all levels of injury.

An analysis of the progress of the regeneration brings out the fact that two distinct periods are to be recognized in rate of regeneration in its relation to level of the cut. During the first two to four days after the operation regeneration is confined to cell migration from the old tissues without cell division. During this period in the frog tadpoles there is no essential difference in length regenerated at the different levels and the specific rate is therefore much greater after shorter than after longer removals. In the second period with the initiation of rapid cell multiplication the rate of regeneration is greater the deeper the level and furthermore is directly proportional to the length removed. As soon as the bulk of material produced by cell division is considerably greater than that which was produced by cell migration there is an approach to constancy in specific length regenerated. This holds for all except the shortest removals. After the shortest removals the total regeneration is so small in amount that a large part of it is made up of the original migrated material. Therefore from these levels the specific regenerated lengths are greater than from the deeper levels even at a late period of regeneration.

A further complication is introduced by the fact that regeneration is not complete. Only a certain per cent of the removed length is replaced and the end of the process is reached sooner after the shorter than after the longer removals. From the deepest levels regeneration is still proceeding when it has stopped from the medium and shallowest ones. When the process is completed in all cases the specific length is therefore slightly greater after both the longest and the shortest removals than after medium ones.

As to the cause of the difference in rate at the different levels

little more can be said than that it does not seem to be due to inherent differences in the cells at the different levels. If differentiation in the tail proceeded from the tip toward the base, the more rapid rate from the more basal levels might be explained by the more embryonic character of the cells at these levels. As the tip is approached the material would become more and more inert. There is however no evidence that differentiation proceeds in this way in this case.

The progressive increase in rate with depth of level of the cut is undoubtedly due to reactions which involve a more central control, a co-ordination of the functional activity as a whole. The period of cell migration probably is only slightly subject to such control. It is a period in which the response is largely local in character and there is correspondingly little if any difference at the different levels. The rate of cell division which is the important factor during the period of rapid increase in length is however undoubtedy under central control.

Summary

1. In frog and salamander larvae with removed tail lengths of one-fifth to two-thirds, the general rule holds that the length regenerated in a given time is proportional to the length removed, or in other words the length regenerated per unit of removed length is a constant.

2. An analysis of the data shows however that this applies only to the material produced by active cell division.

3. During the first four days, in frog tadpoles, when the regenerating part is made up almost entirely of cells that have migrated from the old tissues without division there is no such relation between length removed and length regenerated. The length of new material at this time is not strikingly different for the different levels and the process seems to be a local response of the cells to the injury. The length regenerated per unit of removed length is greater at this time for the shorter than for the longer removals.

4. Since comparatively a large part of the regenerating material after the shorter removals is made up of migrated cells even at the later periods it follows that the specific regenerations from these levels are greater than from the deeper ones.

5. During the later periods the specific regenerated lengths tend to be higher after both the shortest and the longest removals than after medium ones. In the case of the shortest ones this is due to the relatively large part of the whole regenerated tail that is made up of migrated cells. In the case of the longest removals it is due to the fact that regeneration continues for a time after it has stopped in the medium ones.

6. It does not seem probable that the differences in length regenerated at different levels can be due to differences in the original character of the cells involved in the process. Such a well graduated difference in cell capacities is difficult to conceive. The process must be under a more central control, probably connected with general functional activity.

PART IV

THE CHANGE IN RATE OF REGENERATION DURING THE REGENERATIVE PROCESS

The present experiments were undertaken in extension of previous studies on the change in rate throughout the regenerative cycle. This previous work showed that the increase in amount of material during regeneration follows the general rule of increase during an ordinary life cycle. The rate is at first very slow, then increases very rapidly to a maximum, then declines rapidly at first and then more and more slowly as zero is approached.

Frog tadpoles and salamander larvae were used in the present study. Large tadpoles of *Rana clamitans* which remained fairly constant in size during the course of the experiments were found to be the most satisfactory. The results obtained from them were uniform enough for an analysis of the change in rate. The salamander larvae showed a great variation in rate from day to day apparently associated with external factors such as food and temperature. The data obtained from them are however of interest in comparison with the frog tadpole results.

The experiments will be taken up in turn beginning with the series containing the largest number of individuals and giving the most uniform results.

Experiment I Rana clamitans Second regenerations of the tail Series 3676-3765

The tadpoles were collected on December 9, 1911 and first removals were made on December 22 and second removals on January 8. Measurements were taken 4, 6, 8, 10, 12½ and 56 days after the operation. The operations were made at six different levels, the removals approximating 6, 10, 18, 31, 49 and 67 per cent of the tail length. The first of these removals averaged 1.5 mm. and four individuals with completed measurements are available, the next averaged 2.8 mm. with seven individuals, the third 4.9 mm. with five, the fourth 8.4 mm. with ten, the fifth 13.1 mm. with eight and the sixth 18.1 mm. with ten individuals. The rates per day for each level during each period are given in table 55 and in graphic form in figure 52. The maximum

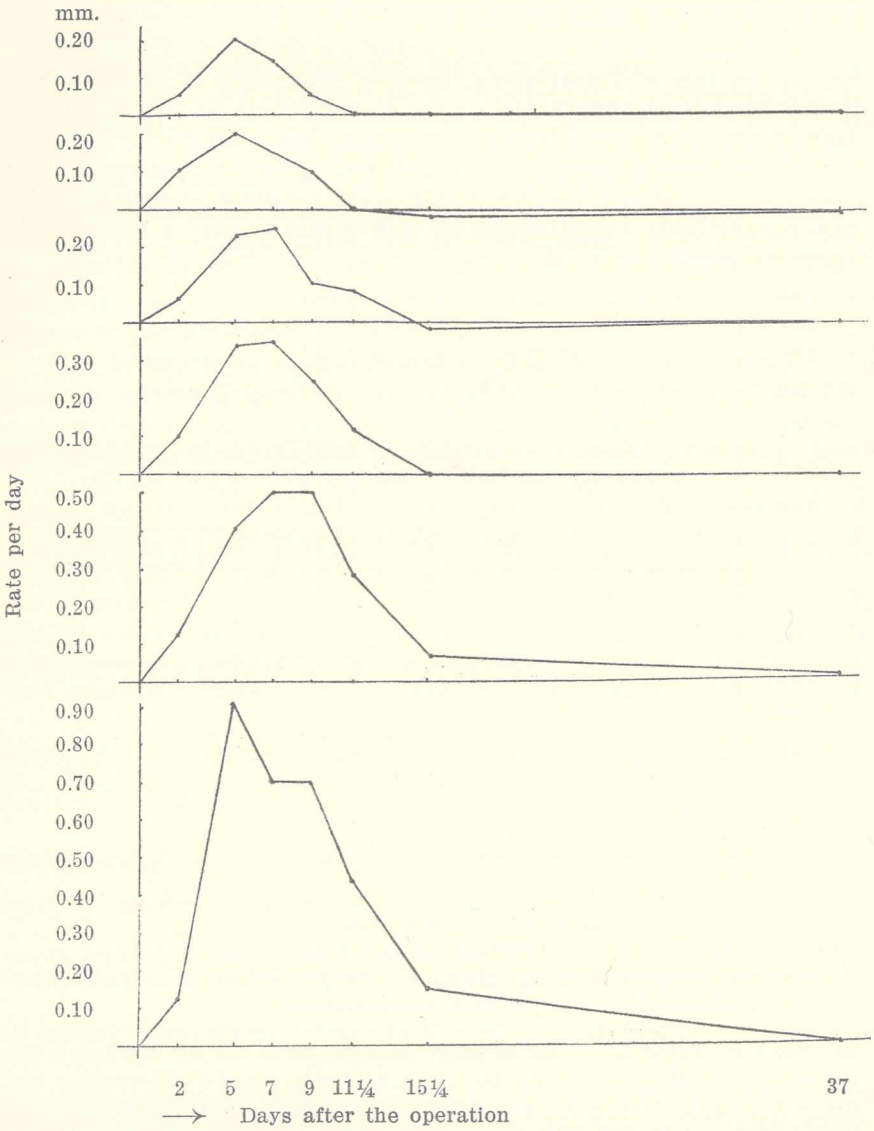

Figure 52 Rates of second regenerations of the tail per day at different times after the operation for six different levels *Rana clamitans* The removed lengths are 1.5, 2.8, 4.9, 8.4, 13.1 and 18.1 mm.

rate is reached during the period between four and six days at three of the levels and between six and eight days at the other three. The rise in rate is very rapid and the decline also rapid.

As discussed in the preceding section on the effect of the level of the cut, the rate of regeneration increases with depth of the level and the increase is such that in general the specific length or length regenerated per unit of removed length is approximately a constant. A reduction of the rates to specific rates therefore gives an opportunity for averaging the different levels together. The resultant average is based upon a sufficiently large number of individuals to give a considerable degree of smoothness in the curve of rate. The data for specific rate

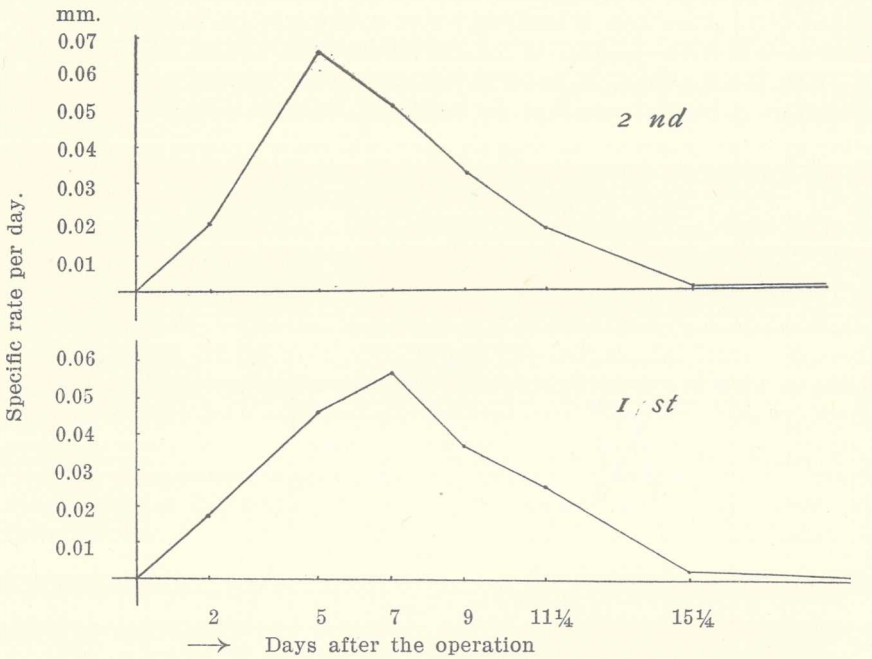

Figure 53. Specific rates of first and second regenerations at different times after the operation *Rana clamitans* Tail regeneration Upper figure, second regenerations; lower, first regenerations.

are given in Table 56. The average specific rates for all six levels together are 0.019 mm. during the 0 to 4 day period, 0.066 during the 4 to 6 day period, 0.051 for 6 to 8 days, 0.033 for 8 to 10 days, 0.017 for 10 to 12½ days, 0.001 for 12½ to 18 days and —0.001 for 18 to 56 days. This change in rate is represented graphically in the upper part of Figure 53. For the four deepest levels the averages are given in a separate column of Table 56. They exclude the two lowest levels which

depart considerably from the others in specific rate. There is however no essential difference in the two sets of values as regards the form of the rate curve.

The change in rate of regeneration or acceleration of rate from any period to the succeeding one is shown in Table 57 in which the period of change is represented by the middle days of the two periods which are being compared. The average of all the levels shows the acceleration to be +0.095 mm. from the 2 to the 5 day period, —0.015 for 5 to 7 days, —0.030 for 7 to 9 days, —0.058 for 9 to 11¼ days, —0.028 for 11¼ to 15¼ days and —0.001 for 15¼ to 37 days. It is only between the first two periods that acceleration of rate is a plus quantity. During all the others it is minus, the most rapid rate of decrease coming between 9 and 11¼ days.

The accelerations of specific rate are more reliable measures for obtaining averages including the different periods. Such values are given in Table 58 and in graphic form in Figure 54. They give a result in the relation of the periods to each other essentially similar to that above. The average accelerations of specific rate are +0.014 for the

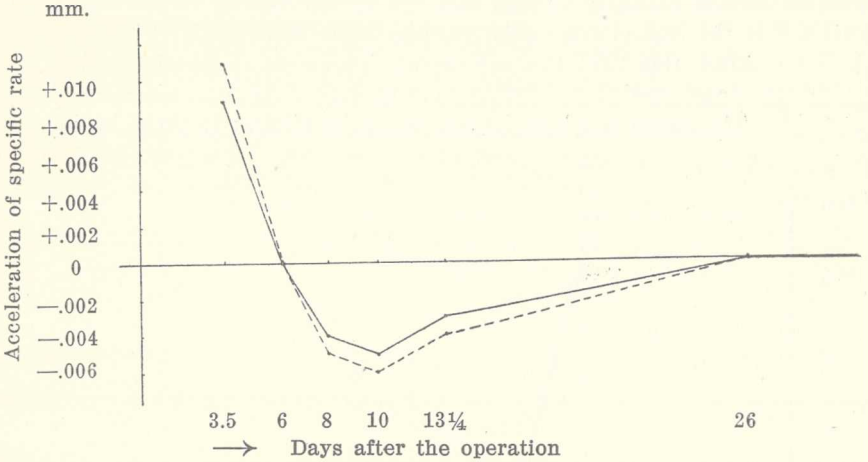

Figure 54. Acceleration of specific rate First and second regenerations of the tail in *Rana clamitans* Unbroken line=First regeneration Broken line=Second regeneration.

2 to 5 day periods, —0.004 for 5 to 7 days, —0.009 for 7 to 9 days, —0.0085 for 9 to 11¼ days, —0.003 for 11¼ to 15¼ days and 0.000 for 15¼ to 37 days. The first period is the only one with a plus acceleration. The greatest minus acceleration comes between the 7 and the 9 day periods instead of 9 to 11¼ days. Averaging only the regenerations for the four deepest levels which show a constant specific rate

the values are respectively +0.011, 0.000, —0.005, —0.006, —0.004 and 0.000, putting the greatest rate of decrease between the 9 and the 11¼ day periods.

An examination of the curves of specific rate and a comparison with the facts of histogenesis shows that acceleration of rate is a plus quantity only during the period before active differentiation of the cells, i. e. until the end of the fifth or seventh day. As soon as tissue differentiation is fairly begun the retarding influence is apparent and by the ninth to eleventh days when muscle fibres and other cells are in full process of differentiation the negative acceleration is at its height.

Following the percentage increment method used by Minot (1908) for ordinary growth and using length instead of weight because the latter could not be determined with sufficient accuracy the results given in Table 59 are obtained. The values for the six periods excluding the first one are 106, 28, 12, 5 and 0. The regenerated material present at the end of four days is made up almost wholly of cells that have migrated from the old tissues and have not as yet undergone division. After the fourth day the additions to regenerated material are almost wholly the result of cell division. From the end of the fourth to the end of the sixth day the material is on the average more than doubled in length each day. After this time the percentage increment decreases rapidly. The change from period to period is represented in graphic form in Figure 55. The curve is a logarithmic one quite similar to that obtained

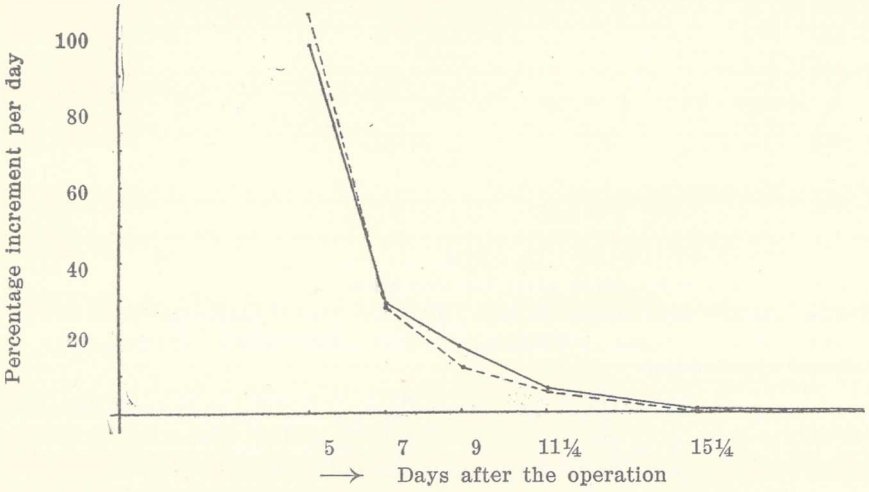

Figure 55 Percentage increment per day at different periods after the operation First and second regenerations of the tail of *Rana clamitans* Unbroken line=first regeneration. Broken line=second regeneration.

by Minot for growth. It should however be pointed out that both regeneration and ordinary growth undoubtedly have a very rapidly ascending branch of the curve if the very beginnings of the processes are included.

TABLE 55

Rana clamitans Series 3676-3765 Second regenerations
Rate of regeneration of tail per day at different times during the regenerative process for six different levels

Percent of tail length removed	6	10	18	31	49	67
Length removed in mm.	1.5	2.8	4.9	8.4	13.1	18.1
No. of individuals	4	7	5	10	8	10
Days 0- 4	0.05	0.10	0.06	0.10	0.12	0.13
4- 6	0.20*	0.20*	0.23	0.34	0.40	0.91*
6- 8	0.14	0.15	0.25*	0.35*	0.50*	0.70
8-10	0.05	0.10	0.10	0.25	0.50*	0.70
10-12½	0.00	0.00	0.08	0.12	0.28	0.44
12½-18	0.00	—0.02	—0.02	0.00	0.07	0.15
18-56	0.00	—0.01	0.00	0.00	0.01	0.00

TABLE 56

Rana clamitans Series 3676-3765 Second regenerations
Specific rates at different levels at different times

Percent of tail length removed	6	10	18	31	49	67	Average of all levels	Average of four longest removals..
Length removed in mm.	1.5	2.8	4.9	8.4	13.1	18.1		
No. of individuals	4	7	5	10	8	10		
Days								
0- 4	0.037	0.035	0.012	0.012	0.010	0.007	0.019	0.010
4- 6	0.135*	0.080*	0.045	0.040	0.045*	0.050*	0.066*	0.045*
8-10	0.035	0.035	0.025	0.030	0.035	0.035	0.051	0.042
6- 8	0.090	0.045	0.050*	0.045*	0.040	0.040	0.033	0.032
10-12½	0.000	0.000	0.025	0.015	0.030	0.030	0.017	0.025
12½-18	0.000	—0.005	—0.004	0.000	0.005	0.009	0.001	0.002
18-56	—0.002	—0.004	0.001	0.000	0.001	0.000	—0.001	0.000

TABLE 57

Rana clamitans Series 3676-3765 Second regenerations
Acceleration of rate of regeneration of tail per day at different times during the regenerative process for six different levels

Percent of tail length removed	6	10	18	31	49	67	
Length removed in mm.	1.5	2.8	4.9	8.4	13.1	18.1	Average of all levels
No. of individuals	4	7	5	10	8	10	
Middle of periods Days							
2- 5	+0.05*	+0.03*	+0.06*	+0.08*	+0.09*	+0.26*	**+0.095***
5- 7	—0.03	—0.02	+0.01	+0.00	+0.05	—0.10	**—0.015**
7- 9	—0.04*	—0.02	—0.07*	—0.05	0.00	0.00	**—0.030**
9-11¼	—0.02	—0.04*	—0.01	—0.06*	—0.10*	—0.12*	**—0.058***
11¼-15¼	0.00	—0.00	—0.02	—0.03	—0.05	—0.07	**—0.028**
15¼-37	—0.00	—0.00	+0.00	—0.00	—0.00	—0.01	**—0.001**

TABLE 58

Rana clamitans Series 3676-3765 First regenerations

Acceleration of specific rate of regeneration of the tail

Percent of tail length removed	6	10	18	31	49	67	Average of all levels	Average of four deepest levels
Length removed in mm.	1.5	2.8	4.9	8.4	13.1	18.1		
No. of individuals	4	7	5	10	8	10		
Days 2- 5	+0.033*	+0.011*	+0.012*	+0.010*	+0.007*	+0.014*	+0.014*	+0.011*
5- 7	—0.020	—0.007	+0.002	0.000	+0.004	—0.006	—0.004	0.000
7- 9	—0.027*	—0.007	—0.014*	—0.006	0.000	0.000	—0.009*	—0.005
9-11¼	—0.013	—0.014*	—0.002	—0.007*	—0.008*	—0.007	—0.008	—0.006*
11¼-15¼	0.000	0.000	—0.004	—0.004	—0.004	—0.004	—0.003	—0.004
15¼-37	0.000	0.000	0.000	0.000	0.000	—0.001	0.000	0.000

TABLE 59

Rana clamitans Series 3676-3765 First regenerations

Percentage increment of regenerating tail per day during each time period for six different levels

Percent of tail length removed	6	10	18	31	49	67	Average of all levels
Length removed in mm.	1.5	2.8	4.9	8.4	13.1	18.1	
No. of individuals	4	7	5	10	8	10	
Days 4- 6	91	53	96	142	80	175	106
6- 8	23	19	36	32	29	30	28
8-10	5	9	8	14	19	19	12
10-12½	0	0	6	5	8	9	5
12½-18	0	—2	—1	0	2	2	0
18-56	—0	—1	+0	—0	+0	+0	0

EXPERIMENT II RANA CLAMITANS FIRST REGENERATIONS OF THE TAIL
SERIES 3676-3765

The tadpoles were collected on December 9, 1911, and the tail removals were made on January 8. Measurements were taken 4, 6, 8, 10, 12½, 18 and 56 days after the operations. The operations were at six levels approximating 6, 10, 17, 30, 48 and 62 per cent of the original tail length. For the first of these levels only two individuals with an average removal of 1.5 mm. are available, for the second five individuals with 2.6 mm., for the third three with 4.6 mm., for the fourth eight with 8.2 mm., for the fifth five with 13.0 mm. and for the sixth five with 16.7 mm. The rates of regeneration per day are given in table 60 and the graphs for the rates in Figure 56.

The specific rates are given in Table 61. Averaging these values so as to include all the different levels for each period the specific rates are 0.018 for 0 to 4 days, 0.046 for 4 to 6 days, 0.057 for 6 to 8 days, 0.037 for 8 to 10 days, 0.026 for 10 to 12½ days, 0.002 for 12½ to 18 days and —0.001 for 18 to 56 days. The graph is shown in the unbroken line in Figure 53. Using only the four deepest levels the average specific rates are respectively 0.013, 0.042, 0.045, 0.036, 0.025, 0.006 and 0.000 giving essentially the same form of curve as for the average of all levels.

The accelerations of rate are shown in Table 62 and the accelerations of specific rate in Table 63 and in the unbroken line of Figure 54. The average accelerations of rate per day are respectively +0.078, 0.000, —0.022, —0.042, —0.025 and 0.000 mm. The average accelerations of specific rate including all levels are respectively +0.011, —0.001, —0.007, —0.0075, —0.003 and 0.000 and including only the four deepest levels, +0.009, 0.000, —0.004, —0.005, —0.003 and 0.000. As for second regenerations the only plus acceleration is between 2 and 5 days and the most rapid decrease takes place between 9 and 11¼ days.

The percentage increments per day are shown in Table 64 and in the unbroken line of Figure 55. The values are respectively 98, 29, 17, 6, 1 and 0 percent per day giving approximately the same form of curve as for second regenerations.

In general the first regenerations agree with the second but on the whole the second regenerations reach their maximum earlier and are more rapid than the first up to the time of maximum rate. The first are more rapid than the second after the maximum.

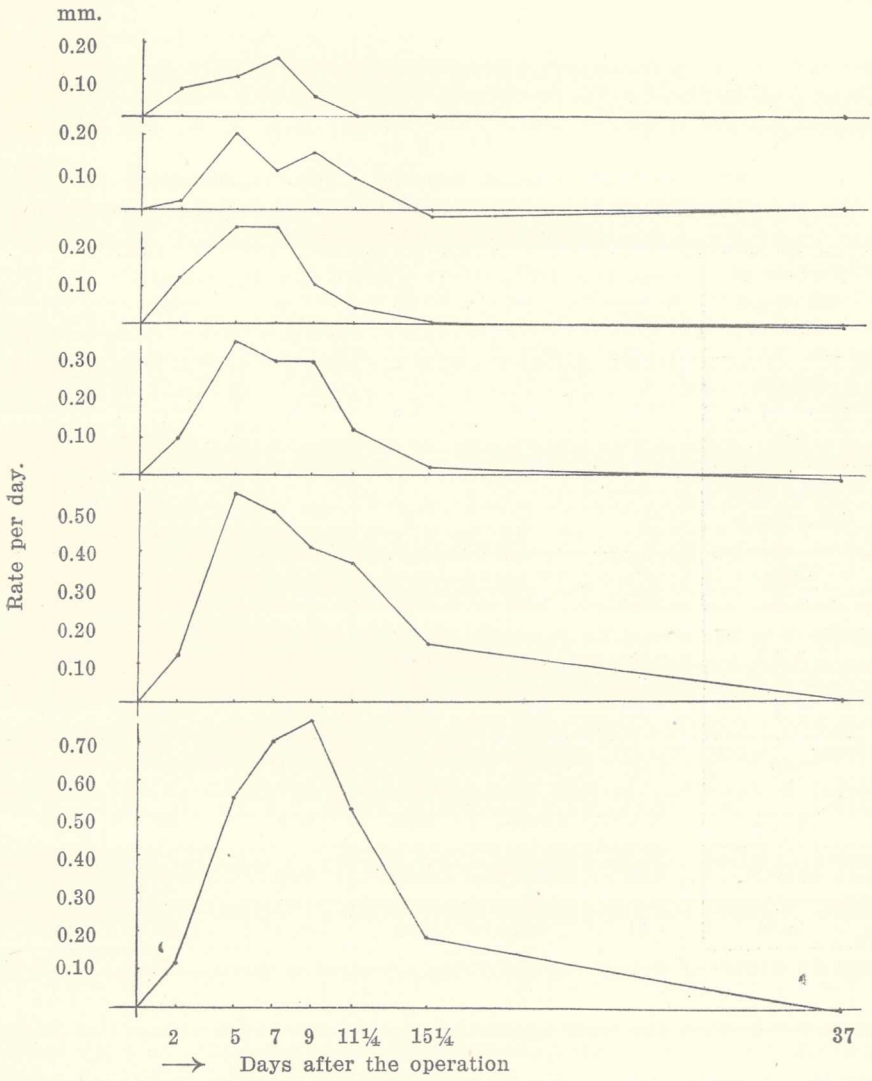

Figure 56 Rates of first regenerations of the tail per day at different times after the operation for six different levels *Rana clamitans* The removed lengths are 1.5, 2.6, 4.6, 8.2, 13.0 and 16.7 mm.

TABLE 60

Rana clamitans Series 3676-3765 First regenerations
Rate of regeneration of tail per day at different times during the regenerative process for six different levels

Percent of tail length removed	6	10	17	30	48	62
Length removed in mm.	1.5	2.6	4.6	8.2	13.0	16.7
No. of individuals	2	5	3	8	5	5
Days						
0- 4	0.07	0.02	0.12	0.10	0.12	0.12
4- 6	0.10	0.20*	0.25*	0.35*	0.55*	0.55
6- 8	0.15*	0.10	0.25*	0.30	0.50	0.70
8-10	0.05	0.15	0.10	0.30	0.40	0.75*
10-12½	0.00	0.08	0.04	0.12	0.36	0.52
12½-18	0.00	—0.02	0.00	0.02	0.15	0.18
18-56	—0.01	0.00	—0.01	—0.01	0.00	0.00

TABLE 61.

Rana clamitans Series 3676-3765 Second regenerations

Specific rates at different levels at different times

Percent of tail length removed	6	10	17	30	48	62	Average of all levels	Average of four longest removals
Length removed in mm.	1.5	2.6	4.6	8.2	13.0	16.7		
No. of individuals	2	5	3	8	5	5		
Days								
0- 4	0.042	0.015	0.027	0.012	0.007	0.007	0.018	0.013
4- 6	0.065	0.040	0.055*	0.040*	0.045*	0.030	0.046	0.042
6- 8	0.115*	0.045	0.055*	0.040*	0.040	0.045*	0.057*	0.045*
8-10	0.025	0.055*	0.020	0.035	0.045*	0.045*	0.037	0.036
10-12½	0.015	0.040	0.010	0.015	0.035	0.040	0.026	0.025
12½-18	—0.002	—0.007	0.000	0.004	0.011	0.009	0.002	0.006
18-56	—0.004	—0.001	—0.001	—0.001	0.000	0.000	—0.001	0.000

TABLE 62

Rana clamitans Series 3676-3765 First regenerations

Acceleration of rate of regeneration of tail per day at different times during the regenerative process for six different levels

Percent of tail length removed	6	10	17	30	48	62	
Length removed in mm.	1.5	2.6	4.6	8.2	13.0	16.7	Average of all levels
No. of individuals	2	5	3	8	5	5	
Middle of periods Days.							
2- 5	+0.01	+0.06*	+0.04*	+0.08*	+0.14*	+0.14*	+0.078*
5- 7	+0.02*	—0.05*	0.00	—0.02	—0.02	+0.07	0.000
7- 9	—0.05*	+0.02	—0.07*	0.00	—0.05*	+0.02	—0.022
9-11¼	—0.02	—0.03	—0.02	—0.07*	—0.02	—0.09*	—0.042*
11¼-15¼	0.00	—0.02	—0.01	—0.02	—0.04	—0.06	—0.025.
15¼-37	—0.00	0.00	—0.00	—0.00	0.00	+0.00	—0.000

TABLE 63

Rana clamitans Series 3676-3765 First regenerations
Acceleration of specific rate of regeneration of the tail

Percent of tail length removed	6	10	18	31	49	67	Average of all levels	Average of four deepest levels
Length removed in mm.	1.5	2.8	4.9	8.4	13.1	18.1		
No. of individuals	4	7	5	10	8	10		
Days 2- 5	+0.007	+0.023*	+0.009*	+0.010*	+0.011*	+0.008*	+0.011*	+0.009*
5- 7	+0.013*	—0.019*	0.000	—0.002	—0.002	+0.004	—0.001	0.000
7- 9	—0.033*	+0.008	—0.015*	0.000	—0.004*	+0.001	—0.007	—0.004
9-11¼	—0.013	—0.012	—0.004	—0.009*	—0.002	—0.005*	—0.007*	—0.005*
11¼-15¼	0.000	—0.008	—0.002	—0.002	—0.003	—0.004	—0.003	—0.003
15¼-37	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

TABLE 64

Rana clamitans Series 3676-3765 First regenerations
Percentage increment of regenerating tail per day during each time period for six different levels

Percent of tail length removed	6	10	17	30	48	62	Average of all levels
Length removed in mm.	1.5	2.6	4.6	8.2	13.0	16.7	
No. of individuals	2	5	3	8	5	5	
Days							
4- 6	33	200	50	87	110	110	98
6- 8	30	20	25	27	31	44	29
8-10	6	21	7	18	23	25	17
10-12½	0	8	2	5	9	12	6
12½-18	0	—2	0	1	3	3	1
18-56	—0	0	—0	—0	0	+0	0

EXPERIMENT III RANA CLAMITANS FIRST AND SECOND REGENERATIONS OF THE TAIL SERIES 3628-3675

For comparison with the data of experiments I and II it is of interest to note the results obtained from this entirely different series of the same species which was designed primarily for the comparison of first and second regenerations. A full description of the experiment is given in the section on the effect of successive removal upon the rate of regeneration. The data of specific value for present purposes are given in Table 65. Measurements were made only at six and at eight days after the operation. Fifty percent in length of the tail was removed in both first and second regenerations. Twenty- one individuals are available for first and sixteen for second regenerations.

The rates per day are 0.52 mm. for 6 to 8 days for first regenerations as compared with 0.50 for the same period in Experiment II and 0.62 for second regenerations as compared with 0.50 in Experiment I. The specific rate per day for 6 to 8 days for first regenerations is 0.049

and for second regenerations 0.057 as compared with 0.050 for forty eight percent removals in the first regenerations of Experiment II and 0.050 for forty nine percent removals in the second regenerations of Experiment I.

The percentage increments per day are 26 for first regenerations as compared with 29 in Experiment II and 28 for second regenerations as compared with 28 in Experiment I.

The close agreement of these values taken from a comparatively large number of individuals strengthens the conclusion as to the validity of the comparisons at different periods and levels in experiments I and II.

TABLE 65

Rana clamitans Series 3628-3765

First and second regenerations of the tail Six and eight days

	No. of individuals	Total length mm.	Tail length mm.	Percent of length removed	Length removed	Regenerated length Six days	Regenerated length Eight days	Rate per day	Specific rate	Percentage increment per day
First regeneration	21	32.7	21.4	50	10.6	2.01	3.06	0.52	0.049	26
Second regeneration	16	33.4	21.8	50	10.9	2.18	3.42	0.64	0.057	28

EXPERIMENT IV AMBLYSTOMA PUNCTATUM TAIL SERIES 4600-5052

Operations were made at five levels approximating 10, 21, 34, 53 and 74 per cent of the original tail length. The removed lengths average respectively 1.1, 2.2, 3.7, 5.8 and 8.1 mm. Measurements were made 2, 4, 6, 8-9, 10-11, 13, 14-15 and 16-17 days after the operation. The rates per day for each of the levels at each of the different times are given in Table 66.

The specific rates are shown in Table 67. The averages for all the levels at each of the time periods are respectively 0.032 mm. for 0 to 2 days, 0.004 for 2 to 4 days, 0.053 for 4 to 6 days, 0.039 for 6 to 8 days, 0.064 for 8.4 to 10.3 days, 0.043 for 10.3 to 13.0 days, 0.012 for 13.0 to 15.2 days and 0.019 for 15.2 to 17.3 days. As in the case of other salamander experiments the data are more irregular than those for the frog tadpoles because of the susceptibility of the salamander larvae to factors which have not so far been brought under control. The character of the food is probably an important factor. The greatest rate comes between 8.4 and 10.3 days after the operation for three of the five levels and also for the average of all levels. This is later than

the maximum for the frog tadpole which comes between four and six days for second regenerations and between six and eight days for first regenerations. The period of decline in rate is also more extended in these salamander larvae than in the frog tadpoles of Experiments I and II.

On account of the irregularity of the data it is not possible to study the acceleration of rate for the present data.

The percentage increments per day are given in Table 68. The values for the seven time periods are respectively 8, 71, 20, 23, 14, 4 and 7. The greatest percentage increment comes between 4 and 6 days as in the case of the frog tadpoles. An earlier period, that between two and four days is represented here. During this period the percentage increment is low. If this value can be accepted the curve here includes the very steep ascending portion discussed above. The irregularities in rate to which the salamander larvae are subject and the fact that the low value during this period does not appear in all the salamander experiments however makes the interpretation doubtful.

TABLE 66

Amblystoma punctatum Series 4600-5052

Rate of regeneration of tail per day at different times during the regenerative process for five different levels

Percent of tail removed	10	21	34	53	74
Length removed. in mm.	1.1	2.2	3.7	5.8	8.1
Days					
0-2	0.05	0.07	0.07	0.23	0.26
2-4	0.01	0.00	0.07	—0.03	—0.06
4-6	0.10*	0.16	0.16	0.14	0.27
6-8.4	0.03	0.07	0.07	0.29	0.21
8.4-10.3	0.05	—0.01	0.39*	0.43	0.37
10.3-13.0	0.10*	0.11	0.07	0.07	0.51*
13.0-15.2	0.01	0.17*	—0.17	0.18	0.09
15.2-17.3	—0.05	0.05	0.11	0.48*	0.41

TABLE 67

Amblystoma punctatum Series 4600-5052

Specific rates of regeneration of the tail at different levels at different times after the operation

Percent of tail removed	10	21	34	53	74	Average of all levels
Length removed in mm.	1.1	2.2	3.7	5.8	8.1	
Days 0-2	0.035	0.035	0.020	0.040	0.030	0.032
2-4	0.020	—0.005	0.015	—0.005	—0.005	0.004
4-6	0.095	0.070	0.045	0.025	0.030	0.053
6-8.4	0.054	0.033	0.029	0.046	0.033	0.039
8.4-10.3	0.100*	—0.011	0.105*	0.095*	0.042	0.064*
10.3-13.0	0.044	0.063	0.019	0.011	0.078*	0.043
13.0-15.2	—0.032	0.081*	—0.036	0.018	0.027	0.012
15.2-17.3	0.000	0.005	0 005	0.069	0.014	0.019

TABLE 68

Amblystoma punctatum Series 4600-5052

Percentage increment of regenerating tail per day during each time period for five different levels

Percent of tail removed	10	21	34	53	74	Average of all levels
Length removed in mm.	1.1	2.2	3.7	5.8	8.1	
Days						
2-4	10	0	50	—6	—12	8
4-6	83*	107*	53*	35	77*	71*
6-8.4	10	16	12	42*	20	20
8.4-10.3	13	—2	49	31	24	23
10.3-13.0	27	17	5	3	23	14
13.0-15.2	1	19	—10	8	3	4
15.2-17.3	—6	4	8	17	11	7

EXPERIMENT V AMBLYSTOMA PUNCTATUM TAIL SERIES 4101-4540

The experiment consists of the regenerations of removed halves of the tail without additional injury in some individuals and with an additional removal of the two forelegs in others. Measurements were made at nine periods, 2, 4, 6, 8, 10, 12, 14, 16 and 19 days after the operation. The rates of regeneration are given in Table 69. The number of individuals for most of the levels is five. The full data are discussed in the section on the effect of degree of injury. The average rate for each of the different times shows that the maximum comes during the eight to ten day period. The high value for the greater degree of injury at 14 to 16 days is due to the death during that period of the two individuals with the lowest values. The result agrees very well with the maximum rate in Experiment IV.

The percentage increments are given in Table 70. The highest value comes during the two to four day period followed by decrease with but little irregularity.

EXPERIMENT VI AMBLYSTOMA PUNCTATUM TAIL SERIES 3962-4004

First, second and third regenerations after removal of approximately one-half of the tail were studied. The complete data are given in the section on the effect of successive removals. Measurements were made at 2, 4, 6, 8, 10 and 14 days. The rates per day are given in Table 71. The maximum rate comes between 8 and 10 days agreeing with the other data for regeneration of the tail in salamander larvae.

The percentage increments are given in Table 72. The highest rate comes at the earliest period, between two and four days, and is followed by a rapid and then a slower decrease.

TABLE 69

Amblystoma punctatum Series 4101-4540

Rate of regeneration per day of tail at different times during the regenerative process for two degrees of injury

Period of regeneration Days	Middle of period Days after operation	Rate of regeneration per day for each period		Average rate
		One-half tail	One-half tail + fore-legs	
0-2	1	0.17	0.13	0.15
2-4	3	0.19	0.27	0.23
4-6	5	0.29	0.25	0.27
6-8	7	0.37	0.47	0.42
8-10	9	0.69*	0.50	0.59*
10-12	11	0.46	0.46	0.46
12-14	13	0.23	0.37	0.30
14-16	15	0.37	0.53*	0.45
16-19	17½	0.16	0.03	0.09

TABLE 70

Amblystoma punctatum Series 4101-4540

Percentage increment per day of regenerating tail at different times during the regenerative process for two degrees of injury

Days	Percentage increment per day during each period		Average
	One-half tail	One-half tail+ fore-legs	
2 to 4	54*	100*	77
4 to 6	40	31	35
6 to 8	28	36	32
8 to 10	33	22	27
10 to 12	13	14	13
12 to 14	5	9	7
14 to 16	8	11	9
16 to 19	3	0	1

TABLE 71

Amblystoma punctatum Series 4101-4540

Rate of regeneration per day at different times during the regenerative process

Period of regeneration Days	Middle of period Days after operation	Rate of regeneration per day during each period			Average
		First	Second	Third	
0-2	1	0.11	0.12	0.13	**0.12**
2-4	3	0.22	0.25	0.37	**0.28**
4-6	5	0.35	0.32	0.18	**0.28**
6-8	7	0.41	0.64*	0.66	**0.57**
8-10	9	0.68*	0.57	0.76*	**0.67***
10-14	12	0.45	0.57	0.47	**0.50**

TABLE 72

Amblystoma punctatum Series 3962-4004

Percentage increment per day at different times during the regenerative process

Days	Percentage increment per day during each period			Average
	First	Second	Third	
2 to 4	100	100	142	114*
4 to 6	53	43	18	38
6 to 8	30	45	48	41
8 to 10	31	21	28	27
10 to 14	12	15	11	13

EXPERIMENT VII AMBLYSTOMA PUNCTATUM FORELEGS
SERIES 4101-4540

The experiment consists of the study of the rate of regeneration of single completely removed fore-legs under three degrees of injury to the individual: without additional injury, with the other fore-leg removed at the same time and with the other fore-leg plus one-half of the tail removed . Measurements were made at 2, 4, 6, 8, 10, 12, 14, 16 and 19 days. The rates of regeneration are given in Table 73. The maximum rate does not come until the 14 to 16 period. The percentage increments are given in Table 74. The highest value comes during the 2 to 4 day period. There is a gradual decrease from this time.

On the whole the data for the leg regeneration show a more extended period than do the tail regenerations.

TABLE 73

Amblystoma punctatum Series 4101-4540

Rate of regeneration per day of fore-leg at different times during the regenerative process for three degrees of injury

Period of regeneration Days	Middle of period Days after operation	Rate of regeneration per day for each period			Average
		One fore-leg	Both fore-legs	Both fore-legs + one-half tail	
0-2	1	0.06	0.08	0.07	**0.07**
2-4	3	0.04	0.10	0.07	**0.07**
4-6	5	0.10	0.08	0.13	**0.10**
6-8	7	0.12	0.15	0.09	**0.12**
8-10	9	0.12	0.25	0.25	**0.21**
10-12	11	0.28	0.18	0.18	**0.21**
12-14	13	0.25	0.29	0.34	**0.29**
14-16	15	0.52*	0.41*	0.39*	**0.44***
16-19	17½	0.27	0.21	0.27	**0.25**

TABLE 74

Amblystoma punctatum Series 4101-4540

Percentage increment per day of regenerating fore-leg at different periods for three degrees of injury

Days	Percentage increment per day during each period			Average
	One fore-leg	Two fore-legs	Both fore-legs + one-half tail	
2-4	34	62*	46*	47*
4-6	45*	23	45	38
6-8	28	28	16	24
8-10	19	30	35	28
10-12	31	9	15	18
12-14	17	18	21	19
14-16	26	19	17	21
16-19	14	10	13	12

Discussion

The results obtained from the present study show that with certain material it is possible to control disturbing factors so as to get data of a sufficiently uniform nature for an analysis of the change in rate. Such material was found in the tails of the tadpoles of *Rana clamitans*. The analysis has yielded results which should be of value in a determination of the factors involved in the stimulation of growth and more particularly those concerned in slowing it down and finally bringing it to a stop. The characteristics of the change in rate have been studied by means of the curves of rate, of acceleration of rate and of percentage increments. The rate is slow at first, increases rapidly until it is near a maximum at about eight days; then decreases, at first rapidly and then more and more slowly as zero is approached. The acceleration of rate is plus only between the first two periods, i. e., up to the fifth day. After that it is minus, reaching its lowest point at ten days. The percentage increment

is very high between the first and second periods but decreases very rapidly at first and then more slowly.

It is evident that there is a close similarity between the change in rate of growth during the regeneration cycle and the change in rate during an ordinary developmental cycle and there is every reason to believe that the factors controlling the one are similar to those controlling the other. The problem of the factors is particularly interesting when it is noted that for widely different levels the rates of regeneration differ in such a way that length regenerated in a given time is proportional to the length removed. The process of regeneration apparently is initiated in a similar manner at each level but is kept under such control that only a certain per cent of the length is regenerated in a given time.

Knowledge of the process is at present insufficient to enable one to discuss with profit the nature of the control of rate of regeneration. All that can be done is to point out the relations of certain phenomena. The initial slow period is coincident with the period of cell migration without cell division, the period of rapidly increasing rate is coincident with the period of rapid cell multiplication without pronounced cell differentiation and the period of rapidly decreasing rate is associated with the appearance of pronounced differentiation in the cells. There is certainly some causal relation between these phenomena.

Summary

1. In second regenerations of the tail in *Rana clamitans* the average specific rates are 0.019 mm. for the 0 to 4 day period, 0.066 for the 4 to 6 day period, 0.051 for 6 to 8 days, 0.033 for 8 to 10 days, 0.017 for 10 to 12½ days, 0.001 for 12½ to 18 days and —0.001 for 18 to 56 days.

2. The average accelerations of rate are +0.095 mm. per day from the first to the second period, —0.015 from the second to the third, —0.030 from the third to the fourth, —0.058 from the fourth to the fifth, —0.028 from the fifth to the sixth and —0.001 from the sixth to the seventh.

3. The average percentage increments between the same periods are respectively 106, 28, 12, 5, 0 and 0.

4. The average accelerations of specific rate for the four deepest levels between the same periods are respectively +0.011 mm., 0.000, —0.005, —0.006, —0.004 and 0.000.

5. In first regenerations of the tail in *Rana clamitans* the average specific rates are 0.018 mm. for 0 to 4 days, 0.046 for 4 to 6 days, 0.057 for 6 to 8 days, 0.037 for 8 to 10 days, 0.026 for 10 to 12½ days, 0.002 for 12½ to 18 days and —0.001 for 18 to 56 days.

6. The average accelerations of rate are +0.078 mm. per day from

the first to the second period, 0.000 from the second to the third, —0.022 from the third to the fourth, —0.042 from the fourth to the fifth, —0.025 from the fifth to the sixth and 0.000 from the sixth to the seventh.

7. The average accelerations of specific rate for the four deepest levels between the same periods are respectively +0.009, 0.000, —0.004, —0.005, —0.003 and 0.000.

8. The average percentage increments between the same periods are respectively 98, 29, 17, 6, 1 and 0.

9. The experiments on salamander larvae show a similar change in rate of regeneration during the process but the number of individuals is too small to allow an analysis of the data.

10. The changes in rate that have been noted bear a definite relation to the histological changes that have been observed during the regeneration of the tail.

PART V

THE EFFECT OF DEGREE OF INJURY UPON THE RATE OF REGENERATION

In a former series of papers the writer gave the results of experiments on the effect of degree of injury upon the rate of regeneration. A number of different species of animals and various combinations of injuries were involved. The results then obtained tend on the whole to show that within certain limits the rate of regeneration from an injured surface is not retarded by simultaneous regeneration in other parts of the body. Where a difference exists between the rates with and without additional injury there is usually an advantage in favor of the part with additional injury. The differences are however often slight and in some of the cases come within the limits of probable error. It is only when the data as a whole are taken that it is possible to judge of the correctness of the general conclusion that within fairly wide limits of additional injury there is certainly no decrease in rate of regeneration but rather a tendency toward an increase.

Some additional data on these points have been obtained in connection with the present study of the factors of regeneration. On the whole they confirm the previous results. The principal experiment (Experiment I) was planned with a view to further analysis of the problem, especially the determination of the effect of additional injury to a like organ as compared with additional injury to an unlike organ.

Experiment I Amblystoma punctatum Series 4101-4540

The young were hatched on March 29-April 4, 1913, and the operations were made on May 4 and 5. The measurements of the control individuals at the time of the operations are given in Table 75. The average total length is 31.3 mm., the tail length 14.4 mm., the average length of the fore-legs 3.6 mm. and the average of the hind-legs 1.5 mm.

The measurements of control individuals at the end of the experiment on May 23 are given in Table 76. The total average length is 42.7 mm., the tail length 20.0, the average of the fore-legs 6.2 and the average of the hind-legs 4.5 mm.

The experiment consisted in the determination of the regenerated length of the right fore-leg under three degrees of injury: when the

right fore-leg alone is removed, when its mate is also removed and finally when its mate and one-half of the tail are removed. In the last two cases the average of the two fore-legs is taken as the proper value for the regeneration of a fore-leg. A large number of individuals, all hatched from the same lot of eggs, were used and a selection of larvae was made so as to make the experimental animals as nearly alike as possible in this respect. In each of the five sets an individual for each degree of injury was killed at two days after the operation, and also at four, six, eight, ten, twelve, fourteen, sixteen and nineteen days. The data are given in Tables 77 to 88. The three degrees of injury may be represented by (1) R, (2) R+L, (3) R+L+½T, in which R=right fore-leg removed, L=left fore-leg removed and ½T=one-half of the tail removed. The second involves the removal of some additional material of the same kind as that removed in the first. The third as compared with the first involves the removal of some of the same kind of material and some of another kind. In every case it is the regeneration of the fore-leg that is used as the basis of comparison.

The additional simultaneous injury and regeneration does not decrease the regeneration of the individual fore-leg. At two days the average regenerated lengths of a fore-leg are respectively 0.13, 0.16 and 0.15 mm. for the three degrees of additional injury; at four days the corresponding values are 0.22, 0.36 and 0.29; at six days 0.42, 0.53 and 0.55; at eight days 0.66, 0.83 and 0.73; at ten days 0.91, 1.34 and 1.24; at twelve days 1.48, 1.60 and 1.61; at fourteen days 1.98, 2.19 and 2.29; at sixteen days 3.02, 3.01 and 3.08; at nineteen days 3.84, 3.64 and 3.90. At only two of the nine periods is the regeneration of the fore-leg without additional injury as rapid as that of a fore-leg with additional injury and at these two times it is less rapid than one of the two other groups. In seven of the nine cases the regeneration of the fore-leg without additional injury is less than either of the two with such injury.

Among the forty individual comparisons in which all three degrees are present the degree with no additional injury has 6⅚ firsts, the degree with an additional fore-leg 15⅚ firsts and the degree with an additional fore-leg plus one-half of the tail has 17⅓ firsts. Among the nine time groups the degree with no additional injury has 1⅓ firsts and each of the additional injury combinations has 3⅚ firsts.

Taking up the lowest positions in the three degrees in the same way, among the forty individual comparisons the degree with no additional injury gives the lowest regeneration in 21⅓ cases while the additional injury combinations each have only 9⅓ lowest regenerations. Among the nine time groups the degree with no additional injury has the lowest value 6 times, the one with an additional removal of the other fore-leg

2½ times while the one with the highest degree of injury gives the lowest regeneration for the fore-leg only ½ times.

These comparisons show very clearly that the regeneration of a fore-leg is not as rapid when the individual is regenerating no other part at the same time as it is when the other fore-leg is being regenerated at the same time. The additional removal of one-half of the tail does not seem to accelerate the regeneration any further because there is no essential difference between the effect of an additional injury of a fore-leg and an additional injury of a fore-leg plus one-half of the tail. It may be that the effect of additional removal is confined to removal of a similar part, the tail removal in this case involving a different kind of organ. Or it may be that the accelerating effect is found only within certain degrees of injury the limit being exceeding by the highest of the three degrees.

TABLE 75

Amblystoma punctatum Series 4101-4540
Experiment I Controls at beginning of experiment

Date	Catalog number	Total length mm.	Tail length mm.	Fore legs			Hind legs		
				Right	Left	Av'age.	Right	Left	Av'age.
5/4/13	4110	35.0	16.4	4.0	4.0	4.0	3.0	3.1	3.05
5/4/13	4210	31.8	14.8	3.6	3.8	3.7	1.4	1.5	1.45
5/4/13	4310	28.1	11.9	3.3	3.3	3.3	1.0	0.9	0.95
5/4/13	4320	33.8	15.3	3.3	3.1	3.2	1.1	1.0	1.05
5/5/13	4410	30.2	13.8	3.6	3.6	3.6	1.0	1.0	1.0
5/5/13	4510	28.7	14.0	3.9	3.8	3.85	1.4	1.2	1.3
	Average	31.3	14.4			3.6			1.5

TABLE 76
Amblystoma punctatum Series 4101-4540
Experiment I Controls at end of experiment

Date	Catalog number	Total length mm.	Tail length mm.	Fore legs			Hind legs		
				Right	Left	Av'age.	Right	Left	Av'age.
	4120	46.7	24.3	6.1	6.1	6.1	5.0	5.0	5.0
	4130	44.7	21.7	6.5	6.6	6.55	5.2	5.1	5.15
5/23	4140	44.5	20.1	6.5	6.4	6.45	5.2	5.1	5.15
	Average	45.3	22.0			6.4			5.1
	4220	43.1	20.1	6.1	6.0	6.05	4.1	4.1	4.1
	4230	45.5	20.6	6.0	6.0	6.0	4.0	5.0	4.5
5/23	4240	43.7	20.4	6.0	5.5	5.75	4.0	4.4	4.2
	Average	44.1	20.4			5.9			4.3
	4330	47.2	21.9	7.1	7.2	7.15	5.3	5.2	5.25
5/23	4340	41.5	19.5	6.1	6.2	6.15	4.0	4.1	4.05
	Average	44.3	20.7			6.6			4.6
	4420	41.0	18.2	7.0	7.0	7.0	4.9	4.8	4.85
	4430	40.5	19.4	5.6	5.6	5.6	4.0	4.1	4.05
5/23	4440	40.4	18.9	6.0	6.0	6.0	4.1	4.0	4.05
	Average	40.6	18.8			6.2			4.3
	4520	36.5	16.0	5.6	5.6	5.6	4.0	4.0	4.0
	4530	40.9	18.5	6.7	6.8	6.75	4.9	4.8	4.85
5/23	4540	40.6	19.1	5.0	5.0	5.0	4.0	4.0	4.0
	Average	39.3	17.9			5.8			4.3
Grand average		42.7	20.0			6.2			4.5

TABLE 77

Amblystoma punctatum Series 4101-4540

Length of regenerated fore-leg in millimeters for different degrees of injury

Two days

Catalog number	Degree of injury		
	One fore-leg	Both fore-legs	Both fore-legs + one-half tail
4101-11-21	0.10	0.22	0.22
4201-11-21	0.10	0.15*	0.11
4301-11-21	0.10	0.15	0.17*
4401-11-21	0.20	0.17	0.20
4501-11-21	0.15*	0.10	0.07
Average	**0.13**	**0.16***	**0.15**

TABLE 78

Amblystoma punctatum Series 4101-4540

Length of regenerated fore-leg in millimeters for different degrees of injury

Four days

Catalog number	Degree of injury		
	One fore-leg	Both fore-legs	Both fore-legs + one-half tail
4102-12-22	0.25	0.25	0.52
4202-12-22	—	0.52	0.22
4302-12-22	0.15	0.37*	0.22
4402-12-22	0.40*	0.30	0.27
4502-12-22	0.10	—	0.20
Average	**0.22**	**0.36***	**0.29**

TABLE 79

Amblystoma punctatum Series 4101-4540

Length of regenerated fore-leg in millimeters for different degrees of injury

Six days

Catalog number	Degree of injury		
	One fore-leg	Both fore-legs	Both fore-legs + one-half tail
4103-13-23	0.40	0.20	0.92*
4203-13-23	0.50	0.87*	0.52
4303-13-23	0.45	0.65*	0.42
4403-13-23	0.45	0.60*	0.47
4503-13-33	0.30	0.35	0.42*
Average	**0.42**	**0.53**	**0.55***

TABLE 80

Amblystoma punctatum Series 4101-4540

Length of regenerated fore-leg in millimeters for different degrees of injury.

Eight days

Catalog number	Degree of injury		
	One fore-leg	Both fore-legs	Both fore-legs + one-half tail
4104-14-24	0.50	0.75	0.97*
4204-14-24	0.80	0.80	0.80
4304-14-24	0.85	0.87*	0.62
4404-14-24	0.43	0.95*	0.75
4504-14-24	0.70	0.80*	0.52
Average	**0.66**	**0.83***	**0.89***

TABLE 81

Amblystoma punctatum Series 4101-4540

Length of regenerated fore-leg in millimeters for different degrees of injury

Ten days

Catalog number	Degree of injury		
	One fore-leg	Both fore-legs	Both fore-legs + one-half tail
4105-15-25	0.25	1.82*	1.60
4205-15-25	0.95	1.10*	1.07
4305-15-25	1.05	1.22	1.32*
4405-15-25	1.20	1.37*	1.07
4505-15-25	1.10	1.20*	1.12
Average	**0.91**	**1.34***	**1.24**

TABLE 82

Amblystoma punctatum Series 4101-4540

Length of regenerated fore-leg in millimeters for different degrees of injury

Twelve days

Catalog number	Degree of injury		
	One fore-leg	Both fore-legs	Both fore-legs + one-half tail
4106-16-26	1.45	1.50	1.77*
4206-16-26	1.35	1.47*	1.44
4306-16-26	1.80*	1.60	1.65
4406-16-26	1.00	1.70*	1.50
4506-16-26	1.80*	1.72	1.67
Average	**1.48**	**1.60**	**1.61***

TABLE 83

Amblystoma punctatum Series 4101-4540

Length of regenerated fore-leg in millimeters for different degrees of injury. Fourteen days

Catalog number	Degree of injury		
	One fore-leg	Both fore-legs	Both fore-legs + one-half tail
4107-17-27	2.60	—	2.25
4207-17-27	1.70	1.97	2.22*
4307-17-27	1.45	1.87	1.95*
4407-17-27	2.25	2.72	2.90*
4507-17-27	1.90	—	2.12
Average	**1.98**	**2.19**	**2.29***

TABLE 84

Amblystoma punctatum Series 4101-4540

Length of regenerated fore-leg in millimeters for different degrees of injury Sixteen days

Catalog number	Degree of injury		
	One fore-leg	Both fore-legs	Both fore-legs + one-half tail
4108-18-28	2.60	2.60	2.70*
4208-18-28	2.40	2.62*	2.22
4308-18-28	2.80	2.67	2.85*
4408-18-28	3.60	3.57	3.65*
4508-18-28	3.70	3.57	3.97*
Average	**3.02**	**3.01**	**3.08***

TABLE 85

Amblystoma punctatum Series 4101-4540

Length of regenerated fore-leg in millimeters for different degrees of injury

Nineteen days

Catalog number	Degree of injury		
	One fore-leg	Both fore-legs	Both fore-legs + one-half tail
4109-19-29	4.00*	3.72	3.95
4209-19-29	3.65	4.05	4.25*
4309-19-29	3.60	2.85	3.60
4409-19-29	4.10*	3.95	3.80
Average	**3.84**	**3.64**	**3.90***

TABLE 86

Amblystoma punctatum Series 4101-4540

Length of regenerated fore-leg in millimeters for different degrees of injury

Summary Two to nineteen days

Days	Degree of injury		
	One fore-leg	Both fore-legs	Both fore-legs + one-half tail
2	0.13	0.16*	0.15
4	0.22	0.36*	0.29
6	0.42	0.53	0.55*
8	0.66	0.83*	0.73
10	0.91	1.34*	1.24
12	1.48	1.60	1.61*
14	1.98	2.19	2.29*
16	3.02	3.01	3.08*
19	3.84	3.64	3.90*
Groups first	0	4	5
Groups last	7	2	0

TABLE 87

Amblystoma punctatum Series 4101-4540

Length of regenerated fore-leg for different degrees of injury

Tabulation of firsts for individual comparisons

Days	Injury		
	One fore-leg	Both fore-legs	Both fore-legs + one-half tail
2	1½	1½	2*
4	1	1	1
6	0	3*	2
8	⅓	3⅓*	1⅓
10	0	4*	1
12	2	2	1
14	0	0	3*
16	0	1	4*
19	2	0	2
Total firsts	6⅚	15⅚	17⅓
Groups first	1⅓	3⅚	3⅚

TABLE 88

Amblystoma punctatum Series 4101-4540

Length of regenerated fore-leg for different degrees of injury

Tabulation of lowest values for individual comparisons

Days	Injury		
	One fore-leg	Both fore-legs	Both fore-legs + one-half tail
2	3	1	1
4	1½	1½	1
6	3	1	1
8	2⅓	⅓	2⅓
10	4	0	1
12	3	1	1
14	3	0	0
16	½	3½	1
19	1	2	1
Total lasts	21⅓	9⅓	9⅓
Groups last	6	2½	½

EXPERIMENT II AMBLYSTOMA PUNCTATUM SERIES 4101-4540

This experiment deals with the same series of individuals as Experiment I. The comparison in this case however is one between the regeneration of the removed half of the tail when it alone is removed and its regeneration when there is an additional removal of the two fore-legs. The data are given in Tables 89 to 99. At two days the regeneration of the tail without an additional injury is 0.35 mm. and with an additional injury 0.27. The corresponding values at 4 days are 0.73 and 0.81, at 6 days 1.32 and 1.31, at 8 days 2.06 and 2.26, at ten days 3.44 and 3.27, at twelve days 4.36 and 4.20, at fourteen days 4.82 and 4.94, at sixteen days 5.57 and 6.00 and at nineteen days 5.90 and 6.06. The regenerating tail with no additional injury is ahead at four times and the one with additional injury is ahead five times. In thirty three individual com-

parisons the group with no additional injury is ahead seventeen times and the additional injury group sixteen times. Taking the individual cases by time groups the individuals with no additional injury are ahead 5½ times and those with an additional injury 3½ times.

These comparisons show no advantage of one combination over the other. The additional removal of the fore-legs does not retard nor does it accelerate the regeneration of the tail. This result strengthens the view that the acceleration in Experiment I is probably due to the additional removal of material similar to that whose rate is being studied.

TABLE 89

Amblystoma punctatum Series 4101-4540

Length of regenerated tail in millimeters for different degrees of injury

Two days

Catalog number	Degree of injury	
	One-half tail	One-half tail + fore-legs
4131-21	0.55*	0.15
4231-21	—	0.35
4331-21	0.35*	0.25
4431-21	0.30	0.30
4531-21	0.20	0.30*
Average	**0.35***	**0.27**

TABLE 90

Amblystoma punctatum Series 4101-4540

Length of regenerated tail in millimeters for different degrees of injury

Four days

Catalog number	Degree of injury	
	One-half tail	One-half tail + fore-legs
4132-22	0.45	1.00*
4232-22	0.90	0.90
4332-22	0.50	0.60*
4432-22	1.10*	0.95
4532-22	0.70*	0.60
Average	**0.73**	**0.81***

TABLE 91

Amblystoma punctatum Series 4101-4540

Length of regenerated tail in millimeters for different degrees of injury

Six days

Catalog number	Degree of injury	
	One-half tail	One-half tail + fore-legs
4133-23	1.60	——
4233-23	0.90	1.00*
4333-23	1.70*	1.65
4433-23	1.10	1.50*
4533-23	——	1.10
Average	1.32*	1.31

TABLE 92

Amblystoma punctatum Series 4101-4540

Length of regenerated tail in millimeters for different degrees of injury

Eight days

Catalog number	Degree of injury	
	One-half tail	One-half tail + fore-legs
4134-24	2.40	2.60*
4234-24	1.80	1.90*
4334-24	1.80	2.26*
4434-24	2.70*	2.30
4534-24	1.60	——
Average	2.06	2.26*

TABLE 93

Amblystoma punctatum Series 4101-4540

Length of regenerated tail in millimeters for different degrees of injury

Ten days

Catalog number	Degree of injury	
	One-half tail	One-half tail + fore-legs
4135-25	3.65*	3.20
4235-25	——	2.55
4335-25	3.20	1.46
4435-25	3.65*	3.20
4535-25	3.25	4.15*
Average	**3.44***	**3.27**

TABLE 94

Amblystoma punctatum Series 4101-4540

Length of regenerated tail in millimeters for different degrees of injury

Twelve days

Catalog number	Degree of injury	
	One-half tail	One-half tail + fore-legs
4136-26	4.10	4.20*
4236-26	4.70*	3.55
4336-26	4.20*	3.50
4436-26	4.60*	4.50
4536-26	4.20	5.25*
Average	**4.36***	**4.20**

TABLE 95

Amblystoma punctatum Series 4101-4540

Length of regenerated tail in millimeters for different degrees of injury

Fourteen days

Catalog number	Degree of injury	
	One-half tail	One-half tail + fore-legs
4137-27	4.80	6.00*
4237-27	4.90*	4.70
4337-27	5.00*	4.95
4437-27	4.90*	4.00
4537-27	4.50	5.05*
Average	**4.82**	**4.94***

TABLE 96

Amblystoma punctatum Series 4101-4540

Length of regenerated tail in millimeters for different degrees of injury

Sixteen days

Catalog number	Degree of injury	
	One-half tail	One-half tail + fore-legs
4138-28	6.50*	5.50
4238-28	5.80	——
4338-28	5.00	6.40*
4438-28	5.00	——
4538-28	8.00	6.10
Average	**5.57**	**6.00***

TABLE 97

Amblystoma punctatum Series 4101-4540
Length of regenerated tail in millimeters for different degrees of injury
Nineteen days

Catalog number	Degree of injury	
	One-half tail	One-half tail
4139-29	6.90*	5.90
4239-29	3.20	6.20
4339-29	——	5.55
4439-29	4.90	6.60*
Average	**5.90**	**6.06***

TABLE 98

Amblystoma punctatum Series 4101-4540
Length of regenerated tail in millimeters for different degrees of injury
Summary Two to nineteen days

Days	Degree of injury	
	One-half tail	One-half tail + fore-legs
2	0.35*	0.27
4	0.73	0.81*
6	1.32*	1.31
8	2.06	2.26*
10	3.44*	3.27
12	4.36*	4.20
14	4.82	4.94*
16	5.57	6.00*
19	5.90	6.06*
Groups first	4	5

TABLE 99

Amblystoma punctatum Series 4101-4540
Length of regenerated tail for different degrees of injury
Tabulation of firsts for individual comparisons

Days	Injury	
	One-half tail	One-half tail + fore-legs
2	2½*	1½
4	2½	2½
6	1	2*
8	1	3*
10	2*	1
12	3*	2
14	3*	2
16	1	1
19	1	1
Total firsts	**17**	**16**
Groups first	5½	3½

EXPERIMENT III AMBLYSTOMA PUNCTATUM SERIES 4005-4008

Experiments III, IV, V and VI comprise merely a few individual comparisons obtained from experiments devised principally for the study of other factors. They are included here under the rule that no valid data on the matter at hand are to be excluded.

In Experiment III the regeneration of the hind-leg is compared under the four conditions of (1) no additional injury, (2) removal of the other hind-leg, (3) removal of the other hind-leg and one fore-leg and (4) removal of the other hind-leg and both fore-legs. The data are given in Table 100.

Three sets of comparisons were made at twelve days after the operations, each with a single individual for each degree of injury. The regenerating hind-leg with no additional injury is distinctly behind the cases with additional injury. The greatest regenerated length comes in one case with an additional injury of one hind-leg plus one fore-leg and in two cases with one hind-leg plus two fore-legs. The averages begin-

ning with the lowest degree of injury are respectively 1.50, 1.73, 1.86 and 1.88 mm.

The additional removals are in every case removals of leg material and the result agrees with that of experiment I in giving an increased rate of regeneration of a part when similar organs are removed at the same time.

TABLE 100

Amblystoma punctatum Series 4005-4008

Length of regenerated hind leg in millimeters for different degrees of injury

Twelve days

Catalog number	Degree of injury			
	One hind-leg	Both hind-legs	Both hind-legs+one fore-leg	Both hind-legs+both fore-legs
4005	1.35	1.90	1.95*	1.75
4006	1.65	1.80	1.82	1.92*
4008	1.50	1.50	1.80	1.85*
Average	**1.50**	**1.73**	**1.86***	**1.84**

TABLE 101

Amblystoma punctatum Series 4005-4008

Length of regenerated fore-leg in millimeters for different degrees of injury

Twelve days

Catalog number	Degree of injury	
	One fore-leg + both hind-legs	Both fore-legs + both hind-legs
4005	3.0*	2.8
4006	3.1*	3.0
4008	3.0	3.15*
Average	**3.07***	**2.98**

EXPERIMENT IV AMBLYSTOMA PUNCTATUM SERIES 4005-4008

In this experiment the regeneration of the right fore-leg is compared under conditions of differing degrees of additional injury. In one combination there is an additional removal of the two hind legs and in the

other of both hind-legs plus the remaining fore-leg. The data are given in Table 101. In two of the three cases the smaller additional degree of injury shows the greater regeneration of the fore-leg. The average is 3.07 mm. for the lesser degree and 2.98 for the greater degree, an advantage in favor of the lesser degree.

It should be noted that this is not strictly comparable with the main issue of Experiments I, II and III. Aside from the small number of cases it is a comparison between two degrees of injury each of which is of considerable extent. It may be that the removal of three of the four legs is near the degree of injury yielding the maximum rate for each removed leg.

Experiment V Amblystoma punctatum Series 4010-4025

A comparison is made between the regeneration of a half of the tail when it alone is removed and when both fore-legs are removed at the same time. Four individual comparisons are made at fourteen days. The data are given in Table 102. The regenerated lengths and specific lengths regenerated are ahead in two of the four cases for each of the degrees of injury. The average regenerated length with no additional injury is 5.1 mm. and with additional injury 5.0 mm. The specific regenerated length is 0.65 with no additional injury and 0.68 with addi-

TABLE 102
Amblystoma punctatum Series 4010-4025
Regeneration of tail for different degrees of injury
Fourteen days

	Degree of injury					
	One-half tail			One-half tail + both fore-legs		
Catalog number	Length removed	Length regenerated	Specific amt. regenerated	Length removed	Length regenerated	Specific amt. regenerated
4014-13	7.7	4.9	0.64	7.0	5.2*	0.74*
4018-17	8.8	5.2*	0.59*	8.0	4.3	0.54
4022-21	8.0	5.3*	0.66*	8.0	5.1	0.64
4025-24	7.0	4.9	0.70	6.6	5.3*	0.80*
Average		**5.1**	**0.65**		**5.0**	**0.68**

tional injury. The data show essential equality between the rates of regeneration under the two conditions of the experiment. This agrees with the data in Experiments I and II which show no increase or decrease in rate of regeneration when unlike material is removed simultaneously with the removal of the organ whose rate is being studied.

EXPERIMENT VI AMBLYSTOMA PUNCTATUM SERIES 4010-4025

Three individual comparisons were made at fourteen days of the right fore-leg, when it alone is removed, when the other fore-leg is also removed and when the other fore-leg plus one half of the tail is removed. The data are given in Table 103. In two of the three cases the individuals

TABLE 103

Amblystoma punctatum Series 4010-4025

Length of regenerated fore-leg in millimeters for different degrees of injury

Fourteen days

Catalog number	Degree of injury		
	One fore-leg	Both fore-legs	Both fore-legs + one-half tail
4011, 12, 13	2.00*	1.77	1.65
4015, 16, 17	2.00*	1.60	1.80
4019, 20, 21	1.95	1.82	2.22*
4023, —, 24	2.00	——	2.00
Average	1.99*	1.73	1.92

with no additional regeneration are ahead of the others. The greater injury gives the greater rate in one of the three. The average regenerated lengths beginning with the lowest degree of injury are respectively 1.99, 1.73 and 1.92 mm. The few cases may be a sufficient explanation of the lack of agreement with the more extended series of Experiment I.

DISCUSSION

The experiments as a whole show that a part regenerates slightly more rapidly when additional material of the same kind is removed than when the part alone is removed. Simultaneous removal of tail material does not accelerate the regeneration of a leg nor does simultaneous removal of a leg accelerate the regeneration of the tail. The rate in these cases however is not decreased by the additional injury. The state-

ment may therefore be made that within limits the regeneration of a part is not retarded by simultaneous removal and regeneration of material in other parts of the body. When this additional material is of the same kind as that whose rate is being studied there may even be an acceleration of regeneration.

In comparison with such a factor as level of the cut this difference in rate is slight and no such quantitative relation as in that case can be made out. It must however be considered that the principal object of the original experiments was to show that additional injury within the given limits tends to *increase rather than decrease* the rate of regeneration. This has been proved for these experiments. The evidence in favor of a definite increase in rate with any certain increase in degree of injury is not so conclusive. It is obvious that in many series of experiments factors whose influence is greater than that of the factor under discussion may obscure the result.

Emphasis should again be placed on the fact that all data obtained by the writer are included. That some of the series, especially those with a few individuals, diverge from the general result is to be expected by anyone in similar work who has attempted to eliminate entirely all of the factors except the one under observation at a particular time.

Summary

1. A comparison was made of the rate of regeneration of a leg or of the tail of an Amblystoma larva when the part alone is removed with its rate when similar or dissimilar parts of the individual are removed at the same time. The data are derived from two principal Experiments, I and II, and from a few scattered observations listed as Experiments III to VI.

2. In Experiment I a comparison was made of the rate of regeneration of the right fore-leg when it alone is removed with its rate when the other fore-leg is removed at the same time and when the other fore-leg and one half of the tail are removed. The result obtained from forty individual comparisons made at different times shows that the rate of regeneration of the right fore-leg in each of the series with additional injury is greater than in the series without additional injury.

3. The rate of regeneration of a right fore-leg when its mate plus one-half of the tail is removed is not essentially different from the rate when its mate alone is removed. The addition of the injury to a dissimilar organ, the tail, does not alter the rate of regeneration of the fore-legs.

4. In Experiment II it is shown that there is no significant difference between the rate of regeneration of a tail one-half of which has been

removed without additional injury to the individual and the rate after the same injury plus a removal of both fore-legs.

5. The data of Experiments III to VI show some departures from the general rule probably because they deal with few individuals. On the whole however they bear out the results obtained from the principal experiments.

PART VI

THE COMPLETENESS OF REGENERATION

One of the striking facts in connection with amphibian regeneration as made out in the present studies is the lack of completeness of the process. When a part of the tail is removed the lost part is never completely restored. Data on this problem are to be found in a number of sets of experiments one of which (Experiment V) was devised especially for the present purpose.

Experiment I Rana clamitans Series 3557-3624

One-half of the tail was removed in the individuals of three groups, A, B and C. After 35 to 39 days, which was sufficiently long so that regeneration had stopped, another removal was made and so on until each individual had undergone five regenerations. The data are given in Table 104. The average removed length as estimated from the measurement of a few individuals was 17.0 mm. The average length of the completed first regeneration is 8.6 mm. or 51 per cent of the removed length, of the second regeneration 8.0 mm. or 53 per cent, of the third 7.5 mm. or 51 per cent, of the fourth 5.5 mm. or 42 per cent and of the fifth 6.4 mm. of 45 per cent. On the average about one-half of the removed length is replaced when one-half of the tail length is removed.

Experiment II Rana clamitans Series 3628-3675

One-half of the tail length was removed in the individuals of this experiment and regeneration was allowed to proceed for twenty days, a sufficient time for bringing it to a stop. The data are given in Table 105. The average original tail length was 21.8 mm., of the removed length 10.6 mm. and of the regenerated length 5.4 mm. The completed regenerated length is thus 51 per cent of the removed length.

Experiment III Rana clamitans First regenerations Series 3676-3765

The data are given in Table 106. The tails were removed at different levels approximating 6, 10, 17, 30, 48 and 62 per cent of the tail lengths. Regeneration was completed at these levels at 12½, 12½, 12½, 18, 18

and 56 days respectively. The regenerated lengths at these times of completion are respectively 61, 46, 39, 33, 42 and 41 per cent of the removed lengths. It will be noted that the two shortest removals give the highest per cents and the two medium ones the lowest per cents. This difference is discussed in Part III on the effect of level of the cut.

Experiment IV Rana clamitans Second regenerations

Series 3676-3765

The data are given in Table 107. The tail was removed at different levels approximating 6, 10, 18, 31, 49 and 67 per cent of the removed lengths. Regeneration was completed for these levels at 10, 10, 12½,

TABLE 104

Rana clamitans Series 3557-3624 Completeness of regeneration Successive regenerations in single individuals One-half of tail removed = 17 mm. on the average First operation Oct. 23, 1911 Second operation Groups A and B Nov. 18 Group C Nov. 28

	Catalog number	First regeneration	Second regeneration	Third regeneration	Fourth regeneration	Fifth regeneration
		Nov. 28	Jan. 3	Feb. 9	Mar. 16	April 24
Group A	3564		9.5	8.5	—	—
	3565		9.8	11.4	7.3	8.2
	3566		10.0	9.3	8.1	6.3
	3567		11.9	9.5	8.0	11.9
	3568		8.4	9.9	11.0	8.5
	3569		10.0	8.7	8.0	8.1
	3570		8.7	8.1	—	—
	Average		9.8	9.3	8.5	8.6
Group B	3578		8.3	9.0	5.8	8.2
	3579		8.2	8.1	11.3	7.0
	3580		11.9	7.3	6.9	8.1
	3581		9.7	8.0	7.6	11.6
	3582		8.3	12.8	7.4	5.4
	3583		8.8	7.4	5.0	6.8
	3584		8.8	9.5	6.4	—
	Average		9.1	8.9	7.2	7.8

TABLE 104 (Continued)

	Catalog number	First regeneration	Second regeneration	Third regeneration	Fourth regeneration	Fifth regeneration
		Nov. 28	Jan. 3	Feb. 9	Mar. 16	April 24
	3586	8.1	—	—	—	—
	3588	6.1	7.5	7.3	5.7	7.2
	3590	8.5	6.6	7.5	5.1	6.5
	3592	7.1	8.0	5.7	4.9	6.5
	3594	8.6	7.8	2.0	5.0	6.1
	3596	9.0	8.6	9.4	6.7	6.8
	3598	10.7	9.7	9.3	6.1	6.2
	3600	8.2	8.0	6.9	5.8	5.9
	3602	9.9	7.7	6.8	4.4	4.7
Group	3604	9.6	7.6	6.6	4.9	5.7
C	3606	7.4	7.8	8.0	5.0	5.9
	3608	9.0	8.0	9.0	5.5	6.9
	3610	8.5	8.9	8.3	5.4	7.1
	3612	7.4	7.0	7.1	4.8	5.5
	3614	8.3	6.6	6.2	4.5	5.2
	3616	8.0	8.3	7.9	6.1	7.9
	3618	9.7	9.5	9.7	7.3	8.0
	3619	—	8.0	7.5	6.6	6.1
	3622	10.2	8.5	—	—	—
	3624	9.3	7.5	—	—	—
	Average	8.6	8.0	7.5	5.5	6.4
Percent of removed length regen. Av.		51	53	51	42	45

12½, 56 and 56 days respectively. The regenerated lengths at these times of completion are respectively 67, 46, 33, 31, 40 and 39 per cent of the removed lengths. As in the case of the first regenerations the two shortest removals give the highest per cent of regeneration and the two medium removals the lowest per cent.

EXPERIMENT V AMBLYSTOMA PUNCTATUM SERIES 6212-6281

The experiments on tadpoles of *Rana clamitans* having shown that only a half or less of the removed length on the average is completed during regeneration it became a matter of interest to see if this might not have been due to the age of the tadpoles, which were obtained in the fall. Accordingly a series of Amblystoma larvae was operated upon within a

few days after they had left the egg envelopes and was kept until the salamanders were well advanced in their metamorphosis. Since in young salamander larvae the border line between old and regenerated tissue is soon obliterated it became necessary to devise another method of testing completeness of regeneration than the direct measurement of the regen-

TABLE 105

Rana clamitans Series 3628-3675

Tail length	Removed length	Regenerated length Twenty days
24.1	13.1	5.9
24.6	13.2	5.2
22.1	11.0	4.9
23.2	11.1	5.5
23.1	11.7	5.9
25.0	12.5	5.8
20.4	9.9	5.6
20.8	10.0	5.2
29.2	15.5	5.9
23.8	10.5	5.6
23.3	10.9	5.6
25.6	10.9	5.9
20.8	10.1	4.7
19.2	9.8	4.6
21.1	11.5	5.5
22.0	11.8	6.1
17.0	8.2	5.6
19.0	9.7	5.5
22.4	9.8	4.3
19.8	10.1	5.2
20.8	8.4	5.1
21.8	9.1	5.0
15.4	7.3	6.0
18.1	9.0	6.0
21.8	10.6	5.4
Percent removed	49	
Percent of removed part regenerated		51

TABLE 106

Rana clamitans Series 3676-3765 First regenerations

Number of cases	Percent of tail length removed Average	Tail length removed in mm. Average	Average maximum regeneration in percent of removed length	Average maximum regeneration in mm.	Days after operation when maximum is reached Average
2	6	1.5	61	0.9	12½
5	10	2.6	46	1.2	12½
3	17	4.6	39	1.8	12½
8	30	8.2	33	2.7	18
5	48	13.0	42	5.5	18
5	62	16.7	41	6.9	56

TABLE 107

Rana clamitans Series 3676-3765 Second regenerations

Number of cases	Percent of tail length removed Average	Tail length removed in mm. Average	Average maximum regeneration in percent of removed length	Average maximum regeneration in mm.	Days after operation when maximum is reached Average
4	6	1.5	67	1.0	10
7	10	2.8	46	1.3	10
5	18	4.9	33	1.6	12½
10	31	8.4	31	2.6	12½
8	49	13.1	40	5.2	56
10	67	18.1	39	7.1	56

erated material. This consisted in a comparison of the ratio between tail length and body length in the operated individuals with that in control unoperated individuals. This was done after regeneration had been going on during the whole larval period. If the $\frac{\text{tail}}{\text{body}}$ period is the same in operated as in unoperated individuals it is proper to suppose that regeneration has been complete. If however the ratio is lower the conclusion that regeneration is incomplete is very probably correct though absolute certainty can not be assumed because of the possibility of the changed ratio being due to regulatory changes in other parts of the individual.

The experiment consists of a comparison of the relative degree of completeness of regeneration of the tail in four groups, (1) with no operation, (2) with one-fourth of the tail removed, (3) with one-half of the tail removed and (4) with three-fourths removed. The operations were made as soon as possible after the animals left the egg envelopes and the experiment proceeded until all four legs were well developed and absorption of the gills had begun. This allowed practically the entire larval period for regeneration. There were seventy individuals at the start but a high mortality reduced the number very considerably. Limnodrilus was used as food.

The data are given in Tables 108 to 112. The average ratio between tail and body length in control individuals at the end of the experiment is 1.09, in individuals with one-fourth of the tail removed it is 1.01, in those with one-half removed 0.93 and with three-fourths removed 0.86. This progressive relative decrease in the tail length as compared with the body length is very probably due to lack of completeness of regeneration even though the whole larval period has been allowed for such completion.

Discussion

Apart from the starting stimulus in regeneration the most interesting problem is undoubtedly that of the stopping stimulus. With the growth once started what are the factors involved in checking it? In general it has been assumed that regeneration goes on until the removed organ is entirely replaced and that over- and under-regeneration occur but rarely. The present data make it probable that incompleteness is more general than has been supposed. The factors at work in bringing regeneration to a close tend to overdo rather than underdo their function.

A further investigation of the problem of completeness of regeneration would be of interest.

TABLE 108

Amblystoma punctatum Series 6212-6281 Controls

	Living lengths Beginning of experiment April 19 1915				Killed lengths End of experiment June 19 1915			
Catalog number	Total length	Tail length	Body length	Ratio, $\frac{\text{Tail}}{\text{Body}}$	Total length	Tail length	Body length	Ratio, $\frac{\text{Tail}}{\text{Body}}$
6218	14.1	6.3	7.8	0.81	27.8	14.5	13.3	1.09
6222	13.7	6.0	7.7	0.78	27.2	14.4	12.8	1.12
6223	13.7	6.0	7.7	0.78	32.4	16.9	15.5	1.09
6233	15.0	6.7	8.3	0.81	33.2	17.4	15.8	1.10
6237	14.3	6.0	8.3	0.72	30.2	15.8	14.4	1.10
6243	13.2	5.4	7.8	0.69	31.4	16.3	15.1	1.08
6247	14.6	6.4	8.2	0.78	27.9	14.9	13.0	1.15
6262	14.9	6.3	8.6	0.73	31.3	16.4	14.9	1.10
6268	15.3	7.0	8.3	0.84	30.5	15.3	15.2	1.01
Average	**14.3**	**6.2**	**8.1**	**0.77**	**30.2**	**15.8**	**14.4**	**1.09**

TABLE 109

Amblystoma punctatum Series 6212-6281 One-fourth of tail removed

	Living lengths Beginning of experiment April 19 1915						Killed lengths End of experiment June 19 1915			
Catalog number	Total length	Tail length	Body length	Tail / Body =	Rem'v'd length	Rem'v'd / Tail =	Total length	Tail length	Body length	Tail / Body =
6234	14.4	6.2	8.2	0.76	1.7	0.27	32.0	16.8	15.2	1.11
6244	13.6	6.0	7.6	0.79	1.7	0.28	26.9	13.3	13.6	0.98
6249	13.3	5.9	7.4	0.80	1.6	0.27	28.5	14.5	14.0	1.04
6265	15.0	6.5	8.5	0.76	2.0	0.31	27.7	13.3	14.4	0.92
Average	14.1	6.1	7.9	0.78	1.7	0.28	28.8	14.5	14.3	1.01

TABLE 110

Amblystoma punctatum Series 6212-6281 One-half of tail removed

	Living lengths Beginning of experiment April 19 1915						Killed lengths End of experiment June 19 1915			
Catalog number	Total length	Tail length	Body length	Tail / Body =	Rem'v'd length	Rem'v'd / Tail =	Total length	Tail length	Body length	Tail / Body =
6239	14.3	6.3	8.0	0.79	2.8	0.44	28.5	14.5	14.0	1.04
6250	14.3	6.2	8.3	0.75	3.7	0.60	23.1	10.7	12.4	0.86
6255	14.6	6.8	7.8	0.87	3.2	0.47	28.7	14.0	14.7	0.95
6264	15.4	7.2	8.2	0.88	3.5	0.49	24.7	11.5	13.2	0.87
Average	14.7	6.6	8.1	0.81	3.3	0.50	26.2	12.7	13.6	0.93

TABLE 111

Amblystoma punctatum Series 6212-6281 Three fourths of tail removed

	Living lengths Beginning of experiment April 19 1915						Killed lengths End of experiment June 19 1915			
Catalog number	Total length	Tail length	Body length	Tail / Body =	Rem'v'd length	Rem'v'd / Tail =	Total length	Tail length	Body length	Tail / Body =
6216	14.1	6.4	7.7	0.83	4.9	0.77	29.3	14.1	15.2	0.93
6221	13.8	6.1	7.7	0.79	4.3	0.70	16.1	7.0	9.1	0.77
6246	14.1	6.1	8.0	0.76	4.6	0.74	25.5	11.9	13.6	0.87
Average	14.0	6.2	7.8	0.79	4.6	0.74	23.6	11.0	12.6	0.86

TABLE 112

Amblystoma punctatum Series 6212-6281 Summary of averages

	Living lengths Beginning of experiment April 19 1915						Killed lengths End of experiment June 19 1915			
Character of operation	Total length	Tail length	Body length	Tail / Body =	Rem'v'd length	Rem'v'd / Tail =	Total length	Tail length	Body length	Tail / Body =
Control	14.3	6.2	8.1	0.77	—	—	30.2	15.8	14.4	1.09
One-fourth tail...	14.1	6.1	7.9	0.78	1.7	0.28	28.8	14.5	14.3	1.01
One-half tail	14.7	6.6	8.1	0.81	3.3	0.50	26.2	12.7	13.6	0.93
Three-fourts tail.	14.0	6.2	7.8	0.79	4.6	0.74	23.6	11.0	12.6	0.86

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